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POSTERS

1. Management and Organisation

1.1 Organisational Issues

P-001

A STUDY OF RELATIONSHIP AMONG INTERNAL MARKETING, ORGANIZATIONAL IDENTIFICATION AND ORGANIZATIONAL CITIZENSHIP BEHAVIORS IN NON-PROFIT ORGANIZATIONS-THE STAFFS OF TAINAN BLOOD CENTER AS AN EXAMPLE

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Background: The Non-Profit Organization can provide entirely flawless service through employees supporting each other. Therefore, the implementation of internal marketing is important for relying on the cohesion of the mission-oriented Non-Profit Organization.
Aims: The purpose of this study was to explore the effect of internal marketing on the organizational identification and organizational citizenship behavior of employees in a nonprofit organization.
Methods: This study used a questionnaire to survey 170 employees in the Tainan Blood Center. One hundred and fifty-two (89%) questionnaires were returned. All the data were analyzed by SPSS 14.0. This study used descriptive statistics analysis, correlation analysis and multiple regression analysis to test all the hypotheses.
Results: The result showed the internal marketing, organizational identification and the average value of equity in organizational citizenship behavior were above the middle level. The internal market had a positive impact on organizational identification and organizational citizenship behavior. There was a positive and indirect effect of internal marketing on organizational citizenship behavior through organizational identification.
Conclusion: Internal marketing was found to have a positive impact on improving employees' organizational identification and organizational citizenship behavior. It is helpful to enforce internal marketing by motivation management, training and communicating in the NPO.

1.3 Cost/Effectiveness

P-002

This abstract has been withdrawn.

P-003

THE ADVANTAGES OF BLOOD DONATION PROGRAM IN DR HASAN SADIKIN HOSPITAL BANDUNG INDONESIA

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Introduction: Until 2009, blood transfused in Dr Hasan Sadikin Hospital (RSHS) was only provided by Bandung Blood Transfusion Service of Indonesian Red Cross (UTDC-PMI Kodya Bandung). By the end of 2009 UTDC-PMI Kodya Bandung has increased the cost of blood per unit, from IDR 130,000 to IDR 200,000. This condition urged RSHS to find another source of blood, by performing blood donation program in RSHS. This decision was supported by RSHS's capability to perform screening test for infectious diseases (HIV, Hepatitis, Syphillis) with ELISA Method, and the availability of potential donor in RSHS such as doctors, nurses, medical students, residences and other hospital staff. Thus, since the beginning of 2010, RSHS has conducted its blood donation program.
Aims: The purpose of this study was to find out how much RSHS's blood donation program contributes to blood availability in RSHS, and how much money can be saved.
Methods: In this retrospective study, we reviewed the amount of blood in the RSHS blood bank which came from UTDC-PMI Kodya Bandung and from RSHS's own blood donation program, and we calculated the amount of money which could be saved, during 2010.
Results: The amount of blood derived from UTDC-PMI Kodya Bandung during 2010 was 50,745 units, while from RSHS's blood donation program was 705 units, which contributed for about 1.4% of all blood available in RSHS. The cost for one unit of WB and PRC production in RSHS is IDR 161,419. Thus, during 2010 RSHS can saved 705 X IDR (200,000–161,419) = IDR 27,199,605.
Conclusion and suggestion: RSHS's blood donation program contributes only a small portion to the availability of blood in RSHS, but at least some savings can be made. We suggest RSHS to increase the frequency of its blood donation program to reduce the dependency to UTDC-PMI Kodya Bandung.

P-004

EFFECTIVENESS OF PCR MULTIPLEX TESTING OF BLOOD DONORS

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Background: At present, Russia adopted Government Decree 1230 from 31 December 2010, for the first time regulate the use of molecular-biological methods of testing for blood donor screening, including a multiplex testing. The use of closed commercial systems of expensive equipment and reagents significantly reduces the economic efficiency of the organization of the PCR laboratories and leads to the impossibility of widespread implementation of nucleic acid testing (NAT).
Aim: Current practice is to use local multiplex open test systems and evaluate their effectiveness in the blood service.
Methods: Total 180,297 donors tested in three regions of Russia: Saratov blood transfusion station has tested 157,220 donors, Pyatigorsk blood transfusion station 10,937 donors, the Department of Blood Transfusion Russian Scientific Center of Surgery 12,140 donors. For the analysis, the test system Central Research Institute of Epidemiology of the Russian Federation: 'AmpliSens HCV/HBV/HIV-FL' AmpliSens HCV-FRT (HCV-FL), AmpliSens HIV RNA-FRT. All samples were examined by PCR in real time amplifier DT-96 by 'DNA technology' (Russia) and Rotor Gene RG-6000 'Gorbett research' (Australia) in the format minipool (5–10 samples in the pool). To assess the cost-effectiveness of testing is necessary to calculate the costs of NAT-tested 180,297 samples of plasma (A) A = A1 + A2 + A3 + A4 + A5 + A6. A1 – the sum of direct costs associated with implementation of laboratory tests: the cost of reagents and other consumables. A2 – wage fund for the period of research laboratory staff. A3 – total overhead costs – maintenance costs laboratories, utilities, etc. A4 – repair fund instrument. A5 – depreciation of laboratory equipment. A6 – cost quality control laboratory.
Results: Of the 180,297 surveyed donors in seronegative window period identified: 10 samples of HCV RNA and a DNA sample HBV. Total cost to identify one HCV RNA positive donor (ELISA negative) accounted for 19,500$. From one dose of whole blood obtained from the donor was possible to prepare three hemocomponents that can be transfused to three potential recipients. The costs of treating hepatitis in three recipients are on average 35,000$.
Conclusions: Improvement of the effectiveness of PCR testing in Russia is achieved through the use of local development of laboratory equipment and test kits for multiplex analysis format minipool. It is important to maintain a balance between the economic component of testing in general and sensitive detection of pathogens.

1.4 Training and Education

P-005

DEVELOPING STAFF CAPABILITY THROUGH EXPERIENTIAL LEARNING IN THE BLOOD BANK, SINGAPORE

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Background: While organizational performance depends on both the professional skills and the personal attitude of staff, training is an influential tool that can improve both performance and morale. As the national blood service, the Blood Services Group (BSG) of the Health Sciences Authority is committed to maintain a high standard of safety and quality in our national blood supply. Training is required for staff to deliver better work performance and keeping abreast of the latest knowledge and skills. Currently, workplace training on Process Excellence Skills is conducted in-house, to strengthen the capabilities of BSG's staff.
Aim: To develop staff capability in process management and improvement skills amongst BSG members
Methodology: Lean Six Sigma Green Belt training with certification with project consulting services was introduced for training. An awareness workshop on an 'Overview of Process Excellence' was also conducted for section leaders and staff. The objective was to provide an overview of the DMAIC (Define, Measure, Analyse, Improve and Control) Methodology through Lean Six Sigma Simulation where participants learned how to identify inefficiencies, eliminate waste and errors and improve quality and productivity. After the awareness workshop, a small team of seven staff from three different sections were selected for training which includes working on

three improvement projects proposed by Management. Staff underwent intensive training and the duration of training is from July to November 2010. Total training period is 19 days, in which 12 days are for classroom training, 5 days coaching and 2 days of workshop. The training included both, DMAIC and Lean methodologies. The process steps of DMAIC ensure that the process analyses are consistent and lean methodology to eliminate waste. Statistical and graphical analysis of data is by using the SigmaXL Statistical software. A high emphasis is placed on Trainee's Project. About 35% of times are set aside for the actual application of learnt tools to be applied to the trainee's project. Training followed a Learn-Apply format. Training includes a very strong project coaching component. Trainees experience not only the lessons from their project but also those of their colleagues as well which is an additional benefit. Finally, a Practical Examination is conducted to find out whether trainees understand how to use the Methodology and Tools.

Results: Staff is able to identify factors that affect processes within the blood bank (section). Training has produced significant results; improving the turn-a-round time for donor donation, sustained a significant rejection rate for platelet clumping from 10% to 3.5% and improving the quality of services provided.

Conclusion: In order for a Lean Six Sigma implementation to be successful, management must fully embrace the Lean Six Sigma philosophy. Training has enabled staff to apply a simple structured approach to problem solving. Towards the end of the project, all teams are motivated to come up with more creative ideas. Through Lean Six Sigma projects, staff has generated a pipeline of supplementary and subsequent projects. Thus, a culture of continuous improvement is instilled over time.

P-006
ENHANCEMENT OF EXECUTIVE ADMINISTRATION ON THE APPROPRIATENESS OF BLOOD FOR CLINICAL USE IN SHENZHEN

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Objective: To improve the optimal use of blood across-the board in Shenzhen.

Methods: On the basis of reviewing medical records of transfusion and of understanding the current situation, a systemic administration in transfusion medicine has been established and a comprehensive plan of training and education on transfusion medicine has been made and implemented. This includes the Shenzhen Committee of Blood Transfusion Administration, a handbook and manuals of basic knowledge of blood transfusion training clinicians on how to appropriately use blood, and quality control by reviewing transfusion record.

Results: The percentages in review of medical records accordance with appropriateness use of RBC and plasma have a raise from 65.26% and 22.94% in 2006 to 86.79% and 33.2% in 2010 respectively. Meanwhile, the requirements for blood components have increased. For instance, the platelet concentrates and the cryoprecipitate rose from 8.19% and 4.56% to 33.2% and 23.3% of the total blood components during the same period.

Conclusion: There appears a rational trend in clinical use of blood components and improvement of clinical knowledge in transfusion medicine.

P-007
ONE WEEK EDUCATION IN APHERESIS AT DHARMAIS NATIONAL CANCER HOSPITAL

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Introduction: In Indonesia, an increasing number of apheresis procedures are performed. Single donor platelets are collected in six Blood Transfusion Services and five hospitals. Also therapeutic apheresis procedures are performed in all of these hospitals also perform therapeutic apheresis procedures. In the last 3 years, in Indonesia in total 448 therapeutic apheresis procedures (TPE, leuco- and platelet reduction, and PBSC collection) were performed of which 146 (32.5%) in the Dharmais Cancer Hospital. More hospitals in East Java planned to start a therapeutic apheresis service and asked for education of physicians and nurses. Therefore, a 1 week educational program to increase the knowledge, skills and capability of apheresis nurses for therapeutic apheresis was developed and performed (August 2010) at the Dharmais Hospital in Jakarta.

Program of the training:

The educational program was build-up of a curriculum including:

1. Basic hematology, including the characteristics, kinetics, physiology and function of blood cells, and blood circulation.
2. Basic apheresis physiology, including calculation of total blood volume, maximal extra corporal blood volume, fluid balance etc.
3. Indications for apheresis procedures, including the aim, advantages/disadvantages of apheresis in specific diseases, the method of apheresis, start/stop sequences, volume to be processed, volume to be removed/replaced, choice of replacement fluids.
4. Criteria for donors and patients for apheresis, including the obstacle and the risk of procedures as well as the actions to be taken in case of side effects, volume to be taken.
5. Introduction to the apheresis machine available (MCS+, Haemonetics) including mechanism of the machine, availability of disposables, how to operate, as well as trouble shooting.
6. Documentation (such as SOPs, working instructions, worksheets).
7. Performance of a donor and patient procedure using the MCS+.

Method: In the first 2 days of the training, four speakers discussed the theoretical topics mentioned above, while on the 3rd day, machine knowledge and machine operating was trained by Haemonetics. The last 2 days, the participants practiced installation of various disposables and participated in donor procedures. At the end of every session, the training was ended with a discussion the daily subjects. To evaluate the success of the complete educational course, a pre- and post-training test was performed by all participants.

Results: In total four nurses and one medical specialist (internal medicine) of a hospital located in East Java participated in our course. Based on the pre- and post-training test, the knowledge of the five participants increasing by 74%, 36%, 61%, 97% and 173%.

Conclusion: We conclude that our 1 week educational program in apheresis is successfull in upgrading the knowledge and the skill of participants in apheresis procedures.

P-008
DONOR MEDICINE, ESTABLISHING A MEDICAL PROFESSION

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Background: Although many physicians are involved in Donor Medicine, the area is not recognized as a separate medical (sub)speciality. Despite many believe that transfusion medicine includes the whole transfusion chain from donors to recipients (vein to vein), there are good arguments to distinguish Donor Medicine as a separate entity. In fact donors rely on the idea that they trust their bodies to entities (blood banks) where a medical responsibility lies for donation and selection procedures, including apheresis, stimulation, immunisation and proper counselling for aberrant test results. The needs and arguments in favour for Donor Medicine are promotion of quality of donations and safety and security of donors and prevent conflicts of interest with physicians involved in the care of recipients.

Aims: The aim of our project is to build and imply a curriculum for Donor Medicine that meets criteria of medical education and competencies (CANMEDS) and independent assessments and qualification.

Methods: The project is divided in several parts:

1. Defining the learning objectives
2. Creating a modular programme
3. Organise and define internships and training on the job
4. Setting up terms for assessment and certification
5. Assuring the quality
6. Defining guidelines for reregistration

Results: Based on defined learning objectives a formal curriculum for donor medicine was created and all physicians involved in donor care were invited to participate in the program. The programme consists of a 2 year period with theoretical modules, internships and training on the job. Several modules were developed such as donor selection and assessment, bleeding procedures and apheresis, blood born diseases, quality control and systems, immunohematology, blood processing, stem cells/tissues and organs, donor counselling en epidemiology. An independent medical certification committee was installed to approve the programme and to assess the individual curricula vitae and requests for qualification and/or exemption of some modules. From January 2005 until January 2010, 48 physicians enrolled in the programme; of these at present 36 have been qualified, four have dropped out for personal reasons and eight are presently in training. Qualified physicians should be requalified every 5 year and should meet the demands of 16 h practice a week and a total of 200 accreditation points. An average of six physicians is starting each year with the programme. The national medical association recognized the programme as a training for a new medical subspeciality Donor Medicine, part of Public Health.

Summary: A competency-based modular professional medical curriculum for Donor Medicine has been build to meet the needs for improvement of quality of medical donor care and prevent conflict of interest with patient care. The programme is recognized as a new subspeciality within Public Health. This development will further stimulate medical care for donors who are health in principle and should remain healthy with respect to medical and social-ethical aspects.

P-009

CONTINUING MEDICAL EDUCATION AT NATIONAL INSTITUTE OF TRANSFUSION MEDICINE-CURRENT EXPERIENCES AND FUTURE EXPECTATIONS

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Introduction: Continuing medical education (CME) is a key factor in a contemporary medicine. The models of CME are different and depend of the practical and theoretical needs of the medical institutions.
Aim: To evaluate current experiences in a process of CME that was organized after the process of reorganization and integration of Blood Transfusion Services (BTSs) into a National Institute of Transfusion Medicine (NITM).
Material and method: The 120 employees divided in three groups were included in CME. The participants were with different educational status and working positions (medical doctors, specialists in Transfusion Medicine (TM), laboratory technicians, biologists and nurses). After the CME, the participants were interviewed to express their opinion and attitudes about the CME. The education was provided by eight educators from NITM (professors, associate professors in TM, quality managers, MD specialist in TM and pharmacist). The program had 13 topics in four modules: blood donation process, control of blood and blood products, storage and distribution of blood as well as quality assurance and quality control. Each module lasted 8 h. There were presentations, group work, discussions and elaboration of personal experiences as case studies. The written evaluation was part of the official rapport together with suggestions, propositions and questions from the participants.
Results and discussion: Beside other forms of education, CME was accepted with positive attitudes from all participants. Specially was positively evaluated the team work, multi professional approach and involvement of all employees to discuss about the different phases in the work. Participants have pointed out some weak points on which should work in a future.
Conclusion: There is a real need of CME at TM. The current experiences showed that CME had positive impact in everyday work at BTSs, especially after the reorganization and transformation of BTSs.

1.5 Risk Models, Standards and Regulation

P-010

EARTHQUAKES AND AFTER EFFECTS

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On February 22nd a 6.3 magnitude earthquake struck the Canterbury region. The earthquake caused widespread damage and multiple fatalities in Christchurch City. Services in the city were severely disrupted.
The purpose of this poster is to describe the impact of the earthquake on the New Zealand Blood Service in Christchurch and to examine the response of NZBS to the disaster. It deals with the events on the day and also in the days of recovery which followed, with the emphasis being on the Donor Centre.
The Donor Centre in Christchurch is in the Suburb of Riccarton which is about a 15 min drive from the Blood Bank in town. With telephone and internet being down directly after the earthquake, communication between the centres was initially impossible.
A meeting of team leaders was immediately called. The Coordinated Incident Management System (CIMS) was called into place to manage the disaster. A timeline illustrates the events and actions taken on a day by day basis. These included the management of staff, delegation of duties post earthquake, checks on the building to ensure structural safety and working facilities. Equipment had to be checked, repaired and calibrated. Contingency plans had to be made to ensure that blood products were available to treat patients at Christchurch hospital. NZBS centres throughout the country were feeling the strain as they had to take on extra work to support and supply Christchurch. The effects of the earthquake on the wellbeing of staff also affected the timeline and the decision to reopen the laboratory.
The disaster has highlighted the need for laboratory emergency protocol to be updated and the need to have ongoing training and drills for staff. Improved emergency plans are in draft and work on these is ongoing. So far, earthquake drills are being planned. The possibility of training in basic disaster management for all staff is being examined, with in depth training for staff in roles of responsibility. Also alternative communication systems (satellite phones) are being considered in case of future telephone and internet failures.
New Zealand is a very seismically active country, and earthquakes can strike any time and in any place. The importance of having disaster management plans in any laboratory is made plain by the experiences of Christchurch. Earthquakes are common throughout the whole of the Pacific Circle. Therefore it would be advisable for all laboratories to have plans in place to deal with natural disasters such as earthquakes. Hopefully, Christchurch's experiences will be useful as a model for future disaster management planning.

P-011

EFFECTS OF A SIMPLE CLEANSING PROTOCOL ON BACTERIAL GROWTH IN PLASMA THAWING WATER-BATH

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Background: Though commercial plasma thawing device was adopted by more and more blood banks in Taiwan, the traditional water-bath was still in use in most blood bank due to its higher speed to thaw frozen plasma component and much lower price to purchase. Unfortunately, documented cases of transfusion-related bacterial infection resulting from contamination in water-bath have been reported in the literature. However, there are no defined criteria for the acceptable colony count in water used in water-bath container. The criteria of <2000 CFU/ml for R.O. water, which has been revised to <200 CFU/ml later, used in hemodialysis might be a more or less similar counterpart. Furthermore, protocol for cleansing water-bath is not yet established.
Aim: This study aims to evaluate the effect of a simple cleansing protocol upon the time-dependent bacteria growth in the water-bath.
Method: The water-bath container was cleaned using R.O. water and kept in use for 24 h. Then the container was filled with 11 l of R.O. water and 1 l of 6% bleach solution with a final concentration of 0.5% per liter. After 15 min, the container is rinsed with clean R.O. water three times and then the water-bath container was filled with R.O. water and kept running for 24 h. The colonies in the water were counted in accordance with the guideline published in 'General Rule for Environmental Microbiology-Bacteria, Environmental Analysis Laboratory EPA, Executive Yuan, ROC. Briefly, 100 ml of R.O. water was collected for culture before use. After the R.O. water is poured into the water bath container, aliquot of 100 ml of R.O. water was collected in duplicate at 5 min (0 h), 4, 8, 12, and 24 h. Each sample was cultured and counted in Mueller-Hinton media. The procedure was repeated in triplicate.
Result: The initial colony count at 0 h was up to 3318 CFU/ml. After cleansing and soaking with 0.5% bleach solution, no colony growth was detected for samples collected at 0 and 4 h. The colony count averaged 11.7 (range: 9–17) CFU/ml, 1058.3 (1045–1065) CFU/ml, and 360,757.6 (27,831–49,091) CFU/ml for samples collected at 8, 12, and 24 h, respectively.
Conclusion: The present research showed that a simple cleansing process using diluted bleach solution effectively decrease the bacteria colony growth in water-bath container for up to 12 h if we used <2000 CFU/ml as acceptance criteria.

P-012

This abstract has been withdrawn.

1.6 Blood Supply Management and Utilization

P-013

This abstract has been withdrawn.

P-014

RETROSPECTIVE INVESTIGATION AND ANALYSIS OF CLINICAL BLOOD TRANSFUSION DURING EMERGENCY

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Objective: To estimate the appropriateness of emergency transfusions of plasma by using technical specifications of clinical transfusion.
Methods: Two separate retrospective surveys of medical records from 12 May 2008 to 12 July 2008 (151 transfused patients) and from 6 June 2009 to 18 August 2009 (17

transfused patients) from three hospitals selected from all hospitals that are involved in emergency rescue were investigated and analyzed.
Results: In total of 538,080 ml of plasma that was transfused in 956 cases, 88% was fresh frozen plasma (FFP) and 12% was frozen plasma (FP). 33.4% (319/956) of plasma transfusions were assessed as inappropriate.
Conclusion: In terms of the technical specifications of clinical transfusion guidelines on component transfusion, there is considerable inappropriate transfusion of FFP and FP during emergency.

P-015
AN APPROACH TO SHORTEN RED BLOOD CELLS SHELF LIFE

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Background: Changing the maximum shelf lives for RBC units would require novel approaches to RBC inventory management. E.g. in Stanford University RBC units spent on average 8.6 days on the shelf with a mean age of 10.2 days at delivery and 18.8 days at issue for transfusion1.
Aims: To evaluate RBC shelf lives in Pirogov Center and our approaches to shorten red blood cells shelf life.
Methods: There were evaluated ages of RBC before delivery and before transfusion in 2009.
Results: Ages of RBC in Pirogov Center are at 25% less than Stanford University (Tables 1 and 2). With the exception of days on shelf of 0 negative cells which in USA are transfused more often in emergencies without crossmatch. We have four blood suppliers and try to have hospital blood supply for 1 week. RBC consumption in previous years and current clinical situation are taking into account for blood order. One procurement consisted from 1 to 77 units. Amount of monthly delivered RBC correlates with the amount of transfused RBC (Table 3).

Table 1: Mean age at delivery and at issue and mean number of days spent on shelf based on ABO/Rh type in Pirogov Centre

Blood type	A+	A−	B+	B−	AB+	AB−	O+	O−	Mean
Age at delivery (days)	7.7	5	8.8	7.4	7.3	9	7.7	4.3	7.6
Age at issue (days)	13.8	14.9	14	12.1	14	13.4	13.9	17.6	14
Days on shelf	6.1	9.9	5.3	5.1	6.7	4.3	6.3	13.3	6.5

Table 2: Mean age at delivery and at issue and mean number of days spent on shelf based on ABO/Rh type in Stanford University

Blood type	A+	A−	B+	B−	AB+	AB−	O+	O−	Mean
Age at delivery (days)	10.5	12.1	14.6	13	20.2	19	8.1	10.3	10.2
Age at issue (days)	20.4	27.7	21	25	29.3	32.7	14.9	21.2	18.8
Days on shelf	9.8	15.6	6.3	12	9	13.8	6.7	10.9	8.6

Table 3: Correlation between monthly quantities of delivered and transfused RBCs in Pirogov Centre

Year	r	p
2007	0.88	<0.001
2008	0.89	<0.001
2009	0.68	0.014
2010	0.84	0.001

Conclusion: Diversification of suppliers and maintain the stock of RBC in the volume of weekly clinical needs can reduce the shelf life of RBC before transfusion. The criterion for the effectiveness of logistics RBC is a correlation number of doses of RBC transfused and delivered monthly.
Reference:
1. Fontaine MJ, Chung YT, Erhun F, Goodnough LT: Age of blood as a limitation for transfusion: potential impact on blood inventory and availability. *Transfusion* 2010; **50:** 2233–2239
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P-016
CONSUMPTION TREND OF BLOOD AND BLOOD COMPONENTS IN AMIR ALLMOMNIN HOSPITAL IN AHWAZ

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Background and objectives: Order for a large number of blood and blood component is a very common practice that leads to great reduction in blood supplies, lower blood unit quality, increase the number of expired units, and imposes heavy cost on blood transfusion centers. To evolution the most common indication for blood order and blood use, we studied Amir Allmomnin hospital to seek a standard pattern for blood transfusion in Ahwaz.
Materials and methods: This is a cross sectional study on 2300 blood order form in blood bank department of Amir Allmomnin hospital in Ahwaz. The data were collected with a questionnaire in all blood order forms from March to May 2011. Data were analyzed in SPSS 16 with the use of chi square and *T* test. To calculate C/T ratio, the number of cross matched units was divided by the number of transfused units.
Results: In this study, 2300 blood order forms were evaluated. Out of the patients, 44.8% were male with the age average of 54. The average number of products ordered for every patient was 2.36 ± 1.70 units and the average number of transfusion unite was 1.97 ± 1.50. From all blood order forms, 54.5% (CI: 95% = 52.1–56.9) were not transfused. The highest rate of blood orders pertained to gynecology (19%), ICU (14%), general surgery (5.8%), orthopedics (7%). Packed cell was the product with the highest order rate of 79.6%.
Conclusion: The result of this study show that the rate of non-transfused ordered blood components in Amir Allmomnin hospital is so high. Thus, we need a regional guideline for blood orders so that we would be able to reduce this rate. We should also reconsider the blood order processing and the blood utilization strategy.

P-017
A STUDY OF BLOOD USAGE IN A TERTIARY CARE HOSPITAL IN SRI LANKA

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Background: In Sri Lanka, blood and blood product usage has been increased significantly during past 5 years due to epidemics of dengue hemorrhagic fever and increasing number of cardiac and transplantation surgeries. Sri Lanka has a nationally coordinated blood transfusion service (NBTS), where 85 hospital based blood banks are managed by National Blood Center (NBC) which is the headquarters in Colombo. This study was done in Sri Jayawardanapura General Hospital which gets all the blood and blood component support from NBC. It has a total bed strength of 1065 which includes transplantation and cardiac surgery units.
Aim: In order to minimize the wastage of Red Cell Concentrates (RCC) and optimize blood usage, retrospective analysis of cross matches and cross match to transfusion ratio (C:T) was obtained with the aim of preparing a Maximum Surgical Blood Ordering Schedule (MSBOS) for the hospital.
Method: Data of number of cross matches and RCC issues for each specialty were collected from the cross match request register and RCC and blood component issue register and analyzed accordingly.
Results: C:T ratio was highest in the gynaecology and obstetrics units, where as is up to the recommended ratio in medical and paediatric units. The overall C:T ratio for the hospital was 4:1 which is showed in detail in Table 1. Total RCC discards are shown in Table 2.

Table 1: Cross match: transfusion ratio

	Request	Cross match	Issues	C:T ratio
Medical	459	386	269	**1.4:1**
Surgical	457	351	144	**2.4:1**
Gyn & Obs	267	227	29	**8:1**
Peadiatrics	6	6	5	**1:1**
Total	1189	970	447	**4:1**

Table 2: Blood discard rates for 6 months

Blood Discards

Month	No of blood packs	Blood discard %
DEC 2011	43	14%
JAN 2011	57	22%
FEB 2011	9	5%
MAR 2011	38	8%
APR 2011	29	20%
MAY 2011	46	9%
Average	**37**	**13%**

Conclusions: Inappropriate reservation of RCC for surgeries which hardly needs blood transfusions results in greater wastage of RCC. Implementation of MSBOS and group, screen and serum saving method should be implemented without any delay in order to assure appropriate clinical use of blood and blood products.

P-018
A SURVEY OF HOSPITAL BLOOD BANK PRACTICE AND INVENTORY MANAGEMENT IN HONG KONG: INSIGHTS INTO BETTER OUTCOME

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Background: Blood stocks are kept in hospitals to ensure adequate and timely supply of blood components for effective and efficient therapeutic use, including unexpected massive transfusions. On the other hand, hospital blood banks (HBB) have the responsibility to minimize blood wastage and outdating.
Aims: To assess HBB inventory management practice and performance based on the data collected in a survey conducted in May 2011.
Methods: Questionnaires were sent to 28 local hospitals including 16 public hospitals managed by Hong Kong Hospital Authority (HA hospitals) and 12 private hospitals (non-HA hospitals). Questions asked included blood stock holding, methods of pre-transfusion testing, application of maximal surgical blood ordering schedule (MSBOS), durations of crossmatch reservation and crossmatching in advance, stock sharing relationship with other hospitals, application of 'First-in-first-out' (FIFO) principles in blood distribution, use of standard operating procedures (SOP) and method for calculating blood order. Two parameters were computed: (i) Issuable Stock Index (ISI): estimation of the number of days of unreserved red cell stock of all blood groups held in the inventory; (ii) Wastage as a Percentage of Issues (WAPI). Hospital ISI, WAPI and other variables asked in the questionnaire were compared. Mann-Whitney test was used to test for differences when applicable.
Results: Response rate to the questionnaire was 100%. During May 2010–April 2011, 217,101 units of red cells were distributed to all hospitals (HA hospital: 89.7%; non-HA hospitals: 10.3%). Nominal stock value or average red cell distribution per day was 594.8 units. Overall ISI and WAPI for all hospitals was 7.27 days and 1.02% (2211 units) respectively. For hospital ISI, the medians and ranges for all (n = 28), HA (n = 16) and non-HA hospitals (n = 12) were 8.21 (3.88–25.72), 7.02 (4.72–13.87) and 12.40 (3.88–25.72) days respectively. For hospital WAPI, the medians and ranges for all, HA and non-HA hospitals were 0.65% (0.00–44.86%), 0.25% (0.00–1.30%) and 12.04% (0.54–44.86%) respectively. Majority of the responses showed the following local blood bank practice: (i) use of type and screen with abbreviated/computer crossmatch method for pre-transfusion testing (n = 21/28, 75.00%); (ii) not using MSBOS (n = 23/28, 82.14%); (iii) crossmatch reservation of 72 h (n = 15/28, 53.57%); crossmatch in advance <24 h (n = 22/28, 78.57%); (iv) not sharing stocks (n = 24/28, 85.71%); (v) FIFO (n = 26/28, 92.86%); (vi) SOP for placing order (n = 26/28, 92.86%); (vii) use both computer and visual count in estimating blood order (n = 17/28, 60.71%). Non-HA hospitals were associated with higher ISI (median: 12.40 days vs 7.02 days; P = 0.0154) and WAPI (median: 12.04% vs 0.26%; P < 0.0001). ISI >7.5 days was associated with higher WAPI (median: 2.47% vs 0.42%; P = 0.0269). Comparison with other variables was not possible due to small sample size of some question responses.
Conclusions: It is advisable to hold a blood stock to an ISI of <7.5 days in order to minimize blood wastage. The application of MSBOS to reserve the optimal quantity of red cell units, shortening crossmatch reservation to 24 h and crossmatching <24 h in advance, sharing stock with clustered hospitals and using FIFO principle for blood distribution may help reduce WAPI and improve performance at hospital blood banks.

P-019
DISTRIBUTION AND UTILIZATION RATE OF WHOLE BLOOD AND BLOOD COMPONENT IN DR HASAN SADIKIN GENERAL HOSPITAL: 6 YEARS REVIEW (2004–2010)

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Background: The purpose of a blood transfusion is to replace lost blood, to increase the flow rate of cardiac output, to increase blood elements, to replace the missing clotting factors and immune system elements. Whole blood and blood component transfusion is a serious event. Therefore, blood or components transfusion must be made within the appropriate indication after careful evaluation of clinical status. It is important that distribution and utilization rates of whole blood and blood component use have to sufficient extent regarding to rational transfusion in daily practice.
Aims: The aim of this study is to determine and evaluate the distribution and utilization rate of whole blood and blood component in Dr Hasan Sadikin General Hospital as the main hospital of West Java province in Indonesia.
Methods: The data collected from January, 2004 to December, 2010 (6 years) in Blood Bank Service Dr Hasan Sadikin General Hospital, which consist of: ABO type of whole blood and blood component, the amount and type of blood component, transfusion rate and the clinic where transfusion have done. Utilization rate is determined as blood to patient ratio and whole blood to blood component ratio.
Results: The amount of whole blood and blood component transfused was 324.539 units for 114.232 patients with ratio blood unit to patient = 3:1. Percentage ABO type of transfused products was as follows: O type (35%), A type (29%), B type (28%), and AB type (8%). Percentage of transfused products was as follows: packed red cell (60.8%), platelet suspension (18.5%), whole blood (9.8%), fresh frozen plasma (5.7%), washed red cell (3.5%), cryoprecipitate (1.4%), and buffy coat (0.4%). Transfusion rate of whole blood and blood components by clinic service type as follows: 12.6% in internal medicine, 15.1% in general surgery, 7.8% in obstetric and gynecology, 13.2% in department of child health and disease, and 51.2% in other service.

Table 1: Distribution of whole blood and the components

Years	Whole Blood	Packed Red Cell	Platelet Suspension	Fresh Frozen Plasma	Washed Red Cell	Cryoprecipitate	Buffy Coat	Total
2004	7007	18547	2279	2452	1161	449	61	31956
2005	6641	21988	5502	3229	1595	376	213	39544
2006	7307	25633	8825	2390	1566	684	390	46795
2007	4520	30647	9464	2796	1411	651	471	49960
2008	4043	32050	10497	2941	1495	724	59	51809
2009	2260	34590	12910	1604	2024	584	33	54005
2010	136	33877	10430	3110	2003	943	25	50524
Total	31914	197332	59907	18522	11255	4411	1252	324593

* Number represented unit of blood bag

Table 2: Transfusion rate by clinic service type

Clinic Service Type	Unit Number
Internal Medicine	49218
General Surgery	25442
Obstetric & Gynecology	41043
Department of Child Health and Disease	42971
Others	165865
Total	**324539**

Conclusions: Utilization rates of whole blood and blood components are effective, proved by decreasing whole blood utilization rates and increasing the use of blood component. Information work always should be increased and the clinicians always should keep in mind that transfusion can lead to serious complication and benefit/risk assessment must take into account each transfusion.

P-020
PACKED RED CELL TRANSFUSION REQUEST PATTERNS IN A TERTIARY CARE WOMEN'S HOSPITAL IN SRI LANKA

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Background: De Soysa Maternity Hospital for Women (DMH), Colombo, is one main hospitals totally dedicated for women's health in Sri Lanka. Thus, it becomes a main centre for referral of patients from all parts of the country needing specialised tertiary care in the fields of Obstetrics and Gynaecology. Therefore the blood bank of DMH gets many requests for transfusions.
Aims: To analyse the patterns of requests for packed red cell transfusions in relation to the clinical indications, to recognise unnecessary blood reservations, and to improve blood bank services to give the patient best possible care with available resources.
Methods: A descriptive retrospective study was carried out by analysing all request forms (excluding neonatal transfusions) for packed red cells, over a period of 6 months from 1 January 2010 to 30 June 2010. A detailed collection of data was gathered and analysed using SPSS data analysis tool.

Results: There were 2388 request forms evaluated during the study, out of which only 91 patients (3.8%) needed transfusions. Seventy-two per cent (1721) of the requests fell in to the 'Obstetric' category. More than 2/3 of all the requests were marked as 'Urgent' cases. Considering the indication for the request, 50.3% were for 'Emergency Caesarean Sections', 21.5% for 'Elective Caesarean Sections', 9.7% for 'Abdominal Hysterectomy', 5.7% for 'Laparotomy', 5.4% for 'Laparoscopy', 4.4% for 'Vaginal Hysterectomy and Repair'. With comparison to other indications, those who underwent myomectomy had the highest probability of needing transfusions (29.7%), followed by 'Dilatation and Curettage' (11.1%) and 'Laparotomy' (9.6%). Although the highest number of red cell reservation requests were for Caesarean Sections, only 2.6% of them needed a transfusion later. None of the Laparoscopic surgeries (128) carried out in DMH needed blood transfusions during the study period.
Summary/conclusions: This study clearly shows that there is a marked discrepancy between packed red cell requests and actual instances of transfusions. Unnecessary cross-match requests overburden the staff of the blood bank. Some minimal risk surgeries such as laparoscopic procedures didn't need a single blood transfusion even though the practise was to routinely cross match and reserve blood. Therefore an evidence based discussion should take place between the ward staff and the blood bank, to revisit and renew blood ordering schedules to reduce wastage of manpower and resources.

P-021
ANALYSIS THE DEMOGRAPHIC CHANGES OF THE BLOOD DONORS IN KAOHSIUNG BLOOD CENTER

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Background: Blood Services Organization should monitor the demographic structure of the donor population and decide the strategy for recruiting donors. Kaohsiung Blood Center (KBC) database provides a unique opportunity for analyzing the demographic changes of the blood donors.
Methods: This study started with data in 2000 to have equal distance between each year. The numbers of donors and donations by sex, age group and donation patterns (first-time (FT) donors or repeat (RP) donors) for 2000, 2002, 2004, 2006, 2008, 2010 were generated from the database. Data on the general population were obtained from the Department of Household Registration.
Results: Over 180,000 volunteer blood donors donated over 400,000 bags of blood in KBC. During the 11-year period, the donor population reduced with an average percentage of 0.15 every year but the general population of age group between 17 and 65 years increased by 0.5% per year. Study results also revealed that 62.5% of the donors were male, and 79.5% were RP donors. Donors of age 20–24 years (35.51%) predominated over the blood donation in 2000, however, donors of 30–39 years (22.31%) predominated in 2010. The proportion of 17–24 years decreased in both FT and RP donors. Male RP donors of age 20–24 years decreased significantly from 15.49% in 2002 to 8.47% in 2010, indicating that it was hard to recruit and maintain the donors with this age stratification. RP donors of 50 years or older increasingly contributed to a larger proportion of the donor pool (3.82% in 2000 to 14.92% in 2010) and made the highest donation frequency. People of these age groups limited by the donor selection criteria will affect donor population within 15 years.
Conclusion: The population structure in KBC is shifting from younger to older age groups. The ageing situation is more severe than the general population trends. Strategies should be focused on the younger age group recruitment and increasing their donation frequency. Therefore we can maintain a stable blood supply.

P-022
THE ANALYSIS OF BLOOD NEEDS IN CHINESE NEW YEAR HOLIDAY

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Background: People travel domestically or overseas during Chinese New Year Holidays. In the holidays, blood donation decreases tremendously, medical need for blood is also uncertain and adequate supply of blood preparations becomes the greatest challenge to the Blood Center.
Aim: This is a retrospective review of the supplies of blood components from Kaohsiung Blood Center database to assess the need for blood and trend for usage, and to suggest adequate management for blood inventory.
Methods: Kaohsiung Blood Center database was queried to analyze the supplies of blood components including RBCs, platelet concentrate, apheresis platelets and plasma between 2005 and 2009. Supply was compared between three intervals: I: Chinese New Year's Eve to the beginning of sixth, II: the week before the Chinese New Year, III: 2 weeks before the Chinese New Year.
Result: A total of 250,175 units were supplied to hospitals by Kaohsiung Blood Center between 2005 and 2009.

Table 1: Supply of blood components between 2005 and 2009 (unit: unit)

	RBCs	Platelet concentrate	Apheresis platelets	Plasma
Interval I	1570 ± 542	1941 ± 752	227 ± 97	1124 ± 625
Interval II	10,019 ± 770	3279 ± 565	389 ± 85	11,318 ± 1213
Interval III	7870 ± 256	3606 ± 992	386 ± 101	8307 ± 777

Conclusion: The need for both RBCs and Plasma in interval II were highest probably because hospitals began to increase the inventory of blood preparations 1 week before Chinese New Year. Accordingly, Kaohsiung Blood Center must be prepared to urge volunteers to give blood prior the peak of need, and increase RBSs and plasma inventory in before the Chinese New Year.

P-023
REQUESTS FROM MEDICAL INSTITUTES UNDER UPCOMING BROAD AREA BLOOD SERVICES IN JAPAN

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Background: Japanese Red Cross Society (JRCS) has been pushing ahead the reorganization of blood service in Japan to accomplish the supply of safety and even blood products in just proportion. This may be called Broad Area Blood Services (BABS). By around FY 2012, Blood Centers (BCs), originally several BCs including Okayama Red Cross Blood Center (ORCBC) had their own laboratories, will be basically reconstructed to seven-Block Area Centers (BACs) having manufacturing facilities and the other regional BCs only having promoting, collection and distribution sections. ORCBC belongs to 'Tyu-Shikou Area (consists of adjacent nine prefectures)' will change to one of regional BCs.
Aims: We aim to find out the needs of medical facilities for regional BCs under BABS by carrying out a questionnaire survey to get the whole picture of local medical institutions.
Methods: In December 2009, we conducted a questionnaire to ask transfusion services of regional medical institutions in the name of Prefectural Government Joint Transfusion Therapy Committee with their agreement. Institutions surveyed (248) were chosen by the usage record of transfusion even a single unit in 2008. The survey items were 114 as following: scale of a facility, amount of blood products used, management system, protocol of transfusion, transfusion-related blood tests, autologous blood transfusion, peripheral blood stem cell transplantation, etc. The item: the effects of BABS were collected as inquiries and requests.
Results: The collection rate was 52.8% (131/248 facilities). The analyzed data indicated that facilities whose beds are <49 require a quick delivery of blood products in acute situations and technical supports of blood type testing when strike on problematic patients. We received 97 matters as inquiries and requests about the effects of BABS. Among them, obtaining compatible blood type and constructing emergent blood distribution systems were most frequent matters, similarly.
Summary/conclusions: We have carried out several approaches to our district medical institutions to achieve the advancement of transfusion therapy and promote the proper use of blood products in collaboration with prefectural government, medical association, hospital association and association of medical technologists. Upcoming BABS, we did a wide questionnaire survey of transfusion therapy to our regional medical institutions. This survey showed us the needs of rapid supply of blood in emergency and technical support of laboratory blood type tests in times of trouble. By these needs, we have started planning a new blood supply station in remote location and presenting some workshops of blood type testing according to their technical levels.

P-024
IMPLEMENTING ARIMA MODEL IN FORECASTING BLOOD DEMAND IN SARDJITO GENERAL HOSPITAL BLOOD BANK, YOGYAKARTA, INDONESIA

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Background: Blood demand in a hospital is dynamic in nature. This requires blood bank manager to manage blood supply and blood bank resource carefully, especially in a relatively young blood bank like Sardjito General Hospital Blood Bank. Blood col-

lection effort and blood bank resource management will gain benefit from an adequate forecast of blood demand.
Aims: This study aims to examine ARIMA model performance in forecasting blood demand.
Methods: The time series consisted of the monthly blood units issued by Sardjito General Hospital Blood Bank from January 2008 to December 2010. Using statistical package Stata 10.1, an ARIMA model was created and subsequently monthly blood demand for the first quarter of 2011 was forecasted. Performance was indicated by the coverage rate of blood demand by the predicted value.
Results: ARIMA model performs accurately to forecast monthly blood demand in the first quarter of 2011 with the median coverage rate of 98.29%, minimum of 95.24%, and maximum of 107.42%.
Conclusion: Over the first quarter of 2011, prediction of monthly blood demand generated by ARIMA model are accurate enough to be of help in the planning of blood collection efforts and blood bank resource management.

P-025

This abstract has been withdrawn.

P-026

IMPROVEMENT OF BLOOD COMPONENT TRANSFUSION APPROPRIATENESS WITH A PROSPECTIVE AUDIT PROGRAM AT A MEDICAL CENTER: A 2-YEAR EXPERIENCE

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Background: Inappropriate transfusion practice can be identified and corrective actions can be taken by monitoring blood component utilization upon request. Useful tools like predetermined transfusion guidelines, pretransfusion approval, and transfusion audits are useful tools in the education of those requesting blood components. The experience of a blood utilization auditing program and its outcome at a tertiary care medical center (1600 beds) in Central Taiwan was here presented.
Aims: A continuous improvement of blood transfusion practice was achieved by reduction of inappropriate use of blood component through blood utilization auditing.
Methods: With the support from the transfusion committee, a prospective audit program had been started since February 2009. A revised indication for blood component transfusion was announced about 1 week before initiation of the blood auditing. With assistance of the hospital information system upon request for blood components, the ordering physicians must indicate the reason for transfusion, related clinical information and any corresponding laboratory results. All above information were first reviewed by specialists of blood bank upon receipt of the request. If there was any doubt or deviation from the transfusion indications, the ordering physicians would be first notified by specialists to check any error in order entry. Otherwise, a second opinion from a transfusion or the third opinion from the chairperson of the Transfusion Committee should be consulted if the ordering physicians insisted that transfusion was necessary. The blood components were issued only when such requests were approved by any supervisor. Blood component utilization was monitored monthly and reported to the Transfusion Committee quarterly.
Results: From February 2009 to April 2011, a total of 2845 requests (4.85%) were subject to notification: 2707 requests (95.15%) granted after enquiring complete information as required for review, 117 requests (31.99%) cancelled after communication, 26 requests (0.91%) modified in amount or type of components and 21 requests (0.74%) subject to consultation. The average decrease of blood utilization per month from February 2009 to April 2011 as compared with the baseline amount calculated from January 2008 to January 2009 was as follows: 40% for whole blood, 6% for packed red cell, 2% for cryoprecipitate, 26% for fresh frozen plasma and 95% for frozen plasma.
Summary and discussion: Effective improvement of inappropriate use of blood components could be achieved by a prospective blood auditing program. Most requests notified because of lacking complete information as required for review. The information system may be improved and helps in reduction of notification and play as an important role in alerting and autoverifying related information upon request.

P-027

REVIEW OF BLOOD AND COMPONENT TRANSFUSION PRACTICE IN A TEACHING HOSPITAL OF NORTH INDIA – A RETROSPECTIVE STUDY

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Background: Availability of component therapy, has transformed transfusion practice in recent years. However, pattern of utilisation differs from one institution to another. Several studies demonstrate wide variations in clinical transfusion practice between institutions. Evaluation of blood and component utilisation patterns defines practice in an institution and permits comparison with other institutions. It also helps in proper inventory management, better planning of resources to reduce wastage and cost. Further, problem areas in transfusion practice that require improvement can be identified. Hence this study was undertaken to assess the blood utilisation pattern in a teaching hospital of a developing country.
Aims: 1. To study prevailing transfusion practices.
2. To analyse component utilization patterns.
3. To identify gaps in prescription.
Methods: The study was conducted in a 300 bedded, teaching hospital of India with newly established component separation unit. Request forms for a period of 3 consecutive months in the year 2011, were analysed retrospectively. A total number of 868 request forms were included. Data related to patient demographics, specialty, diagnosis, emergency or routine transfusion, type and quantity of components ordered, were extracted from the request forms. The data was coded and a database was created in MS Excel. SPSS VER.16 was used for analysis. Transfusions were analysed to determine, proportion of component use by speciality, indication, type and volume of component requests, patient demographics, proportion of single unit transfusions, and quantity of component demand per episode of transfusion.
Results: Sex distribution of transfusion requests showed that 70% were males and 30% were females. Breakup of requests by age showed that most heavily transfused age category was, 16–30 years, followed by 31–40 years, together accounting for 50% of all. Most requests came from the department of surgery – 29%, followed by medicine at 22%. The department of Paediatrics utilised 10% of all transfusions. Demands from Orthopaedics and Obstetrics were similar at 6%. Emergency ward utilised 6% of transfusions. 15.36% requests were from other hospitals. Analysis of component demands showed that most were for PRBC – 61%, followed by demand for whole blood – 24%. FFP was at 10% and Platelets at 5%. Out of all PRBC requests, 46% were for single units, followed by two unit demands at 39%. Similarly within Whole Blood, 52% was for single units. Most requests for Platelets too were for single units – 39%. However for FFP proportion of one and two unit requests were same at 32%. Platelets were ordered mostly by departments of medicine and paediatrics for indications like leukemia, hemetemesis, clotting disorder, hepatosleenomegaly, acute liver disease. FFP was used mostly by departments of surgery and paediatrics for ascites, intestinal obstruction, liver disease, TB and hematemesis.
Most prevalent blood group was B Positive followed by O, A and AB. Negative groups were approximately 9% of all.
Summary/conclusions: Surgical specialities constituted the bulk of all transfusion requests. Some inadequacies in transfusion practice like improper dose and indication of Platelets and FFP could be addressed by dissemination of guidelines among clinicians. Continuous monitoring and review of requests by hospital transfusion committee is necessary.

P-028

AN ANALYSIS OF BLOOD USAGE IN SURGICAL UNITS OF GENERAL HOSPITAL, AMPARA

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Introduction: Human blood is an invaluable limited resource that needs to be use appropriately with minimizing the wastage. The preoperative cross-matching of blood units for surgical patients is performed in anticipation of a potential need. The lack of proper guideline for blood ordering leads to requesting unnecessary cross-matches. Consequently, it consumes blood bank resources unnecessarily, increases the blood stocks that must be maintained, and increases the number of units that become outdated.
Objectives: To analyze pre-operative blood ordering practices in the surgical units of General Hospital Ampara and to propose suitable strategies for appropriate blood usage.
Material and methods: General Hospital Ampara has one general surgical unit and one obstetrics and gynecology unit. This was a retrospective analysis of cross-match requests and operation theatre records. The blood ordering and usage data was ana-

lyzed over a 9 month period from 1 January 2009 to 30 September 2009. The cross-match to transfusion ratio (C: T), transfusion index (TI) and range of units transfused are calculated for each procedure as they can be used as indicators of blood requirement and potential severity of bleeding.
Results: Data was analyzed for 27 surgical procedures in 1598 patients. One thousand eight hundred and thirty-one units cross-matched for these patients and only 232 (12.6%) units were transfused. Overall C: T was 7.9; 23.8% cases were from general surgical unit and C: T was 6.9; 76.2% were from obstetrics and gynecology unit and C: T was 8.1; 26 (96.3%) procedures had C: T > 2.5; 23 (85.2%) procedures had TI < 0.5 only four procedures had C: T ≤ 2.5 or TI ≥ 0.5.
Conclusions: For majority of surgical procedures, the 'group and saving' method can be applied instead of routine cross-matching. The results of this study can be used for drafting Maximum Surgical Blood Order Schedule (MSBOS), and the hospital transfusion committee should review schedule and adjustments should be made according to blood usage data.

P-029
This abstract has been withdrawn.

1.8 Quality Management

P-030
COACHING AND AUTOMATION RESULT IN SIGNIFICANT PROCESS IMPROVEMENTS IN ONE TRANSFUSION SERVICE
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Background: Our facility provides full-service support to a large, metropolitan area and includes 1300 inpatient beds. We deliver multi-dimensional care and related services and dispense approximately 53,000 blood products yearly. Our organization is not unlike most others in that we have tried various methods for process improvement and multiple ways to engage personnel and make changes. We have achieved some success with process changes but faced ever increasing complexity of care and processes, difficulties with timely communication, and work processes and automation that didn't support the workload.
Study: In November of 2010, we implemented new automation for pretransfusion testing. In addition to training all personnel in the use of the new instruments and manual gel methods, personnel were introduced to lean thinking concepts and the principles of flow and creating value for the end customers of the process, our patients. Rather than using strictly didactic learning and mandating process changes, we chose to set up focused coaching sessions for not only the personnel learning the new technologies but also for the middle managers who would be monitoring the new processes and encouraging adoption of new ways of thinking about the work. Key personnel were engaged to help design and implement standardized work areas for specimen receipt and processing, automated and manual testing, and specimen storage. One-on-one training and coaching sessions were set up with each technologist, to make sure they were comfortable with the new testing methods and analyzers. Daily huddles were introduced to enhance communication and foster teamwork, and daily assignments were rotated among technical leaders to monitor and support flow of specimens and personnel.
Results: Because personnel were involved with every level of the changes, they felt that their ideas and concerns were being heard and respected. The coaching approach we took ensured involvement and engagement at all levels of our transfusion services. Some of the results we achieved through coaching for process improvement are in the table and include >95% reduction in pretransfusion testing clerical errors and customer complaints, 20% savings in physical space, 79% reduction in routine T&S TATs, 68% reduction in labor needed for pretransfusion testing, 77% reduction in RBC waste, and >$39,000 reduction in special unit charges due to personnel suggesting and implementing Rh phenotyping on analyzer.

Table 1: Summary of results from coaching and automation

Category	Baseline	Post-Coaching for P.I.	% Reduction or Change
Routine T&S TAT	5:40:24	1:15:00	79%
Space Savings			20%
Aver. Batch Size	35 - 40	9	74%
Testing Labor Savings	-	-	68%
Clerical Errors	8-14/mo	0	>95%
Customer Complaints	18/month	<1	95%
Savings from Fewer Orders for Special Units			>$39.000
Expired RBCs	30/month	<7	77%

Conclusion: The coaching approach moved our employees from a less engaged group to a fully engaged team, focused on serving our patients more effectively and optimizing resources.

P-031
OPERATIONS, QUALITY MANAGEMENT AND RISK MANAGEMENT IN THE TRANSFUSION SERVICE
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Background: For general healthcare, the difference between quality and safety has been unclear for measurable patient outcomes. In contrast, in the Transfusion Service (TS) the relationship between quality and safety has been direct and demonstrable.
Methods: Case studies are described to illustrate the relationship between operations, quality management and risk management in the TS.
Results: Blood availability for elective surgery:
Over three audited intervals, the incidence of patients undergoing elective surgery without available crossmatched blood that had been requested was 1:333, 1:328, and 1:225 for pre-QI, post-QI, and subsequent post-intervention audit assessment, respectively.
Event discovery reports (EDRs):
Over 2 years, incidence of Biologic Product Deviation Reports (BPDRs, FDA-reportable) was successfully reduced from 60 BPDRs (12%) of 507 EDRs in 2009 to 42 (12%) of 336 EDRs in 2010.
Wrong blood in tube (WBIT):
One hundred and two WBIT specimens were identified (by a change in patient's ABO/Rh) from 176,711 type and screen/crossmatch specimens received over a 5 year interval, detected either by previous patient record of ABO/Rh, or by a second specimen for blood type confirmation requirement, since implemented in our TS for the last 3 years. No known cases of 'mismatched' RBC transfusion have occurred during this interval.
Conclusion: There is an inverse relationship between resources/time expended on quality and risk management relative to volumes of operations in the TS. Laboratory-based initiatives that improve patient safety and clinical outcomes, need to have resources aligned with the personnel and time required for quality management and risk management.
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P-032
PROCESS IMPROVEMENT IDENTIFIED IN HKRCBTS QUALITY CONTROL LABORATORY WITH QUALITY TOOLS FROM LEAN MANUFACTURING
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Background: Q.C. testing of blood and blood components ensures consistent product quality and efficacy. The frequency of quality control adopted is 1% of all units with a minimum of 4 units per month, and if less, each unit. In 2010, all Q.C. parameters tested were 16,740 for 604,750 units of blood products manufactured. The Q.C. specimen sampling time in pre-examination phase was 357 h per annum. The need to identify and eliminate waste (non-value-added activities), led the BTS to seek for improvement opportunities that could be identified using quality tools from LEAN manufacturing.
Aims: To apply LEAN tools to uncover areas of process waste in blood product Q.C. pre-examination phase such as unnecessary conveyance, inventory, waiting/idle time, inspection and rework, and to produce exponential results aiming at 30% reduction of current processing time.
Methods: Adopting DMAIC model in five stages: (i) Define problems of Q.C. specimen sampling process, (ii) Measure and quantify the capability of the existing process, (iii) Analyze data, (iv) determine and implement Improvement, and (v) establish Control to ensure improvement continuity. SIPOC identification (the acronym stands for supplier, input, process, output and customer) was the first LEAN tool applied to define the boundaries of current process. Flow of materials and movement of operators between sub-processes were tracked in a Spaghetti Chart to reveal inter-laboratory layout problems, crossing over, bottlenecks, redundant moves and other inefficiencies. The major causes of these problems were further identified and verified by Cause and Effect Diagram. Proceeded with Value Stream Mapping (VSM), improvement opportunities were identified across the entire pre-examination phase. Current state value stream map (cVSM) was created to determine the total value-added time (VAT) and total non-value-added time (NVAT). Every sampling step was scrutinized to reduce/eliminate the NVAT. In Improve Phase, 'Pull-system' was applied to ensure immediate availability of required Q.C. products on site during sampling; 'Cell-line' to ensure readily accessibility of necessary equipment. Finally, future state value stream map (fVSM) was constructed to evaluate and monitor process improvement.
Results: The results of cVSM and fVSM were tabulated and analyzed as follows. Re--designed work flow, work areas and improved equipment accessibility contributed 11% sampling time reduction, 100% reduction in waiting/idle time, and 25% reduction in transportation time. The total VAT was 11% reduced; total NVAT was significantly 71%

reduced, resulting annually 99 h saving in pre-examination phase, i.e. overall 27% time saving was achieved through this LEAN manufacturing operation. See Table 1.

Table 1: Comparison of process times and distances

Process Category	Current Process Time	Current Distance Travelled	Improved Process Time	Improved Distance Travelled	% Reduction in Time
Sampling	28 min.	48 meters	25 min.	0 meter	11%
Waiting/ idle	6.5 min.		0 min.		100%
Transportation	4 min.	132 meters	3 min.	54 meters	25%
Total VAT	28 min.		25 min.		11%
Total NVAT	10.5 min.		3 min.		71%
Total VAT & NVAT reduction	38.5 min.		28 min.		27%

Conclusions: The LEAN quality tools were effective in identifying opportunities for improving work processes and the flow of Q.C. specimens and reduction in valuable operator process time in pre-examination phase. The Q.C. specimen sampling time was reduced by 27%, i.e. 3% less than the 30% reduction target. Further improvement opportunities would be identified using the LEAN quality tools. We consider this LEAN manufacturing operation a valuable experience in BTS's sustainable development; will extend LEAN application scopes in future planning of work area and work flow designs prior to moving into our new blood centre.

P-033

REDUCING ERROR POTENTIAL IN UNEXPECTED ANTIBODY DETECTION TESTING WITH AUTOVUE INNOVA

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Background: Automation can greatly reduce the need for human intervention and overall error potential in pretransfusion testing. The right automation coupled with lean thinking can ensure optimization our most scarce resource – human experience and intellect.
Aims: United Christian Hospital in Hong Kong wanted to use lean tool applications to evaluate pretransfusion testing processes post-implementation of the ORTHO AutoVue® Innova (AVI) analyzer and evaluate the error potential reduction with AVI for antibody screening. The overarching business goals of our facility continue to be to decrease error potential and simplify current work processes, enhance services while absorbing increased workload, and decrease employee stress levels. The aim of this case study was to use lean thinking applications to identify continued improvement opportunities and evaluate the effectiveness of the AVI integration for antibody screening only, to impact our business goals.
Method: Lean principles and tools were used to assess work practices and identify improvement opportunities to optimize personnel, the physical space and the investment in the AVI instrumentation. The main tools used were product and operator value flow analyses and defect and error potential analysis. Work practice assessment included the people, processes, equipment, physical plant, and reagents and disposables required. The testing operations associated with manual test tube and grouping plate methods, manual column agglutination technology, and AVI for antibody screening were analyzed. Also analyzed were specimen receipt and processing and donor unit confirmatory grouping.
Results: Lean application of value analysis and error potential analysis revealed multiple areas for continued improvement, which could be achieved by a simple layout redesign and additional consolidation of testing platforms. Results and projected results are shown in Table 1. Projected improvements with testing consolidation included a 40% reduction in group and screen (G&S) cycle time from specimen receipt to released results by automation the ABD testing as well as the antibody screen. Also projected were a 58.1% reduction in operator time for G&S testing and an 83% reduction in operator time for overall testing and 88% reduction in hands-on time. Other benefits included 98% reduction in error potential for G&S testing and improved flow of specimens and operators with re-location of AVI analyzer.

Table 1: Summary of projected results from lean and AVI

Category	Pre-AVI Lean Assessment	Post-AVI for Antibody Detection Lean Assessment	Projected w. Integration of AVI for >90% of Testing	Projected Continued % Reduction w. Integration of AVI for >90% of Testing
Process Steps	37	18	3	91.9%
Defect Opportunities	507	3	3	99.4%
Time from Receipt to Result Release	70 min.	75 min.	45 min.	35.8%
Required Labor Units for G&S	-	67%	-	58.1%
Total Hands-on Time for Testing	-	533 sec.	66 sec.	87.6%

Summary: The application of lean tools provided an effective way to uncover ongoing opportunities for process improvement and AVI optimization in order to accomplish our business goals.

P-034

APPLICATION OF LEAN SIX SIGMA TO STREAMLINE WORKFLOW OF TRANSPORTATION, RECONCILIATION AND SORTING OF PRE-PROCESSED WHOLE BLOOD UNITS IN A BLOOD TRANSFUSION SERVICE

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Background: Due to space constraint, bins containing pre-processed blood units are stored in a cool room on the second floor (2/F) of the building. After overnight-hold of whole blood units, moving carts located on the ground floor (G/F) are brought to 2/F to transport blood bins to the processing laboratory on G/F. Upon reconciliation of blood units, empty bins are returned to another room on 2/F for cleaning but the moving carts must be returned to G/F for storage and other usage. Blood components have to be processed within 24 h of blood collection. Time spent on transportation, reconciliation and sorting of pre-processed blood determines time-line of workflow in component preparation. Any delay in an element of this process will affect overall component preparation. The effect will be remarkable when total quantity of blood units collected is high.
Aims: The aim of this study is to identify process improvement opportunities through Lean Six Sigma tools so as to decrease total time for transportation, reconciliation and sorting of pre-processed whole blood: targeted at a 10% total time reduction. Expected benefits obtained from this improvement are smoothening of production workflow, release the time strain for component preparation within 24 h and decrease in staff overtime work.
Methods: Lean Six Sigma tools were applied to analyze various work tasks and identify process improvement opportunities. The main tools used were process mapping (SIPOC: Supplier, Input, Process, Output and Customer), Flow Process Chart, Ishikawa Diagram and Pareto Chart. Variation was assessed in five aspects: operator, method, environment, material and machine. Lean concept on reduction in various forms of waste was used to cut excess motion, waiting time, transportation time and distance travelled. A pilot study was conducted to eliminate doubts that skipping an apparent non-value-added step might affect quality of blood units.
Results: Ishikawa Diagram, Pareto Chart and Variation Analysis revealed multiple possible root causes and multiple areas for improvement that could be achieved by purchasing sufficient utensils, re-locate storage area for moving carts. Non-value-added procedure was reduced by a pilot study to ensure good quality of packaging by eliminating plastic bag wrapping coolants and work instruction was updated accordingly. Total process steps were reduced by 19.4%, total distance travelled was reduced by 37.1% and total travel/waiting time was reduced by 41.7%. The time for the whole process (workflow of transportation, reconciliation and sorting of unprocessed whole blood units) was successfully reduced by 12.1%. Moreover, the more the units collected, the more the time would be saved as the time reduction was calculated in terms of minutes per blood unit.
Conclusions: Through application of Lean Six Sigma tools, tasks were analyzed in details and process improvement opportunities were identified to aid speeding up the processes of reconciliation of pre-processed whole blood units. In the end, not only such processes have been streamlined to achieve a target of decreasing the whole process time by 12.1%. Additional benefits which included addressing the OHS (occupational health and safety) issues and enhancement of environmental green were also obtained.

P-035

THE POWER OF LEAN PLUS AUTOMATION TO INCREASE OPERATIONAL PRODUCTIVITY AND SAFETY IN PRETRANSFUSION TESTING

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Background: Business needs at our transfusion service included optimizing the investment in automation, enhancing process effectiveness, capacity and overall quality of services, and optimal placement of new instrumentation for pretransfusion testing.
Aims: Our transfusion service, Prince of Wales Hospital in Hong Kong, set about applying lean principles and tools to the main operations in the transfusion services related to pretransfusion testing: specimen receipt and processing, blood grouping and screening, donor unit confirmatory testing, and compatibility testing. The case study aim was to use lean applications to identify improvement opportunities in all areas and to identify and make improvements in the specimen receipt and testing areas quickly and integrate pretransfusion testing automation with optimal placement of the instrument.
Method: Lean principles and tools were used to assess work practices and identify improvement opportunities with and without ORTHO AutoVue® Innova automation (AVI). The main tools used were product and operator value flow analyses and defect and error potential analysis. Work practice assessment included the people, processes, equipment, physical space, and reagents and disposables required. The testing operations associated with manual test tube methods, manual column agglutination technology, and AVI were analyzed. Also captured and analyzed were specimen receipt and processing, compatibility testing, and donor unit confirmatory grouping.
Results: Lean application of value analysis and error potential analysis revealed multiple areas for improvement that could be achieved by a simple layout redesign and consolidation of testing platforms. Projected improvements with testing consolidation are shown in the table below as related to Group and Screen testing (G&S) and included an 18% reduction in test result cycle times without automation and a 52% reduction with the AVI. Reductions in the overall labor required to support testing was projected to be 84.9% with integration of the AVI to support >90% of routine pretransfusion testing. Other benefits of the lean analysis and AVI integration included 83.3% reduction in process steps and a 98% reduction in error potential along with a layout that minimized congestion and maximized specimen and operator flow.

Table 1: Summary of projected results with lean and AVI

Category	Pre-Lean and AVI for Routine G&S	Average % Reduction Post-Lean and AVI
Receipt in Transfusion Service to Results Release	1:33:44	52%
Labor Requirement for G&S Testing	-	84.9%
Process Steps	18	83.3%
Defect Opportunities	132	98%

Summary: The application of lean tools provided an effective way to uncover significant opportunities for process improvement. When coupled with the use of the AVI instrumentation, exponential improvements were identified.

P-036

AUTOMATED PRETRANSFUSION TESTING ENHANCED BY LEAN THINKING TO DRIVE OPERATIONAL EFFICIENCIES

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Background: Business needs in transfusion services include optimization of personnel, increased service capability, enhanced process effectiveness and overall quality of services, and optimal placement of equipment. The specific needs in one site were to decrease the stress on personnel, optimize the investment in automation and other resources, and continue to provide good service to clinicians.
Aims: This transfusion service is located in Canberra, Australia, and serves a wide geographical area. We wanted to apply lean principles and tools to the main operations of specimen receipt and processing, pretransfusion testing, and donor unit confirmatory grouping tests. The case study aim was to use the lean applications to identify improvement opportunities and recommend optimal usage and placement of ORTHO AutoVue® Innova (AVI) analyzers for pretransfusion testing.
Method: Lean principles and tools were used to assess work practices and identify improvement opportunities with AVI and instrument placement. The main tools used were product and operator value flow analyses and defect and error potential analysis. Work practice assessment included the people, processes, products, equipment, physical space, and reagents and disposables required. The testing operations associated with manual test tube and column agglutination technology methods and the AVI were analyzed. Process focus was on group and screens (G&S) and donor unit confirmatory grouping tests. Point-to-point diagrams were constructed to follow the flow of both specimens and personnel.
Results: Multiple areas for improvement were identified with value-added lean analysis and error potential analysis. These improvements could be achieved by a simple layout redesign and consolidation of testing platforms. Table 1 shows a summary of the results of the lean application and integration of AVI. These results included a 98% reduction in error potential, 32% reduction in G&S cycle time from specimen receipt to released results and a 35% reduction in donor unit confirmatory grouping. A 72% reduction in operator time was projected with the inclusion of donor unit confirmatory grouping on the AVI. Other benefits included reorganization of supplies in testing areas, decreased visual noise, consolidation of functional activities to eliminate congestion of walk patterns, and optimal location of AVI analyzers.

Category	Cycle Time	Average % Reduction Post-Lean
G&S Total Cycle Time from receipt to Results	1:06:30	32%
Total Cycle Time for Segment Prep and Testing of Donor Units	1:25:02	35%
Labor Requirement for Donor Unit Grouping	-	72%
G&S Process Steps	16	81.2%
G&S Defect Opportunities	107	97%

Summary: Lean tools provided an effective way to uncover significant opportunities for process improvement. When coupled with the use of the AVI instrumentation, exponential improvements were identified. In addition, the lean tools drove best placements for the two AVI analyzers by identifying current and optimal flow of the patient and donor unit specimens as well as the people.
Table 1: Results from lean and AVI optimization

P-037

ASSESSTMENT OF ERROR REPORTS AT SHEBIN EL KOM RBTC DURING 2010

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Background: Shebin El Kom RBTC is situated at Menoufia Governorate and it's about 60 km far from Cairo.
Shebin El Kom RBTC is a large Regional Blood Transfusion center which is renovated and reconstructed through the Egyptian – Swiss project for developing Blood Transfusion Services.

Table 1

A) According to Type of errors

Type	No. of errors	% of total reports
1. Laboratory	11	39.3 %
2. Safety	5	17.9 %
3. Documentation	5	17.9 %
4. Technical	3	10.7 %
5. Clerical	3	10.7 %
6. Others	1	3.5 %

B) According to Departments

Type	*No. of errors*	*% of total reports*
Patient Care	*10*	*35.7 %*
Donor care	*8*	*28.6 %*
Blood Component	*3*	*10.7 %*
Serology	*3*	*10.7 %*
House Keeping	*2*	*7.1 %*
BED	*1*	*3.6 %*
Finance Department	*1*	*3.6 %*

Some new activities and services was offered at our center after new opening at January 2008.

These activities include the establishment of Quality Department, Training and Research Department and Information Technology Department. Since then we started to ensure applying national blood transfusion standards and recording error reports.

Aim: Categorization and evaluation of errors happened at Shebin El Kom RBTC during 2010 started from 1 January 2010 to 31 December 2010.

Materials and methods: 1. Documents of recorded error reports (From 1 January 2010 to 31 December 2010).

2. Data analysis.
3. Statistics and charts.

Results: Analysis of recorded error reports during 2010 showed the following results:

1. Total No of error reports was: 28 errors.
2. Categorization of errors based on types of errors and Departments committed for these errors was illustrated in the following tables.

Conclusion: We conclude from this study that the highest % of errors according to types was recorded to be laboratory errors (39.3%) and according to the committed department was the patient care Dep. (35.7%) followed by Donor care Dep. (28.6%).

Recommendations: 1. All staff must follow safety measures and SOPs.

2. All Documents must be used.
3. Retraining and Refreshment for staff members especially PCD and DCD.
4. Revision and Follow up held by Head of Departments and physicians.

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P-038

APPLYING QUALITY ASSURANCE TO CLINICAL INTERFACE OF BLOOD TRANSFUSION THROUGH ESTABLISHMENT OF HOSPITAL TRANSFUSION COMMITTEES IN SRI LANKA

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Background: Sri Lanka has a centrally co-ordinated National Blood Transfusion Service (NBTS), where 85 hospital based blood banks are managed and monitored through 16 cluster centers by the National Blood Center (NBC) in Colombo. When achieving the target of good transfusion practices, it is necessary to establish hospital transfusion committees (HTC) in all the hospitals covering the entire country. HTCs act through a peer-reviewed process aimed at improving safety, enhancing education, developing policies and monitoring appropriateness of blood component use.

NBTS of Sri Lanka initiated action to implement HTCs in the year 2005. The success of this programme until the year 2010 is debatable as the number of hospitals where HTCs were established from 2005 to 2009 was <10 where active participation took place only in one or two.

Objective: NBTS has decided to achieve its aim of 100% functioning HTCs in the country by strengthening already established HTCs and taking steps to establish new HTCs in all hospitals island wide.

Method: A focal point was established In NBC to coordinate this project with the Administration. Following areas were taken in to primary consideration. When establishing HTCs in a resource poor country like Sri Lanka strategies adapted by the NBTC were

1. Changing the context of the existing programme.
2. Getting an authorization from ministry of health.
3. Training medical officers in charge (MOIC) on management in organizing and conducting meetings.
4. Establishment of a direct link with the CEO of the NBTS on the project.
5. A simple, effective and attractive agenda to support the clinical and administrative staff of the hospitals to address issues promptly.
6. Acquisition of funds for a sustainable programme.
7. Recommendations to upgrade the blood bank performance and transfusion practices were sent to relevant authorities after each committee meeting by the National Blood Centre.
8. Guidance and resource persons were provided for all awareness programmes required by the end users of blood and blood components.

Review of statistics for previous 6 months was presented in each Hospital Transfusion Committee meeting that lead to interactive and informative discussions at the meetings. This served as a very good platform to review of performance of each hospital blood bank and blood usage of clinicians. A model for this review was provided by the NBTS, which includes following critical poits

1. Analysis of blood collection.
2. Analysis of blood and blood component production and usage.
3. Cross match transfusion ratio for each speciality.
4. Data of Heamovigilance and necessary corrective actions.
5. Monitoring of wastage rates.

The results of the review were used by the transfusion committee to recommend changes in practice by the hospital staff to improve patient care.

Results: The objective of implementation of HTCs in all hospitals with all clinical specialities throughout the country was achieved. The table below shows the achievement results.

Table 1: Achievements gained through implementation of HTCs

	Before implementation of HTC(Before 2010)	After implementation of HTC(in 2011)
Blood Discard Rates	20%	4%
Level of critical blood stock maintenance	76%	94%
Expansions of Haemovigilance Network	5%	100%
Knowledge of bedside transfusion practices	9%	75%

Table 2: Number of HTC Held

	2006	2007	2008	2009	2010	2011
Number of HTCs	3	3	4	5	40	48
Number of provinces covered	1	1	3	3	8	9

P-039

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P-040

This abstract has been withdrawn.

P-041
STRINGENT SPECIMEN-LABELING REQUIREMENTS AND DISPOSAL DECREASE THE RATE OF 'WRONG BLOOD IN TUBE' IN TRANSFUSION PRACTICE

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Background: Wrong blood in tube (WBIT) was one of the important causes of transfusion errors. Since the implementation of quality management system, such as ISO 15189, CAP Laboratory Accreditation Program or COLA Laboratory Accreditation, much progress has been made to the accuracy of specimen identification in Taiwan. Though the American Association of Blood Bank's guideline suggest that in case of discrepancy or doubt, another sample shall be obtained, but the criteria of specimen rejection and the following disposal of the rejected specimen are essentially different among hospitals. For example, some will return the specimen to the person who collected the specimen, and others will retain the rejected ones and ask a newly collected specimen for testing. These different levels of operative specification might have undesirable effect on transfusion safety, or more specifically wrong specimen for compatibility testing before transfusion.
Aim: A prospective observational study was conducted to evaluate the effect of a stringent labeling requirement upon the rate of wrong blood in tube in real transfusion practice.
Method: From 1 January 2008 to 31 May 2011, each specimen that did not fulfill the requirement of acceptance for pre-transfusion testing (rejected specimens) was retained, and a new legible specimen was requested and used for compatibility testing according to routine procedures. The retained specimen was tested for ABO and Rh typing, and was recording on a special sheet not for routine use. The results of the rejected specimens and the newly collected accepted specimens were compared. Incident reports of wrong blood in tube were also collected. Rates of wrong blood in tube among accepted specimen and rejected specimen were calculated, and were compared by Fisher's exact test. A P value of <0.05 was considered statistically significant.
Result: A total of 57,181 consecutive specimens were received for compatibility testing. Among them, 1151 (2%) failed to meet the defined criteria of acceptance in the laboratory. The reasons for rejection included wrong name or hospital patient identification number (i.e., did not match requisition form), lack of at least one signature of phlebotomist on the specimen tube, etc. Overall, the rate of WBIT was 0.61% (7/1151) and 0.004% (2/56,030) for rejected and accepted specimens, respectively. Compared to the rate of WBIT in accepted specimen, the rate in rejected specimens was 170-folds higher. ($P < 0.05$)

Table 1: Frequency of ABO and/or Rh discrepancies

Table 1 Frequency of ABO and/or Rh discrepancies

	Specimen typed	Discrepancies	%
Correctly labeled	56030	2	0.00357%
Mislabeled (rejected)	1151	7	0.60817%
			P<0.05

Conclusion: A stringent policy for labeling requirement including retained the rejected specimen would significantly decrease the rate of wrong specimen for pre-transfusion testing (i.e., WBIT).

P-042
IMPROVEMENT OF TRANSFUSION ACQUISITION ACCURACY USING PROSPECTIVE BARRIER ANALYSIS

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Background: Transfusion acquisition errors would not only waste the time of the blood bank technologist to resolve it, but also potentially lead to the delay of issue the delivery of life saving blood product to the critical patient. Barrier analysis was first developed by Trost and Nertney and was introduced into healthcare organization with root cause analysis in recent decade. A barrier is a control measure designed to prevent harm to vulnerable or valuable objects. The prospective barrier analysis has been applied to improve the safety of drugs usage, and is demonstrated an easy, simple and rapid assessment tool to detect safety barrier defects and switch on the fail-safe mechanism.
Aim: This study is to use the prospective barrier analysis to detect and modify the defense barrier defect, and to evaluate its effect on transfusion acquisition accuracy.
Method: Transfusion acquisition errors were retrieved from rejection records of specimen and transfusion request form. All errors were categorized according to the transfusion acquisition process. Corresponding current barrier/control/defense mechanism was recorded and its importance to safe practice and power of failsafe was analyzed by consensus of blood bank technologist, nursing practitioner and quality management staff. Then possible new B/C/D mechanism was proposed to counteract each of the hazards, and the potential importance to safe practice and power of failsafe. The implementation cost and responsible department or person was also defined. After the implementation of the new mechanism, the numbers of transfusion errors was compared to those before.
Result: Before this project, a total of 1641 transfusion acquisition errors were recorded. The rejection of transfusion acquisition includes patient's blood type was not written, lack of patient's information, the time of transfusion and blood product counts that were not written, etc. Seventeen hazards were identified and six of them (84% of all errors) were selected for barrier analysis, and six additional barriers were proposed and implemented. The transfusion acquisition errors reduce 74.7%.
Conclusion: Prospective barrier analysis is simple and easy to use and is effective in reducing transfusion acquisition errors. Successful application to other area of transfusion process would be expected.

P-043
COMPARISON OF QUALITY PARAMETERS OF PLATELET CONCENTRATES PREPARED BY DIFFERENT METHODS

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Background: There are two types of platelet concentrates namely Recovered or Random Donor Platelets (RDP) and Single Donor Platelets (SDP) or Aphaeresis Platelets (AP-PC). There are two basic methods of producing Recovered/Random Donor Platelets (RDP) from whole blood donations, one is the Platelet Rich Plasma-Platelet Concentrate (PRP-PC) method and the other is the Buffy Coat Derived Platelet Concentrate (BCD-PC) method. Single Donor Platelets (SDP) or Aphaeresis Platelets (AP-PC) collected from voluntary thrombocytaphaeresis donors with the help of an automated cell separator.
Aim: Study is designed to compare different types of platelet concentrates prepared at National Blood Centre, Sri Lanka based on standard Quality monitoring parameters.
Method: Study was based on an analysis of platelet preparations performed and tested from 1 January 2008 to 31 March 2009. A set of 323 data were available for PRP-PC, 428 for BCD-PC and 691 for AP-PC.
Results: In the present study the mean volume of PRP-PC, BCD-PC and aphaeresis-PC was 62.87 + 12.63, 62.17 + 10.97 and 191.72 + 52.46 ml and ranged from 20–104 and 28–114 and 96–448 ml respectively. Out of these units, 315 are for PRP-PC, 417 for BCD-PC and 679 units are for AP-PC. Their mean pH was 7.25 + 0.57 (mean + SD), 7.15 + 0.44 (mean + SD), 6.66 + 0.57 (mean + SD) and ranged from 5.74–8.82, 5.66–9.16, 5.11–8.10 respectively. The mean platelet count of PRP-PC, BCD-PC and AP-PC was, 39.38 + 25.41, 59.89 + 28.99 and 253.62 + 89.33 $\times 10^9$/unit and ranged from 10–134, 11–159 and 22.9–601 $\times 10^9$/unit respectively. The mean WBC count in PRP-PC, BCD-PC and AP-PC units was 0.0467 + 0.342, 0.0435 + 0.054, and 0.0625 + 0.012 $\times 10^9$/unit and ranged from 0–6, 0–0.55 and 0–6 $\times 10^9$/unit respectively. There is no significant difference between three types ($P > 0.05$). The mean RCC count in PRP-PC (n = 321), BCD-PC (n = 427) and AP-PC (n = 690) units was 0.0467 + 0.342, 0.435 + 0.054 and 0.0625 + 0.262 $\times 10^9$/unit and ranged from 0.00–0.03, 0.001–0.082 and 0.00–3.00 $\times 10^9$/unit respectively.
Conclusion: There is no significant difference between PRP-PC and BCD-PC in terms of the mean values of volume, platelet count per unit, pH, WBC count per unit and RBC count. All three types of platelet products have required pass rate in relation to NBTS standards with regard to pH and RBC count per unit. This study also highlights the importance of the routine quality monitoring, in order to minimize the variation in end products produced and reduce the likelihood of releasing the low quality products, which can occur due to variation in blood donors, collection and component production processes.

P-044
'INTERNAL AUDITS' – ARE WE DOING IT PROPERLY?

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'Internal Audit' (IA) is an independent examination of a quality system which measures the effectiveness of an organisation's quality management system. In ISO 15189 accreditation program, it is required to audit all 15 management and eight technical elements internally during a calendar year. It is a documented and systematic tool and should be done periodically by independent and qualified people. IA allows an organization to find and resolve non-compliances before the certification body/customer finds them.

'Auditor' is a person with the demonstrated personal attributes and competence to conduct an audit. The auditor should never be aggressive no matter what happens.
Audit process consists of five stages namely, preparation and planning, performance of the audit, closing meeting, submitting an audit report and the follow up. It is very important to remember that, 'Proper Planing and Preperation Prevent Poor Performance'. Firstly, the annual Audit schedule is given to respective Laboratory Management. Then appoint trained personnel to perform the internal audits. Audit team should consist of following four categories, and they should not attach to the laboratory being audited. The categories are, Lead Auditor, two or three Audit team members, technical experts and observers. Auditee should be notified at least 1 week in advance.
Development of a audit checklist by obtaining and reviewing all relevant documentation such as previous audit reports, quality manual, relevant SOP and working instruction is a very important step, as it controls the continuity and act as a memory aid, guide and a controller to the depth of the audit. It can either be high level (Generic or specific) or low level (detailed) one. Checklists have lots of advantages, if developed for a specific audit and used correctly. They promote planning, ensure a consistent approach, and act as a sampling plan and time manager an easy way to take notes during the audit process, and ensure that the adequate evidence is obtained. Auditors need to be trained to use it to obtain maximum information. It also helps to ensure systematic and comprehensive audit.
Usually audit should be started with a brief opening meeting in which lead auditor do the introduction, explain the objectives and scope of the audit, give the timetable and discuss the general administrative arrangements and limitations. During the performance objective evidence are gathered to verify compliance or non-compliance. This is done by inspecting documents, asking questions or by observing procedures performed. Following completion audit team will evaluate the evidence and agree on non-conformances found in order to make a list of non compliances.
The lead auditor explain the outcome of the audit and ensure the auditee understands and accepts the findings, which can be categorise as major/minor non-conformities or as an observations in closing meeting. Then agree for the final release of the audit report and dates for correct of non-conformities. After submitting an audit report follow up should be done with regard to see a satisfactory response from auditee and their commitment to correct for any deficiency.

P-045
IMPROVEMENT IN LABORATORY SERVICES AND BETTER UTILIZATION OF LIMITED RESOURCES THROUGH APPLICATION OF BASIC LEAN PRINCIPLES

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Background: The lack of adequate laboratory space is a concern facing transfusion services. Congested physical space for performing key functions can lead to increased stress levels and higher error potential.
Aim: To make better use of limited space and resources, our transfusion service wanted to use some basic lean concepts and tools to try and reduce our turnaround times and identify alternative layouts to create better workflow in the laboratory.
Methods: We applied lean principles and tools on two separate occasions to identify improvement opportunities, first in our Type and Screen (T&S) and, second, in our Crossmatch (XM) testing processes. We followed the path of the personnel as well as the blood samples, established a baseline measurement, and identified key points where we could make changes.
After identifying areas for improvements in our processes on our first assessment, we then set about changing our floor plan to improve workflow. After a period to allow staff to acclimatize with the new lay out and processes, we applied the lean tools a second time. We measured our processes to calculate the improvements, and these new measurements now serve as our new baseline when we need to re-evaluate our status in the future.
Results: Our baseline data showed the distance travelled by our technicians, who operate the ORTHO AutoVue® Innova (AVI), was significant. With process redesign and the implementation of a new LIS system, it was projected that we could reduce the distance travelled by the technician by up to 97% and reduce the operator hands on time by 81%.
Historical data shows our average turnaround for T&S plus XM testing was 180 min. Post lean implementation, our data indicates that our current turnaround time is 120 min, a reduction of 33%.
In March 2011, we then looked to further reducing our turnaround times by applying the tools on our current XM method (manual gel card). Through operator analysis, we calculated that by automating our XM tests on the AVI, and further layout reconfigurations, we could project a 24% reduction in operator hands on time and a 23% reduction in process steps.
Summary: Lean thinking and tools provided unbiased, data-driven evidence to support change within our laboratory and enhance services. We were able to reduce operator hands-on time, which has led to reduced turnaround times and decreases error potentials. The new laboratory layout has led to a better working environment with less stress. Lean, along with automation, has given our laboratory increased capacity with the same amount of resources.

P-046
SATISFACTION SURVEY OF BLOOD DONORS IN NATIONAL CENTER FOR BLOOD IN ABIDJAN, COTE D'IVOIRE

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The National Blood Transfusion Center (CNTS) is responsible for the execution of the blood policy in Côte d'Ivoire. Make quality, is simply to do what you planned to do, to measure the effectiveness of our action, to make the necessary corrections or in the execution, or in the forecast. Now all quality system shall satisfy the customer. Therefore, any quality system require to measure the degree of customer satisfaction. The purpose of this survey report focuses on measuring the satisfaction of the blood donor at the fixed site of the CNTS in Cote d'Ivoire.
Methodology: Our study was conducted on the CNTS site fixed of Treichville in Abidjan, Côte d'Ivoire. This is a prospective study of systematic random type which took place from 2 February to 1 March 2010. The study population consisted of volunteer blood donors aged 18–65 of both sexes and of any origin.
With the help of a questionnaire completed by the donors we evaluate all the services offered to the blood donor reception, consultation, collection, collation. Nine hundred and one blood donors have been interviewed.
Results: Donors are mainly young with a median age of 32. Eighty-five per cent of respondents are regular donors. Seventy-seven per cent of donors were satisfied with opening hours of the site which is at 7:30 min. Text messaging reminder of the date of donation is appreciated by our donors (over 90% in favor). Our donors have acclaimed the comfort of the reception room in 98%. Our host is rather popular (81% favorable). The cleanliness of our premises is advocated by the donors. About 20% of donors are not satisfied with the information we give them during and after the donation. The satisfaction rate is modest (77%) for the waiting time for a blood donation. The reception staff fixed site is satisfactory at all levels of the process (91%). Doctors consultation are very close to the plebiscite. Collection staff is deemed competent by 88% of donors interviewed and deemed insufficient by 35% of donors. The donors have acclaimed the comfort of the room collection. Thirty-one per cent of donors are not satisfied with the post donation snacks we provide. The arrangements for reporting the results of biological gift are considered satisfactory by 79% of donors. Eighty-eight per cent of blood donors say they talk about blood donation in their communities.
Conclusion: Satisfaction rate exceeds the lowest 60%. Points satisfactions existed, and they are many.

2. Blood Donation
2.1 Blood Donor Recruitment

P-047
THE IMPACT OF IMPLEMENTATION OF THE H1N1-RELATED DEFERRAL CRITERIA ON BLOOD DONORS IN SINGAPORE

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Background: Study on blood donors who came to donate blood in Blood Bank at HSA, Singapore during H1N1 epidemic period.
Aim: To analyze the total number of donors deferred due to the implementation of H1N1 related deferral criteria and to look into the return rate of deferred donors within a 6-month period from their date of deferral.
Method: This is a retrospective analysis of all the donors deferred due to the newly implemented H1N1 related deferral criteria. The period of study was from 29th April till 14th July 2009. The donors who have traveled to H1N1 affected countries were deferred for 2 weeks. The deferral was lifted on 7 July 2009 after many countries were affected including that of Singapore. The donors who were symptomatic for influenza, or diagnosed to have H1N1, or asymptomatic with close contact with H1N1 patients were deferred for 3 weeks.
Result: Total 206 donors were deferred. Seventy-seven (37.4%) of them were new donors and 129 (62.6%) were repeat donors. Only 7 (9.1%) of new donors returned for a donation and 70 (90.9%) did not. For repeat donors, 70 (54.3%) returned for a donation and 59 (45.7%) did not.
Conclusion: The repeat donors were generally more committed to the blood donation program while the new donors tended to drop off from our donor pool if they were deferred on their first attempt.

P-048
This abstract has been withdrawn.

P-049
TYPES OF DONOR SOLICITATION AND THEIR EFFECTIVENESS ON BLOOD DONATION RATES IN EASTERN TAIWAN

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Background: The decreasing eligible age-group blood donors in the widely dispersed population in eastern Taiwan demanded the Blood Center to increase individual donations as its top priority goal.
Aims: It is the purpose of this study to find out the most effective method of blood solicitation among different age-group donors.
Methods: The survey was conducted on whole blood donors at four fixed blood donation stations, six randomly selected blood drive event, and seven mobile stations, which were chosen based on the blood donation rate of each station from June to September 2010. A total of 1400 questionnaires were handed out and all data were analyzed by SPSS.18.
Results: The three major source information for blood donation was postcard notification (35.0%), passing through the event (17.2%), encouraged by family and friends (15.6%). The top three most effective method of solicitation regarded by the donors are postcard notification (41.9%), e-mail notification (19.1%), and text message notification (12.5%). The fact that only 1.7% of donors participated in the event through website notification was relatively low, methods to enhance promotion through website is worthy of further study. It was also noted that 67.7% repeat-blood donors needed to be reminded of the blood donation event.
Conclusions: A cross analysis of the donors' basic information showed that the most suitable and effective method for solicitation varies among donors with different attributes. The best way for donors with high numbers of donation and over 40 years old can be solicited more effectively through postcards and those who are younger with higher education and lower frequency of donation can be more effectively reached through e-mail or text messaging. However, to confirm if an effective donation solicitation will yield a higher rate of return requires further extensive research in the future.

P-050
THE EFFECT OF MAJOR DISASTERS ON NEW DONORS – A CASE STUDY ON 921 EARTHQUAKE AND TYPHOON MORAKOT

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Background: Blood drives in Taiwan are now entering their 37th year. According to the statistics published by the Taiwan Blood Services Foundation, over the years, the 'Annual Blood Collection', 'Donation Rate', and 'Donation Frequency' have been on a steady rise. This shows that citizens of Taiwan have developed the concept of regular blood donation.
However, there is one phenomenon worth close scrutiny. A gradual decline in new donor rate appeared in recent years, which was unprecedented in the recent decade (please see Table 1). People in Taiwan are often motivated with enthusiasm to donate blood when a major disaster hits Taiwan. To further explore whether a major disaster acts as a catalyst to New Donors, this research sets its objective to study the connection between 'New Donors' and the two factors.
Methodology: Based on the statistics published by the Taiwan Blood Services Foundation, this research made a comparison on the New Donors rate in the respective month of the 921 Earthquake 1 and Typhoon Morakot 2 to the rates recorded in the month before and after the disasters.
Results: 1. The 921 Earthquake hit Taiwan on September 21st 1999. The New Donor rate during that month was10.11%, which registered a 3.17% of increase compared to the previous month (August, 6.94%) and a 0.42% increase compared to the following month (October, 9.69%). The statistics revealed that the New Donors rate of the month of the disaster is significantly higher than the rate recorded in the month prior and after to the disaster, which indicates that the disaster effectively encouraged New Donors.
2. Typhoon Morakot hit Taiwan on August 8th 2009. The New Donors rate was 8.51, which registered a 2.83% of increase compared to the previous month (July, 5.68%) and a 1.62% increase compared to the following month (September 6.89%).

Table 1: TBSF recent decade New Donor rate Unit: %

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
New Donor rate (%)	29.16	24.28	21.71	20.25	17.81	17.40	17.45	16.65	15.80	15.26

Conclusion: When these two disasters hit Taiwan, the New Donors rate saw a significant surge, compared to the month before and after the events. Both disasters, the 921 Earthquake and Typhoon Morakot, caused high casualties, and the continuous in-depth media coverage motivated many people to join in a blood drive.
Since blood has a relatively short preservation period, the risk of having to abandon excess blood is an issue demanding close attention. Another issue arising from a sudden surge in blood donation is the problem of blood testing. Therefore, the best strategy lies in health education. Encouraging people to stay healthy and donate blood regularly would be the best strategy.
Notes: 1. 921 Earthquake (21 September 1999): 2415 deaths, 29 missing persons, and 11,305 injured persons.
2. Typhoon Morakot (8 August 2009): 673 deaths, 26 missing persons, and 34 injured persons were recorded.

P-051
THE STRATEGY TO INCREASE THE RATE OF FIRST TIME DONORS RETURN

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Background: According to Taipei Blood Center 2003–2004 statistics, the first time donors showed no significant gender differences, their ages concentrated in 17–20 years, while the type of career were chiefly students, about 50%. The percentage of first time donors was account to 12.3% of total blood donors and about 60,000 persons per year. More complex and advanced therapeutic treatments have led to increasing blood utilization. Blood centers are challenged to maintain a safe and adequate blood in the face of increasing blood demand. Donation at first time donors is critical to building a cadre of committed repeat donors. First time donors are of great importance because they represent the continuity of the blood supply.
Aim: The aims of this study are to interview first time donors with telephone questionnaire to understand the motives of their donation, the reasons why they do not continue to give blood and the adverse reactions after blood donation.

Methods: Between 1 April 2006 and 31 May 2007, a total of 840 blood donors who only donated blood once in 2005 were interviewed. Another 840 donors, matched in donation time, age and sex with study group were selected as control, there was no telephone questionnaire were given to them.
Results: The motives for the first time donors were as follows, convenient place to donate (39.6%), requests by organizations (22.9%), responsibility to help others (19.1%), and recruited by relatives or friends (11.8%). The reasons why they did not continue to give blood were busy (42.1%), do not know where to donate blood (30.3%), and health problem (16.5%). As for the adverse reactions, the most was bruise (2.2%), followed by feeling of fatigue after blood donation (1.6%) and arm sore (0.2%). The total donor reaction rate was 6.9%. Finally the rate of return in the study group was 14.0% and that of control group was 17.7%.
Conclusions: Convenient place to donate, requests by organizations, responsibility to help others, and recruited by relatives or friends appear to influence first time donors return. The return rate of study group is 14.0%, compared to 17.7% in control group. There are no statistically significant differences between the study group and control group. Further long-term follow-up and evaluation are necessary to assess the effectiveness. Our findings may assist blood centers in optimizing their efforts in recruiting and retention of first time donors.

P-052
THE INFLUENCE OF HIGH-PRICE SOUVENIR ON THE BLOOD DONATION RESULTS IN NORTH TAIWAN

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Background: The blood donors get used to receive various souvenirs after donations. These souvenirs usually affect the achievements of blood drives a lot according to their designs and values especially during the economic depression era. Sometimes they may also influence the first time donor rate and laboratory exam results.
Aims: To understand the changes and influences of blood drive achievements, unqualified donation ratio and anti-HIV EIA positive ratio by the high-price souvenirs.
Methods: We gave every donor who successfully donated blood a voucher for exchanging a free beefsteak set (value: 500NTD/set) contributed and sponsored by local chain restaurant during the Chinese Lunar new year which brought the most blood shortage period in Taiwan each year. There were total 1500 free vouchers which were sent in 17 different blood donation sites in this study on 2 February 2010 near the Chinese Lunar New Year.
Results: There were 3339 persons successfully donating their blood while the achievement rate was 185% higher than usual. Among the 3339 donors (include 1500 voucher winners), the first time donor rate was 24.1% (annual = 7.6%). The age portion rate of 17–24 years old was 43.7% (annual = 15%). The total unqualified donation rate in laboratory was 3.11% (annual = 1.56%) which was approximately near two times than annual. The anti-HIV 1 + 2 EIA positive rate was 0.24% (annual = 0.10%) which was approximately near 2.4 times than annual. Among the 1500 persons who got the beefsteak vouchers successfully, the first time donor rate was 32%, the age portion rate of 17–24 years old was 28.4%, the total unqualified donation rate in laboratory was 2.66%, the anti-HIV 1 + 2 EIA positive rate was 0.4% (six persons) which was four times higher than annual.
Conclusions: The high-price souvenirs actually not only influenced the donation achievement remarkably but also affected the donors' motive especially the young group of 17–24 years old and the unqualified donation ratio in the laboratory exam. We are now facing the impacts of both decreasing birth rate and aging of population, therefore, we still have lots of efforts to promote, inspire, recruit, educate and even attract the young generation to donate their blood with correct knowledge of HIV prevention and protection in the near future.
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P-053
IMPACT OF PROMOTION TO THE MOTIVATION OF THE DONORS IN A LARGE BLOOD DONATION EVENT

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Background: Kaohsiung Blood Center is responsible for the blood supply for the areas of Kaohsiung, Pingtung, and Penghu. More than 2000 events annually, blood donation provides as much as 57.7% of the blood the center offers the hospitals in the areas. It attracted our attention that 4415 bags of blood were given by the donors in a town with 850,000 populations in an 10-h donation event. The aim of the study was to define the motivation of the donors participating in a large blood donation event.
Methods: A total of 2993 volunteers took part in the Blood Donation sponsored by a local company (Kuo-Hsing Poultry & Livestock Feeds Co., Ltd) in 2 January 2011. Among them, 2245 volunteers were studied to evaluate their motivation for blood donation. With dimensions of Motivation and Behavior to examine the impact of promotion, a questionnaire was developed including basic demographic, experience of blood donation, and reasons for donating. Descriptive analyses and Student t-tests were used to test the differences.
Results: Among the 2245 volunteers who were studied, 1316 were male (58.6%), 929 were female (41.1%). Most of them were local residents (83.2%). Blood were given most frequently at the blood donation vehicle (37.4%), and followed by Pingtung Blood Center (31.7%). 660 volunteers (29.8%) were motivated by the company image of Kuo-Hsing Co., Ltd, and 594 (26.8%) were urged by the family and friends. Direct-mail promotional cards were ranked most efficient material to attract people to give blood, whereas broadcasting via promotion car was ranked least. However, the differences (3.61–3.95) were not significant. Female donors demonstrated significantly higher degree of acceptance toward promotional cards direct mailed ($P = 0.022$) and promotion text paged to them ($P = 0.047$). When compared with donors in Kaohsiung city, donors in Pingtung area preferred significantly the promotion banner ($P = 0.032$). On the attitude to routine donation, there was no difference between male and female donors, nevertheless, female donors were inclined to give blood in a particular activity ($P = 0.004$). Study results also revealed that 23.5% of the regular donors only took part in the activities sponsored by Guo-Hsing. In addition, only 7.3% of the donors gave their blood for the first time, yet the average percentage of first-time donors was 15% in those areas.
Conclusion: Especially female, donors in the Pingtung area are willing to participate and are satisfied with the blood donation activities sponsored by specific company (i.e. Kuo-Hsing Poultry & Livestock Feeds Co., Ltd). With or without remuneration or gimmick, company image reinforces the motivation of volunteers to give blood.

P-054
DETECTION OF HEMOGLOBIN CONCENTRATION FOR ANALYZING BLOOD LOSS AND RECOVERY IN TAIWAN

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Background: Blood donation plays an important role in each health care system. Hb concentration is estimated, taking gender, weight and height into account. Therefore, the purpose was to investigate the recovery of Hb after standard blood donation.
Aims: Hb serves as a mere indicator of iron status in this study. The research analyzed Hb concentration before and after blood donation.
Methods: The study was approved by Ethics Committee of TBSF, with two groups to be tested in this research: one group donating 250 cm^3 and a second group donating 500 cm^3 The Hb concentrations of 42 blood donors were analyzed before and up to 3, 6, 8, 14 and 30 days after blood donation. Body weight and height of each donor was measured immediately before donating blood. For each donor, the decrease in Hb concentration on various days was estimated by fully automated system. The data are presented as the means and standard deviations for continuous variables. For differences between values of baseline and days 3, 6, 8, 14 and 30, mixed models with fixed effect of time and BMI and random effect of subject were used to analyze the data. The test revealed two-tailed P values and a P value <0.05 was considered statistically significant. All analyses were performed with the statistical package SAS for Windows (Version 9.2; SAS, Cary, NC, USA).
Results: Hb concentration decreased until day 6 for donors donating 250 cm^3 blood, prior to starting to increase slowly. For blood donors donating 500 cm^3 blood, Hb concentration decreased until day 3 before showing slow recovery. All test subjects remained healthy during the observation period.
Conclusion: The main finding of this study is that Hb as a direct measure of the blood system is restored in about 30 days after 250 and 500 cm^3 whole-blood donation. The results of this study confirm the minimal, recommended donation intervals (60 days for 250 cm^3 donation, 90 days for 500 cm^3 donation) as adequate when, for the first-time, being judged upon by Hb as a direct marker of hematologic recovery in Taiwan.

P-055
GIFT: THE INFLUENCE ON BLOOD DONOR'S CHARACTERISTICS IN TAIWAN

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Background: The weather is changeable, freezing and cold weather attacks continuously during winter season, while heavy rain and hot, humid weather hits in summer. The preceding causes leading to the public's less willingness to donate blood had resulted in lower level of blood donation behavior during the year. Therefore, the scheduled quantity of blood supply usually can hardly be achieved. Some business

corporations will sponsor certain valuable and attractive gifts (i.e. discount coupons of famous restaurant) to draw donors' attention and willingness during this period.
Aims: The purpose of this study was to determine whether the incentives for blood donation have impacts on the donors' characteristics.
Methods: The implementation of an incentive-based blood donor recruitment program on 2 February 2010 drew participation from 1081 blood donors. The incentive for blood donation was to provide donors with discount restaurant coupons. Goodness of fit test was used to examine whether the distributions of donor characteristics for this incentive-based recruitment program were different from those of donor population in 2010. The donors' characteristics applied in this study include gender, age group, employment status, blood groups, volume of donation and donor status. All analyses were performed with the statistical package SAS for Windows (Version 9.2; SAS, Cary, NC, USA).
Results: Our research findings revealed that the distributions of age group, donation volume, donor status and employment status between donors recruited from the incentive-based donation program were statistically different from those of donor population in 2010 (all $P < 0.05$). The donors recruited from the incentive-based donation program had higher proportions of 21–30 year age group, blood volume of 250 cm^3, first-time donor and students from gift-providing donors than donor population in 2010 (59.9% vs 30.2%, 89.3% vs 66.1%, 24.0% vs 11.8% and 73.9% vs 22.6%, respectively).
Conclusion: Business corporations can provide better, valuable gifts to draw blood donors of different characteristics, as well as increase first-time donor rates. However, the public still needs to be informed and instilled the importance of blood donating behavior in long term, making sufficient blood supply match the needs from hospitals.

P-056
DONATION FOLLOW UP STUDY OF FIRST TIME DONOR IN TAINAN

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Background: According to TBSF statistics in 2003, 43.5% of new donors did not donate again. Why did they not keep donating? That issue had influenced the promotion of blood donation remarkably, so we want to investigate the reasons.
Aims: To identify factors that influence the first time donors returning.
Methods: One thousand five hundred and twenty-one persons are selected randomly from 3282 first-time donors in Tainan area during March 2007. We designed a semi-structure questionnaire and mailed to the objects. The data are analyzed by using SPSS ver 17.0. They are grouped by gender, occupation, education, religion, marital status and so on. Analysis of variance is used to compare the intention by first-time donors who return to donate or not. We compared them via chi-squares and *t*-test method.
Results: Among 1521 mailed persons, 120 persons were withdrawn due to incomplete address, 350 persons replied validly. The reply rate is 25.5%. By the result of correlation matrix of continuous variables, we found the satisfied donation experience and the attitude in blood donation shows a significant positive correlation ($r = 0.403$); the attitude in blood donation and the knowledge of blood donation shows positive correlation ($r = 0.132$), age and the attitude in blood donation has negative correlation between ($r = -0.149$). Other variables showed no significant differences, but there are significant differences between the two variables, namely the shortage of blood supply and the age of the first-time donor. Comparing the person who think the supply of blood is not enough and return to donate after August 2008, has 4.388 times than those think the supply of blood is enough. About age, the odds ratio was 1.100, it meaned that each additional 1 year of age may increase the probability of repeat donation by 10.0%.
Conclusion: 1. Perception of insufficient blood storage will motivate donors to donate. Adequate message release is important to attract donors to give blood.
2. The first-time donors do not donate again after August, 2008 are caused by several factors. The first factor is 'do not find appropriate blood donation sites' (41.6%). It indicates the promotion for blood donation sites must be convenient to the first donors.

P-057
DONORS CHARACTERISTIC ANALYSIS OF MASS BLOOD DRIVE IN TAINAN

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Background: It is an important goal to balance the blood donation and supply for each blood center. Tainan blood center demands 900–1000 U (250 cm^3) of bloods for medical transfusion every day. Initiation mass blood drive on weekend is the major blood donation recruitment
Aims: The purpose of this study was to analyze the donors' personality character, response to service, satisfaction and the subsequent donation at mass blood drive.
Methods: We designed a questionnaire that questions included the service quality assessment, the donors' satisfaction with services, personality characters and the tendency of subsequent donation. The questionnaires were collected at two mass blood drives, sponsored by 'Huwei Lions Club' and 'Yi Cheng Charity Organization'. There were 2035 donors and 1205 donors individually. One thousand one hundred fifty-six donors at HLC and 745 donors at YCCO filled the questionnaire respectively. The rate of participation is 58.67%. We used SPSS14.0 software to analyze the data which were showed with descriptive statistics, frequency distribution, correlation analysis, multiple regression analysis.
Results: Among these 1901 donors, male/female ratio is 69%/31%. 35.1% of donors aged 21–30 years in HLC, by contrast, 31.5% of donors aged 31–40 years in YCCO. In occupation, most donors are students (27.4%) in HLC and most donors are labors (31%) in YCCO. The major cause of this time donation is friend's invitation (19.1%) in HLC, however the major cause of this time donation is regular donation (31.3%) in YCCO. Small size donation (250 cm^3) is higher in HLC than YCCO.
In the service quality assessment the highest score is empathy in HLC (88.87) and YCCO (90.47). In satisfaction, donors expressed 83.11 score in HLC, and 87.79 score in YCCO. In personal characteristics, the highest score is agreeableness (81.04) in HLC, and it is conscientiousness (81.57) in YCCO. The willingness of subsequent blood donation is 78.04 in HLC and 78.25 in YCCO.
Among the factor analysis, we analyse the factor by Standardized coefficients. In the mass blood drive of 'HLC', (i) 'conscientiousness personality character' got the highest β value ($\beta = 0.214$, $P < 0.0005$) and it means that it is the greatest influence on subsequent blood donation. The following factors were (ii) 'extraversion personality character' ($\beta = 0.137$, $P < 0.0005$), In the mass blood drive of 'YCCO', (i) 'blood donor satisfaction' got the highest β value ($\beta = 0.268$, $P < 0.0005$) and represents that it is the greatest influence on subsequent blood donation. The following factors were (ii) 'extraversion personality character' ($\beta = 0.191$, $P < 0.0005$).
Conclusion: At the two mass blood drives, results of regression analysis indicated that the positive factors for blood donors' subsequent donation are service quality at the donation, donors' satisfaction and their personality characters. Adjusted by the regression model, their explanatory power (Adjusted R^2) are 0.287 and 0.324, respectively. There are significant but the explanatory power is not high enough. This findings show that there are other factors to affect blood donors and further research is needed to understand whether other intervening variables affect subsequent donation behavior.

P-058
WAITING TIME IN THE PROCESS OF BLOOD DONATION ON CAMPUS

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Background: School is one of the important blood donation sites for each blood center. In 2007, Hsinchu Blood Center has conducted a survey of how the campus donors feel about the procedure of blood donation. It was found that people complained about the waste of time when they were standing in line waiting too long before blood donation. Those who have experienced a sense of emptiness, anxiety, and dissatisfaction become less willing to donate again. Researchers have found that the cost to business organizations is unexpectedly high when customers give up because the waiting line is too long. To improve the situation of standing in line for blood donation on campus, this study has performed a time-and-action analysis of the service procedure. Using the waiting line theory, this study seeks to discover the statistical distribution of the donor's waiting time during the service hours.
Method: 1. The analysis is based a sample of 1994 donors.
2. The concept of six-sigma management is used to define blood donation procedure, to observe the actual donation process as it took place on site, and to establish the core procedure and to identify key donors.
3. To construct a chart for the service procedure, the study divides the procedure into six steps: (i) entrance; (ii) filling out the forms; (iii) waiting for donation; (iv) interview and screening; (v) blood collection; and (vi) exit. For each step, those who stayed and those who left were separately recorded.
4. SPSS software is used to understand the distribution of the amount of time the donor spent in each step.
5. Situational simulation is used to produce a chart in which the percentage of donors in each half-hour is shown.

Results: 1. About 10% donors who left without donating blood. Of them, 48% donors left immediately because they had no strong intention in donating blood, moreover, too many people had been queuing up. There were 10% of them who liked to donate but 10 min waiting was the limit they could endure.
2. The donors spent most of their time waiting outside the bloodmobile. There is much variation, however, in the amount of time each step took. What caused the prolonged waiting was Step 2 (the time spent in filling out the forms) and Step 4 (the time needed for interview and screening). Thus, to shorten the waiting period for donors, the donation service should simplify the forms they are to fill out and add more staff members during the peak hours for interview.

P-059

A STUDY TO EVALUATE THE EXPERIENCES OF REGULAR VOLUNTEER BLOOD DONORS IN ORDER TO ELIMINATE THE MYTHS ABOUT BLOOD DONATION

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Background: National Blood Transfusion Service (NBTS) of Sri Lanka, adhering to the recommendations of World Health Organization encourages the voluntary non remunerated regular blood donors. Many programs are conducted for the motivation recruitment and retention of volunteer non-remunerated donors. Celebration of World Blood Donor Day is one of the main attractions where felicitation of regular blood donors is done annually at selected cluster center blood banks all over the country. There are many myths regarding blood donations established in the society. In spite of these common myths blood donors attended to the felicitation programs has been regularly donating blood.
Aims: To evaluate the attitude of voluntary regular blood donors towards the common myths about blood donation.
To utilize the experiences of regular blood donors to eliminate the myths on blood donation.
Method: A questionnaire was developed taking in to account the most common myths regarding blood donation. Donors were requested to mark one of the three responses according to their experience.
Age, Sex and number of blood donation also were included in the questionnaire and no questions were made to reveal the personal identification details of the donor.
Along with the World Blood Donor Day Calibrations; Blood donors who have donated more than twenty times (20) were invited for the felicitation ceremony organized at the National Blood Center Sri Lanka. While the donors were attending for the calibration this questionnaire was offered to them and the responded papers were evaluated.
Results: A total of 60 (sixty) donors had responded to the questionnaire. Out of the responders 25 donors had donated blood for 20–40 times. Eighteen donors had donated blood for 41–60 times. Fifteen donors had donated blood more than 61 times. Responses of these regular blood donors for the above questions are summarized in the given table. Evaluation reveals that out of the responded blood donors 80% considers blood donation has no effect on alteration of body weight, 88% consider blood donation has no effect on alteration of physical fitness, 95% consider blood donation has no effect on alteration of sexual performances and 82% consider blood donation has no effect on alteration of the exposure to diseases.

Table 1

No.	Question Being a regular blood donor it has affected to:	Responses to each question		
		Increased	Decreased	No change
01	Alter the body weight	12 (20%)	00	48 (80%)
02	Alter the physical fitness	07 (12%)	00	53 (88%)
03	Alter the sexual performances	03 (05%)	00	57 (95%)
04	Alter the exposure to diseases	00	11 (18%)	49 (82%)

Summary/conclusions: In this study regular blood donors have demonstrated that the act of regular blood donation has no significant effect on alteration of their body weight, physical fitness, sexual performances or the exposure to diseases. National Blood Transfusion Service can use the results of this study for the awareness programs on donor motivation and retention eliminating the myths on blood donation.

P-060

USE OF DONOR DEFERRAL EVALUATION FOR OPTIMIZING RECRUITMENT OF BLOOD DONORS

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Introduction: A healthy donor population is the greatest asset of any blood transfusion service. The Sri Lankan National Blood Transfusion Service is heavily dependent on voluntary non remunerated blood donors and blood donors undergo basic screening tests of weighing and hemoglobin measurement and are counseled and examined by trained medical officers to check the suitability to donate. Donors not suitable for donation are deferred temporarily or permanently. Deferral of a large number of donors who present voluntarily to donate blood can adversely affect the donor population and blood collection. Therefore every effort should be made to minimize the donor deferral due to preventable causes.
Aims: To evaluate the donor deferral patterns in donor population of blood bank, Base Hospital, Wathupitiwala and identify the factors which could help to improve the donor recruitment in future and minimize the preventable deferrals.
Methodology: A retrospective study was done by retrieving data from donor deferral registers and donor declaration forms of blood bank, base hospital, Wathupitiwala, Sri Lanka from 2008 to 2010. Donors were categorized as permanent and temporary based on the time period of deferral and reasons for deferral and donation history were analyzed.
Results: A total of 7609 donors were recruited during the 3 years from 2008 and 887 were deferred with a deferral rate of 11.65%. Out of the deferred donors 57% (n = 506) were between 18 and 30 years of age and 568 (64%) were males. Low hemoglobin level was the commonest reason for deferral (n = 242, 27.3%) followed by chronic disease such as hypertension, diabetes mellitus and ischemic heart disease (n = 81, 9.1%), high blood pressure at the time of examination (n = 72, 8.1%) and infected wounds (n = 60, 6.8%). There were 563 (63.5%) first time donors among the deferred population and the commonest reasons for deferral among them were low hemoglobin level (n = 195, 34.6%) followed by chronic disease such as hypertension, diabetes mellitus and ischemic heart disease (n = 38, 6.7%), taking antibiotics for upper respiratory tract infection (n = 38, 6.7%), and inadequate overnight sleep (n = 32, 5.6%). Out of the 324 deferred donors who had donated blood at least once before 41 (12.6%) were deferred as they have not completed the minimal interval of 4 months before the next donation.
There were 111 (12.5%) permanent deferrals and 776 (87.5%) were deferred temporarily. Commonest reasons for permanent deferral were chronic disease such as hypertension, diabetes mellitus and ischemic heart disease (n = 81, 72.9%), epilepsy (n = 19, 17.1%) and valvular heart disease (n = 7, 6.3%). A significant number of females (n = 191, 78.9%) compared to males (n = 51, 21.1%) were deferred due to low hemoglobin level ($P < 0.05$)
Conclusion: Large number of deferrals due to low hemoglobin levels in both first time and repeat donors has a significant effect on donor pool and blood collection. Education of donors on simple dietary modifications should be done during counseling and supplementary iron therapy should be considered. Large number of repeat donors getting differed due to non-completion of 4 months after the last donation should be addressed by educating the donors with appropriate post donation information.

P-061

THE CHARACTERIZATION ANALYSIS OF BLOOD DONORS IN BLOOD DRIVE BY TASTY RESTAURANTS: A CASE STUDY OF TAINAN BLOOD CENTER

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Background: In order to maintain sufficient blood in the blood bank, Tasty restaurants, it is popular for youth, held massive blood drive to attract more blood donors.
Aims: Blood center is responsible for meeting the blood demand in a region. The blood drives sponsored by Tasty restaurants usually attracted passive blood donors. This research seek to understand the features of these donors and find ways to recruit them as regular donors.
Methods: This research incorporates data analysis towards the differences between the blood drive sponsored by Tasty restaurants on January 20th to 21st (study group) and other seven drives in the same month and region (control group). The variables include their ages, genders, occupations, volume, frequencies, and the qualified rate of their blood screen. This research uses the statistics software, SPSS ver 17.0, to conduct a *t*-test and a chi-square test for difference analysis ($\rho < 0.05$).
Results: The statistical analysis shows: For the study group, the average age of the donors is 27.5 ± 9.0 years, M: F = 55.5%: 44.5%, while control group has a mean age of 34.7 ± 11.7 years, M: F = 63.0%: 37%.

1. There is a significance difference between two groups regarding ages, genders, occupations, volume, and first-time donor. The donors in the study group are

(i) younger, (ii) more female population, (iii) more students, (iv) more 250 cm^3 donation, and (v) more first-time donors.
2. There is no significant difference in their blood screen results.
3. There is a significant amount of students in the study group than control group (49.4% vs 21.8%), but not much difference in other occupations.

Conclusion: This research shows that blood donors in the study group are mainly students compared to the control group, yet there is no significant difference between two groups regarding other occupations. Special marketing approaches would attract different blood donors. However, the clustering effects and the instability features of student donors are the challenges towards recruiting these donors and become regular donors.

P-062
THE CHARACTERISTIC ANALYSIS OF BLOOD DONORS IN BLOOD DRIVE IN TEMPLES

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Background: Taiwanese people have the tradition to visit temples during the Chinese New Year, praying for a peaceful and harmonious year. Some people believe the superstition that donating their blood can avoid potential accidents on themselves. The Tainan Blood Center held blood drives in three temples during the Chinese New Year, contributing to the blood supply.
Aim: The study seeks to understand the features of blood donors in the temples, and attracting them to become regular donors.
Methods: This study adopts data analysis to distinguish the differences between (i) the study group: the blood donors in three temples, the South Kunshen Dai Tianfu Temple, the Orthodox Luerhmen Matsu Temple, and the Dongshigan Temple, during the 2011 Chinese New Year (February Fourth to Seventh) and (ii) the control group: the other donors in Tainan within the same time period. This research uses the statistics software, SPSS ver 17.0, to conduct a *t*-test and a chi-square test in the difference analysis ($\rho < 0.05$).
Results: 1. The statistical analysis shows: For the study group, the average age of donors is 36.0 ± 10.5 years, M:F = 64.4%: 35.6%, while control group has a mean age of 35.1 ± 11.6 years, M:F = 64.3%:35.7%. The result reveals there are significant differences in ages ($t = 2.39$), occupations ($\chi^2 = 99.47$), and blood screen ($\chi^2 = 17.18$).
2. No significant difference between two groups in genders ($\chi^2 = 0.005$), donation volume ($\chi^2 = 2.09$), and first time donors ($\chi^2 = 0.41$).
3. The donors in the study group are (i) elder, (ii) identifying as labors in occupations, (iii) higher qualified rate in the blood screening.

Conclusion: Taiwanese people practice the tradition to visit temples during the Chinese New Year, and the blood drives in the temple can help to meet the blood demand. This research reveals that the ages and occupancies are significantly different in the study group. However, the blood tests in the study group have significantly lower qualified. This study assumes the irregular lifestyle during the long vacation period contributing to the result, yet this assumption needs further study.
This study suggests that the blood drives during the Chinese New Year can supply the usual blood shortage over the same time period. Different marketing strategies should be practiced to attract these donors.

P-063
NEW ATTEMPTS TO DEVELOP BETTER SURROUNDINGS OF BLOOD DONATION IN JAPAN RED CROSS TOKYO METROPOLITAN BLOOD CENTER

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Background: Our blood business is supported by volunteer civilian as blood donor. So we have to treat them with great gratitude. From such standpoint, our hearty hospitality is getting more and more important.
Aims: For aims of stable blood supply, we give donors high satisfaction for contribution and secure them as unshakable supporter.
Method: At first, we established some new donation rooms which suit the town and people who live in or visit there as the place where donors share moments of donating contribution. In these new rooms, we entrust promotion of blood donation, which have been under the leadership of us, to the partners (which means cooperating companies, schools, and ordinary donors as volunteer in 'industrial-academic cooperation'. The partners circulate information about new room's comfort and interest, donation's easiness and helpfulness. That gives more people opportunity to join the donation volunteer. In addition, from the standpoint that 'The leader in blood donation surroundings is a donor', we have been pursuing hearty hospitality. Our hospitality aim to encourage the donor's feeling that 'Blood donation enrich my heart' or 'Blood donation makes me a tender-hearted'. Moreover, we are exploring the new way to promote blood donation utilizing the power of design and art. Under the theme 'Design, Art and humanitarian assistance', we built an interactive relationship with Art University and confer with its professors and students about what should be a place to share moments of donating contribution and what should be a space design.

Figure 1: The entrance of 'Yurakucho donation room'

Figure 2: 'akiba: F donation room' themed 'near future'

Results: The attempt above attracted many attentions of Media all over the world. It highlighted blood donation especially in young people. As one of the result of that effect, donors increased by about 23,000 person from 2008 to 2010 in Akihabara area. And 'akiba: F', which is one of the new room, were awarded '2010 Good Design Award' (in the category of Society/Public, Cultural, and Educational Facilities). It's a proof that our idea and method were recognized as supporting the future of transfusion medicine.
Conclusion: Under our motto of 'feeling better for donor', 'warmth in heart for donor' and 'more timely for donor', we keep improving the surroundings of blood donation. Then our first mission is to give more people opportunity to participate in donation volunteer. And the second mission is to provide the satisfaction for their contribution by our hearty hospitality. Then the final mission is to make donors become aware that they are not just 'participants', but 'the supporters to save the patients'. We have to encourage our partners who kindly understand our blood business. And we have to co-work with them to secure stable blood supply. We and partners also have to be conscious strongly that, the most important thing, all the blood donation is for patients.

P-064
INFECTIOUS MARKERS AMONG BLOOD DONORS IN A PROVINCIAL HOSPITAL OF THAILAND: ANALYSIS FOR SURVEILLANCE AND IMPROVEMENT OF BLOOD RECRUITMENT

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Background: Procurement of blood for safety transfusion includes recruitment and retention of low risk donor selection and proper screening for infectious markers in donated blood.

Aims: This retrospective study was carried out for surveillance and improvement of blood recruitment by analysis for infectious markers among blood donors at Kamphangphet Hospital, Kamphangphet province, situated in the central part of Thailand.
Methods: Retrospective study for prevalence of HIV, HBV, HCV and syphilis infections detected in donated blood during 2005–2008 was performed. The prevalence of infectious markers in accordance to sex, age and frequency of donation (first time and repeated) were analyzed.
Results: There were 28,373 units of blood collected from 24,040 blood donors consisting of 59% (14,219) male, 41% (9821) female, 39% (9437) first time and 61% (14,603) repeated donors. The total prevalence of blood transmitted in the 3 year period including HIV, HBV, HCV and syphilis as screened by ELISA for anti-HIV/HIVAg, HBsAg, anti-HCV and syphilis by RPR testing were 0.26%, 2.36%, 0.71%, and 0.44%, respectively. None of the HIV infected donor positive for HIVAg only was found. The highest prevalence of anti-HIV (0.39%), HBsAg (3.05%) and anti-HCV (1.12%) were demonstrated in 2008 with statistically significant difference ($P < 0.05$). Male donors had higher prevalence than female in all of four infectious markers. The prevalence of HCV infection in male donors was increased by age. We also confirmed the higher prevalence of HIV, HBV and HCV infectious markers in first time donors compared to repeated donors. In addition, collected blood from previous positive infected donors was found increasingly, mostly at mobile units.
Conclusion: This study reveals that HIV, HBV and HCV infections remain health problems in Thailand, which need education for prevention of blood and sexual transmitting pathogens for general population at all age group including school children. Pre donation counseling including donor informed consent should be intensively implemented in all of first time and repeated donors. Post donation counseling for infected blood donors is also necessary for care and monitoring of their health status especially prevention of transmission and stop blood donation. For improving safe blood recruitment and cost effectiveness, the active data system with efficient process management is important to exclude repeated donation from previous positive infected donors. The effort to recruit and retain female donors which is lower risk should be encouraged.

P-065
A STUDY OF DONOR DEFERRAL PATTERNS IN SOUTHERN REGIONAL BLOOD CENTER – KAMBURUGAMUWA, SRI LANKA

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Background: Sri Lanka has a cluster based Blood Transfusion Service which is centrally co-ordinated by the National Blood Center. In the Southern Region of the country, our blood center is the main Blood collector with a contribution of 17,495 units in 2010. The ultimate goal of a blood transfusion service is to provide blood from 100% voluntary, non-remunerated donors. The deferral pattern of the voluntary donors has a major impact in achieving the above targets.
Aim: To study the donor deferral patterns according to age, gender and to locate the group of donors who should be address and educate. This will support in policy formulations to promote voluntary blood donors and to develop strategies to call up deferred donors as soon they become eligible to donate blood.
Methods: The data was collected from in-house and mobile donor deferral registers maintained at Southern Regional Blood Center – Kamburugamuwa. The time period considered was from 1 January 2010 to 31 December 2010. Relevant donor declaration forms were traced and data was verified. The information abstracted includes age, gender and reasons for deferral. Further, reasons for deferral have divided mainly as temporary and permanent.
Results: Total blood collection for year 2010 was 17,459 units and out of which 1279 (7.3%) were deferrals. Out of the total deferrals, 864 (67.6%) were males and the balance 415 (32.4%) were females. A total of 744 (58.2%) were temporary deferrals and 534 (41.8%) were permanent deferrals. Of the temporary deferrals most of the donors had brief medical illness which totals to 199 (20.7%) and other reasons like alcohol ingestion, lack of sleep amounts to 197 (20.5%). Among other temporary reasons anaemia 29 (3.9%), use of antibiotics 150 (20.2%), surgical procedures 32 (4.3%), last donation <4/12 78 (10.5%) were categorized.
With the analysis of permanent reasons the majority fall in to chronic medical illness 463 (86.7%) as other reasons were history of hepatitis 87 (16.3%), high risk behavior 67 (12.5%) and age >60 years 17 (3.2%).
Age distribution of donors is shown in the table below.

Table 1

Age	Number		Percentage (%)	
	Temporary	Permanent	Temporary	Permanent
<24	303	77	79.7	20.3
25–34	300	132	69.4	30.6
35–44	151	154	49.5	50.5
45–54	82	129	38.9	61.1
>55	7	36	16.3	83.7

Summary: Main concerned should be on temporary deferral group as most had brief medical illness or had taken antibiotics recently. They should be educated regarding these issues and make strategies to call them back when they become eligible for donations. Regular donors should be addressed effectively on the time period between two donations.
Anaemia in females has shown to be significant compared to males. They should be referred to medical clinics and encourage seeking treatment with haematanics.
Also temporary deferrals are more in young donor population and permanent deferrals are more in ageing population. So the main concentration should be to address the young population in order to increase the blood donor numbers.

P-066
This abstract has been withdrawn.

P-067
A STUDY ON COMPARISON OF SERVICE QUALITY PERCEPTION BETWEEN SERVICE PROVIDERS AND BLOOD DONORS

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Background: In Taiwan, blood collection centers operate as non-profit and private organizations to collect blood from volunteers to be supplied to regional hospitals. With the decline of birth rates, the donation rates of new donors drop year by year. Moreover, as the age increasing or health problems the repeat donors gradually cannot to donate blood. It's important to boost the motivations of donating blood and to recruit new blood donors continuously. Taichuang blood collection center have already made much effort, including good personnel training, well-appointed blood collection room, delicious biscuit and practical gifts, to facilitate the regular recruitment of blood donors to meet the demand of hospital patients.
Aims: The purposes of this study are: (i) To evaluate the donors' satisfaction of service quality with Taichung blood donation center; (ii) To evaluate the service providers' satisfaction perception of service quality; (iii) To understand the service quality gap between service providers and blood donors; and (iv) To develop a high reliability and validity scale of service quality as an evaluation instrument of service quality in our blood collection center.
Methods: We conducted a cross-sectional study, and a questionnaire was designed according to SERVPERF (Q = P) model modified to the specific service quality requirements of the blood collection center. The survey instrument consisted of 31 items grouped according to five dimensions of quality service: tangible, reliability, convenience, responsiveness and benefit, on a 5-point Likert scale ranging from very dissatisfactory (score = 1) to very satisfactory (score = 5). The participants were blood donors donating blood in 1 year and the service provider that are blood collection staffs to which the participants were expected to respond anonymously. The inter-reliability Cronbach's α used in this research indicated good inter item reliability and the test–retest reliability that showed a good consistency obtained in blood donors and service provider.
Results: A total of 407 blood donors and 99 of service providers were tested in this research, and the data showed both groups are close to the degree of satisfaction in the dimensions of tangible, convenience and benefit. It means the blood donation service quality of the three dimensions had a satisfied level to blood donor. In the dimensions of reliability and responsiveness, the blood donors had a lower score of service satisfaction than that of the service provider that means there is a service quality gap between blood donor and service provider.
Conclusions: Related policies should be enacted in response to catch up on what blood donors have expected. In addition, this study established a high reliability and validity scale of service quality to as an evaluation instrument of service quality.

P-068

A STUDY OF ASSESSING THE ATTITUDES OF MOBILE ORGANIZERS TOWARDS THE MOBILE TEAM OF NATIONAL BLOOD CENTRE SRI LANKA

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Background: National Blood Centre (NBC) Sri Lanka (SL) is the headquarters of the blood transfusion service of the nation which coordinates 85 hospital blood banks. Annual blood collection of NBC mainly depends on the mobile drives. The mobile organizers are a very important category for the better provision of blood service as they are the stakeholders for donor motivation and retention. Mobile organizers' attitudes towards the blood service are of paramount importance to any blood transfusion service.
Aim: To assess the attitudes of mobile organizers towards the mobile blood collection team of NBC.
Method: A cross sectional study was done by sending a questioner by post to all mobile organizers of the NBC SL in 2009. It was filled by the organizers and posted back to the centre anonymously. Questioner contained the attitudes regarding, (i) Punctuality and initiation of mobile, (ii) Team friendliness towards the donors and team discipline, (iii) Ending of mobile, (iv) Any delay in procedures (registration, counseling and donor bleeding) and (v) Team adequacy. Attitudes were analyzed under each category.
Results: Total of 166 mobile organizers had responded within the period of 3 months. Sixty per cent of the organizers were satisfied about the punctuality and initiation of the mobile while 36% were unsatisfied and 4% had not responded. Ninety-five per cent of organizers were satisfied about the team friendliness towards the donors and team discipline while 5% were unsatisfied. Satisfactory attitude of organizers on ending of mobile was 92% while 8% was unsatisfied. Seventy-six per cent of the organizers said that there was no delay in registration while 19% were unsatisfied and 5% had not responded. Eighty-four per cent of organizers were satisfied about the counseling while 8% said counseling was delayed and 8% of organizers did not respond to the question. Eighty per cent of organizers said there was no delay in donor bleeding while 5% said it was delayed and 15% did not respond. Eighty per cent of the organizers were satisfied about the team adequacy and 18% were unsatisfied while 2% were not responding.
Out of them overall satisfactory rate was 81% where as 14.14% results were unsatisfactory. On the other hand 4.86% were non responders.
Conclusion: Overall satisfaction of organizers towards the mobile team is good, but the punctuality and the initiation of mobile is a little less than other factors considered. This must be due to the fact that the location of the mobile is far away from the center, it takes a lot of time to travel from NBC to an out of city mobile due to the distance and also due to the heavy traffic with in Colombo limits. To solve this problem the organizers can get the help of the closest peripheral blood bank. All other factors considered in the questioner have got satisfactory levels reflecting the efficient professional attitude of the staff of the NBC SL.

P-069

WHY WE DEFER OUR VALUABLE DONORS

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Background: Annual blood collection of Sri Lanka is around 300,000. National Blood Center of Sri Lanka contributes around 20% of annual blood collection by mobile and in house blood collection. It is important to study causes for donor deferral and donor deferral patterns as it has a significant impact on blood collection.
Aim: 1. To study donor deferral patterns.
2. To identify preventable causes of donor deferral.
Material and method: A retrospective study was done in a period of 6 months from 1 April 2010 to 31 September 2010. The data collected from the donor deferral registers of four mobile blood collecting teams at National Blood Center, Sri Lanka.
Results: From 1 April 2010 to 31 September 2010 total mobile blood collection at National Blood Center was 25,948. There were total 2037 donor deferrals, of which 1484 (73%) were temporary deferred while 553 (27%) were permanently deferred.
Analysis of donor deferral criteria shows that, unfulfilled basic requirements (age, weight, Hb, adequate sleep,4 months from previous donation) consisted 256 (13%), acute infections 433 (22%), past infections 204 (10.2%), on treatment for a systemic illness 317 (15.5%), undiagnosed systemic diseases 31 (1.5%), blood pressure abnormalities 329 (16%), past history of surgery 69 (3.3%), high risk behaviors 31 (1.5%), tattooing, ear piercing, acupuncture treatments 64 (3%), recent foreign travel 63 (3%), and others 226 (11%).
Of those due to unfulfilled basic requirements, 41% were deferred due to low hemoglobin levels.
Of the total number of deferral, 997 (49%) donors were first time donors and 1040 (51%) donors had donated more than once.
Conclusions: Acute infections were the leading cause for donor deferral in our donor population. A significant percentage of our donor population was deferred due to low hemoglobin. Public education about blood donation and causes of donor deferral may lower the donor deferral rates since significant percentage of donors were deferred due to unfulfilled basic requirements and tattooing, ear piercing and acupuncture treatment. As most of the donors were temporary deferred and nearly half of deferred donors were first time donors, counseling after deferral and provision of next date for blood donation is important to maintain adequate amount of blood collection in the country.

P-070

SEASONAL FLUCTUATIONS OF VITAMIN D3 IN A COHORT OF BLOOD DONORS IN UPPER AUSTRIA

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Problem: Vitamin D3 (calcidiol, 25-OH-vitamin D3) is generated by UV-irradiation and stored in the human body, in summer more than in winter. Reduced sun exposure in winter can result in a decrease of vitamin D3. We decided to detect seasonal variations of vitamin D3 in voluntary blood donors to evaluate the impact of this parameter for our donors.
Patients and methods: Samples were taken from 105 blood donors (18–65 years) before and after winter and stored immediately without light exposure. After centrifugation, freezing and thawing the vitamin D content was tested with the testing kit '25-OH-Vitamin D3/D2' (Chromsystems, Germany) according to manufacturer's instructions on a HPLC instrument (LC20; Shimadzu, Japan). The results of vitamin D3 were categorized in normal range (20–70 μg/l), undersupply (10–20 μg/l) and deficiency (<10 μg/l). Vitamin D2 was not interpreted because the tested cohort did not supplement vitamin D in this period.
Results: The difference of vitamin D3 concentration before and after winter was significant. The mean reduction was above 40% and does not depend on gender. Vitamin D3 deficiency was present in 1.9% of the samples before winter and in 21.9% afterwards. 17.1% of the cohort had an undersupply at the first point of testing. This group increased to 50.5% after the winter. Only 27.6% of the donors were in the normal range at the first as well as at the second sampling.
Conclusions: The prevalence of an undersupply/deficiency of vitamin D3 of 0.190 before winter and 0.724 afterwards implicate that reservoirs of vitamin D3 are emptied in winter due to reduced solar irradiation. Our results suggest that the parameter vitamin D3 could be a gratification for our voluntary blood donors in or after wintertime. In case of undersupply/deficiency a supplement therapy could be recommended.

2.2 Blood Collection including Apheresis

P-071

EVALUATION OF BLOOD DONOR DEFFERAL

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Background: The goal of selection of blood donors is to ensure safety of blood donor as well as the recipients. To accomplish this donor deferral criteria must be precise and uniformly used by the blood transfusion service of the country.
Aim: To evaluate the reasons for blood donor deferral during mobile blood collection programs organized by National Blood Transfusion Service, NBC, Colombo, Sri Lanka.
Material and method: Retrospective analysis of whole blood donor deferral registers at the NBC – Sri Lanka during August 2010 to December 2010. Prior to evaluation, potential blood donors are screened using haemoglobin level, blood pressure, pulse rate and medical examination according to the Stranded Operative Procedures (SOP) prepared by quality management section of NBC.
Results: The total number of prospective blood donors was 25,564 for the study period. 19,429 (76.0%) were male donors and 6135 (23.99%) were females. One thousand three hundred eighty-four prospective donors were differed. Out of this 955 (69.0%) were male donors and 429 (30.99%) were female donors.
While analyzing the age of female differed donors' majority 181 (42.19%) were in 31–45 years age group and among male donors major contribution 487 (50.99%) was from 18 to 30 age group.
The total number of deferred donors, volunteered for the first time for blood donation was 751 (54.26%) [300 female (69.9%) and 451 (47.22%) male].
The deferral was temporary in 1097 (79.26%) cases [female 348 (81.1%) and 806 (84.39%) male].
The most frequent causes for temporary deferral was high blood pressure, donation interval between last donation is <4 month, inadequate sleep and infected wounds.

The leading causes of permanent deferral were unsatisfactory medical conditions, chronic illnesses and infectious diseases.
Conclusion: The donor deferral is a complex process. According to this study 54.26% of donors were first time donors and 79.26% were differed temporary. So it is mandatory to provide clear explanation about the cause of the deferral to increase return rate for future donations.

P-072
OPTIMIZATION OF THE TIME FROM ARRIVAL AT WORKPLACE TO 1ST VENEPUNCTURE IN MOBILE BLOOD COLLECTION UNIT BY USING LEAN SIX SIGMA METHODOLOGY

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Background: Lean Six Sigma is a methodology to improve the performance excellence for the organization. It uses a systematic approach to identify defects and eliminate the waste by using data-driven structured strategy and root causes analysis. The mobile blood collection unit in Hong Kong Blood Transfusion Service usually takes about 60 min to start the 1st venepuncture for blood donation after arrival at workplace. The project adopted the core value of Lean Six Sigma for the continued quality improvement to optimize the time of starting the first venepuncture in the mobile blood collection team.
Aims: The goal statement is to reduce the time span from the arrival of mobile unit at the workplaces to the start of the 1st venepuncture by 15%.
Methodology: The approach includes the various phases of Define, Measure, Analysis, Improve and Control (DMAIC).
Y = f (X) Y is the output measure of the time to start the 1st venepuncture where as the X(s) are all the variables from the high level process flow (Supplier, Input, Process, Output and Customer) that possibly contributed to the output Y. The data collection included the workflow study, the audit plan and the time study.
Result: The time study showed the mean of time spent from the arrival of mobile team at the donation site to the time of starting the 1st venepuncture was 56.49 min. Three variables from the workflow study were identified to show their associations with the time span to start the 1st venepuncture time. They are pre-donation preparation of donation group (P = 0.0066), the set-up sequence (P = 0.0013) and the time to start 1st health screening (P = 0.03).
An improvement plan based on the findings was then initiated. The result showed that the time to start the 1st venepuncture after arrival of the donation site was reduced by 14.57%. The compliance of the most interventions was from 73% to 100%
Conclusion: In general, the compliance rates of most interventions were satisfactory. The evaluation revealed that mobile jobs without sign-up were the major obstacle for compliance of the interventions. If the data was analyzed by deleting those MU drives without sign-up, the result showed that the time span from MU arrival to 1st venepuncture would further be reduced by 17% which could achieve more than the goal statement set in the project. The improvement plan showed its effectiveness to optimize the time to start the 1st venepuncture to donor. However, pre-arrangement of sign up is highly recommended for the collection drives.

P-073
PLASMAPHERESIS AND CLOTTING ACTIVATION

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Introduction: Recently, flocculation and/or clots have been observed in a substantial percentage of defrosted FFP delivered by the Dutch blood transfusion organisation. Activation of platelets can initiate activation of the clotting cascade. Therefore, it has been suggested that the root cause of the flocculation/clots is activation of the clotting system by activated platelets due to the apheresis equipment used. In the Netherlands, all FFP is collected by apheresis applying Fenwal and Haemonetics equipment.
Aim of the study: To study activation of the clotting system in donors and in apheresis derived plasma using Fenwal's Autopheresis C and Haemonetics' PCS2.
Materials and methods: After informed consent, six repeat plasmapheresis donors were asked to donate two times a unit of 650 ml of plasma with an interval of 4 weeks. Three donors donated at day 1 with the Autopheresis C (Fenwal) and 4 weeks later with the PCS2 (Haemonetics) and vice versa. Prior to, and directly after the plasmapheresis procedure, an EDTA sample was drawn from the donor for whole blood cell counts, measurement of thrombo-elastography (TEG), CD62P, annexine-V, PAC-1, thrombin-antithrombin complex (TAT-complex), prothrombin fragment 1 + 2, D-dimers, FVIII-antigen, FVIII-activity and total protein. Since one donor refused the 2nd donation, results from five donors were analysed. Prior to the freezing of the apheresis plasma, and after thawing (after 12 days), thrombin-antithrombin complex (TAT-complex), prothrombin fragment 1 + 2, D-dimers, FVIII-antigen, FVIII-activity and total protein were measured.
Statistics: To compare the laboratory results in the donors, a paired t-test was used; A P-value of <0.05 was considered to be significant.
Results: In 11 plasmapheresis procedures, no statistical differences in tests results performed with donor blood drawn prior to, and after the apheresis procedure were observed. No differences were observed between the collected plasma parameters in the paired study in five donors donating with Autopheresis C or with PCS2. In none of the 11 plasma units, activation of the clotting system was observed.
Conclusion: In 11 plasmapheresis procedures with six donors, no activation of the clotting system in donors and derived products was observed. No changes caused by the apheresis procedure were seen. A root cause for the flocculation/clot formation in the plasma could not be resolved. Additional research is needed.

P-074
EXAMINATION OF BLOOD FERRITIN IN LONG TERM, REGULAR PLATELETPHERESIS DONORS

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Background: The major side effect of whole blood donation is iron depletion. For regular plateletpheresis (PH) donors, less was discussed. It is worthwhile to study the blood ferritin level as well as iron deficiency rate (ID rate) in apheresis donors.
Methods and results: This study was conducted from January to December 2007. Venous blood was collected for ferritin examination from long-term, regular, repeat PH donors. These were donors who donated platelet by apheresis 20–24 times per year. First-time PH donors were also selected simultaneously as a control group. A total of 325 cases were collected, of which 125 were first-time donors and 200 were repeat donors. Of the 200 repeat PH donors, group (A) were 50 cases who had a history of 20–50 donations, (B) 50 cases of 51–100 donations, (C) another 50 cases of 101–150 donations, (D) 25 cases of 150–175 donations and (E) 25 cases of 176–200 donations. Amongst the first-time donors, there were 75 men and 50 women, and 40% were 21–30 years old. This gender and age distribution were similar to that of whole blood donors. For the 200 repeat PH donors, however, there was male predominance of more than 90%, and the peak age group was 51–60 years (34.5%). The average ferritin level amongst male donors was 67.8 ng/ml for first-time donors. For repeat male donors, the ferritin levels were 47.3 ng/ml in A, 48.5 ng/ml in B, 38.8 ng/ml in C and 43.2 ng/ml in D for the different groups. The proportion of cases whose ferritin levels dropped below 20 ng/ml was 6.7% amongst first-time donors. For repeat male donors, low ferritin levels occurred in 9.1% of the cases with 20–50 PH donations, 13.6% of the cases with 51–100 donations, 19.6% of the cases with 101–150 donations and 26.0% in the cases with 151–175 donations. Apparently, the occurrence of iron deficiency increased with an increased number of donations. Comparison of female donors were not done, because of limited cases.
Conclusion: Repeat, long-term, regular plateletpheresis could cause iron depletion. Iron deficiency increases with the years of donation and the number of apheresis. Two Hematonic tablets, with elementary iron 100 mg on the day of apheresis, is effective in preventing iron depletion as well as the decrease of hemoglobin. Regular iron study, such as ferritin examination, is necessary for repeat donors, especially those with a history of apheresis of more than 100 times within 5 years.

P-075
CORRELATION OF DONATION FREQUENCY WITH CAPILLARY AND VENOUS BLOOD HEMOGLOBIN

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Background: To avoid inadequate blood obtained from donation, it is essential to know whether there are differences among the results analyzed by different methods to quantify hemoglobin (Hb) and ferritin, and if donation frequency affects donors.
Aims: This study attempts to compare the results gained from three methods (CuSO4 gravimetric method, HemoCue system, and CBC test) frequently used to test for Hb level, to examine the level differences between Hb and Ferritin on target variables, and to understand if donation frequency affects Hb level and Ferritin level.
Methods: Blood samples were obtained from 1512 volunteers to test for Hb; capillary fingerprick sample analyzed by both the $CuSO_4$ gravimetric method and the HemoCue system, venous sample analyzed by the CBC test. The Hb level obtained from CBC test is defined as 'true' value. Additional 5989 volunteers entered the study during 6 July and 16 October 2009, and were further grouped based on gender, age, first-time donors, and regular donors. Data were recorded and analyzed with SPSS 18.0.
Results: When comparing the Hb level analyzed by the $CuSO_4$ gravimetric method with that analyzed by the CBS test, the 'true' rate was 63.5% and 85.8% for female and male, respectively. The Hb level analyzed by the HemoCue system was consistent with that analyzed by the $CuSO_4$ gravimetric method. Age impacts upon Hb level but not

Ferritin. Aged male donors had significantly lower Hb level than younger donors ($P = 0.001$). Instead, elder female donors had higher levels of both Hb and ferritin ($P < 0.001$). First-time donors had higher ferritin level than regular ones ($P < 0.05$). For both female and male donors, donation frequency influenced on the ferritin level significantly ($P < 0.001$).

Conclusions: The 'true' rate of the Hb level analyzed by $CuSO_4$ gravimetric method is low. Donation frequency has less impact on Hb level but more on ferritin level. That ferritin levels of the older female donors are higher than younger ones might be attributed to menopause.

P-076
CHANGES IN SERUM FERRITIN LEVEL IN APHERESIS DONORS

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Background: In order to understand the changes of serum ferritin in apheresis donors, blood center started the detection of serum ferritin for the repeated apheresis donors every 6 months since July 2008.

Aims: In this study, retrospective analysis of serum ferritin changes in apheresis donors will be performed, so that we can provide sustainable health care for blood donors.

Methods: This study was a retrospective study, to analyze 212 (male) repeated apheresis donors who received serum ferritin examination in 2010 May–June were selected as the study sample and all of them had been done five times ferritin detection. According to the frequency, blood donors were divided into four groups: (Group A) First time of apheresis donors, (Group B) <100 times, (Group C) 101–200 times, (Group D) more than 201 times. We used SPSS ver 18.0 software to analyse the association between ferritin, blood donation interval and frequency (significant difference as $P < 0.1$).

Results: 1. Average age of 212 apheresis donors was 44.1 ± 9.6 years, average serum ferritin was 50.3 ± 39.9 μg/l. The statistical results of four groups: Group A (n = 11) mean age was 41.3 ± 9.7 years, mean serum ferritin was 45.9 ± 55.7 μg/l, previous donation interval was 114.4 ± 23.1 days, Group B (n = 109) mean age was 40.9 ± 9.3 years, mean serum ferritin was 56.0 ± 44.5 μg/l, previous donation interval was 36.4 ± 30.8 days. Group C (n = 49) mean age was 46.2 ± 7.9 years, mean serum ferritin was 46.3 ± 33.2 μg/l, previous donation interval 31.6 ± 22.9 days. Group D (n = 43) mean age was 50.3 ± 8.7 years, mean serum ferritin was 41.6 ± 27.5 μg/l, previous donation interval was 37.3 ± 38.1 days.

2. 15.92% of repeated apheresis donors were low serum ferritin (<20 μg/l).

3. The frequency of repeated apheresis donors was negatively affected to serum ferritin ($\beta = -0.168$, $P = 0.017$), it showed long-term sustainability of the platelet donation has a downward trend of serum ferritin. The interval between blood donation and serum ferritin was positively ($\beta = 0.117$, $P = 0.065$), that means the longer the interval between blood donation, blood ferritin values would be higher.

4. In group D of repeated apheresis donors the serum ferritin was lower than previous other groups, but statistical analysis among the three groups was no significant difference.

Conclusion: The study found the serum ferritin was significantly related to the interval between blood donation and the frequency, Page, E. A. (Transfusion Medicine **20**, February 2010) presented the same results. The results show consistent positive correlation for the iron content decrease and interval between blood donation. Care for blood donors' health, some countries have continued to supplement oral iron, the results of this study also provide a reference for oral iron supplementary.

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P-077
THE EFFECT OF TRIMA ACCEL SYSTEM ENABLING PLASMA-ASSISTED RINSEBACK FEATURE ON REPEATED PLATELET-APHERESIS DONORS

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Background: Three cell separators, MCS, Trima, and Fenwal, were compared in 2007. It is found that Trima Accel system without plasma-assisted rinseback left the most residual blood volume. We requested the agent to enable the function of plasma-assisted rinseback in Trima Accel system to improve the performance. In this study, the effect of plasma-assisted rinseback was evaluated.

Aims: We recruited the donors harvesting platelets by Trima Accel system for investigation the changes of residual blood volume in Trima kits and serum ferritin level of donors after enabling plasma-assisted rinseback feature.

Methods: In the first stage, 31 platelet-pheresis donors (all male) were recruited, and we evaluated the changes of residual blood volume, HCT, packed RBC in kits and their experiences after enabling plasma-assisted rinseback feature. In the second stage, 48 platelet-pheresis donors who the sequences of donation were more than 15 times 1 year were traced, and we evaluated the changes of serum ferritin level after enabling plasma-assisted rinseback feature.

Results 1. The results of 'process time' and 'actual ACD volume' weren't significantly different.

2. The experiences of the donors after enabling the feature were the same as before.
3. The results of 'residual blood volume', 'HCT' and 'packed RBC' in kits were significantly lower. The decrease of 'residual blood volume' was 13.8%. The decrease of 'HCT' was 51.3%. The decrease of 'packed RBC' was 59%, the reducing volume was to a certain degree of 36 ml whole blood.
4. Before enabling plasma-assisted rinseback feature, the mean of serum ferritin level was 28.06 ± 20.26 (SD) ng/ml, and the frequency of Iron deficiency (serum ferritin <20 ng/ml) was 39.6%. After enabling plasma-assisted rinseback feature, the mean of serum ferritin level was 52.77 ± 25.07 (SD) ng/ml, and the frequency of Iron deficiency (serum ferritin <20 ng/ml) was 6.3%.

Conclusions: After enabling plasma-assisted rinseback feature in Trima Accel system, the blood loss of donors was reduced, and the serum ferritin level of donors was increasing significantly. The plasma-assisted rinseback feature was beneficial for repeated platelet-apheresis donors.

P-078
ADEQUATE LENGTH OF THE NEEDLE FOR WHOLE BLOOD DONATION

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Background: In the whole blood donation, a blood collection needle of 36 mm in length is generally used. The needle makes not only the blood donor but also nurses scary. Aims:

The aim of this study is to examine the adequate length of the whole blood collection needle.

Methods: Whole blood collection bags Kawasumi ABQ-200M7NF, ABQ-400M7NF and Terumo BB-ZQM207J, BB-ZQM407J were used for this study. Each of the bags has a same 17G needle of 36 mm in total length. Ninety-six of Sumo wrestlers who are thought representative of the good physique in Japan and 1032 of usual voluntary donors were examined and donated whole blood as an ordinary manner. The needle length inserted in the skin (inserted needle length) was calculated by measuring the needle length out of the skin (outer needle length) during blood donation.

Result: The body weight of the groups of Sumo wrestlers vs usual voluntary donors were 119 ± 28 kg (range 60–200 kg) and 65 ± 11 kg (40–120 kg), respectively. The height of those were 178 ± 7 cm (range 153–192 cm) and 168 ± 8 cm (147–188 cm). Then the inserted needle length of Sumo wrestlers was 15.3 ± 4.1 mm (range 6–27 mm). On the other hand that of usual voluntary donors was 15.2 ± 2.9 mm (5.6–26.5 mm). There is no significant difference between the two groups on the inserted needle length.

Summary: It was clarified in this study. First, the length of the needle necessary for whole blood donation was not different in the body weight and height. Second, the average length of the needle inserted at the blood donation was around 15 mm. Third, the length of the needle does not need over 27 mm. Based on these findings, needles for whole blood donation can be shortened. According to the Poiseuille equation, the fourth power of the radius of the needle is in inverse proportion to the length. So a smaller needle will become acceptable for whole blood donation.

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P-079
STUDY ON NUMBER OF CYCLES AND YIELD PERFORMANCE AMONG DONORS WITH HISTORY OF FAILURE TO MEET PLATELET YIELD PERFORMANCE

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Background: Platelet demand is high and recruitment of apheresis donors in order to meet daily usage is our primary goal. There is a small group of apheresis donors who have 150,000–190,000 × 10^9/l platelet count which in turn results in low platelet yield performance.

Aims: The aim of this study was to obtain a 3.0 × 10^{11} platelet dose from these donors paying particular attention to the duration of the process, post platelet count, platelet depreciation and acceptance by donors.

Methods: The study was conducted for a period of 6 months from the month of June to December 2009. All donors met the apheresis criteria and given written informed consent. Eighty apheresis donors with an average pre-platelet count of 150,000–190,000 × 10^9/l and with history of failure to obtain the desirable platelet yield were recruited into the study. The procedures were carried out on the Haemonetics MCS+ cell separator machine using the SDP protocol.

In this study, donors donated platelets consistently for 6 months instead of doing alternate donation between plasmapheresis and plateletpheresis with an interval of 2–3 months. The target yield was decreased from 3.6 to 3.0×10^{11} which corresponds to the decrease on the number of cycles in the procedure. The post platelet count was also monitored to assess platelet depreciation.

We started our recruitment in June and used the results collected in that month as a baseline data. We only adjusted the target platelet yield from 3.6 to 3.0×10^{11} from July onwards (3.6×10^{11} is our centre set target). Within the duration of the study, pre platelet count was taken and the collection of their platelet was based accordingly to donor's exact platelet count instead of taking the average three previous consecutive donations to obtain estimated platelet count.

Results: The average platelet depreciation during the study was $17{,}000 \times 10^9$/l as compared to $44{,}000 \times 10^9$/l before the study. This was demonstrated on 53 (92%) of the donors but for the four (8%) of the donors there seem to show a fluctuation in their platelet depreciation. We noticed a gradual increase in the donors' platelet count after 4 months onwards among all the different age group. Through the immediate availability of pre-platelet count, we are able to set the precise process volume and cycles needed to obtain the target yield of 3.0×10^{11}. (As opposed to setting a higher processed volume based on previous average counts.)

Summary/conclusion: This small study suggests that by decreasing the platelet yield target from donors with these pre platelet counts is possible without compromising donor safety and efficacy of the platelet concentrates. The platelet concentrates obtained satisfy the international guidelines. With close monitoring of these donors we are able to increase our apheresis pool of donors and meet daily targets.

P-080

PREOPERATIVE AUTOLOGOUS BLOOD DONATION USING A DOUBLE-UNIT RBC COLLECTION SYSTEM IN ORAL AND MAXILLOFACIAL SURGERY

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Background: As the life expectancy increases, blood shortage is becoming a matter of great concern worldwide. Recently, double RBC collection system is adopted in many countries to procure more blood from healthy blood donors. Pre-operative autologous blood donation (PABD) aims to meet future blood transfusion requirements and has the advantage of safety because it is free from transfusion transmitted infection and alloimmunization.

Aim: We conducted double-unit PABD for patients who underwent scheduled elective oral and maxillofacial surgery to find out if the procedure is safe and effective.

Methods: We compared laboratory and clinical parameters of patients who underwent double-unit RBC collection using ALYX system (n = 117) with 82 patients who donated one unit of blood every week for 3 weeks before surgery.

Results: No significant adverse reaction was found in the double-unit PABD group. The hemoglobin (Hb) and hematocrit (Hct) before autologous blood deposit, the preoperative Hb, and 24-h postoperative Hb and Hct didn't show significant differences between two groups. Double-unit PABD group showed higher preoperative Hct level (ALYX, 39.7 ± 3.2%; control, 38.6 ± 2.7%; P = 0.024) and less decrease in Hct (12.3 ± 5.9%) when compared to control group (14.8 ± 5.6%) (P = 0.008). Duration of hospitalization showed no significant differences between two groups, however, double-unit PABD group showed a shorter interval from the first day of collection to the time of admission (ALYX, 14.4 ± 4.7 days; control 20.8 ± 3.8 days; P < 0.001). The percentage of patients requiring additional allogeneic blood transfusion was not significantly different between two groups.

Conclusions: PABD using double-unit RBC collection system was convenient since the patients visited our blood bank only once for pre-operative deposit. Clinical and laboratory parameters were comparable to the conventional one-unit PABD. In conclusion, double-unit PABD is considered as a safe and efficient method for patients undergoing autologous transfusion for scheduled elective surgery.

P-081

PREVALENCE OF HIGH TITRE IGM ANTI A/B AMONG VOLUNTARY BLOOD DONORS IN SRI LANKA

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Background: The National Blood Centre (NBC) is the headquarters of the National Blood Transfusion Service and it contributes to 21% of total annual whole blood collection. One hundred per cent of the whole blood collection is processed into blood components. Platelet concentrate is prepared either from Buffy coat derived or PRP method from 81% of the whole blood collected by the NBC.

Providing ABO compatible platelets may, at times become a difficult task because of the increasing demand for platelets and its limited shelf life. ABO incompatible platelet transfusion is shown to have a varied outcome.

A major mismatch platelet transfusion may show a reduction in platelet increment whereas a minor mismatch transfusion may show a severe haemolytic transfusion reaction due to presence of high titre IgM Anti A or Anti B in the donor plasma.

In Sri Lanka practice of transfusing minor incompatible platelet is not a standard practice. The advantage of using minor incompatible platelet is to minimize the wastage of platelets that expires 5 days after collection.

Aim: The purpose of this study is to determine the titre of IgM Anti A and Anti B among group A, group B and group O voluntary blood donors at the National Blood Centre – Sri Lanka.

Method: A descriptive study conducted at National Blood Centre – Sri Lanka in 2 months from April 2011 to May 2011. One hundred and fifty donor samples selected by stratified sampling method.

The anti A titre of group B, anti B titre of group A and anti A and anti B titre of group O donors were tested. Serial twofold dilutions of plasma were prepared in PBS. Samples were tested in parallel, for anti A and Anti B by direct agglutination using 3% pooled freshly prepared A1 and B cells. O cells were used as a negative control. The titre was recorded as reciprocal of the highest dilution giving 1 + clumping. The cut off value to determine the donor as high titre is taken as a positive result at 128 or more in saline agglutination.

Results: Out of 150 donor samples 50% of the donors were group O, and 82% of them were male. Forty-four per cent of the donors were between the age 28–37 and 34% of them were between the age18–27. Thirty-eight per cent of the donors were high titre positive and 62 of them were high titre negative. Sixty-three per cent of the high titre positive donors were group O. Forty-four per cent of female donors were high titre positive.

Conclusion: This study shows that the prevalence of high titre donors among Sri Lankan voluntary blood donor population was 38% and it is highest among group O donors. There was no significant rise in high titre positive donors among females.

It is recommended that it is important to consider the risk and the benefit before deciding out of group platelet transfusion.

P-082

USAGE OF THROMBOCYTAPHERESIS (SINGLE DONOR PLATELET) IN BANDUNG BLOOD DONATION SERVICE, INDONESIAN RED CROSS

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Background: Thrombocytapheresis is the separation and collected the platelet components and the remainder blood component are returned to the donor automatically using a special machine. Can be conducted continuously or intermittently.

Single Donor Platelet is more benefit than Multi Donor Platelet but due to the price is more expensive therefore still limited usage.

In Bandung Blood Donation Service, Single Donor Platelet has been done since 2003, using MS 3000 machine which is continuously flow with two access.

Since 2006, Bandung Blood Donation Service use Haemonetics machine MCS 3p and MCS+ with intermittent flow using one access.

Until now the usage of plateletpheresis (SDP) still on request from the physician clinicians.

The participation of the government with more affordable prices is highly expected.

Aims: (i) Increase the usage of plateletpheresis (SDP) by socialization to hospitals, (ii) To gain attention from government to give participation with affordable prices.

Situation analysis: 1. The number of doctors who have been trained are four people 2. Trained operators are six people

3. Number of Haemonetics machine available are: 1 unit MCS 3p and 2 unit MCS+

Methods: (i) Interviews with operators and technicians, (ii) Data collection.

Result: In 2003: 2 SDP, 2004: 3 SDP, 2005: 6 SDP, 2006: 10 SDP, 2007: 14 SDP, 2008: 23 SDP, 2009: 54 SDP, 2010: 211 SDP and from January to May 2011: 177 SDP.

Conclusion: There was an increase in usage, especially since 2010, the average demand for 13 SDP/month and from January to May 2011 average request are 35/month.

P-083

PLASMA PRODUCTION BY A NEW CELLULAR SEPARATOR: A GENERAL ASSESSMENT OF NIGALE NGL XJC 2000

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Background: The Nigale NGL XJC 2000, distributed by Hemotrans (Pomezia, Italy), is a new cellular separator exclusively dedicated to plasma production by apheresis. This

instrument, characterized by a discontinuous blood flow with a single venous access, adopts the technology developed by Latham, namely the separation of blood components according to their density gradient by applying an appropriate centrifugal force. In the Nigale's kit, a blow moldeld bowl is included: Thanks to the structure of this kind of bowl the coming in blood does not go through stratified hemocomponents, moreover the greater rotation speed respect to Latham bowl (7.000 vs 4.800 r.p.m.) permits to obtain a plasma with a lower cellular contamination. Moreover, compared to the previous version, a needle-fistula for collection, a satellite bag for donor's sampling, a spike for clinical use of plasma collected and a device for quality controls on product have been included in new kit.
Aims: We evaluated, in terms of effectiveness and efficiency, the qualitative and quantitative performances of this equipment.
Methods: Between November 2010 and May 2011, 200 plasmapheresis were performed with this new instrument, of course after informed consent, on 150 periodic blood donors with at least one previous experience in apheresis donation. In order to evaluate the operability and manageability of this cellular separator, the quality of its kits and the compliance of donors, we have recorded all adverse reactions to donation, any interruptions in the procedure due to malfunctions of instrument or abnormalities of kits.
Results: We did not detect any serious accident, only in 20 out of 200 plasmapheresis (10%) were recorded minor inconveniences. We have observed 18 mild adverse reactions due to citrate toxicity (9%), resolved by changing the pump speed or with a little break, but completing the procedure in all cases; in one case the donation was interrupted by a fainting (0.5%), an adverse reaction of moderate grade; while in the last case the apheresis failed for a prolonged low-pressure in the collection (0.5%) due to difficulties in venipuncture, but without consequences for the donor. Serious reactions were not observed, thus no one procedure has been interrupted for malfunctions of instrument or for defects or disruptions of the kits.
Conclusions: Italy is still far away to obtain the self-sufficiency in plasma-derivates, for this reason the collection of plasma by apheresis must be implemented. Nigale NGL XJC 2000, in our experience, is characterized by an intuitive management software and by simplicity in the mounting kit. The donation can be customized according to the parameters of the donor and the amount of plasma to produce. The safety in the process is guaranteed thanks to the various sensors connected to alarms, moreover it is possible to re-infuse saline solution at will to achieve an optimal hemodynamic compensation. Also considering the low costs of kits and the few mild observed adverse reactions, in our experience, the benefits of this new cell separator are related to safety, reliability and efficiency.

P-084
USING LEAN TOOLS TO IMPROVE DONOR TURN AROUND TIME

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Background: The need to optimise scarce resources and reduce waste, without sacrificing quality and patient safety led our facility to apply lean tools to look for improvement opportunities. Being the only blood bank in Singapore a high volume of donors come each day to donate and time is the motivating factor that propel donors coming forward. We recognised that time wasted with a poor process was time. Tools from lean six sigma were introduced in our work area from registration to donor room and the main goal of these tools is to identify activities in our workflow which will help improve donor turn-around time which in turn affects service delivery.
Aims: The aim of this study was to identify ways to enhance efficiencies in our workflow using lean six sigma tools to improve donor turn-around time.
Methods: With the help from a business partner, routine work processes from donor registration to donor room were analysed. The lean six sigma tools were applied to evaluate every second of a process and how long and how fast the donor goes from one station to another station of the whole donation process. The analysis also seek to determine how much of any donor's time is spent adding value to the donor vs how much time is spent performing registration, medical screening, haemoglobin testing and donation.
Results: Analysis showed that process timing for each station was consistent regardless of day of week. Average turn-around time was around 45 min on a non busy day.. From the study we noticed if there was a centre booking it effects the turn-around time. Relationship between waiting time and staff ratio at screening and donation showed the higher the ratio, the longer the wait. Waiting time at all stations affected the overall turn-around time of up to 90%. Based on the analysis, we incorporated haemoglobin testing together with medical screening which demonstrated a drop in the turn-around time.
Summary/conclusion: The lean quality tools were effective in identifying opportunities for minimizing or eliminating waste while conserving scarce resources. The tools pointed to more efficient design for work areas and reduction of donor traffic in already congested area.

P-085
HEMOGLOBIN PRE-DONATION SCREENING USING A NOVEL NON INVASIVE METHOD

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Background: Current Standard Operation Procedures worldwide require performance of Hemoglobin (Hb) levels measuring as a prerequisite for blood donation. At present, tests are performed using different invasive techniques, which have a number of disadvantages including donor's discomfort and pain, risk of infectious disease transmission, significant quantities of bio-hazardous waste and the need for very well-trained personnel. An innovative, non-invasive method was employed, which is based on Occlusion Spectroscopy technology in the red/near-infrared range, where a new bio-physical signal is generated following an over-systolic pressure which is produced at the finger base.
Aims: To evaluate the feasibility and validate the performance of a novel non-invasive method for Hemoglobin detection (NBM-200 device; OrSense Ltd. Israel) on a volunteer blood donors population in a national blood services set-up.
Methods: A total of 120 healthy volunteer blood donors (75 male, 45 female) in the age range 18–66 years participated in the study, which was conducted at the Blood Services Center fix-site donor's room. Upon receipt of informed consent from the donors, measurements were performed by placing an annular, multi-wavelength probe with pneumatically operated cuffs on the volunteer's thumb. Venous samples were taken from the same donors, before donation, and evaluated on a Cell-Dyn Ruby (Abbott) blood analyzer ('Reference values'). Results were compared by using Means, Standard Deviations (SD) and t-test.
Results: Hemoglobin levels measured on the NBM-200 device ranged from 10.6 to 16.4 g/dl, with an average of 14.1 ± 1.4 g/dl. These values were in accordance with the 'reference values', which ranged from 10.0 to 17.1 g/dl, with an average of 14.2 ± 1.4 g/dl. The difference between the Hb readings on the NBM-200 and the venous references samples is statistically insignificant (P = 0.65). The mean difference calculated as an absolute value, was 0.8 ± 0.6 g/dl. About 67% of the differences between the two sets of data were <1 g/dl, and 97% of the differences were <2 g/dl. The device did not cause any discomfort to the study participants, was safe and well tolerated.
Conclusions: The Hb measurements obtained by the NBM-200 (OrSense) new method were in relatively good agreement with venous blood reference values. The operating staff found the NBM-200 easy to use, reliable and appreciated by the donors. The technique reduces the need for invasive finger prick or venous blood sampling, thereby enhancing safety, and improving the overall experience of blood donation.

2.3 Donor Adverse Events

P-086
MILD ANEMIA CORRECTION IN REGULAR APHERESIS DONORS AS A SHORTEST WAY BACK TO DONOR'S ACTIVITY

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Depletion of iron stores is a frequent side effect not only for blood donors, but also for regular plasma donors (RPDs), donating plasma by apheresis up to 20 times per year. Mild anemia is common reason for temporary deferral and in most cases, nutritional recommendations directed on increase of hemoglobin and completion of iron stores don't lead to their normalization and deferred donors leave blood bank for a long time.
Aim: To develop effective scheme for anemia correction for regular plasma donors.
Methods: Within 6 months at Moscow Blood Bank (MBB) to all RPDs deferred due to decrease of hemoglobin (Hb), red blood cells (RBCs) and haematocrite (Ht) follow therapy was administered for 30 days: complex iron medication (in one capsule – iron sulfatis 150 mg, ascorbic acid 50 mg, riboflavinum 2 mg, tiamin mononitrate 2 mg, nicotinamid 15 mg, pyridoxine hydrochloride 1 mg, pantotenic acid 2.5 mg) – one capsule daily, and additionally – Ascorbic acid 100 mg three times daily and Folic acid 100 mkg three times daily. Upon 30 days of therapy, the control blood examination was performed in all cases.
Obtained results were evaluated by Biostatistica (version 4.03) and statistically significant differences were observed for $P < 0.05$ (Mann–Whitney test).
Results: Total number of RPDs, donated plasma during research period was 2613, in which 671 (25.7%) women and 1942 (74.3%) men. Totally 76 (2.9%) persons were

deferred due to mild anemia – 46 women and 30 men. Thus, frequency of this complication at female donors was 6.9%, at male – 1.5%.
Initial Hb level at the moment of deferral in female group was 115.0 ± 2.8 g/l, RBCs count 3.83 ± 0.21 × 1012/l, Ht – 34.28 ± 2.8%, in male group – 123.3 ± 4.5 g/l, 4.27 ± 0.49 × 1012/l and 37.2 ± 2.29%, respectively. After 30 days of therapy, it has appeared that 10 women and nine men have ignored received recommendations. Thus, they composed comparative group of study, whereas donors, who received the therapy – main group. In main female group, Hb concentration has increased to 124.6 ± 4.0 g/l, RBCs up to 4.2 ± 0.27 × 1012/l, Ht up to 37.4 ± 1.21%; in main male group Hb raised up to 136.7 ± 5.4 g/l, RBCs up to 4.9 ± 0.44 × 1012/l, Ht up to 41.3 ± 2.28% ($P < 0.05$ in comparison with initial value in all cases).
In comparative groups upon 30 days significant increase of analysed parameters wasn't noted: in female group Hb level was 114.2 ± 8.6 g/l, RBCs 4.03 ± 0.14 × 1012/l, Ht – 34.1 ± 2.43%, in male – 127.7 ± 2.3 g/l, 4.67 ± 0.46 × 1012/l and 37.8 ± 1.03%, respectively ($P > 0.05$ in comparison with initial value in all cases).
Conclusion: It is obvious that proposed therapy is highly effective for RPDs deferred for mild anemia and allows them to return to donation in shortest terms. It is necessary to consider, that MBB donors, being inhabitants of a huge megacity, are chronically stressed, in most cases can't keep healthy diet and suffer from hypovitaminosis. Therefore, despite presence at a complex preparation both iron and vitamins, ascorbic and folic acids which deficiency is most often observed at Moscow inhabitants, were additionally administered. Taking responsibility in maintaining of donor health, specialists of blood bank should work out effective schemes for anemia prophylaxis and criteria for it's administration.

P-087
HB A2 LEVELS AMONG INDIVIDUALS WITH BETA-THALASSEMIA TRAIT

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Background: Level of HbA2 is a well established screening test for beta-thalassemia trait (BTT). Some reports suggested that iron deficiency in BTT patients causes HbA2 to be lower than expected. However, conflicting reports have led to confusion amongst clinicians as to the reliability of HbA2 measurement when screening for BTT in iron deficient individuals.
Methods: Out of 3000 blood donors at Regional blood transfusion center, Alexandria, Egypt, accidently discovered patients with hypochromic microcytic anemia where screened for presence of BTT, and confirmed by molecular testing, variability in HbA2 was assessed. Thirty eight cases were found and classified as 'iron deficient' or 'non-iron deficient' based on their serum ferritin, (serum ferritin < 15 μg/l) and data was analyzed independently. The association of HbA2 levels with gender, iron deficiency or beta-thalassemia mutation type was evaluated using a two-sample T-test. The relationship of HbA2 with gender, hemoglobin level, Hb F percentage, reticulocyte percentage and/or the natural logarithm of serum ferritin was evaluated using single and multiple linear regression analysis.
Results: HbA2 ranged from 1.8% to 7.8%, with a mean of 5.2%. Mean HbA2 in females (4.9%, SD 0.7%) was lower than that in males (5.5%, SD 0.8%) ($P = 0.002$). Ten cases had a HbA2 level below 3% (range 2.0–3%), all of whom had serum ferritin 15 μg/l. The 10 cases with serum ferritin <15 μg/l had evidence of iron-deficient hematopoiesis. However, HbA2 was lower (5.3%) vs HbA2 in non-iron deficient individuals (5.5%; $P = 0.04$). A significant association of low HbA2 with low ferritin ($P = 0.04$) and beta+-thalassemia mutation type ($P < 0.001$). There was no significant association with gender ($P = 0.1$), hemoglobin ($P = 0.2$), reticulocyte count ($P = 0.08$) or hemoglobin F level ($P = 1.0$).
Conclusions: Serum ferritin <15 μg/l was associated with a small but significant decrease in HbA2 (mean HbA2 of 5.3%, vs 5.5% in non-iron deficient individuals; $P = 0.04$). Although ferritin under 15 μg/l was associated with lower HbA2 levels, beta+-thalassemia mutation type explained more of the variability than did iron status in individuals with lower HbA2 values. HbA2 remains a reliable test for BTT screening, even in the presence of iron deficiency. In patients known or suspected to have BTT, but in whom HbA2 is found to be <3.5%, testing for delta-globin abnormalities should be pursued.

P-088
A RETROSPECTIVE STUDY OF VASOVAGAL REACTION IN BLOOD DONORS: CONTRIBUTORY ROLE OF GENDER, AGE, WEIGHT, DONATION STATUS AND BLOOD PRESSURE

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Background: Vasovagal reactions are renowned adverse events related to blood donation. Although vasovagal reactions occur in small number of donors, these reactions may have negative effect on return donation and disrupt blood collection activities.
Aim: The objective of this study was to determine the input of age, weight, mean Arterial Blood Pressure, gender and donation status in vasovagal reaction in blood donors.
Study design and method: A retrospective study involved 11,314 blood donors from center and 11,361 blood donors from mobile sessions during June 2009 to December 2009 in Singapore. One-way ANOVA was used to study the significance of each variable contributed to vasovagal reaction in blood donors.
Results: It was occurred in 60 out of 11,314 (0.5%) donors had vasovagal reaction after blood donation in center and 555 out of 11,361 (4.9%) donors in mobile sessions respectively. First time donor status had a greater influence on developing vasovagal reaction compare to repeated donors, 3% vs 0.3% in center and 7.8% vs 3.36% in mobile sessions respectively. In the same time, age under 30 years had a higher chance of getting vasovagal reaction compare to those of 30 years and above, 1.2% vs 0.18% in center and 7% vs 2% in mobile accordingly. After analyzing with one-way ANOVA, age, donation status (first time donor), weight, gender, meanABP (except in center) were observed to have significant effect ($P < 0.001$). The most important variables in center, in descending order, were donation status (first time donor), age, weight, and gender. On the other hand, age, donation status (first time donor), weight, gender and meanABP were graded in descending order at mobile sessions.
Conclusion: Donation-related vasovagal syncope reactions are a multifactorial process largely influenced by age, donation status (first time donor) and weight.

P-089
SERUM FERRITIN MONITORING IN PLATELETPHERESIS DONORS WITH UNEXPECTED PROCEDURE TERMINATION

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Background: Serum ferritin is a good lab. test revealing iron storage in bone marrow. It had also been used in nutrition and health survey in Taiwan (1993–96) and in Jiangsu, China (2002). Ferritin together with hemoglobin and/or transferrin saturation were used to evaluate anemic status in these two surveys. In blood donors, ferritin has been used to evaluate the iron status of repeated apheresis blood donors in large scale. However, it has been found difficult to apply ferritin data in blood donors, due to wide normal range from 20 to 300 μg/l. We evaluated a group of blood donors who inadvertently lost hundreds c.c. blood due to unexpected procedure termination upon plateletpheresis. The series ferritin follow-up every 6 months as schedured, allowed us look into ferritin change longitudinally in individual donors.
Methods and results: Thirty-eight plateletpheresis donor's records from 2009 July to 2010 June, were reviewed. Of whom 34 were male and four female. Serum ferritin data were retrospectively found 42–68 days prior to the unexpected procedure termination, and 100–150 days post the event, upon regular plateletpheresis . Expect two of these 38 donors who were found no change of ferritin level before and after the event, 25 donors (21 males and 4 females) had ferritin level decreased from 36 to 25 (100 days) and 27 μg/l (150 days). Interestingly, 11 males had ferritin level increased from 46 (42 days before) to 76 μg/l (126 days after). Four hundred and sixty-two plateletpheresis donor's ferritin data were reviewed from October 2009 to April 2010. Half of these donors had elevated ferritin level, instead. Blood loss ranged from 100 to 474 c.c. (mean 230 and median 287 c.c.), estimated in the discarded collecting device. Half of the donors used MCS apheresis machine and half used Trima machine. Hematocrit (Hct) in the bowel of MCS collecting device was about 1.4 times of donors Hct, and 1.2 times in the cassette of Trima collecting device. The reason of ferritin increment post blood loss was not clear. Unfortunately, the diet habit and/or iron supplement taken were not clear in these donors.
Conclusion: From this retrospective study, we can approximately estimate ferritin decrement of 14.08 μg/l in male and 8.21 μg/l in female upon 100 c.c. blood loss in the discarded collecting device of 25 donors. The ferritin change following blood loss or donation need further prospective, detailed study of these group donors, which is underway.

P-090
DETECTION OF *STREPTOCOCCUS BOVIS* IN AN APHRESIS DONOR DIAGNOSED AS HAVING COLON CANCER

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Background: Back to 1950–1970, clinical researchers spent time and effort trying to understand the etiology of septisemia in patients. The colon bacterias were found adhere to the colon tumor and spread into blood circulation by enriched lymphatics and neovasculized vessels. *Strptococcus bovis* was found adherent to the receptor of tumor and suspected the most probable bacteria to cause bacteremia.

Methods and results: All plateletpheresis blood products were routinely cultured by aerobic BactT/ALERT method. A long term plateletpheresis donor was found repeated positive cultures in 1 and half years. The bacteria was *Bacillus* Sp. for the first time (October 2007) and Gr. D Enterococcus at the second and third times, *Streptococcus bovis* for the fourth time. The doctor interviewed the donor upon the third positive culture reported. The donor claimed living well without bloody stool noticed. Skin sterile technique performed on this donor 12 times, without positive reports. Gauzes and cotton swarbs were sent for culture without positive reports, neither. Colonoscopy and endoscopy were suggested by the doctor, however, refused by the donor. Three months after the fourth positive culture, the donor called and stated colon cancer diagnosed post bloody stool. Pathological report and operation report were refused hand in by the donor. The donor had blood cultures 2 weeks after each plateletpheresis positive cultures and revealed negative at all.
Conclusion: The bacteria adhered to colon tumor can spread into blood circulation upon pressure change in the colon, such as constipation. The same mechanism might play a role upon the donors blood inflow and outflow during apheresis. Streptococcus could spread into blood circulation passively during the procedure. This also explained the negative blood culture 2 weeks post apheresis.

P-091
INTRAVENOUS FLUID CHALLENGE FOR BLOOD DONORS WITH SEVERE DONOR REACTIONS

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Background: The blood donors with severe complications post whole blood or aphrresis donation, such as nausea, vomiting, dizziness, cold sweeting, lethargy, rigidity, convulsion and transient conscious loss, used to be sent to emergency room nearby, if they were unable to leave the donation site. Since May 2010, the intravenous solution Dextrose 2.5%/Saline 0.45%, 500 cm^3, was added into first-aid box. The doctor started giving verbal order from central office as full-run of such IV solution at donation site for these donors with severe complications. Comparison of these two groups was carried out retrospectively.
Methods and results: From March 2008 to April 2011, 11 such severe complications donors were sent to emergency room accompanied by nurse. Laboratory tests and IV solution at regular rate were given by doctor. Eight of these 11 donors stayed at emergency room from 1 h 20 min to 7 h 45 min, average 3 h 28 min, until the donors were able to walk with assistance and sent home. From May 2010 to May 2011, nine such donors received IV Dextrose 2.5%/ Saline 0.45% full-run at donation site and finished 250–500 cm^3 in 1–1.5 h. They were able to leave donation site after rested for 20–30 min.
Conclusion: By giving IV at donation site for those severe complications post donation is as effective as emergency room management. In addition, the donor and nurse can be benefit from time and stress relief.

P-092
FREQUENCY OF ADVERSE EVENTS IN PLATELETPHERESIS DONORS IN REGIONAL TRANSFUSION CENTRE IN NORTH INDIA

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Background: Although automated cell separators have undergone a lot of technical refinements, attention has been focused on the quality of platelet concentrates than on donor safety. We planned this prospective study to look into donor safety aspect by studying adverse events in normal healthy plateletpheresis donors.
Study design and methods: The study included 500 healthy, first-time (n = 301) and repeat (n = 199) plateletpheresis donors after informed consent. The plateletpheresis procedures were performed on Trima Accel (5.1 version, GAMBRO BCT) and Amicus (3.2 version, FENWAL) cell separators. The adverse events during procedure were recorded and classified according to their nature. The pre and post procedure hematological and biochemical profiles of these donors were also assessed with the help of automated cell counter and analyser respectively.
Results: A total of 18% (n = 90) adverse events were recorded in 500 plateletpheresis donors, of which 9% of were hypocalcaemia in nature followed by hematoma (7.4%), vasovagal reaction (0.8%) and kit related adverse events in (0.8%). There was significant post procedure drop in Hb, Hct, platelet count of the donors ($P < 0.0001$) whereas WBC count showed a statistically significant rise ($P < 0.0001$). Divalent cations (iCa+, TCa+, TMg+) also showed a statistically significant decline after donation ($P < 0.0001$). However there were no statistically significance difference between adverse events in Trima Accel (5.1 version, GAMBRO BCT) and Amicus (3.2 version FENWAL) cell separators.
Conclusion: Donor reactions can adversely affect the voluntary donor recruitment strategies to increase the public awareness regarding constant need for blood and blood products. Commonly observed adverse events in plateletpheresis donors were hypocalcemia, hematoma formation and vasovagal reactions which can be prevented by pre-donation education of the donors and change of machine configuration. Nevertheless, more prospective studies on this aspect are required in order to establish guidelines for donor safety in apheresis and also to help in assessing donor suitability, especially given the present trend of double product apheresis collections.

P-093
COMPARE DETECTION OF SYPHILIS IN DONOR POPULATION AT SOUTHERN REGIONAL BLOOD CENTER – KAMBURUGAMUWA, SRI LANKA FROM 2006 TO 2010

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Background: Syphilis is one of the transfusion transmitted infections which is checked in donor blood. Syphilis disease itself has various stages. Primary and secondary Syphilis can have symptoms but tertiary or latent Syphilis lacks the typical symptoms and also can cause multi-organ disease. Early detection is important as this is a curable disease with prompt treatment. A sensitive detection system should be available to prevent the disease being spread via blood transfusions.
Aim: To screen all blood donors for Syphilis by a sensitive method as to identify donors who are infected but in different stages.
Method: Data was collected from registers maintained at the laboratory at Southern Regional Blood Center – Kamburugamuwa, Sri Lanka from 2006 to 2010.
Results: Total of 74,690 blood units were screened at the center during the period from 2006 to 2010 and all underwent Venereal Disease Research Laboratory (VDRL) test. Test results are shown in the Table 1.
The confirmatory test TPPA (Treponema Pallidum Particle Agglutination) was performed on all donors who were positive for VDRL test and the results are shown in the Table 2.
All blood units with positive serological results were discarded. Further donors who were positive from TPPA were contacted and referred to the Sexually Transmitted Disease (STD) clinic at General Hospital, Matara, Sri Lanka for further management.
Summary: The number of donors detected with Syphilis has a considerable variation thought out the duration under consideration. It is important to address this in a systematic manner as syphilis is a curable disease. It is recommended to implement proper early detection and reference for treatment systems as a priority. Also steps should be taken to educate young donor population regarding the above issues.

Table 1

Year	Number of donors became positive of VDRL test
2006	21
2007	40
2008	29
2009	10
2010	18

Table 2

Year	Number of donors became positive of TPPA test
2006	4
2007	1
2008	4
2009	1
2010	5

2.4 Rare Donor Programme

P-094

ROLE OF PULSE OXIMETRY PLETHYSMOGRAPHIC WAVEFORM VARIABILITY IN PREDICTING ACUTE BLOOD LOSS

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Background: Patients with acute blood loss must be treated with aggressive fluid infusion. But no reliable non-invasive monitor tool or parameter were available for assessing the condition. A lot of research papers showed positive correlation between change of stroke volume and pulse oximeter amplitude variation before and after fluid challenge in septic patient who was sedated with ventilator support. But there are few relevant materials investigating spontaneous breathing patients with acute blood loss by pulse oximeter.
Study design and methods: This research imitate the class 1 hemorrhagic shock patient with spontaneous breathing (blood loss 10–15%), via voluntary blood donor. Cardiac sonography was used as the standard of assessment, before and after blood donation. We accessed the change of cardiac stroke volume; pulse oximetry amplitude variation (ΔPOP); serum biomarkers before and after blood donation.
Results: Thirty-two repeated blood donors and 1 first blood donor as volunteers (male, 15) were included in this study. Their mean age was 30.6 ± 6.6 years (range, 22–53 years). Body weight was 68.91 ± 4.79 kg. Average blood loss was 12 ± 1.17% as estimated by blood volume vs 7% of body weight and blood drawn duration was 10.31 ± 3.27 min. Bleeding rate was 0.79 ± 0.19 ml/min/kg. There was no significant difference in systolic arterial pressure, diastolic arterial pressure, and respiratory rate between the baseline and post blood donation conditions. However, cardiac output, stroke volume decreased and heart rate increased after blood donation. The occult hypoperfusion biomarkers such as Pro-BNP, Lactic acid, venous blood Gas Base Excess, were the same between pre and post blood donation. The hs-CRP was decreased and venous blood Gas pH increased post blood donation, which necessitate further exploration. A significant difference (P = 0.0299) in ΔPOP was found before and post blood donation, and the mean ΔPOP were 12.93 ± 4.05% and 14.93 ± 5.61% seperately.
Conclusions: This is a pilot study of a larger group healthy voluntary blood donors. The physiological change in acute blood loss patient is quite different from blood donor. But this, if having achievement after further research and analysis, and tools using skillfully, can become theoretical foundation for clinical research in the future.

P-095

RARE BLOOD DONORS IN TAIWAN

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Background: A rare blood group is defined as the blood groups absence of a high prevalence antigen or absence of several antigens within a single blood group system giving a prevalence of 1/1000 or less in the general population. As a blood provider in Taiwan, we have maintained a rare donor panel and storage of frozen RBCs of rare blood groups. To increase the number of rare donor, a multi-year project of screening of donors for minor blood system including Rh, Kidd, Lewis, Duffy and MNS systems were performed since January 2005.
Aims: The aim of the study is to investigate the phenotype frequencies of various blood group systems and to characterize the rare donors found in the donor population.
Methods: The data from the screening project carried out between January 2005 and March 2011 were analyzed. A total of 332,443 random voluntary blood donors were screened for the red cell antigens Rh (D, C, E, c, e), Kidd (Jka, Jkb), Lewis (Lea, Leb) and MNS (M) using monoclonal and polyclonal antibodies with automatic analyzers (Olympus PK 7300; Center Valley, PA, USA). In addition, 2499 donors from foreign blood donor group were analyzed for Duffy (Fya, Fyb) by manual tube method.
Results: During the period, 1,425,825 red cell typing were carried out on 332,443 blood donors. A total of 2335 (0.7%) donors were identified as rare. Of these rare donors, 2059 were determined as Rh (D–), giving a breakdown for the combinations of antigens as 1111 for rr, 728 for rr', 144 for r'r', 46 for rr'', 25 for r'r'' and 5 for r'ry. Furthermore, 263 donors negative for high-incidence antigens and 13 donors of rare RzRz phenotypes were identified. As regards the donors negative for high-incidence antigens, the donors identified for the phenotype were 128 for Jk (a-b–), 100 for Fy(a-b+), two for Fy(a-b–), four for Di(b–), 14 for i(–) and 15 for k(–). In the recent 2 years, we have collected 1469 rare blood units, of which 234 were stored in frozen. Further, these rare donors are characterized as below. Rare donors were found 64.8% from male and 35.2% from female donors. There were 67.2% of the rare donors at the age between 17 and 40. Only 41.7% of rare donors gave blood every 3 months and 37% of them did not give subsequent blood for over 2 years.
Conclusions: At present, we are able to provide adequate number of multi-antigen-negative and Jk(a-b–) bloods for the blood demand in Taiwan. However, the Fy(a–), Di(b–) and Rh null donors remain short in our donor panel. The project of screening for rare donors should be continued and the procedures to approach these donors should be addressed.

3. Blood Products
3.1 Blood Processing, Storage and Release

P-096

QUALITY CONTROL OF BUFFY COAT DERIVED PLATELET CONCENTRATIONS USED FOR TREATMENT OF CANCER PATIENTS

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Introduction: National Cancer Institute is the pioneering treatment centre for oncological treatment in Sri Lanka. Transfusion of platelets to thrombocytopenic patients is a method of optimizing their clinical condition. In component preparation, two different methods are used for preparation of buffy coat derived platelet concentrate. In the first method, buffy coat derived platelets are prepared by high speed centrifuge at 4000 rpm for 9 min and separation followed by overnight hanging and separation of platelets without mixing using the 'OptipressTM' machine. The second method of platelet preparation was to mix the overnight hanged platelets (prepared as same as above) and then centrifuging it again followed by separation of platelets. The quality of the final platelet concentrate is determined by the preparation method which has a large number of steps involved. Improvement of the quality of components is the primary task of the blood processing laboratories in transfusion service.
Aim: This study was conducted to evaluate and compare the quality of buffy coat derived platelet concentrates using the two separate methods of platelet preparation.
Method: In each method 150 random samples were selected. The volume, platelet count, red cell contamination, leucocyte contamination, pH, and sterility were recorded. All samples were analyzed using 'Orphee' hematological analyzer. The pH was determined by a pH meter and sterility assessed by culture of each sample.
Results: The standard volume of platelet pack falls between 55 and 75 ml. The average volume of 78.68 ml in the unmixed platelet packs (the first method) was slightly higher than this range. However mixed and separated packs (second method) showed average volume of 55.34 ml, which falls in to standerds. The leucocyte contamination of first method was 1.032 × 109. This was higher than the standard cut off of 0.05 × 109. On the other hand second method showed reduced leucocyte count of 0.072 × 109 per unit. Considering the platelet count per pack, unmixed and separated packs (first method) showed 83.9 × 109 per pack, whereas mixed and separated packs (second method) showed a platelet count of 70.39 × 109 which was slightly less than first method. The pH value fell between 7.3 and 7.5 in both methods. None of the platelet packs showed bacterial contamination. The amount of red cell contamination was 1.33 × 109/ml and 0.05 × 109 in unmixed and mixed packs respectively.
Conclusion: Values and the results of the quality control indicate reduced contamination of leucocytes and red cells in the mixed and centrifuged method (second method). However by achieving leuco-reduction and reduced red cell contamination, mixed and centrifuged method produces less number of platelet per pack than the unmixed method.

P-097

A NEW PRESTORAGE LEUCOCYTE REDUCTION FILTER FOR RED BLOOD CELL CONCENTRATES LEADS TO SIGNIFICANT LOWER LEUCOCYTE CONTAMINATION

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Background: Prestorage white blood cell (WBC) filtration of red blood cell concentrates (RCC) reduce alloimmunisation and non haemolytic transfusion reactions and may prevent transmission of leucocyte-associated viruses, bacteria or prions. Therefore the reduction efficacy of a filter system is of importance. We validated a new WBC

filter, Leucoflex LCRD2, with two additional filter layers, and compared it with historical validation data of our routine WBC filter LCRD (both Macopharma, Tourcoing, France).
Methods: Whole blood donations (450 ml) were stored overnight at room temperature (RT) and centrifuged with 4200 *g* for 13:30 min at RT the next day. Afterwards blood was automatically separated, filtration (LCRD2) of buffy coat removed RCC was done with 50, 60, 70 and 80 cm filter height (distance between filter and storage bag) and filtration time was recorded. RCC (n = 215) were analysed for haemoglobin (Hb) and flow cytometry based residual WBC content. The following storage parameters were measured 1, 3 and 6 weeks after donation (n = 45): haemolysis rate, pH, glucose and lactate. Reference data were ascertained equally 3 years before (LCRD, n = 101) with exception for the filter height (solely 80 cm).
Results: Residual WBC could be reduced by half from 0.08 (LCRD) to 0.035 (LCRD2) $\times 10^6$/U (mean). The filter height had no impact on the filtration efficacy (0.03, 0.02, 0.03, and 0.05 for 50, 60, 70, and 80 cm respectively), but the filtration time was extended from 22 min (LCRD, 80 cm) to 35, 42, 46, and 56 min for LCRD2 at 80, 70, 60, and 50 cm respectively. Hb content did not differ significantly (LCRD 51.4, LCRD2 50.7 g/U). Haemolysis rate was higher with LCRD2 compared to LCRD (mean 0.35% vs 0.21%) but was within the guidelines of the Council of Europe. The other storage parameters pH, glucose and lactate did not differ significantly.
Conclusion: The new filter for RCC Leucoflex LCRD2 is significant more efficient in reducing WBC which could be an advantage in terms of preventing transmission of leucocyte-associated infectious agents. Quality controls and storage parameters are within Council of Europe guidelines. The filter height has no impact on the filtration efficacy.

P-098
COMPARATIVE STUDY OF THE KINETIC PROCESSES OF GROWTH FACTORS RELEASE FROM PLATELET GELS PREPARED BY TWO METHODS

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Background: Platelet growth factors (PGFs) released by thrombin-Ca or $CaCl_2$ activation of autologous platelet-rich plasma (PRP) are used as platelet gels (PG) in clinics for wound healing and tissue repair. But the kinetic process of growth factors released from the PG has not been thoroughly studied, and should be learned in order to provide more reference for the clinical application.
Aim: To learn the time course of the release of growth factors from PGs prepared through two methods, that thrombin-Ca and $CaCl_2$.
Method: Platelet-derived growth factor-AB (PDGF-AB), and transforming growth factor-β1 (TGF-β1) were measured before activation and at different time points of 1, 2, 4, 6 h after PG formation that prepared respectively by thrombin-Ca and $CaCl_2$.
Results: Mean PDGF-AB, TGF-β1 concentration increased to 104 ± 40, 101 ± 26 ng/ml respectively after $CaCl_2$ activation for 6 h, and the release amount at the first hour were approximately 65%, 80% of the maximum respectively. Whereas the two factors increased to 139 ± 48, 102 ± 31 ng/ml respectively after thrombin-Ca activation for 6 h, and almost compeletly released at the first hour. In addition, PDGF-AB, TGF-β1 levels and platelet counts showed a certain linear relationship. In the $CaCl_2$ group, the correlation coefficient r were 0.794, 0.932 respectively, whereas 0.9, 0.988 in the thrombin-Ca group.
Conclusion: There is a fast release of PDGF-AB and TGF-β1 from PG at the first hour after gel formation, especially the thrombin-Ca group. And $CaCl_2$ maybe an ideal choose to institute thrombin-Ca. This study may provide useful information for clinicians to improve PG clinical application and to design improved blood-derived biomaterial with controlled release of growth factors.

P-099
DETERMINATION OF FREE HEMOGLOBIN: COMPARATIVE STUDY OF THREE WAVELENGTHS METHOD AND ORTHOTOLIDINE METHOD

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Background: Free hemoglobin (FHb) is the conventional indicators of quality during blood preservation process, which is associated with the distress severity of red blood cells. Transfusion blood with elevated levels of FHb could induce adverse effects of blood transfusion, in particular, would produce harmful effect on the nervous system and circulatory system function. As a result, national Standard of China has put restriction on the concentration of blood deserved to be transfused. Traditional methods for FHb concentration is the chemical approach, including all kinds of benzidine and its improvement, in which the orthotolidine method is the most common one. However, the orthotolidine method enjoys obvious disadvantages, such as tedious operation and poor stability. So another non-chemical approach, the three-wavelength method, which is based on the light adsorption of hemoglobin itself, is beginning to be used more widely among the blood centers.
Aims: The aim of the present work is to have a comparative study of the accuracy, Linearity, stability, precision, recovery rate and ease of operation of the three-wavelength method and orthotolidine one used for measuring FHb. In addition, the advantages and disadvantages of the two methods are evaluated.
Methods: First, to detect the FHb concentration of standards and whole blood samples with the two methods mentioned above, and then do the precision, linearity, precision, recovery, stability experiments in turn, finally compare and analyze the results.
Results: The results obtained by detecting the FHb concentration of FHb standards was in line with the real value, the accuracy could reach 106%. Moreover we gained the results by direct calculation. Recovery rate and precision, which were 1.47% and 109.16% (RSD) respectively, were in accordance with methodological requirements. The color development was stable when detection was in progress. While the results could be obtained by orthotolidine method only when the reference standard was available. The precision (RSD = 5.01%) was no better than the three-wavelength method and recovery rate was 82.87%. The color development was extremely instable. Besides, there was linear relationship between the absorbance (A) and the concentration of FHb only in a certain range (CFHb < 600 mg/l). When the concentration of FHb is higher than 600 mg/l, the plasma sample should be diluted to make its final concentration lower than 600 mg/l. The results obtained by detecting 30 plasma samples with three-wavelength method, orthotolidine method with dilution as well as without dilution were showed as follows, C1 (432.65 ± 115.66 mg/l), C2 (461.13 ± 92.76 mg/l), C3 (688.68 ± 161.35 mg/l). And there is significant difference between C1 and C3 ($P < 0.01$), C2 and C3 ($P < 0.01$).
Conclusions: The FHb results obtained with the three-wavelength method is easy to operate and as well as of high authenticity and stability; the orthotolidine method enjoys obvious disadvantages: tedious operation steps, narrow linear range, many matters needing attention and poor stability. Thus three-wavelength method has more broad application prospects comparatively.

P-100
THE EXPERIENCE OF THE BLOOD COMPONENTS PROCESSING BY AUTOMATED BLOOD COMPONENT EXTRACTORS IN KAOHSIUNG BLOOD CENTER

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Background: Blood component extractors are used to extract components in a semiautomated manner, whereas with multiple functions, the automated Buffy-coat (BC) method is used to prepare pooled platelet concentrates from whole blood in Europe. Instead, the platelet-rich plasma (PRP) method is used to derive platelet concentrates with entirely manual work in Taiwan. Taiwan Blood Server Foundation plans to bring in blood component extractors to improve the quality of preparation of blood components in 2011.
Aims: The aim of the study was to test the outcomes of blood components processed by two systems, Fresenius Kabi Compomat® G4 Automatic Blood Component Processing System and Lmb Technologie GmbH Dual Press.
Methods: Blood samples were collected and prepared for blood components by the staff of Kaohsiung Blood Center between July 2009 and July 2010. Data collected for the study included processing time, components weight, RBC Hct, and PLT. RBC Hct and PLT count of the PLT concentrate was analyzed by Sysmex XT 1800i Automated Hematology Analyzer. Weight of components obtained from the extractor and manual work was compared. The processing time in the double bag preparation was also compared.
Results: A total of 590 samples were collected to prepare for components. The preliminary results of the analyses of RBC, Plasma and PLT weight showed no significant differences between processing methods. However, the processing time was significantly longer for the automatic extractor. The QC tests for RBC Hct all revealed 'qualified' within the acceptance range. The QC acceptability rate for both 250- and 500-ml PLT concentrates were 81.3%, 91.9% and 76.7%, 96.7% for Fresenius Kabi Compomat® G4 Processing System and Lmb Technologie GmbH Dual Press, respectively.
Conclusions: Though it takes a much longer time for processing, the automated processing systems reduce daily workload of routine preparation of blood components and offers constant products with standard quality.

P-101
APPLICATION OF DATA LOGGER TO MONITOR THE TEMPERATURE OF BLOOD COMPONENTS DURING TRANSPORTATION AND STORAGE

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Background: The data logger is often used for monitoring the temperature of blood components during storage and transportation. We conducted the monitoring operation on blood components during storage and transportation according to SOP approved by TBSF.
Aims: This study aim to find the data loggers offered by three companies are applicable to monitor the temperature of blood components or not.
Methods: We validated the system through running the program to find if it affected the operations of the donation system as well as the computer. An accuracy test on the data loggers were then conducted for blood storage equipment. Afterwards, we respectively put the three tested data loggers which are currently used in our Center, and the standard components of outsourced calibration implementation (C****K®) under the environment of refrigeration (−20°C), cold storage (1–6°C), and normal temperature (20–24°C) to compare their read-out values with the standard components; we cross-referenced these differences with the eligibility criteria of the SOP, stating that the errors must be ≤1. Similarly, we conducted another accuracy test on the data loggers for blood components during transportation under the environment of refrigeration (−5°C), cold storage (1–10°C), and normal temperature (20–24°C).
Results: All three thermograph programs were running without affecting the donation system as well as the computer. We then compared the errors between the three tested thermographs plus thermographs in use and the standard components and found that. For L**T**®, there is one value >1°C for blood storage equipment while there is no value >1°C for blood components during transportation by comparing. For T***T***4®, there is one value >1°C for blood storage equipment and there is also one value >1°C for blood components during transportation. For E****T®, there is no value >1°C for blood storage equipment, but instead, there is one value >1°C for blood components during transportation.
Conclusion: The test results revealed the fact that all the three data loggers from different companies running without affecting the donation system as well as the computer, and artificial alterations cannot be carried out against those read-out values; their reliability is thus maintained. For the errors >1°C, there is a need for us to proceed to other discussions to find whether these errors are associated with a high product defect rate or insufficient test samples. To conclude, all the three data loggers could be applicable to monitor the temperature of blood components during storage and transportation if precluding the part of 'imperfect' errors >1°C.

P-102
MANAGEMENT OF TRANSPORT, PACKAGING AND THERMAL INSULATION MATERIAL FOR SMALL QUANTITY OF BLOOD PRODUCT

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Background: When the hospital receives small quantity of blood product, it should ensure the temperature control quality and confirm the temperature during the transport process. Meanwhile, the packaging material management mechanism is also planned, and the application efficacy is reinforced too.
Method: Igloo 9L thermal container is used to carry one unit of blood product, and test is then performed in accordance with different quantity of Thermal Stabilizer and placement locations. Right above and below the blood product, it is placed with Datalogger, and the temperature of the blood product is recorded every 5 min for a recording period of 4 h. The temperature control conditions of all kinds of blood products are: For red blood cell concentrate, it is 1–10°C, for plasma, it is below −5°C, for platelet it is in the range of 20–24°C, for at least 2 h.
Thermal container is numbered and registered for management, and 'borrowing list' is used as receipt for the withdrawal and recycling by the hospital, meanwhile, we will go to hospital to get back the overdue borrowed thermal container and evaluate the efficacy of the management method.
Results: When the environment temperature is higher than 25°C, the result using Igloo 9L thermal container to transport all kinds of blood products is as follows: (i) Red blood cell concentrate is covered by four layers bubble cloths, and above it, it is placed with a refrigerating Thermal Stabilizer, then the temperature is maintained in the range 1–10°C for 2 h and 45 min, (ii) Plasma is covered by two layers of bubble cloths, and one refrigerating Thermal Stabilizer is placed respectively in the upper and lower side, then the blood product is maintained below −5°C for at least 2 h and 10 min, (iii) Platelet is placed within thermal container without the need of adding Thermal Stabilizer, and it is maintained at temperature of 20–24°C for at least 2 h and 30 min.
Igloo 9L thermal container is labeled with 'Hsinchu Blood Center', 'Number', and withdrawal time, and it is also added with the hint of 'It should be sent to the blood warehouse within 2 h'. Use 'Blood product transport and thermal container borrowing form' for inspection and follow-up, for a trial of 3 months, and the return rate rises from 40% before the setup of control mechanism to 90%, that is, the control efficacy is pretty significant.
Conclusions: In the past, polyfoam box is used to package blood product, due to ineffective control, polyfoam box needs to be purchased regularly. In addition to the waste of money in the blood center, the wasted polyfoam box will also become a big load to the environment. After it is changed to durable Igloo 9L thermal container, in addition to easy identification in its appearance, the considerate hint also shows the cooperative mind of the hospital and the professional and responsible image of the blood center.

P-103
EXPERIENCE IN INVENTORY OF BLOOD GROUP NEGATIVE BLOOD

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Background: The need of specific blood group negative blood is increasing gradually. To establish inventory of red cell antigen negative blood is necessary for provision of need timely.
Aims: Establishing inventory of specific blood group negative blood that need often to speed up the process of blood supply.
Methods: We analyzed the frequency of distribution of blood group negative blood in 2009 and chose the types that needed frequently to establish stock. By searching the data base of blood group negative blood files, the specific blood was selected and stock daily but which have been stored for 14 days will be transferred to ordinary stock. The efficiency of inventory management was measured and adjusted.
Results: There were 1698 units of blood stocked and 881 units were supplied for transfusion in third quarter of 2010. The provisions of blood group negative blood were 1416 units totally, and this stock fulfilled 62.2% of demand. The details of blood distributed are in the Table 1.

Table

Blood group	Rh				M		Kidd		Lewis		Duffy
Antigen negative	D	C, e	E, c	e	M	M^{s}	JK(a-b+)	JK(a-b-)	Le(a-b+)	Le(a-b-)	Fy^{b}
No. of stock	360	301	819	52	102	10	14	11	14	9	6
No. of distribution	120	211	465	38	30	0	4	3	0	4	0
proportion	33.3%	70.1%	56.8%	73.1%	29.4%	0	28.6%	27.3%	0	44.4%	0

Conclusions: There were around 23% of Blood which is multiple antigen negative or high frequency antigen negative provided by other blood centers. The situation was no change before or after this project implemented but this is the important point to remedy. It took about 15 min to pick out a unit of desired blood from ordinary blood warehouse but only 5 min is needed to complete the whole distribution process if the blood was from the negative blood inventory. It meant that 62.2% change is a great improvement because we not only speeded up the distribution process but also saved 8800 min of work time per quarter. Moreover, the time of working under a low temperature environment was shortened and the working quality was improved also.

P-104
THE UTILIZATION OF BLOOD PRODUCTS IN TAIWAN

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Background: TBSF was founded in 1974 and had provided blood related services for the past 35 years. The blood donation volume has grown significantly from 3817 bags in 1974 to 2,397,401 bags (250 ml) in 2008.

Aims: Similarly, the supply of blood products has grown from 3794 to 5,354,992 units/year in 2008. With the continuous support from enthusiastic donors, the blood supply has not only been able to meet the demand from hospitalized patients, but also those interested in the therapeutics aspects of blood products. However, the future of blood donation remains uncertain as we continue to find new sources of blood and new applications for blood products.
Methods: This study is based on the number of clinical and hospitalized cases within the National Health Insurance database provided by the National Health Research Institutes. Briefly, one million patients were randomly selected. Base on their medical histories and annual usage of blood products, we can deduce the optimal use and distribution of the donated blood to meet the annual demand. This retrospective study is analyzed by SAS software. Detailed description of the hospital fee, hospitalization cost, and other medical organization cost were factored in for meta-analysis. The blood products were divided into P-RBC, FFP+FP, Platelet concentrate, Platelet Apheresis. Statistical analysis of the data were categorized by the various divisions within NHI, hospitals, medical department, and the incidence of major diseases.
Results: Out of one million patients in 2006, 28,837 units of blood products were used. P-RBC had the highest consumption with 17,668 units (61.27%) and FFP+FP in second with 6197 units (21.49%). Under the NHI, Taipei division consumed the most units with 8973 units (33.11%) while central Taiwan area consumed 19.10% of total units. For the consumption of each blood product, Taipei area leads in consumption in P-RBC with a percentage higher than all other area ($P < 0.05$). Consumption of P-RBC from the Gastroenterology (13.33%), Pulmonology (12.27%), and Orthopedics (12.24%) were similar. As for FFP+FP, it is mostly used by patients from the Gastroenterology (19.96%) and Surgery (16.83%). These two blood products demonstrated different patient applications within different medical departments ($P < 0.05$). Medical centers are the largest users consumers of blood products (44%), and P-RBC accounted for the largest proportion (6831 units). Platelet concentrate (1896 units) is more often used than platelet Apheresis (1254 units).
Conclusion: Based on our analysis, we have viewed the expansion in blood product applications, including disease applications, from different areas and levels of the hospital in Taiwan. This study not only updated us on the current status of blood transfusion, but also clear how to optimize operations of a blood centers. This includes management of blood source and allocating blood products supply. After the new healthcare policy, TW-DRG, was mandated in January, 2009, we shall continue to monitor and analyze the utilization of blood products in Taiwan.

P-105
THE EFFECT OF LYOPHILIZATION ON THE ANTIGENS AND CD MARKERS OF RED BLOOD CELLS

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Background: Lyophilization is an important technique to preserve and long-time storage of red blood cells (RBCs). However, little is known whether the blood group antigens and CD markers of the freeze-drying RBCs are changed.
Aims: Our study aims to investigate the effect of lyophilization on the antigens and CD markers of RBCs.
Methods: The blood group antigens (ABO, Rh, MNSs, Kell, Duffy, Lewis and P blood group systems) were detected by serology; the levels of 2, 3-DPG and ATP of RBCs were determined by ELISA; the cytomembrane CD35, CD44, CD45, CD47 and CD71 of RBCs were measured via flow cytometry before and after freeze-drying, respectively.
Results: After lyophilization, the antigens of ABO, Rh, MNSs, Kell, Duffy and P blood group systems remain the same except Lea and Leb antigens of Lewis blood group system. Before and after lyophilization, there were no statistical difference on the levels of 2, 3-DPG and ATP of RBCs and the cytomembrane CD markers of CD35, CD44, CD45, CD47 and CD71.
Summary: Our study suggests that RBCs functions may remain unchanged after lyophilization. These findings may have important implication for the clinical use of freeze-drying RBCs.

P-106
CONSISTENCY BETWEEN THREE TEMPERATURE MONITORING SYSTEMS AND THE CORE TEMPERATURE OF STORED BLOOD COMPONENT

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Background: Continuous temperature monitoring of blood component storage is important to blood component quality and is required by the Standards of AABB. Commercially available storage device is equipped with a weekly pen and chart recorder, and a standalone thermometer immerged in glycerol solution is also used to manual monitoring of storage temperature periodically. With the introduction of centralized temperature monitoring system, many blood banks adopt the system due to its ease to use for continuous and real-time online monitoring of storage temperature and the advantage of a possible central alarm function to alert the designated staff not in the vicinity of the equipment once an alarm is activated. However, the discrepancy among the centralized temperature monitoring system, the weekly pen and chart recorder, and the standalone thermometer is not infrequently observed.
Aim: This study aims to assess the consistency between three temperature monitoring system and the core temperature of stored blood component.
Methods: The core temperature of packed RBCs was measured by a thermometer inserted halfway into the blood bag. The thermometer was calibrated and traced to NML/ROC national standards. The blood bag was placed into a refrigerator (Sanyo, MBR-1404GR) equipped with a weekly pen and chart recorder (system C) and alarm for deviation of temperature. A standalone thermometer immerged in glycerol solution (system B) was placed just next to the blood bag. A commercially available centralized temperature monitoring system (system A) was setup according to the recommendation of manufacturer. After stabilization of the refrigerator temperature for 60 min, the initial core temperature of the packed RBCs was recorded as well as the temperature of the three systems. Then the refrigerator door was open for 10 min to let the temperature exceed the acceptable ranges for storage. The core temperature and the temperature of the three systems were recorded every minute. The process was repeated three times. Correlation between temperature of the three monitoring system and the core temperature was assessed using Pearson's correlation.
Results: The average core temperature of blood bag fluctuated between 4.5 and 8.2°C. The corresponding temperature recordings ranged from 3.7 to 9.8°C for system A, from 4.0 to 9.5 for system B, and from 3.8 to 13.0 for system C. Compared to core temperature of blood bank, the bias was between −1.1°C and +1.6°C for system A, −0.5 and +1.5 for system B, and −0.8 and +5.0 for system C. Using core temperature as the standard, the Pearson's correlation is 0.964, 0.954, and 0.796 for system A, system B, and system C, respectively.
Conclusion: Both manual monitoring using standalone thermometer and the centralized monitoring system showed good correlation with the core temperature of stored component. All the three systems have a significant positive bias, especially the pen and chart recorder system. However, with regard to the consideration of blood product quality, this bias is acceptable. In addition, the centralized temperature monitoring system has the advantage of continuous recording and remote alarm function, and is easy to observe, as well as the potential to be paperless.

P-107
AN EXPERIENCE OF A DEVELOPING COUNTRY ON MAINTENANCE OF COLD CHAIN DURING PACKED RED CELL TRANSPORTATION

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Background: To ensure the quality of the transfused red cells it is mandatory to maintain them at proper storage temperature of 4 ± 2°C. However during transportation it's allowed a temperature range of 2°C to 10°C for up to 12 h. In most developed countries these standards are ensured through properly insulated containers and commercially prepared cooling elements. In most developing countries these standards may not be maintained due to limited resources. As a developing country Sri Lanka lacks these facilities to the optimum level. Therefore fiber cool containers and ice packs are being used for this purpose, since transportation of red cells within the country is important to ensure the swift provision of blood. This paper describes an experience on maintenance of cold chain during transportation of red cells in National Blood Transfusion Service, Sri Lanka.
Aim: Study was done to validate the cold chain maintenance exist in the system and to develop standard instructions for cold chain maintenance.
Methodology: In a 48 × 30 × 40 cm sized cool container 2, 3, 5, 6, 10 and 20 red cell packs were arranged at different combinations of initial temperatures and number of ice packs, each weighing approximately 550 g. The red cell packs were arranged avoiding direct contact with ice packs by keeping thick hardboard on top of the blood packs and place ice packs on top of it. The temperature of the blood packs were monitored hourly using a calibrated thermometer with a probe which kept inside the box. The number of hours which has taken to reach the maximum temperature of 10°C was recorded.

Results:

Table 1

Table-1				
No. of Red Cell Packs	Initial Temperature (°C)	No. of Ice Packs	Weight of Ice Packs (g)	No. of Hours Taken to Reach the Maximum Temperature
2	5.0	2	1100	12
2	4.5	1	550	12
5	4.0	2	1100	>12
6	4.2	2	1000	10
10	4.9	2	1100	5
10	4.5	4	2280	12
10	7.6	6	3360	4
20	6.9	3	1650	4
20	5.4	4	2250	12
20	5.0	5	3000	>12
20	4.7	5	3030	>12

Table 2

Table-2	
No. of Red Cell Packs	Weight of ice Packs (- 60 °C Frozen)
1–2	500 g
3–5	750g
6–10	1000g
11–15	1500g
16–20	2500g
21–25	3000g

Conclusion: There are mainly two factors affecting the duration of maintenance of required temperature range (2–10°C) during transportation.

1. The ratio between red cell packs and ice packs.
2. Initial temperatures of red cell packs.

Based on this study a table of formula has been formed as a general guide to decide the number of ice packs required during transportation.

In addition following recommendations were also made;

1. Pre transport temperature of red cell packs should be maintained between 3 and 50°C to ensure optimum cold chain maintenance during transportation.
2. If starting temperature is <20°C, there is a risk of freezing of blood packs due to the cooling effect of ice packs.
3. If starting temperature is >50°C, the period of cold chain maintenance (4–100°C range) would be <10 h.

P-108

THE IMPACT OF DONOR HEALTH STATUS ON THE QUALITY OF APHERESIS PLATELETS

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Background: The quality of apheresis platelets (APs) is affected by many conditions including method of collection, type of storage container and duration of collection. But we don't know whether the donor health status such as body mass index (BMI) and lipid profiles can affect the quality of APs.

Aims: Our aims were to determine whether the donor health status has effects on storage of APs and try to find methods to improve quality of blood products.

Methods: According to donor's BMI and lipid level, the plateletpheresis donors were divided into two groups; group I (BMI < 23, TG < 160, HDL > 40) and group II (BMI > 27, TG ≥ 160, HDL ≤ 40). After plateletpheresis, the blood samples from APs bags were collected on day 1 and 4, and then blood gas (pH, PCO_2, PO_2, HCO_3^- and BE), LDH and K^+ were analyzed.

Results: A total of 80 plateletpheresis donors were enrolled and divided into two groups (35 in group I and 45 in group II), the value of pH, PCO_2, PO_2, HCO_3^-, BE, LDH, and K^+ on day 1 and 4 (between group I and II) are shown in Table 1. Significant lower pH, BE, HCO_3^- and higher LDH, K^+ were noted in group II donors.

Table 1: The value of blood sample between grade I and II

	Day 1		Day 4		
	Group I	Group II	Group I	Group II	p-value
pH	7.106±0.028	7.088±0.043	7.235±0.059	7.210±0.065	0.000
PCO_2	62.74±7.14	59.64±5.40	28.86±1.77	27.84±2.25	0.000
PO_2	119.28±11.00	115.36±13.56	118.05±9.91	116.25±16.84	0.968
$HCO3^-$	19.27±1.93	17.59±1.40	12.00±1.41	10.97±1.61	0.000
BE	-11.16±1.73	-12.88±1.79	-13.89±2.40	-15.26±2.68	0.000
LDH	272.62±124.62	288.62±136.49	299.91±167.52	355.36±182.67	0.044
K^+	4.17±0.53	4.23±0.36	4.49±0.45	4.60±0.29	0.000

Conclusions: Obesity with abnormal lipid level did negatively impact on storage of APs. In order to improve quality of APs, blood center should pay more attention to blood donor health promotion. Further evaluation on other blood products should be arranged.

P-109

This abstract has been withdrawn.

3.2 Blood Components

P-110

OUTPATIENT TRANSFUSIONS – OUR EXPERIENCE

Mihic-Tomic B

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Introduction: Anemias are categorized as very frequent pathological conditions occurring both individually and as a consequence of many other diseases.

Aim: Presentation and analysis of red blood cells consumption at the Outpatient Treatment Clinic of the CHC 'Dr Dragiša Mišović-Dedinje' during 2010–2011, as a contribution to resolving professional, organizational and technical issues associated with the treatment of anemias in conditions found in the outpatient clinics.

Material and methods: Data regarding red blood cell consumption were collected using blood request forms forwarded to the Blood Transfusion Department, as well as blood/blood component issuing charts, patients' admission and release protocols and histories of the disease.

Results: During 2010–2011, total of 46 patients were transfused with red blood cells at the Outpatient Treatment Clinic of the CHC 'Dr Dragiša Mišović-Dedinje'. There were totally 97 half day hospitalizations, or 2.1 visits in the average. Among them, 30 (65.2%) were female and 16 (34.8%) were male patients, mean age around 70 years (the youngest patient was 37, and the oldest one was 92 years old). The lowest recorded haemoglobin value before transfusion was 3.7 g/dl, and the highest was 8.9 g/dl. At the admission to the Outpatient Treatment Clinic, all had obvious clinically expressed symptomes and parameters of (mostly) severe anemia confirmed by laboratory findings. In most patients, anemia developed as a consequence of a malignant disease and associated with radio and/or chemiotherapy treatment (I group, 37 or 80.4%), or as a consequence of haematological or hematooncological diseases (II group, 5 or 10.9%) and finally as a consequence of renal or cardiac diseases (III group, 4 or 8.7%). No unfavourable reactions were noted throughout the transfusion administrations. Since the patients from the first and the second group were polytransfused in most cases, 40 or 60 mg of I.V. urbason was administered prior to each transfusion for prevention purposes. Some patients from the third group were administered 1–2 ampoules of lasix in order to prevent circulation overload.

Conclusion: An ever increasing number of patients requires blood transfusion due to severe anemia (haemoglobin value below 4.0 g/dl). Capacity of the Outpatient Treatment Clinic of the CHC 'Dr Dragiša Mišović-Dedinje', where the outpatient transfusion administration takes place, do not even closely satisfy the actual needs. That is why it is necessary to increase the number of the outpatient treatment clinics/hospitals where blood transfusion would be administered, and to consider the need to reestablish the practice of blood transfusion administration at patients' homes.

P-111

PREPARATION OF SINGLE DONOR PLATELET WITH LOW ANTIBODY TITERS FOR ALL PATIENTS

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Background and aim: Platelet concentrates from ABO-identical donors are the components of choice for patients. However, since inventories are generally insufficient and because there is usually a relative abundance of group O donors, perfect matches are not always possible. It is therefore the accepted practice for platelets to be transfused out of the ABO group when ABO-identical platelets are unavailable. In the current study, group O single donor platelets (SDPs) were modified after collecting the platelet pellet in a bag. The AB plasma was added instead of the donor's own plasma and stored as universal SDP.
Materials and methods: A total of 107 modified SDPs were studied. The direct agglutination titers of anti-A/anti-B in the original group O SDPs' plasma (pre-preparation) and the residual of anti-A/anti-B in the modified group O SDPs' were performed.
Results: The pre-preparation titers ranged from 1:4 to 1:1024. The prevalence of high titers (i.e. at least 1:64 in our study) was relatively high, approximately 63% for anti-A and 78% for anti-B. The titer of residual anti-A/anti-B ranged from negative to 1:8. In most of the modified SDPs anti-A/anti-B could not be detected in the plasma (58.9% and 52.3%, respectively).
Conclusions: The results indicate that our modified SDPs have very low titers; that is, acting as a universal SDP which is safe for all ABO patients. This modified SDP form is a more convenient way to overcome the risk from incompatible plasma or loss of platelets during the process of volume reduction.

P-112

QUALITY OF PLATELET CONCENTRATES

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Background: Platelet concentrates (PCs) from whole blood (WB) can be prepared as random donor platelets (RDP) or leukocyte poor platelets concentrate (LPPC). Quadruple bag preparation for LPPC can be done as (a) top and bottom or (b) top and top systems. The difference between the two systems would be in the quality of the LPPC. The current study compared LPPCs and RDP for the quality of PCs (i.e. platelet yield, residual white blood cells) and cost per therapeutic dose.
Methods: We studied a respective 606 and 77 LPPC from (a) top and bottom and (b) top and top and 65 units of RDP. For the LPPC preparation, four buffy coats (BC) 'negative for infectious markers and unexpected antibody' were pooled using a sterile connecting device. The pooled BC were mixed at 150 rpm for 15 min and spun at 2300 rpm for 4 min. After separating the LPPC into a platelet storage bag, a platelet sample was collected from the tubing in order to determine the platelet yield and residual WBC. The unit cost per therapeutic dose was then calculated.
Results: The respective yield of platelets in LPPC (a), (b) and RDP were 6.6, 5.7 and 1.4 units. The respective residual WBC in the three groups were 0.8, 0.8 and 0.3×10^8 cells/unit. The platelet yield in the LPPC was significantly higher in (a) than (b) ($P < 0.05$), but had no significant residual WBC ($P > 0.05$). The respective unit cost of the three groups was 71, 76 and 102 USD.
Conclusion: The four BC estimates of the platelets in LPPC was similar to one therapeutic dose. The LPPC from the top and bottom system had a platelet yield higher than the top and top system. LPPC have a lower residual WBC than RDP as well as a lower preparation cost than one therapeutic dose. It is, therefore, cost effective to prepare LPPC instead of RDP and safer from the perspective of platelet refractoriness and/or alloimmunization.

P-113

USE OF PLATELET GEL WITH BONE GRAFTING FOR RECONSTRUCTION OF UPPER JAW DEFECTS

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Background: The use of platelet gel (PG) in tissue regeneration is a developing area and has been employed in various fields of surgery. Although the mechanisms involved are still poorly understood, easy application of PG in clinical practice and possible beneficial outcome, including bone regeneration, reduction of bleeding and rapid tissue healing, hold promise. The platelets must be activated to release the con-tent of granules, while the clot providing a vehicle to contain the secreted proteins and maintain their presence at the site of application. Combining PG with bone or bone substitutes present significant faster radiographic maturation and denser bone regeneration.
Aim: To evaluate the effect of PG mixed with autogenously iliac crest graft on bone regeneration in different upper jaw defects.
Materials and methods: Twenty patients with upper jaw defects underwent iliac bone grafting harvested from iliac cancellous bone. The bone graft incorporated with PG (PG group) in 10 of them while others serve as control group. They include two patients with tumor resection, 16 with secondary alveolar cleft repair, and two with segmental post-traumatic bone loss. We used standard triple packs containing citrate phosphate dextrose for blood collection at least 24 h before PG application. Platelet poor plasma is mixed with calcium gluconate (1/0.2 rate) at 37°C for 15–30 min; the supernatant is full of thrombin precursors. In sterile Falcon tube, platelet concentrate, thrombin and calcium gluconate were mixed in the following proportions: 3:1:0.5 respectively. The suspension is exposed to slow shaking with the caution to complete 10–12 times a 360 0 tube revolution, and it is left to rest for about 15 min before mixing with bone graft. Quantitative evaluation of re-generated bone was made with dental CT scans preoperatively and postoperatively at 3, 6, and 9 months interval. Parameters that were assessed included: bone defect volume (preoperatively), graft volume (by multiplying bucco-lingual, mesiodistal, and vertical dimensions), overall bone density, gap thickness (between the grafted area and the adjacent normal bone) and post operative complications such as infection, collection, oro-nasal fistula.
Results: A mixed design ANOVA was calculated to evaluate the effects of bone graft type (PG graft vs control grafts) and time of measurement (0-, 3- and 9-month) on the volume of regenerated bone. The average of the volume ratio of regenerated bone of reconstructed upper jaw defects in cases with PG was statistically higher than in controls at 9-month postoperative assessment ($P = 0.043$). However, there was no statistically significant difference between them re-garding the bone density ($P = 0.89$).
Conclusion: PG is a safe and cost-effective source for growth factors and easy to extract. It could enhance the osteogenesis of alveolar bone grafting in patients with different upper jaw defects and may be useful for sub-sequent orthodontic therapy.

P-114

SURFACE MODIFICATION OF POLY (BUTYLENE TEREPHTHALATE) NONWOVEN FABRICS USED FOR BLOOD FILTRATION AND THEIR BLOOD COMPATIBILITY STUDY

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Background: It has long been reported that during blood component transfusion therapy, leukocytes can cause many adverse reactions, including nonhemolytic febrile transfusion reaction, platelet refractoriness, immunosuppression. Leucocytes are also known to accelerate the rate of storage lesion. Therefore, removal of leukocytes to low sufficient levels is necessary to prevent undesired reactions particularly in freshly whole blood.
Aims: The purpose of this study was to increase leukocyte retention and erythrocyte recovery rates through surface modification, using polyvinylpyrrolidone (PVP) which was grafted by a plasma grafting method. In addition, the blood compatibility of the modified poly (butylene terephthalate) nonwoven fabrics (PBTNF) were also evaluated.
Methods: The PVP was introduced to the PBTNF surface by the oxygen plasma treatment method. The immobilized PBTNF was characterized by X-Ray photoelectron Spectroscopy (XPS) and critical wetting surface tension. Tive types of PBTNF-PVP with different wettability were prepared to investigate the leukocyte filter efficiency and blood compatibility. Then blood filtration experiments have been performed on the laboratory scale Different parameters have been studied: the effect of the wettability of PBTNF on the retention of the blood cells, the morphology of blood cells after filtration, the free hemoglobin in whole blood before and after filtration, the complement activation and the hemolysis assay.
Results: The XPS spectra confirmed the creation of new functional groups on the PBTNF surface. The hydrophilicity of the PBTNF surface was improved greatly by covalent immobilization of PVP. And the wettability of PBTNF-PVP was also increased as the oxygen plasma treatment power increased. After filtration, the leukocytes were reduced significantly, while the erythrocytes were reduced slightly. All five types of PBTNF-PVP's FHb were <80 mg/l, which was consistent with the Chinese Medical Vocation Standard <YY0329-2009 > Leukocyte reduction filters for single use. The hemolysis ratio of origin PBTNF, PBTNF-P15, PBTNF-P25, PBTNF-P50, PBTNF-P100 and PBTNF-P200 were about $1.00\% \pm 0.50\%$, $0.30\% \pm 0.32\%$, $0.25\% \pm 0.22\%$, $0.31\% \pm 0.10\%$ and $0.45\% \pm 0.50\%$. This implies that the five types PBTNF-PVP all are compatible with erythrocyte. All the C3a and C5a results imply that the original PBTNF and five types of PBTNF-PVP may have no influence on complement activation.
Conclusions: The grafting of PVP on the PBTNF could improve its leukocyte-removing efficiency and blood compatibility, suggesting that PVP-modified PBTNF is a very promising blood filter for selective removal of leukocytes.

P-115
EVALUATION OF PLATELET FUNCTION DURING STORAGE

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Background: Today, platelet components are general produced from whole blood by centrifugation or by plateletpheresis. Platelets are enucleated cells derived from bone marrow megakaryocytes. They play vary important role in hemostasis, blood clotting, and thrombosis. The life span for platelets in the human circulation is estimated to be about 10 days. However, after 5–6 days of in vitro storage, platelets lose their biological function and display morphologic change from discoid to sphere shape.
Aim: The objective of this study was to determine the platelet activation, apoptosis and metabolism for apheresis derived platelet concentrates (APCs) and platelet concentrates (PCs) stored for 7 days in a platelet storage bag.
Methods: In this study, APCs and PCs collected from ALT abnormal (ALT > 45 IU/l) disqualify components. These platelet components under controlled concentration and volume per bag were stored in plasma up to 7 days at 22 ± 2°C with horizontal agitation. Swirling score was judged by squeezing the bag while holding it in front of a light source on Days 1, 5 and 7 for APCs, Days 5 and 7 for PCs. Samples were drawn aseptically from components with sampling site couplers inserted in a spike entry port of the component bags. Samples for platelet counts, pH, activation and apoptosis were drawn aseptically during storage on Days 1, 5 and 7 for APCs, Days 5 and 7 for PCs. Platelet activation (CD62P) and apoptosis (annexin V) were determined with a triple labeling method on BD FACSCalibur flow cytometry. Bacteria contaminations were performed with BacT/ALERT blood culture system.
Results: We collected 51 PCs bags, forty-one by APCs and 10 by PCs in this study. Twenty-three of the 41 APCs prepared from Haemonetics MCS+ED instrument, four of the 23 were leukocyte reduced APCs components and 19 of the 23 were non-leukocyte reduced APCs components. Ten of the 41 APCs prepared from Fenwal CS-3000 plus Blood Cell separator, these were non-leukocyte reduced APCs components. Eight of the 41 APCs prepared from Trima Accel Collection system, these were leukocyte reduced APCs components. The results indicated, the mean APCs volume levels ranging from 245 to 287 ml and PCs volume levels about 65 ml. All mean platelet counts were more than 1.0 × 106/ml and all mean APCs yield were more than 3.0 × 1011/bag. On day 7, no significant difference were observed in swirling score and CD62P (–)/annexin V (+) expressed but pH, CD62P (+)/annexin V (–) and CD62P (+)/annexin V (+) expressed increased in APCs and PCs during storage. No bacteria contamination was detected by BacT/ALERT blood culture system.
Conclusions: Our results indicated, CD62P (+)/annexin V (–) and CD62P (+)/annexin V (+) expressed in platelet concentrates on days 5 and 7 rises to levels that could compromise the quality of the platelet units. Development of platelet storage lesions influenced especially by storage conditions and platelet concentration in products.

P-116
EVALUATION OF THE BPFA PLUS POST-PROCESS LEUCOCYTE DEPLETION FILTER FOR PACKED RED CELLS

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Background: Leucocyte reduction has been demonstrated to reduce the risk of febrile non-haemolytic transfusion reactions, cytomegalovirus transmission and human leucocyte antigen alloimmunisation in individuals considered to be at high risk for such complications. Leucoreduction can either be performed following storage of red cell units at 4°C or room temperature depending on individual Blood Bank processing flow. As such, leucoreduction filters should be able to perform efficiently in both conditions. We chose to evaluate BPFA Plus post filtration filter to identify if they can meet our needs for leucofiltration at 4°C and room temperature.
Aim: To evaluate the BPFA Plus post process filter in terms of filter performance when used to filter red cell units stored either at 4°C or room temperature.
Method: Whole blood units were collected into (JMS, 450 ml) collection sets, from healthy volunteer donors (n = 60). Units were held for 2 h at room temperature prior to Top/Top processing to generate PRP platelets. The red cell units were held for either <8 h at room temperature (N = 30) or, 1–2 days at 4–6°C (N = 30) prior to sterile connection and filtration with the BPFA Plus (Pall Medical). Filtration was performed at room temperature at a height of approximately 1.2 m using gravity prime. Pre- and post-filtration sampling was performed to assess filter performance and resultant component quality. Filtration times were also measured.
Results: Table 1 displays data for post-filtration red cell units.
Summary/conclusion: All parameters measured met the Council of Europe Guidelines for leucocyte depleted red cell units except for one outlier which exhibited an extended filtration time at room temperature (78.8 min) and 1.45 × 10^6 Leucocytes/Unit. The cause of the high residual leucocytes in the outlier is indeterminate. The remaining results were consistent in both storage groups giving a high level of confidence in the filtration performance of the filter as shown by the median and standard deviation. The filtration times allowed processing within routine processing conditions.

Table 1

Test Parameter	<8 hours Room Temperature		1-2 days 4-6°C	
	Median	Std Dev	Median	Std Dev
Filtration Time (Minutes)	15.3	12.4	17.2	8.3
Leucocyte Count (x10⁶/Unit)	0.049	0.264	0.017	0.022
Total Haemoglobin (g/Unit)	54.2	5.9	53.8	6.2

P-117
OPTIMAL PLASMA VOLUME FOR BLOOD COMPONENT USED FOR EXCHANGE TRANSFUSION

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Background: In Japan, blood component for exchange transfusion (BET) is mainly used for hemolytic disease of the newborn due to ABO incompatibility. BET is a mixture of O-typed red cells and AB-typed plasma. Currently, BET is prepared from washed red cells derived from 400 ml whole blood. The red cells are then added with 240 ml plasma, which yields a hematocrit of 35%. The low hematocrit of BET often causes problems such as anemia in transfused patients. Moreover, its storage period is limited to 24 h after processing.
Aims: Optimal plasma volume for BET and the possibility of prolonging its storage period were investigated by examining the in vitro quality and stability of BET with varying plasma volume.
Methods: Irradiated BETs (Ir-BETs) were prepared as follows: red cells derived from a 400 ml whole blood were γ-irradiated, washed, and added with 90, 120, or 160 ml of plasma. Ir-BETs were stored at 2–6°C for 72 h. Samples were taken every 24 h and subjected to in vitro testing. The volume of Ir-BETs, hematocrit, protein concentration, supernatant hemoglobin, ATP, 2,3-DPG, supernatant potassium, total supernatant potassium and the coagulation factor activity were measured.
Results: After adding 90, 120, and 160 ml of plasma, the volumes of Ir-BETs were 284.5 ± 16.9 ml, 299.8 ± 7.0 ml, and 336.7 ± 8.6 ml and the hematocrit values were 56.3 ± 1.4%, 49.7 ± 0.9%, and 44.0 ± 1.0%, respectively. The supernatant hemoglobin concentration did not increase markedly and the percent hemolysis after 72 h of storage was below 0.2% in all the treatment groups. Regardless of the plasma volume, the ATP concentration remained the same and did not change during the 72 h of storage. Regarding the 2,3-DPG concentration, at least 50% of the initial value was maintained in all the treatment groups during the 72 h of storage and no effect of plasma volume was found. The supernatant potassium concentration increased during the 72 h of storage in all the treatment groups and was highest in Ir-BET with 90 ml of plasma among the three treatment groups. On the other hand, the total supernatant potassium in Ir-BET with 120 ml of plasma after 48 h of storage was comparable to that in Ir-BET after 24 h of storage, which is the storage period of the current component. The coagulation factor activity modestly decreased during the 72 h of storage and no effect of plasma volume was found.
Conclusions: It was suggested that a plasma volume of 120 ml was suitable for (Ir-) BET derived from a 400 ml whole blood and it is possible to extend its storage period to at least 48 h.

P-118
AN ANALYSIS OF THE FACTOR VIII CONTENT IN FRESH FROZEN PLASMA

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Background: The research in content of factor VIII of ABO group started in 1964. This study aimed to estimate the factor VIII of fresh frozen plasma from Taiwan.
Aims: This study aimed to analyze changes in content of factor VIII levels of ABO blood group before donation, processing double blood bag of whole blood (250 and 500 ml respectively), and storage after 30 days.
Methods: We used KC-4Delta blood coagulation analyzer (Sigma/Amelung label) to test the factor VIII levels in different donation volume and centrifugation conditions for 125 blood donors.
Results: We used t-test to analyze the different centrifugation that showed no significant difference ($P > 0.05$). We found that factor VIII level was significantly lower in group O than that in group A, B or AB individuals ($P < 0.05$), and the average of factor VIII level was also significantly reduced during the period from blood donation, processing procedure to refrigeration ($P < 0.05$). These three average value showed significantly different ($P < 0.05$), and had decreasing trend.
Conclusions: Although the different donation volume and centrifugation conditions will not directly affect the content of coagulation factor VIII, the content of coagulation factor VIII will be reduced after processing and storage, but the content still in the normal range.

P-119
IMPROVEMENT OF THE RED BLOOD CELL RECOVERY OF 1 UNIT RBCS FOR LEUKOCYTES-REDUCED

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Background: RBCs Leukocytes-reduced (RLR) provided by blood centers were prepared from two units of packed RBC. According to the quality control of AABB, the Red blood cell (RBC) recovery exceeding 85% will be qualified. (The 16th AABB Technical Manual definition). For the request of the hospitals, RLR was prepared from post-storage one unit (250 ml) of packed RBC. After this procedure, the mean value of RBC recovery went down to 78% that did not meet the minimal qualified standard in 85%.
Aims: The study aims to improve the RBC recovery and confirm the standard operational procedure of pre-storage 1 unit RLR.
Methods: Ten 250 ml double-bags of Whole blood (WB) were collected from random donors who were processed by hard-spin centrifugation into a RBC concentrate and a Fresh frozen plasma (FFP) according to SOP. Then the White blood cell (WBC) filter and packed RBC were connected by a sterile connection device (TSCD-II; Terumo, Tokyo, Japan), and the FFP container was still connected with the packed RBC bag; later they were filtrated at the ambient temperature (20–24°C). After the first filtration, add plasma about 30 g in the packed RBC bag, mixing, to increase the RBC recovery by increasing the RBC in the bag, the filter and the blood tubing and flushing them. Then filter them together again. These procedures must be completed within 8 h after WB collection process. Hematocrit (Hct) value was measured using an automated cell analyzer (Sysmex KX-21N; Toa, Tokyo, Japan). Volume of blood components were determined by dividing the net weight with standard specific gravity values, then calculate the RBC recovery, while residual WBC count was preformed by using the Nageotte hemocytometer.
Results: The mean (± SD) of RBC recovery value was 91 ± 3%, the Hct mean value was 66.6 ± 9.7%, the mean number of residual WBCs per unit was 0.08 ± 0.03 × 106, and the FFP weight mean value was 122 ± 2 g. All results meet the AABB standard. Conclusions: The RBC recovery increased from 78% to 91% after adding the plasma in the RBC concentrate bag. Though the Hct value was reduced by 3.3–66.6%, it's still maintained between 55% and 80%. The RLR was prepared from pre-storage one unit of packed RBC; it could efficiently remove leukocyte from the packed RBC to decrease the cytokine level and the bacterial contamination risk. The product will provide hospitals real requirement, and ensure the quality of blood components.

P-120
THE FEASIBILITY OF PREPARING FRESH FROZEN PLASMA AND PLATELET CONCENTRATE FROM WHOLE-BLOOD DONATIONS USING A 24-H HOLD PROCEDURE

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Background: Since the blood collection sites are dispersive, the time of transporting blood from collection sites to blood center usually affected by traffic situations. The delay compressed the time for preparing fresh frozen plasma and platelets because of an 8-h limitation from blood collection. We needed more manpower to complete the work in confining period. This study compared the quality of blood components prepared from WB stored for 8 and 24 h in 20–24°C before processing with the PRP method.
Methods: After collection, blood was stored and delivered in ambient temperature of 20–24°C. For the control group, blood components were prepared in 8 h from blood collection. The experimental group composed components which are prepared in 24 h after collection. There are 30 bags selected for each group. The quality of plasma and platelet concentrate were compared in the activity of factor VIII before plasma frozen and after 30 days storage in freezer, the residual platelet in plasma, the platelet count of platelet concentrate, the pH of platelet concentrate of near expiration, and phenomena of swirling of platelet.
Results: The codes of FP8 and PL8 were refered to control group, on the other hand, FP24 and PL24 represented the experimental group. The average activities of factor VIII of 30-day stored FP8 and FP24 were 81.43% and 71.58% respectively. The activity remained after 30 days of storage comparing to itself before freezing, FP8 had 84.3% of activity remained and FP24 had 86.1% of activity remained. The difference of plasma factor VIII activity of two group was examined by t test. There were no statitically significant difference in both situations which are before freezing and after 30-day storage. The residual platelet of all plasma were under 50×109/l.
The platelet count of all the PL8 and PL24 were over 3×1011/unit and the pH are over 7.0 in all platelets of near expiration. The phenomena of swirling is obvious in every platelet concentrate visually.
Conclusions: Although the average activity of Factor VIII of FP24 is lower than FP8 by 12.1%, 71.58% in average of FP24 still met the requirements of fresh frozen plasma set up by Taiwan Blood Services Foundation and the standards of Council of European that 70% of activity remained after freezing and stored for 30 days. Not only PL8 but also PL24 were superior to the standards that platelet count should be higher than 2.75×1010/unit and pH should be higher than 6.2. All data showed that prolonging the time of whole blood storage from 8 to 24 h didn't affect the quality of plasma and platelet concentrate significantly. However, increasing WB storage time to 24 h is logistically attractive.

P-121
REDUCE PLATELET REJECTION RATE DUE TO PLATELET CLUMPING BY USING LEAN SIX SIGMA

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Background: The Component Processing Laboratory prepares platelet concentrate using the Platelet-Rich Plasma method. These platelet concentrate units are visually inspected by the laboratory staff before being issued to hospitals for patient use. On average, about 7% of the total platelet concentrates units prepared monthly is rejected due to the presence of platelet clumps. Our objective is to study the possible root causes of platelet clumping, the relationship of causes and subsequently, to improve the rejection rate.
Methods: By using Lean Six Sigma Define, Measure, Analysis, Implement, and Control (DMAIC) as a studying tool, seven areas of possible cause were identified and focused for the study. The team studies the relationship of the high platelet clump rate with (i) the collection sites, (ii) the type of blood bag, (iii) the centrifuges that the unit was processed in, (iv) the collection by day of week, (v) the time taken for blood collection, (vi) platelet resting time, (vii) the inspector's standard of rejection. Data was collected for the month of May to July 2010 and from 3 August to 14 August 2010. Statistical analysis was performed using SIGMAXL software. Gage R&R study was conducted to assess the inspector's skill on Repeatability and Reproducibility of platelet rejection.
Results: The statistical analysis using hypothesis testing showed no relationship between the high platelet clump rates with all the areas studied ($P > 0.05$). In the Gage R&R Study, inconsistency was observed on most of the inspectors assessed. Rejection rates of platelet units due to platelet clumps varied within and among the inspectors. Some inspectors are also observed to be rejecting units of platelets that are deemed to be satisfactory.
Conclusions: The platelet preparation process is demonstrated to be intact. The major driver for the high rejection rate of platelet units due to platelet clumps seems to be human related. Individual inspectors have varied rejection criteria. Standardization of a rejection criteria have helped to reduce the platelet rejection rate due to platelet clumps from 7% to an average 4%.

P-122

AN AUDIT OF FRESH FROZEN PLASMA USAGE IN KING KHALID UNIVERSITY HOSPITAL, RIYADH

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Background: Fresh frozen plasma (FFP) is commonly used in the treatment of patents with coagulopathies who are bleeding or at risk of bleeding, and where a specific therapy or factor concentrate is not appropriate or unavailable. However, up to date there are few firm indications and guidelines to its use which is frequently practiced without seeking laboratory evidence of the degree of the coagulopathy before transfusion and whether improvement in these tests has occurred post-transfusion. As results there is growing concern that FFP is used inappropriately and without scientific rationale.
Aim of this study: This is look-back review of the practice of FFP transfusion by clinical departments in a teaching hospital attempting to throw light on appropriateness of FFP transfusions.
Materials and methods: Blood Bank records of 494 consecutive patients from the following clinical departments at King Khalid University Hospital, Riyadh: Surgery (n = 102), Pediatrics (n = 87), Medicine (n = 306). The results of the coagulation screening test was recorded before and after FFP transfusion.
Results: Total 1620 units were issued for 526 patients and the results obtained were as (data expressed as prevalences):
1. Pre- and post-FFP transfusion prothrombin time (PT) and activated partial thromboplastin time (APTT): 56% in the Department of Surgery, 28.5% the Department of Medicine and 32% in the Dept of Pediatrics.
2. No pre- or post-transfusion PT and APTT testing: Department of Surgery 6.6%; Department of Medicine:8.7% and in the Department of Pediatrics 26%.
3. Only post-FFP transfusion PT and APPT was noted as follows: 33.3% in the Department of Surgery; 51% in the Dept of Medicine and 34% in the Dept of Pediatrics.
4. Plasma fibrinogen: Pre and post-FFP transfusion fibrinogen was noted in 39% in the Department of Surgery 6.6%; Department of Medicine: 8.7% and in the Department of Pediatrics 26%.
Conclusion: There is wide disparity in the resort to coagulation testing before and after the transfusion of FFP. Other than education of clinicians the employment of a transfusion nurse to monitor use of FFP (and other blood products) would definitely pave a way to proper evidence-based transfusion practice and could avoid the inappropriate use and wastage of FFP.

P-123

TRANSFUSION PRACTICE OF CRYOPRECIPITATE-REDUCED PLASMA IN ONE INSTITUTION IN TAIWAN

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Background: Under economic pressure, cryoprecipitate (cryo)-reduced plasma was not only used for correcting deficiency of coagulation factors (except fibrinogen, factor VIII and von Willebrand factor), but also as a source of proteins responsible for osmotic pressure of plasma and maintenance of blood volume. The transfusion practice of cryo-reduced plasma was rarely reported.
Methods: Transfusion practice of cryo-reduced plasma in January 2011 was reviewed. Information about age, sex, clinical conditions, international normalized ratio (INR) of prothrombin time (PT), activated partial thromboplastin time (APTT), and serum albumin level nearest before transfusion were collected. The reason codes of transfusing cryo-reduced plasma were: (i) for managing coagulopathy, (ii) for managing severe hypoalbuminemia (albumin ≤ 2.5 g/dl) in patients with ascites, pulmonary edema, pleural effusion or shock, (iii) for managing mild hypoalbuminemia (albumin 2.6–3.9 g/dl) in patients with ascites, pulmonary edema, pleural effusion or shock, (iv) for preventing shock in patients with burn injury or undergoing paracentesis or thoracocentesis.
Results: In January 2011, a total of 116 times of transfusions with cryo-reduced plasma were analyzed. The dose of cryo-reduced plasma was 1 unit in 98 times of transfusions, and 2 units in 18 times of transfusions. These transfusions were administered to 72 patients (20 female, 52 male, age 20–94 years, median age: 75 years). Plasma transfusions were given to patients in intensive care units (27/72 = 37.5%) or medical ward (31/72 = 43.1%) or surgical ward (14/72 = 19.4%). One hepatoma patient with massive ascites undergoing paracentesis received 10 times of daily plasma transfusions. Numbers of patients in each reason code with various times of transfusions were shown in Table 1. Transfusion reaction (allergic reaction with urticaria) was found in 1 (1/116 = 0.9%) transfusion. A total of 28 patients (code 1: 0 patient, code 2: 11 patients, code 3: 13 patients, code 4: 4 patients) died of their underlying diseases during hospitalization.

Table 1. Numbers of patients in each reason code with various times of cryo-reduced plasma transfusions

Codes of reasons*	Times of transfusions				
	1	2	3	4	10
(1)	1	2	0	0	0
(2)	20	9	2	0	0
(3)	21	5	1	1	0
(4)	4	2	1	2	1

* Codes of reasons: (1) coagulopathy; (2) albumin ≤ 2.5 g/dL with ascites, pulmonary edema, pleural effusion or shock; (3) albumin 2.6~3.9 g/dL with ascites, pulmonary edema, pleural effusion or shock; (4) preventing shock.

Conclusion: In one hospital practice, the main reason for giving cryo-reduced plasma was to manage hypoalbuminemia in critical patients with ascites, pulmonary edema, pleural effusion or shock.

3.3 Plasma Products

P-124

CHARACTERIZATION OF PLASMA PROTEIN ACTIVITY IN CRYOSUPERNATANT

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Background: The administration of cryosupematant plasma (CSP) has increased dramatically in recent years without sufficient evidence to support such change.
Aims: The study evaluated the differences between fresh frozen plasma (FFP) and CSP as regards their content of the protein in vitro, and to show the possibility using CSP instead of FFP as a replacement therapy in some clinical situations.
Methods: The study included 60 blood units obtained from healthy donors attending from six blood centers in China. FFP and CSP were prepared from the same blood units and aliquots from them were used to perform the following assays: total protein, fibrinogen assay, coagulation factors II, V, VII, VIII, IX, X, XI and XII activity assays, non activated partial thromboplastin time (NAPTT), antithrombin III (AT-III) activity assays, and the von Willebrand Factor cleaving protease (ADAMTS13) assay. The content of each bag was calculated and the results were tested for statistical significance.
Results: The average percent protein retention for CSP were: Total Protein, 57.7 g/l, Fibrinogen, 166.9 mg/dl, Factor II, 101.8%, Factor V, 82.6%, Factor VII, 83.6%, Factor VIII, 18.6%, Factor IX, 67.7%, Factor X, 94.7%, Factor XI, 61.6%, Factor VII, 100.2%, NAPTT, 270 s, AT-III, 83.3%, and ADAMTS13, 97.8%. The mean fibrinogen and factor VII levels in CSP were significantly less than in FFP. On the other hand, CSP appeared to be a useful source of coagulation factors II, VII, X, XII, AT-III and ADAMTS13 as it contained adequate amounts of these proteins.
Conclusions: CSP appears to be a satisfactory alternative to FFP in those situations in which the presence of sufficient amounts of factor VIII, von Willebrand factor (vWF), fibrinogen, factor IX and Factor XI in the substitution product are not required. Further validation of these results will encourage the increase of using cryopematant.
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P-125

PRELIMINARY ASSESSMENT OF COAGULATION FACTOR? IN THE CHINESE MARKET

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Background: In the Chinese market, there are three human plasma-derived coagulation factor VIII (F VIII) and one recombinant factor VIII, for the treatment of hemophilia patients. Comparative assessment of these products has not been reported.

Objective: To study these F VIII products in China, a preliminary analysis is made on the active ingredients as well as impurity profile. The research on these factor VIII products' efficacy and safety serves to establish new standards for product quality and provide some theoretical basis for applications.
Methods: A self-made Human F VIII, Green Cross' F VIII, CSL's Aleviate, Shanghai RAAS' F VIII and Bayer's Kogenate® FS are evaluated through the determination of F II, F V, F VII, F IX, F XI and F VIII clotting capability, as well as preliminary evaluation of vWF:Ag content. Non-reducing and reducing SDS-PAGE electrophoresis is employed to assess purity, while protein content is determined by three different assays.
Results: The results in the clotting activity, protein concentration and purity indicate that there are qualitative differences among these factor VIII products.
Conclusion: Preparation by different processes may lead to different product quality Qualitative evaluation of coagulation factor products may be influenced by different methods, equipment and test kits employed. The present study show one of the key quality parameters of F VIII– specific activity in these products to be greater than the WHO requirements for a high-purity F VIII (10 IU/mg) as well as the Chinese Pharmacopoeia 2010 (1 IU/mg) requirements.

P-126
ISOLATION AND PURIFICATION OF HUMAN A1-ANTITRYPSIN BY A NEW TYPE OF IMMUNOAFFINITY CHROMATOGRAPHY

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Background: α1- antitrypsin is a primary for the α1-AT deficiency replacement therapy biological products, now more than two-steps chromatography purification from human plasma. One step affinity chromatography with the preparation of α1-AT have not been reported at present.
Objective: To develop a much more inexpensive process for isolating and purifying α1-antitrypsin from Cohn Fraction IV by a new type of immunoaffinity chromatography and to achieve an improved utilization of the valuable source material fresh frozen plasma.
Methods: Cohn Fraction IV was dissolved with Tris solution in a ratio of 1:12 for 0.5 h at 24°C, and the suspension was centrifuged for 15 min (24°C, 4750 *g*). After added HL-380 (a fumed silica) and centrifuged, the supernatant was loaded through 1.2 μm filters sequentially. With a new type of immunoaffinity chromatography medium- 'Alpha-1 Antitrypsin Select', α1-antitrypsin was purified further. Characterization of α1-antitrypsin was performed by SDS-PAGE and Western Blot. Total protein content was determined by the method of Bradford under UV absorption at 595 nm. The activity of α1-antitrypsin was determined using chromogenic substrate assay.
Results: The purified α1-antitrypsin concentrate showed two protein bands on gels by SDS-PAGE, One of the molecular weight was calculated 54 kD; the others was calculated 140 kD approximately, After reducing SDS-PAGE, large molecular weight polymer protein band of α1-antitrypsin was depolymerized, the result of Western Blot analysis could be related to changes in reaction. The immunoaffinity chromatography step achieved a 90% yield. Thus, an overall 95% in purity and a 41.88% yield was obtained approximately from Cohn Fraction IV (or a 13.96% yield was obtained approximately from human plasma).
Conclusions: The high purity and specific activity of α1-antitrypsin achieved with this process which is could be an efficient and scale-up method for α1-antitrypsin purification from Cohn Fraction IV.

P-127
This abstract has been withdrawn.

P-128
STANDARDIZATION IN DETERMINATION OF CHYLOMICRONEMIA PLASMA OF DONOR BLOOD

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Background: There are six Blood Centers in Taiwan, which are Taipei, Hsinchu, Taichung, Tainan, Kaohsiung and Hualien These centers are in charge of blood collection, blood components preparation and the provision of components to hospitals. The prevalence of donors with chylomicronemia was analyzed in the period of 2003–2007. We found that the prevalence can be in 10 times difference among blood centers. It was interested to figure out the causes but the first step was to eliminate the difference in judgment. The objective of this study was to develop a consistent operational principle and method for standardizing the determination of chylomicronemia among blood centers.
Methods: The experienced personnel from six blood centers were invited to join the consensus meeting. The method for determining the chylomicronemia has to be developed under the premise that no destruction of the blood bag. The agreement was achieved that is to develop a light box with a background which could provide a clear cut of plasma with or without chylomicronemia.
The second meeting was held after the prototype of the light box was made. Plasma of different titer of chylomicronemia vs varieties of backgrounds were tried to determine the best model.
A training course was arranged after the light boxes according to the model chosen were made and the standard operation procedure of chylomicronemia determination was written. The same personnel joined together to get training and the evaluations of competence were performed. Those who were qualified are designated to be the final judgment makers of chylomicronemia of plasma of their blood center. The SOP and the equipment were validated in August 2010.
Results: The variance of the incidence rate of chylomicronemia plasma among blood centers was reduced. The variance was decreased from 47.8% to 29.8% after 4 months of carrying out the standard. It showed that this standard worked well in practice.
Conclusions: We standardized the thickness of plasma in reading area, the luminosity of light box and the cut off value of chylomicronemia. The effect of minimizing the difference of judgment in chylomicronemia among blood centers revealed in the first few months after the standard was put into practice. The practice of the standard has to be checked occasionally to make sure it doesn't lead to a deviation and still meets hospital's need. Besides, the incidence of chylomicronemia should be observed for a longer period of time to figure out the trends.

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This abstract has been withdrawn.

P-130
PRODUCTIVE PLASMAPHERESIS BY NIGALE NGL XJC 2000: QUALITY CONTROLS ON PLASMA COLLECTED

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Background: The Nigale NGL XJC 2000, distributed by Hemotrans (Pomezia, Italy), is a new cellular separator exclusively dedicated to plasma production by apheresis. This instrument, characterized by a discontinuous blood flow with a single venous access, adopts the technology developed by Latham, namely the separation of blood components according to their density gradient by applying an appropriate centrifugal force. In the Nigale's kit, a blow moldeld bowl is included. Thanks to the structure of this kind of bowl the coming in blood does not go through stratified hemocomponents, moreover the greater rotation speed respect to Latham bowl (7.000 vs 4.800 rpm) permits to obtain a plasma with a lower cellular contamination.
Aims: In our Transfusion Centre, we evaluated, in terms of effectiveness and efficiency, the qualitative and quantitative performances of this equipment.
Methods: Between November 2010 and May 2011, two hundred plasmapheresis were performed with this new instrument, of course after informed consent, on 150 periodic blood donors with at least one previous experience in apheresis donation. In order to evaluate the quality of produced plasma, on all samples, collected from all units before freezing, factor VIII and fibrinogen were assayed, moreover sterility controls were performed and, at the end, the cellular contamination with erythrocytes, leukocytes and thrombocytes was evaluated.
Results: Mean factor VIII ± SD on collected plasma units was 90.31 ± 10.23% (normal range 73.1–137.0). Mean fibrinogen ± SD was 334 ± 43 mg/dl (normal range 246–527). All sterility controls resulted negative. Cellular contamination was not significant (mean erythrocytes = 0.01 × 106/μl; mean leukocytes = 0.1 × 103/μl; mean thrombocytes = 11 × 103/μl, with a minimum count of two and a maximum of 31).
Summary/conclusions: The last report of Superior Institute of Health show that Italy is still far away to obtain the self-sufficiency for plasma production and consequently for plasma-derivates. For this reason one of major objectives of Italian Transfusion Centres must be to implement, in addition to classic whole blood donation, the collection of plasma by apheresis. In this context we have evaluated qualitative performance of this new instrument and our data (on factor VIII and fibrinogen dosage, microbial and cellular contamination) show that plasma collected by Nigale NGL XJC 2000 respect both Italian and European quality parameters. At the end the availability of another cell separator dedicated to plasmapheresis, also because offered at extremely attractive costs, may allow a greater number of targeted donations.

3.4 Pathogen Inactivation

P-131
STUDY OF REDUCTION FACTOR OF HEPATITIS B VIRUS DURING PREPARATION OF FACTOR VIII CONCENTRATE BY ELISA METHOD

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Introduction: Hemophilia is one of the prevalent X-linked hereditary disease due to deficiency of factor VIII which causes bleeding in patients. Factor VIII concentrates are used to treat these patients. In spite of therapeutic advantages of human plasma proteins, there is a risk of transmitting blood-borne viruses to the recipients. The previous studies have shown that the risk of hepatitis B virus is high in the factor VIII concentrates. Therefore, if different steps of purification lead to significant reduction of viral load it would be important to manufacturers. In this study, viral reduction of HbsAg was determined by ELISA method.
Method: Highly positive sample of HBsAg was prepared and the starting material of fresh frozen plasma was virus-spiked. The factor VIII concentrate was prepared by precipitation (PEG) and ion-exchange chromatography (DEAE-Sepharose). For determination of OD, the serial dilution (10-fold dilution factor) was prepared. Evaluation of virus titer was performed by comparing the logarithmic dilution of starting material with final material by ELISA method.
Results: The OD of results were entered to Excel program and the HBsAg removal curves were demonstrated and the reduction factors were calculated. In this study different steps of precipitation and ion-exchange chromatography showed 1.2 log and 3.1 log of virus reduction respectively.
Discussion and conclusion: Our experiments indicated that concentration of PEG, temperature, type of gels, flow rate, elution profile (PH and ionic strength), resin porosity and ligand density, proportion of height to diameter of column, volume of washing buffer and the number of washing steps and also virus size, symmetry, membrane structure, surface topography and its surface charge are important parameters for virus removal operation. The results showed that application of this method of viral removal method can totally remove 4.3 log during preparation of factor VIII concentrate.

P-132
PATHOGEN INACTIVATION EFFICACY OF MIRASOL PRT SYSTEM AND INTERCEPT BLOOD SYSTEM FOR PLATELET-RICH PLASMA DERIVED PLATELETS SUSPENDED IN PLASMA

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Background: Pathogen inactivation (PI) technology for platelets suspended in additive solutions has been implemented in some countries. Platelet additive solutions are not in use in Korea.
Aim: This side-to-side comparison study was conducted to evaluate the efficacy of pathogen inactivation in platelet-rich plasma derived platelets suspended in plasma using the Mirasol PRT System (Caridian BCT) and the Intercept Blood System (Cerus).
Methods: Thirteen to 15 units of platelets were pooled using the Acrodose PL system (Pall) and eparated into three aliquots, 50 ml for control and 275 ml each for Mirasol and Intercept system treatment. Platelets were inoculated with the respective virus or bacteria. Four replicates of each viral strain were used for the evaluation. For bacteria, both low titer (102 CFU/unit) and high titer (>107 CFU/unit) inoculation with two replicates for each bacterial strain was used. Pre- and post-treatment titer of each pathogen was evaluated. Platelets inoculated with a low bacterial titer were stored for 5 days and culture was performed with the BacT/ALERT system (Biomerieux).
Results: The levels of inactivation expressed as log-reduction for the Mirasol and the Intercept system for viruses were as follows: human immunodeficiency virus-1 (HIV-1), ≥4.19 vs ≥4.23; bovine diarrhea virus (BVDV), 1.83 vs ≥6.03; pseudorabies virus (PRV), 2.73 vs ≥5.20; porcine parvovirus (PPV), 0.28 vs 0.38; and hepatitis A virus (HAV), 0.62 vs 0.76. Inactivation efficacy for influenza A virus H1N1 could not be evaluated, because the virus was not detectable after inoculation into the platelet units. The levels of inactivation expressed as log-reduction for the Mirasol and the Intercept system for bacteria were as follows: Escherichia coli (E. coli), 5.45 vs ≥9.22; Staphylococcus aureus (S. aureus), 4.26 vs ≥10.11; and Bacillus subtilis (B. subtilis), 5.09 vs ≥7.74. Post-treatment bacterial growth in platelets inoculated with a low titer of S. aureus or B. subtilis was detected only in platelets treated with the Mirasol system.
Conclusion: Pathogen inactivation efficacy of the Intercept system for enveloped viruses such as HIV-1, BVDV and PRV was shown to be satisfactory and comparable with previous study results. The Mirasol system showed satisfactory inactivation efficacy for only HIV-1. Non-enveloped viruses were not inactivated by both systems. For bacteria, inactivation efficacy of the Intercept system was more robust for all bacteria tested at high or low titers.
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P-133
REAL-TIME PCR FOR EVALUATING THE EFFECTIVENESS OF PATHOGEN REDUCTION TECHNOLOGY

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Background: Although the safety of blood transfusion has improved dramatically in the past decade, the risk of transfusion-associated infection still persists. Photochemical principles have been used to achieve pathogen inactivation of blood products. Because of lack of in-vitro infectivity assays for assessment of PRT for emerging viruses, we have established quantitative real-time PCR with long amplicons for three viruses including HHV-8, the human Parvovirus B19 and the Dengue virus. HHV-8 is a cell-associated double-stranded DNA virus with capsid, Parvovirus B19 is a non-enveloped single-stranded DNA virus, and Dengue virus is an enveloped mosquito-born single-stranded positive RNA virus.
Aim: The objective of this study is to evaluate the effectiveness of pathogen reduction technology (PRT) using Mirasol System for whole blood.
Methods: Cell line BCP-1 (ATCC, Manassas, VA,USA) was used as the source of HHV-8, human plasma with high titer of Parvovirus B19 was used as the source of Parvovirus B19, and cell culture supernatant was used as the source of Dengue virus.
Optimization of the real-time assays included evaluation of widely employed hot start enzymes (Roche's FastStart and Applied Biosystems' AmpliTaq Gold) and other polymerase enzymes that may be particularly efficient in generating long PCR products, such as Invitrogen's Accuprime Taq DNA Polymerase High Fidelity, Invitrogen's Platinum PCR SuperMix High Fidelity, Sigma's AccuTaq LA, Applied Biosystems' rTth DNA polymerase and Stratagene's PfuUltra Hotstart DNA Polymerase. We also compared annealing temperature, annealing time, magnesium, primer and dNTP concentrations, extension temperature and time.
Primers were designed for HHV-8 and Dengue, spanning 233–1550 bases and 695–1327 bases, respectively. The primers for Parvo, published by Hokynar et al, Journal of General Virology (2000), 81, 1017–1025, spanned from 689 to 1975 bases.
Mirasol treatment: After establishment of the assay, we evaluated the efficacy of Mirasol treatment on virus spiked to 250 µl of PBS and plasma. For each sample type, three conditions were investigated: untreated, riboflavin only and Mirasol (riboflavin + UV). The effectiveness of pathogen reduction was evaluated based on the delta C_t between amplifications of Mirasol-treated spiking samples and untreated controls.
Results: The optimization data of the Dengue primer pairs is shown in Table 1.
For HHV-8, we have generated eight primer pairs with amplicons of: 233, 515, 627, 821, 978, 1043, 1294 and 1550 bps.
For Parvo B-19, we have generated 9 primer pairs with amplicons of: 689, 706, 743, 1064, 1078, 1244, 1307, 1658, 1975 bps.
We saw a 2-log viral reduction after subjecting the HHV-8 to Mirasol treatment relative to untreated controls.
Conclusion: We succeeded in creating assays of long-product real-time PCR for assessment of PRT for whole blood for viruses to which culture inactivation assays are not available or practical.

Table 1. Optimization results of Dengue primer pairs

Primer Pair	Amplicon Size, bp	Neat	1:10	1:100
		Mean Ct*, n=2	Mean Ct*, n=2	Mean Ct*, n=2
TS4F/D41R	695	17.4	20.9	24.9
D1F/D4R	754	18.2	21.6	25.3
D4SF/DVIR	920	21.6	24.6	28.2
D4CF/DSP4R	940	18.3	21.6	25.3
D4SF/DSP4R	1327	20.5	24.0	27.6

*Ct or threshold cycle value is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal significantly above the background fluorescence. The lower the Ct value, the higher the copies of amplicons generated.

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IMPACT OF SOLVENT/DETERGENT TREATMENT OF PLASMA ON THE GROWTH OF TRANSFUSION-RELEVANT BACTERIA

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Background: A solvent/detergent-filtration (S/D-F) process performed in a single-use bag system has recently been developed for viral inactivation/pathogen reduction of plasma for transfusion [1]. Bacterial contamination of blood/plasma may occur at the time of venipuncture, and is usually due to improper cleaning of the donor's arm. It is not known whether this new S/D treatment carried out at 31°C may affect or promote bacterial growth or may impair bacterial inactivation by the complement. To our knowledge, the impact of S/D treatment on bacteria has not been studied before.
Aim: Our goal has been to study the impact of this S/D treatment on the growth of eight transfusion-relevant Gram-negative or Gram-positive bacteria, including spore-forming strains, that were spiked to plasma prior to the S/D treatment.
Methods: Apheresis plasma was obtained from four donors and pooled. Plasma (P) was spiked with high titers (>10^7/ml) of eight transfusion-relevant bacterial strains. It was then treated with 1% tri(n-butyl) phosphate and 1% Triton X-45 at 31°C for 90 min and extracted by 10% soybean oil at 31°C for 70 min to remove most of the S/D agents. In addition, decomplemented plasma obtained by heating at 56°C for 30 min (DP) and PBS were used as control test materials (TM). Samples were taken at different stages of the process to determine the bacterial count by doing serial dilutions and using a plate assay method. Bacteria growth inhibition tests were also performed using wafers soaked with the various TM, in the presence of the S/D agents or not.
Results: Inactivation of the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* by the complement was not altered by the S/D treatment and reached >8.75, 4.71, and 4.12 log, respectively. Interestingly, the Gram-positive spore forming bacteria *Bacillus cereus* and *Bacillus subtilis* were inactivated by the S/D treatment of P (>7.04 and 1.6 log, respectively) while *Staphylococcus aureus* and *Staphylococcus epidermidis* also showed some inactivation by S/D in PBS (1.94 and >2.02 log, respectively) but not in P or DP. There was no multiplication of *Enterobacter cloacae* during the S/D treatment and oil extraction. Growth inhibition test confirmed the inhibition of the Gram-positive bacteria in all TM containing the S/D agents.
Conclusion: This S/D-F process mantains the functional activity of the complement, does not increase the risk of bacterial growth and can contribute to the inactivation of some Gram-positive bacteria, an observation that has not been reported before. The procedure also includes a terminal sterilizing filtration step that further secures the bacterial safety of such S/D-F plasma.
Reference:

1. El-Ekiaby M, Sayed MA, Caron C, Burnouf S, El-Sharkawy N, Goubran H, Radosevich M, Goudemand J, Blum D, de Melo L, Soulie V, Adam J, Burnouf T: Solvent-detergent filtered (S/D-F) fresh frozen plasma and cryoprecipitate mini-pools prepared in a newly designed integral disposable processing bag system. Transfus Med 2010; 20:48–61.

P-135

This abstract has been withdrawn.

P-136

A QUALITATIVE TEST OF FRESH FROZEN PLASMA AFTER METHYLENE BLUE AND WHITE LIGHT TREATMENT IN CHINA

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Aim: Investigate the Characterization of plasma protein activity in Methylene Blue and white light (MB-PCT) treated fresh frozen plasma in China, by examining the plasma sampled from three blood centers.
Method: Each 10 samples of MB-PCT treated were separately provided by Chongqing Blood Center, Suzhou Central Blood Station and Yancheng Central Blood Station. The paired, untreated plasma units was served as control and stored in −30°C with treated plasma, and the protein retention of blood coagulation factor, IgG, IgM, protein C, protein S and vWF were inspected after 4 weeks using standard coagulation assays. Results were compared with the control.
Results: In comparison with control, The average of percent protein retention in MB-PCT treated plasma after 4 weeks were: Factor II, 93.7%; Factor V, 88%; Factor VII, 90.3%; Factor VIII, 81.3%; Factor IX, 94.3%; Factor X, 90.7%; Factor XI, 78%; Factor XII, 86%; Fibrinogen, 91.9%; IgG, 98.2%, IgM, 94.5%, protein C, 106.7%; protein S, 86.3%; vWF, 82.3%.
Conclusion: Plasma treated with MB-PCT, shows reduction in activity of blood coagulation factors and protein contents. Our result also suggests different procedures of plasma collection and storage could affect the activity of blood coagulation factors and protein retention significantly.

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HIGHLY EFFECTIVE INACTIVATION OF HEPATITIS C BY MB/LIGHT TREATMENT OF PLASMA AS SHOWN BY A NOVEL IN VITRO HCV INFECTION SYSTEM

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Background: Photodynamic treatment using methylene blue (MB) and visible light is a well established method for virus inactivation of human plasma. It has been shown to inactivate a broad range of different DNA and RNA viruses. Due to the absence of a cell culture system for propagation of the hepatitis C virus (HCV), the inactivation efficacy against HCV had up to now to be extrapolated from studies using a model virus (bovine viral diarrhea virus, BVDV). Although HCV is known since 1989, only recently a HCV infection system allowing the propagation of infectious HCV in cell culture has been developed.
Aim: Aim of the current investigation was the investigation of the inactivation capacity of the THERAFLEX MB-Plasma system by using this in vitro HCV infection system.
Methods: HCV Jc1 virus was generated by electroporation of 10 µg RNA into Huh7.5 cells. Cell culture supernatants were harvested 48, 72 and 96 h post transfection. Leukodepleted plasma was prepared from whole blood using standard blood banking technology. MB/light treatment was done using the MacoPharma Theraflex MB-Plasma bag system and the MacoTronic B2 illumination device. Plasma (n = 3; 285 ml) was spiked with cell-culture grown HCV (30 ml). Samples were taken at different steps of the process (Table 1). After illumination with 40 J/cm^2 the plasma was re-spiked with an additional 30 ml of virus suspension to increase the sensivity of the assay and to test for robusness of the inactivation process. Infectivity was determined by immunohistochemistry-based endpoint titration and large volume plating on Huh 7.5 cells. The titer which causes a positive result in 50% of all infected cultures ($TCID_{50}$) was determined according to the method of Spearmann and Kaerber.
Results: The virus titer was determined at a dilution that was neither cytotoxic nor did interfere with the viral titer. Results of the titrations are given in Table 1. HCV in plasma was efficiently inactivated by the MB/light treatment below the limit of detection already with a light dose of 20 J/cm^2. After the second spike again treatment with a light dose of 20 J/cm^2 was sufficient for an inactivation of HCV below the limit of detection.

Table 1: Inactivation of HCV by MB/light treatment

sample / cumulative light dose	$\log_{10}$ $TCID_{50}$ ± SD	cumulative $\log_{10}$ reduction factor
first spike, before addition of MB	6.09 ± 0.39	
0 J/cm^2 after addition of MB	5.85 ± 0.34	0.24
20 J/cm^2	≤1.58	≥ 4.51
40 J/cm^2	≤1.58	≥ 4.51
second spike	5.97 ± 0.50	
60 J/cm^2	≤1.58	≥ 8.90
80 J/cm^2	≤1.58	≥ 8.90

Conclusions: In routine use, MB/light treatment of plasma using the THERAFLEX MB-plasma system (MacoPharma) is done with a light dose of 120 J/cm^2. In the current investigation it was shown that a dose of only 60 J/cm^2 is sufficient to effectively inactivate HCV with a cumulative reduction factor of ≥8.9 log steps. The procedure therefore has the potential to significantly reduce transfusion-transmitted HCV infections.

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COMPARATIVE EFFECTIVENESS OF PRESTORAGE VS POSTORAGE RIBOFLAVIN AND ULTARVIOLET-LIGHT TREATMENT ON THE QUANTITY AND FUNCTIONALITY OF FRESH FROZEN PLASMA CONSTITUENTS

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Background: Treatment of fresh frozen plasma (FFP) by Pathogen-Reduction-Technology (PRT) system – using riboflavin and ultraviolet (UV) light (Mirasol-treated FFP) – blocks the nucleic acid replication process and leads to inactivation of residual white blood cells (WBCs) and viruses, bacteria, fungi or other up till now unknown pathogen agents in blood.
Aims: The goal of this research was to determine the comparative effects of riboflavin and UV-light treatment on the FFP protein quantity and quality in prestorage vs poststorage inactivation setting.
Methods: This study included ex vivo investigation of 20 prestorage vs 20 poststorage Mirasol-treated FFP units. Samples for research were taken before (control groups) and after both prestorage vs poststorage (freezing/thawing) Mirasol-treatment procedures. Plasma biochemical, immune-response (immunoglobulins and complement) and hemostatic activity monitoring (coagulation factors, antitrombin (AT)-III and protein C – pC) were measured using multi-laboratory techniques and equipments.
Results: Mean corrected final concentrations (correction factor = 0.15) of coagulation factors and inhibitors (IU/ml) in prestorage vs poststorage Mirasol-treated FFP samples were: FII = 0.77; FVII = 0.78; VIII = 0.52; FIX = 0.71; FX = 0.71; ATIII = 0.79 and pC = 0.85 vs FII = 0.87; FVII = 0.77; VIII = 0.51; FIX = 0.72; FX = 0.69; ATIII = 0.84 and pC = 0.91, respectively. Final corrected plasma levels of proteins, immunoglobulins and complement components were: TP = 83.1; Alb = 31.1; IgG = 5.32; IgM = 0.52; IgA = 0.93; C3 = 0.67; C4 = 0.15 vs TP = 59.3; Alb = 37.1; IgG = 8.14; IgM = 0.73; IgA = 1.6; C3 = 0.82; C4 = 0.19 in prestorage vs poststorage Mirasol-treated FFP samples. Between Mirasol-treated FFP vs control group no significant differences in plasma constituent levels and functionality were found. In addition, no intergroup (prestorage vs poststorage) significancy was observed, although the obtained levels for plasma proteins in poststorage Mirasol-treated setting were evidently higher.
Summary/conclusion: This study confirmed that in prestorage vs postorage Mirasol-treatment no significant differences in plasma constituent quantity and functionality was observed. Thus, Mirasol-treated FFP remains protein content activity and consequently can be used in clinical settings. Lastly, previously frozen plasma or quarantine FFP units (despite the reduced quarantine period after NAT or PCR-testing) could be also effectively and safely inactivated (postorage setting) after storage/thawing, directly before clinical application.

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EFFECT OF MIRASOL PATHOGEN REDUCTION TECHNOLOGY SYSTEM ON QUALITY OF PLATELETS STORED IN PLASMA

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Background: Pathogen reduction in blood products may be an important tool to reduce risk in blood transfusions. The goal of pathogen reduction process is to inactivate pathogens without compromising features or longevity of the blood product. One of the several methods available for this goal is Mirasol pathogen reduction technology system (Mirasol PRT system). Riboflavin is added to the blood product. The product is then illuminated with UVA light. Photo toxic effect of Riboflavin light treatment is to destroy the nucleic acid in pathogens by photo-oxidation of guanine.
Aim: The purpose of the in vitro study is to evaluate the effect of riboflavin-light treatment on the functions of platelet concentrates made by aphaeresis.
Study design: Eleven double therapeutic units of platelet concentrates were harvested from voluntary donors by using Hemonetics MCS+. Double units were then divided in to two Single therapeutic units ($227 \pm 33 \times 10^9$ platelets). One of the units from each donor was processed in Mirasol PRT system while the other unit from the same donor was used as control. The experimental units were treated with 35 ml of riboflavin and UV rays (6.24/ml, 265–370 nm) by using Mirasol illuminator (Caridian BCT). All units were stored at 22°C on agitator. Samples were taken before and after Mirasol treatment with riboflavin-light and also next day (day 1), 4, 5 and 6. Various variables measured were; platelet count, pH, glucose, lactate and CD62. Swirling was graded from 3 (best) to 0 (absent). Sample for aerobic and anaerobic bacterial culture were taken from all units on day 7.
Results: We observed 2.8% loss of platelets due to pathogen reduction by Mirasol PRT. Bacterial cultures were negative for all units. Results of other variables are summarised in Tables 1 and 2.

Table 1: Results during storage of platelets after treating with Mirasol pathogen reduction technology (n = 11)

	Day 1	Day 4	Day 5	Day 6
pH	7.4 ± 0.06	7.11 ± 0.18	6.8 ±0.23	6.59 ± 0.26
Glucose consumption rate; mmol/10^{12} platelets/hour	0.039±0.01	0.042±0.007	0.042±0.006	0.040±0.007
Lactate consumption rate; mmol/10^{12} platelets/hour	0.084±0.015	0.083±0.011	0.082±0.01	0.090±0.012
Swirling	3.0±0	2.64 ± 0.5	2.36±0.67	1.73±0.9
P-selectin/CD62; Percent positive cells	16.86±6.92	21.52 ± 9.68	20.93±10.34	29.76±11.44

Conclusion: Swirling and pH showed significant decrease while metabolism and activation of platelets was increased in Mirasol-PRT treated units as compared to control units during storage. However, all the variables remained within the acceptable values defined in Guide to the preparation, use and quality assurance of blood components (Council of Europe), during storage for 5 days. Loss of platelets due to pathogen reduction process was much less than platelets loss reported in other pathogen reduction technologies available at present.
Table 2: Results of control platelet units during storage (n = 11)

	Day 1	Day 4	Day 5	Day 6
pH	7.5 ± 0.05	7.5 ± 0.11	7.45 ± 0.11	7.4 ± 0.16
Glucose consumption rate; mmol/10^{12} platelets/hour	0.036±0.02	0.036±0.01	0.032±0.01	0.040±0.01
Lactate consumption rate; mmol/10^{12} platelets/hour	0.054±0.013	0.068 ± 0.015	0.075±0.015	0.064±0.02
Swirling	3.0±0	3.0±0	3.0±0	3.0±0
P-selectin/CD62; Percent positive cells	9.88±7.8	17.9 ± 10.1	15.3±5.75	18.75±6.24

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VERIFICATION OF IN VITRO QUALITY OF PATHOGEN INACTIVATED RBC USING THE S-303 TREATMENT SYSTEM

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Background: A new pathogen inactivation (PI) system for red blood cells (RBC) has been developed using S-303 to crosslink nucleic acids and prevent replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench non-specific reactions. A recent Phase 1 clinical study successfully met the primary endpoint of 24-h recovery per the FDA criteria. Subsequent development studies have focused on expanding the S-303 PI process and optimization of the disposable sets to show compatibility with RBC prepared by practices routinely used in the US and EU.
Aims: In previous studies the input RBC for the S-303 PI process was derived from pooled RBC which were sampled frequently during storage. The purpose of this study was to verify the quality of stored PI RBC using unpooled conventional RBC with reduced sampling during storage.
Methods: On the day (D) of donation, leukocyte depleted (LD) SAGM RBC were prepared from buffy-coat depleted whole blood (500 ml). The volume range of RBC was 250–290 ml (N = 7). Each RBC unit was treated with the S-303 PI system by combining RBC with GSH and a proprietary diluent solution followed by addition of S-303 for a final concentration of 20 and 0.2 mM respectively. After an 18 h room temperature hold, RBC units were centrifuged and the diluent solution was replaced with SAGM. RBC units were sampled prior to storage, then stored at 4°C for 35 days and sampled at the end of storage for meaurement of parameters in Table 1.

Results: After PI on D1 post donation, Hb was 53.1 ± 4.6 g and extracellular protein 44 ± 16 mg/dl. The Hct on D1 was 62.3 ± 1.7% and did not change significantly over 35 days of storage. Hemolysis, K+, lactate and MCV increased from D1 to D35, whereas pH, ATP, Na+, and glucose decreased over storage. MCHC was similar on D1 and D35. Hemolysis on D35 ranged from 0.23% to 0.38% for six of the seven units while the remaining unit had 0.80% hemolysis The relative standard deviation was <10% for all parameters except hemolysis and ATP on days 1 and 35 post donation which we postulate shows donor to donor variability.

Table 1: RBC in vitro function (mean ± SD, n = 7)

Parameter	Day 1	Day 35
Hematocrit (Hct, %)	62.3 ±1.7	63.9±2.0
Hemolysis (%)	0.18 ±0.05	0.38 ±0.19
pH (37°C)	6.776 ±0.033	6.348 ±0.056
Total ATP (µmol/g Hb)	7.06 ±0.92	3.98 ±1.51
Extracellular potassium (K+, mmol/L)	1.58 ±0.06	52.62 ±4.57
Extracellular sodium (Na+, mmol/L)	144.7 ±0.9	103.9 ±3.6
Extracellular glucose (mmol/L)	26.3 ±0.8	15.6 ±1.2
Extracellular lactate (mmol/L)	6.1 ±0.5	24.4 ±1.5
Mean corpuscular hemoglobin concentration (MCHC, g/dL)	33.0 ±1.1	31.8 ±0.7
Mean cell volume (MCV, fL)	87.8 ±5.0	91.6 ±5.4

Conclusion: S-303 pathogen inactivated RBC met current EU and AABB guidelines for LD RBC with respect to Hct and Hb as well as hemolysis after 35 days of storage.

P-141
IN VITRO EVALUATION OF PATHOGEN INACTIVATED RBC USING THE S-303 TREATMENT SYSTEM

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Background: A new pathogen inactivation (PI) system for red blood cells (RBC) has been developed using S-303 to crosslink nucleic acids and prevent replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench non-specific reactions. A recent Phase 1 clinical study successfully met the primary endpoint of 24-h recovery per the FDA criteria. Subsequent development studies have focused on expanding the S-303 PI process by optimization of the disposable sets to show compatibility with conventional US and EU RBC.
Aims: The purpose of this study was to demonstrate the quality of stored PI RBC using the optimized disposable set design.
Methods: One day post donation whole blood (450 ml), stored overnight at 4°C, was leukocyte-depleted (LD) and separated into platelet poor plasma and RBC. The RBC concentrate was suspended in SAGM; ABO matched pairs of SAGM RBC were combined and split into units of 247–312 ml (N = 6). For each replicate, Control units (C) were handled as conventional RBC, stored at 4°C, and Test units (T) were treated with the S-303 PI system. The S-303 PI process involved combining a proprietary diluent solution containing GSH with RBC followed by S-303 (final concentrations: 20 mM GSH and 0.2 mM S-303). After an 18 h room temperature hold, RBC were centrifuged and the treatment solution replaced with SAGM. RBC were stored at 4°C for 35 days and sampled throughout storage for parameters in Table 1. On days 35–36, parameters in Table 2 were measured on aliquots of T and C which had been rejuvenated (using a pyruvate, inosine, phosphate, adenine solution), washed in saline, and resuspended in SAGM. Satistical significance was assessed by repeated measures ANOVA as differences between T and C (P-value < 0.05) throughout storage.
Results: After PI, T units had 52.2 ± 2.1 g of Hb; 1.7 ± 1.5% of Hb was lost during PI processing. The S-303 PI process reduced extracellular protein 20-fold in T (37 ± 10 mg/dl) compared to C (743 ± 123 mg/dl). After 35 days of storage ATP, K+, Na+, MCHC and MCV were not different between T and C. Hct was significantly lower in C, whereas hemolysis was marginally higher in C. T units had lower pH, glucose, lactate than C. On Day 2, post PI, 2,3 DPG was significantly higher in C (11.16 ± 1.49 µmol/g Hb) vs T (1.09 ± 0.66 µmol/g Hb). Evaluation of rejuvenated T and C cells, a method used to model clinical transfusion, showed that T and C cells were able to generate 2,3 DPG and ATP and had equivalent p50 values (Table 2).

Table 1: Day 35 RBC in vitro function (mean ± SD, n = 6)

Parameter	Test	Control	p-value
Hematocrit (Hct, %)	65.7 ±1.0	61.7 ±1.4	<0.0001
Hemolysis (%)	0.19 ±0.03	0.27 ±0.07	0.0536
pH (37°C)	6.381 ±0.042	6.473 ±0.040	<0.0001
Total ATP (µmol/g Hb)	3.56 ±0.79	3.53 ±0.66	0.1286
Extracellular potassium (K+, mmol/L)	48.57 ±2.75	46.58 ±2.34	0.9304
Extracellular sodium (Na+, mmol/L)	103.2 ±3.7	108.2 ±3.4	0.2529
Extracellular glucose (mmol/L)	19.0 ±1.0	19.4 ±1.2	0.022
Extracellular lactate (mmol/L)	21.5 ±1.8	29.1 ±2.8	0.032
Mean corpuscular hemoglobin concentration (MCHC, g/dL)	28.7 ±0.7	28.5 ±0.6	0.2720
Mean cell volume (MCV, fL)	95.6 ±5.1	96.8 ±5.1	0.8944

Table 2: Post rejuvenation in vitro RBC function on day 35–36 (mean ± SD, n = 6)

Parameter	Test	Control
2,3-DPG (µmol/g Hb)	6.48 ±1.53	8.69 ±1.68
Total ATP (µmol/g Hb)	7.93 ±0.61	7.31 ±0.39
P50 (mm Hg)	26.5 ±1.4	27.0 ±1.4

Conclusions: There was minimal loss in hemoglobin due to processing. After S-303 PI units had >40 g of Hb and Hct within 50–70%; hemolysis was <0.8% after 35 days of storage. S-303 PI RBC met current EU and AABB guidelines for LD RBC with respect to Hct, Hb, and hemolysis. Rejuvenation of RBC after 35 days of storage resulted in measurable 2,3-DPG and similar p50 values between Test and Control RBC. All measured in vitro parameters of S-303 treated RBC indicate suitability for transfusion.

P-143
TREATMENT OF PLATELET PRODUCTS WITH RIBOFLAVIN AND UV LIGHT: EFFECTIVENESS AGAINST CLINICALLY SIGNIFICANT BACTERIAL CONTAMINATION

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Background: Bacterial contamination of platelet products has been identified as a significant risk associated with the transfusion of blood components. The storage of platelet products at 22°C provides a media and storage conditions that can sustain bacterial growth. Thus, even though the contamination levels of bacteria are believed to be extremely low, bacteria can proliferate to high titers prior to transfusion resulting in complications including morbidity and mortality.
Aims: The goal of this study was to evaluate the Mirasol® PRT System at preventing bacterial growth in platelet products treated it either plasma or platelet additive solution (PAS).
Methods: A panel of organisms identified in prior hemovigilance programs was selected for evaluation in this study and included the following species: *S. epidermidis*, *S. aureus*, *P. acnes*, *S. mitis*, *S. agalactiae*, *S. pyogenes*, *S. marcescens*, *A. baumannii*, *Y. enterocolitica*, *B. cereus* (spore forming agent), *E. coli*, *E. cloacae* and *K. pneumoniae*. This included several different strains of the same organism, providing a total of 22 strains of bacteria in the study. A priority was placed on selecting bacterial strains that had been isolated from blood. Units of both platelets stored in plasma and platelets stored in PAS were spiked with clinically relevant bacteria loads of 5–100 CFU/product. All products were allowed to rest for 2-h on a platelet incubator shaker at 22°C prior to treatment. On day 7 post treatment, the Mirasol treated units were sampled and screened for bacteria growth by BacT/ALERT. Positive and negative growth controls were also included to ensure the reliability of the results.
Results: When platelet units suspended in plasma were inoculated with an initial bacterial load of 20–100 CFU per product and treated the Mirasol PRT process was 91% effective at preventing bacterial growth at day 7 of storage. At lower titers (<20 CFU per product), the effectiveness of the Mirasol PRT process in the presence of plasma increased to 98% on day 7 of storage. When platelet units treated in the presence of PAS were challenged with an initial bacterial load of 20–100 CFU per product the Mirasol PRT process was 98% effective at preventing bacterial growth at day 7 of storage.

Conclusion: Overall, when weighted for frequency of occurrence of bacterial contamination reported in hemovigilence, Mirasol-treated platelets were 91–98% effective in maintaining units negative for bacterial growth when tested against a list of bacteria of interest. The Mirasol PRT System results presented compare favorably to bacterial screening. Published performance suggests that bacterial screening is only around 50% effective at detecting the presence of bacteria in a contaminated platelet product.

P-144
PHOTOCHEMICAL INACTIVATION OF SELECT ENVELOPED VIRUSES USING THE MIRASOL® PRT SYSTEM FOR PLASMA, PLATELETS IN PLASMA AND PLATELETS IN PAS

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Background: Viruses are categorized into two major groups, enveloped and non-enveloped viruses. Enveloped viruses, in addition to their viral protein capsid, are surrounded by a lipid envelope. This lipid envelope makes enveloped viruses susceptible to solvent detergents and other pathogen reduction technologies that can dissolve or modify this envelope. However, since red blood cells and platelets also contain a lipid envelope these types of blood products cannot be treated by detergent based reduction technologies.
The Mirasol pathogen reduction technology (PRT) inactivates a wide range of bacteria, viruses and parasites using the naturally occurring vitamin B2 (riboflavin) and light. The process works through direct modification of the genomic material, thus its mode of action is independent of the presence of a lipid envelope.
Aims: The goal of this study was to test the effectiveness of the Mirasol PRT process against the following enveloped viruses: Vesicular Stomatitis virus (VSV), Hepatitis B virus (HBV), Murine Cytomegalovirus (MCMV), Human Immunodeficiency virus (HIV), West Nile Virus (WNV), Sindbis (SINV), Influenza A (FLUAV), and Pseudorabies virus (PRV). WNV and SINV are model viruses resembling Hepatitis C.
Mehods: Standard blood bank units of plasma and platelets, suspended in both plasma and PAS, were used to test the effectiveness of the Mirasol PRT System against a wide range of enveloped viruses. All viruses except for HBV were evaluated at third party laboratories. Nominal sized platelet and/or plasma units were used to test VSV, HIV, WNV, SINV, FLUAV and PRV. These viruses were tested using standard infectious dose assays, which included both plaque and TCID50 assay types.
HBV was tested in a scaled down treatment bag designed for treatment of smaller volume products. The virus was measured using classic PCR technique looking for the presence of a 3.1 kb PCR product both pre-treatment and post-treatment.
MCMV was also tested in a scaled down system, which utilized a 48-well culture plate as the illumination vessel. The small treatment volumes were necessary because the pre-treatment and post-treatment samples were tested for their ability to transmit MCMV in an in vivo mouse model. Both cell free MCMV and MCMV infected white blood cells (WBCs) were evaluated in this study.
Results: The observed reductions for VSV, HIV, WNV, SINV, FLUAV and PRV are as follows: ≥6.3, 5.9, ≥5.1, 3.2, ≥5.0 and 2.5 log/ml. The reduction observed using the classical PCR method for HBV was 4.0 log gEq/ml with an assay detection limit of 2.5 log gEq/ml. In the in vivo murine model, mice were challenged with either 6.0 log CFU of cell-free MCMV or 6.0 log of infected murine WBC's. In both cases the Mirasol PRT treatment prevented transmission of MCMV after transfusion.
Conclusion: The Mirasol PRT System is effective at reducing the viral loads of VSV, HBV, HIV, WNV, SINV, FLUAV and PRV. Additionally the Mirasol PRT system was able to prevent MCMV transmission of both cell-free MCMV and MCMV infected WBCs.

P-145
PHOTOCHEMICAL INACTIVATION OF SELECT PARASITES USING THE MIRASOL® PRT SYSTEM FOR PLASMA AND PLATELETS

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Background: For many years the spotlight in blood safety has been focused on preventing virus transmission via tainted blood products through the development of immunological and nucleic acid based assays. To a lesser degree, a secondary focus has been on the prevention of transfusion transmitted bacteria, primarily through the introduction of bacterial screening of platelet products. Parasitic infections which may be transmitted by blood represent yet another threat to blood supply safety and quality. The threat posed by parasitic infections arises primarily due to the presence of parasitic vectors which exist in broad geographical regions. These regions are expanding due to both vector migration and the migration of human populations, thus promoting the spread of disease transmission. A rise in the presence of these agents in the general population translates to increased potential of parasitic disease transmission via blood. Unfortunately many of the techniques employed to screen out units containing viruses, such as nucleic acid testing (NAT) and conventional serology, are not highly effective for the detection of parasites. This is because many parasites have prolonged periods where they are below the level of detection for these assay methods or reside inside host cells where they may escape detection by these approaches.
Aims: The effectiveness of the Mirasol PRT System for platelets and plasma was evaluated for reduction of the following parasites: *Plasmodium falciparum* (malaria), *Orientia tsutsugamushi* (scrub typhus), *Leishmania donovani* (leishmaniasis), *Babesia microti* (babesiosis), and *Trypanosoma cruzi* (Chagas' disease).
Methods: Standard blood bank units of plasma and platelets in plasma were used to test the effectiveness of the Mirasol PRT System against a wide range of parasites. All parasites were evaluated at third party laboratories (American Red Cross Holland Labs, Walter Reed Army Institute of Research, and the US Centers for Disease Control).
Units of plasma and platelets were inoculated with high concentrations of infectious parasite. An initial pre-treatment sample and a sample after the Mirasol PRT process were collected. The *P. falciparum* samples were evaluated using a thin film microscopic technique to look for viable parasites in RBCs. The parasites *O. tsutsugamushi* and *B. microti* were evaluated using in vivo animal models, mouse and hamster respectively. Finally, both *L. donovani*, and T. cruzi were evaluated using microscopy in cultured samples to look for viable organisms pre- and post-treatment.
Results: The observed reductions for *P. falciparum*, *O. tsutsugamushi*, *L. donovani*, *B. microti*, and *T. cruzi* were as follows: ≥3.2, ≥5.0, ≥4.0, ≥4.0 and ≥5.0 log/ml. In all cases, parasites were reduced to the limits of detection of the assay and no infectivity was observed in animal models.
Conclusion: The Mirasol PRT System for both plasma and platelets is effective at significantly reducing the parasitic loads of *P. falciparum*, *O. tsutsugamushi*, *L. donovani*, *B. microti*, and *T. cruzi*. The broad reduction of parasites using the Mirasol PRT System for platelets and plasma could provide an effective method for the prevention of transfusion transmitted parasitic infections.
Table 1: Parasite reduction

Parasites	
Agent	Reduction Value
Orientia tsutsugamushi	≥ 5.0
Trypanosoma cruzi	≥ 5.0
Leishmania donovani infantum	≥ 4.0
Plasmodium falciparum	≥ 3.2
Babesia microti	≥ 4.0

3.5 Biologicals

P-146
THE INCIDENCE OF HEMOLYSIS AND TEMPERATURE CHANGES DURING LONG DISTANCE BLOOD TRANSPORT IN EASTERN TAIWAN

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Background: Temperature control and maintaining blood quality during long distance transport are major problems in blood center, these include packaging methods, personnel deployment, blood products allocation and storing facilities. This study is to set a local standard on the different types of packaging using different transport containers by monitoring temperature changes during shipment.
Aims: After blood collection and without prior refrigeration, our goal is to gradually lower blood products temperature, no lower than 1°C during transport, to conform with the AABB requirement of maintaining blood products temperature (except Platelet products) during transport at 1–10°C. To ensure the quality of blood products, detection of red cell hemolysis was determined by checking hemoglobin levels in plasma.
Methods: Using three different types and sizes of transport containers: MG (34 × 26 × 34 cm), LP (57 × 37 × 33 cm), LR (61 × 36 × 36 cm) with different amount of ice packs, blood products were packed accordingly and temperature were monitored during the entire period of transportation using Data-Logger. Hired temperature-controlled transport vehicle were used for blood products shipment. Blood products were

tested for hemolysis before and after shipment to determined significant level of hemolysis according to the standard of the Council of Europe which should be below 0.8%.
Formula on Computed hemolysis: Hemolysis % = Plasma Hb (g/dl) × (1-Hct)/Total Hb (g/dl) × 100%
Results: Shipment of blood was on the same day of blood collection with or without prior refrigeration. Temperature recording showed that MG container showed an average of 10.6°C on arrival to the Blood Center which is the best achievement compared to the other two containers with an average of 15–17°C. Probable reason is that there are more free spaces on the two other containers that can't be filled-up with ice-pack. Paired t test showed no significant hemolysis of red cell using the three different containers and packing methods. Results of hemolysis were all below 0.8%.
Conclusion: This study proved our hypothesis that with proper packaging and using temperature-controlled vehicle for shipment of blood, red cell hemolysis wasn't increased during long hour shipment. There was gradual lowering of the temperature curve and the lowest temperature didn't exceed the recommended lowest temperature of 1°C. Future studies could be designed to study the effect of long hour shipment on the various chemical properties of the blood cells.

3.6 Novel Blood Products

P-147
RECOMBINANT SOLUBLE THROMBOMODULIN IN SEPTIC DISSEMINATED INTRAVASCULAR COAGULATION

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Background: Disseminated intravascular coagulation (DIC) is a serious complication in patients with sepsis which is resistant to antithrombotic therapy and occasionally fatal.
Aims: We have evaluated the therapeutic effects of recombinant soluble thrombomodulin (rTM) recently developed for patients with septic DIC.
Methods: The patients with sepsis who met the Japanese diagnostic criteria for acute DIC and showed a level of antithrombin (AT) lower than 70% were recruited and divided into two groups, one group (Control group) treated with AT products and Gabexate mesilate (GM), and the other group (TM group) treated with rTM in addition to AT and GM. The differences between TM and Control groups were investigated to see the effect of rTM on the clinical course through DIC scoring, and haemostatic and inflammatory markers on days 0, 3, 5, 7.
Results: Twelve patients were included in the TM group, and 16 patients in the Control group. There were no differences in the APACHEII score, DIC score, or mortality between the groups. The effects of the rTM were as follows; (i) Patients in the TM group showed earlier DIC resolution at day 5 than Control at day 7. (ii) Hypercoagulability state expressed by the increased levels of soluble fibrin monomer (SF) or thrombin-antithrombin complex (TAT) was improved more quickly in TM group compared with Control group. Because the levels of SF at day 3 and 7 in TM group were lower than those in Control group, rTM may suppress thrombin production. (iii) AT was increased much more at day 3 after AT product administration in TM group than in Control group, which also can be explained by suppression of thrombin production by rTM. (iv) Increased levels of the complex of α2 PI/plasmin, D-dimer, and fibrin/fibrinogen degradation product were also improved earlier in TM group than Control group. (v) TNF-α, IL-6, HMGB1 were decreased significantly at day 3 compared with day 1 in TM group. These anti-inflammatory effect can also be evoked through decreased thrombin production by rTM, because thrombin is a strong pro-inflammatory molecule.
Summary/conclusion: rTM may be beneficial in improving septic DIC via its anti-thrombotic and anti-inflammatory properties.

P-148
THE EFFECTS OF FREEZING-THAWING ACTIVATED PLATELET RICH PLASMAS (PRP) ON THE PROLIFERATIONS OF *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA* USING DISC DIFFUSION METHOD

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Background: Fresh platelet rich plasma (PRP) gel has been reported to have antibacterial properties, although its mechanism is not fully understood. The form of gelatinous mass of PRP gel might work as a physical barrier against bacteria. However, it has not been studied whether antibacterial ability of PRP is still present if PRP is not in gel form. Also the effects on the antibacterial ability of freezing-thawing treatment of PRP are not studied although the treatment is an activation method for the prepared PRP as well as storage tool.
Aims: We investigated antibacterial effects on *S. aureus* and *P. aeruginosa* of activated PRP (APRP) and activated platelet poor plasma (APPP) by freezing-thawing procedure and bovine thrombin treatment.
Methods: Twenty healthy volunteer donors were recruited in this study and 10 ml of whole blood was drawn in CPDA-1 anticoagulation from each donors, PRP and PPP were prepared by double centrifugation and the volume of PRP was adjusted to 0.5 ml. After freezing at −70°C and stored for more than one day, PRP and PPP were thawed at 37°C and treated by bovine thrombin and $CaCl_2$. Immediately after activation, they were treated with heparin to prevent gel formation. Antibacterial effects on *S. aureus* and *P. aeruginosa* were evaluated using disc-diffusion and direct dropping method. The inhibited diameters were measured after incubation of 16–18 h at 37°C.
Results: The mean platelet count of donor whole bloods was 142.3 ± 28.3 (×109/l) and that of concentrated PRP was 804 ± 293% of them, representing high platelet yields of the procedures. The inhibited diameters on *S. aureus* and *P. aeruginosa* using disc-diffusion and direct dropping method were zero for all the 20 sets of PRP, PPP, APRP and APPP.
Conclusions: In our study, we could not find any antibacterial effects on *S. aureus* and *P. aeruginosa* of PRP, PPP, APRP and APPP. This result may be due to the freezing-thawing treatment and/or no gel form of PRP, because they might influence the antibacterial effects of PRP. However, it is still not exclusive the methodological insensitivity of disc diffusion method for the determination of antibacterial ability of PRP and PRP.

P-149
PROCESS OF HUMAN PLATELETS LOADED WITH TREHALOSE BEFORE FREEZE-DRYING

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Background and objective: The aim o f this research was to study the technology and methods of loading lyoprotectant-trehalose into cytoplasm of human platelets before lyophilization, to optimize experimental conditions of loading trehalose, to investigate the changes of platelets response to agonists and activation after incubation of platelets for 4 h at 37°C in the presence of lyoprotectant-trehalose, to protract the figures of loading efficiency and intracellular trehalose concentration vs incubation time, temperature and external trehalose concentration, to optimize loading parameters.
Materials and methods: The response of platelets to different agonists thrombin, ADP, collagen and ristocetin were measured respectively by APACT2 aggregometer before and after loading trehalose into platelets; the expressions of CD62p and PAC-1 on platelet membranes in the presence and absence of reversible platelets activation inhibitors were measured by flow cytometry respectively before and after loading trehalose into cytoplasm of platelets.
Results: The results showed that the loading efficiency was linear to incubation time (2 h later) and incubation temperature (rang from 30 to 40°C), respectively. The loading efficiency almost reached 60% when the platelets were incubated at 37°C for 4 h. The intracellular trehalose concentration was higher with the increase of the extracellular trehalose concentration (<50 mM). Compared to untreated groups, the values of MPV and aggregation to different agonists in treated groups showed no significant difference, respectively (P > 0. 01). After incubation of platelets for 4 h, the expression o f CD62p increased to some exten, thowever, the expression of CD62p decreased again when the reversible platelets activation inhibitor PGE-1 and adenosine were added to the incubation buffer.
Conclusion: It is concluded that 37°C, 4 h and the extracellular trehalose concentration <50 mM are the optimal conditions for loading with trehalose. The processing of loading with trehalose before platelet lyophilization has no significant effects on response of platelets to agonists and activation.

4. Transfusion Transmitted Infections
4.1 Screen Strategies for TTI

P-150

This abstract has been withdrawn.

P-151

SURVEY OF THE SERO PREVALENCE OF HUMAN T-LYMPHOTROPIC VIRUS I/II IN HEMODIALYSIS PATIENTS IN HEMODIALYSIS CENTERS IN SUEZ CANAL AREA

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Background: Human T-lymphotropic viruses, or human T-cell leukemia viruses I and II (HTLV-I and II) are two closely related blood borne viruses of retroviridae family, considered to be the causative agent of adult T-cell leukemia and a tropical spastic paraparesis. Transfusion of blood components is one of the modes of viruses transmissions.

Aim of the work: Blood donors screening is performed in countries with high prevalence of HTLV-I/II and also in some European countries. As little research has been conducted in Egypt regarding the prevalence of the HTLV-I/II antibodies in blood donors, hemodialysis patients and in at-risk groups, the aim of our study was to determine the prevalence of anti-HTLV-I/II antibody among hemodialysis patients in Suez Canal area, considered a high risk group for contracting blood born infections.

Methods: In the current study, we chose patients with chronic renal failure on regular hemodialysis. Both age-matched males and females were included in the study with mean disease duration of 4.5 years. Patients were chosen from three cities (Ismailia, Suez and El-Areesh) representing different geographical regions. Samples were screened by ELISA technique (Diapro HTLV-I + II). Initially reactive samples were shipped under standard preservation procedures to SRL Company in Tokyo, Japan to be rechecked using Western Blot technique (PROBLOT HTLV-I).

Results: Among 328 patients, four patients' sera were initially reactive with ELISA (Diapro-Italy) where microplates were coated with HTLV I/II-specific peptides (gp46-I, gp46-II and p21-I). The prevalence was 1.2% with no statistically significant differences between positive and negative HTLV I/II patients regarding age, sex and disease duration. Confirmation of the results was done in Tokyo, Japan by Western Blot for HTLV-I (PROBLOT HTLV-I), where nitrocellulose strips contain proteins p19, p24, p53 and gp46. Only one sample was positive for P24. The other three samples were all negative for p19, p53, p24 and gp 46, suggesting the low prevalence of the disease in Suez Canal area.

Conclusion: The present study recorded only four transfusion dependent hemodialysis patients out of 328 (1.2%) to be seropositive for HTLV-I/II antibodies and were negative by the immunoblotting technique for HTLV-I suggesting the low prevalence of the disease in Suez Canal area.

P-152

STUDY OF PREVALANCE OF TRANSFUSION TRANSMISSABLE INFECTION AMOUNG DONOR POPULATION IN SOUTHERN REGION OF SRI LANKA

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Background: TTI has become a major challenge in safe blood transfusion. More and more new techniques have been introduced to overcome this problem globally such as Nucleic acid testing and pathogen inactivation. But all of them give and additional cost to the health sector and introducing such technique should well justified.

Southern region Blood center is the main blood collecting institute in southern region of Sri Lanka handles around 17,000 volunteer donors annually. Currently in Sri Lanka each donor pack is tested for HIV1 and 2, HBV, HBV, Syphilis and Malaria.

Aim: When compared with other countries in the region, Sri Lanka used to have a very low prevalence of TTI especially HIV. So in this study I have tried to analyze the prevalence of TTI in past 3 years, which will help in deciding the need of an expensive pathogen identification technique.

Method: The date was collected from total voluntary and replacement donors from 2008 to 2010. The screening tests were done at Regional center and the confirmatory tests at National Blood center. Screening methods for HBV and HIV were Enzyme-linked immunosorbent assay (ELISA), for Syphilis Carbon antigen. Confirmatory testing method for HIV was Western Blot, for HBV was ELISA and for Syphilis was Treponema Pallidum Particle Agglutination.

Results: The prevalence (per 1000 donations) of each disease is as follows.

Table 1

	Syphilis		HIV		HBV	
	No	Prevalance	No	Prevalance	No	Prevalance
2008	5	0.28	1	0.05	20	1.12
2009	4	0.21	2	0.10	21	1.14
2010	11	0.62	2	0.11	27	1.54

Conclusion: During past 3 years the prevalence of HBV and Syphilis gradually increased but the prevalence of HIV remains static and also at very low level. So the Southern part of Sri Lanka is still a low prevalence area for HIV.

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EFFECTIVENESS OF TRANSFUSION TRANSMITTED INFECTION (TTI) SCREENING TESTS OF DONOR BLOOD IN SRI LANKA

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Background: National Blood Transfusion Service is the sole provider of blood and blood components to the state sector and nearly all private sector health institutions in Sri Lanka. It consists of the National Blood Centre (NBC) which is the central body, 16 cluster centers and 67 hospital based blood banks. TTI screening is exclusively done at NBC and cluster centers by specially trained technical staff. Currently all donated blood is tested for HIV, Hepatitis B, Hepatitis C, syphilis and malaria. HIV, HBV, HCV screening are done in duplicate using Enzyme Immunoassay (EIA), Enhanced Chemiluminescence Immunoassay (ECI), particle agglutination and rapid methods in cluster centers while, NBC uses only ECI and EIA. Positive samples from cluster centers are sent to NBC for repeat testing after discarding the positive packs. At NBC all the screening positives samples are tested with a different method and only repeat reactive blood is discarded. Though other TTIs are confirmed Hepatitis C confirmation cannot be done due to unavailability of HBC PCR. Syphilis is screened with VDRL and confirmed by TPPA and only confirmed positive samples are discarded. By 2012 TTI screening is expected to be upgraded with NAT testing.

Aim: To assess the effectiveness of Hepatitis B, C and HIV screening test methods of Donor blood in Sri Lanka.

Method: Retrospective study was done at NBC using TTI screening records from March to August 2010 and analyzed 1883 screening positive samples of HIV, HBV, HCV and Syphilis from NBC and all other cluster centers.

Results: For HIV screening, cluster centers have used EIA, ECI, rapid and particle agglutination tests 70.24%, 24.47%, 4.73%, 0.56% respectively. Out of them 98.04% of ECI, 70.64% of EIA, 16.66% of rapid and 0% of particle agglutination positives were repeat reactive.

In NBC 88.89% of HIV testing done with EIA and 11.11% with ECI. One hundred per cent ECI and 46.97% EIA positives were repeat reactive.

In cluster centers 23.61%, 74.07%, 1.39%%, 0.93% of HCV testing were done with EIA, ECI, rapid and particle agglutination respectively. From that 95% of ECI, 66.67% of EIA and 0% of rapid and particle agglutination positives were repeat reactive.

In NBC 63.42% of HCV testing done with EIA and 36.58% with ECI. 97.25% ECI and 51.32% EIA positives were repeat reactive.

For HBV screening cluster centers have used EIA, ECI, and rapid tests 17.57%, 79.73%, 2.7% respectively. Out of them 71.19% of ECI, 46.15% of EIA and 100% of rapid test positives were repeat reactive.

In NBC EIA and ECI were used for HBV testing 76.96% and 23.04% of the time respectively.92% of ECI and 43.11% of EIA positives were repeat reactive.

Conclusions: NBC uses EIA and cluster centers use ECI for screening most frequently. Positive predictive value of ECI method is higher than that of other screening methods. Considering the blood and blood product discard, repeat testing and work load ECI is an effective method of screening TTIs compared to other tests.

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STRATEGY OF VIRUS NUCLEIC ACID AMPLIFICATION TEST (NAT) FOR DONOR SCREENINGS OF PLATELETAPHERESIS POPULATION

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Background: In order to ensure blood safety, we extended our NAT screening assay to include plateletapheresis donors (PDs). A retrospective analysis showed that 3726 PDs had given a total of 13,838 platelet concentrates (PC) (2010.03–2011.07). Based on China's State criteria, a single donation interval for whole blood donors (BDs) is every 3 months and for PDs is 15 day, but limited to 12 PC donations in a year. In practice, our blood screening strategy is: if ELISA is (+), the donated blood is discarded and the donor is ineligible to donate for life. If the results are ELISA(−)/NAT reactive, the blood is discarded, and the donor is still eligible to donate. And if the results are ELISA(−)/NAT(−), blood components qualify to be distributed to clinics. Because BDs and PDs have different donation frequencies, we need to develop a new strategy for PC donations.
Method: Our screening tests involved parallel ELISA assays using kits from two different manufacturers against HIV, HCV and HBV. In addition, an individual detection (ID) NAT assay was implemented using ULTRIO reagents in the TIGRIS system. If the results are ELISA(−)/NAT reactive, confirmatory tests are performed for HIV, HCV and HBV detection respectively.
Results: The reactive rate of NAT screenings was 0.91% (34/3726). Three donors with ELISA(+)/NAT reactive results were ineligible to donate for life. Thirty-one donors with ELISA(−)/NAT reactive results had confirmatory tests. Seven of those 31 donors were determined to be HBV DNA infected. The positive rate of confirmatory testing was 0.188% (7/3726). According to our present donor strategy, donors who are ELISA(−)/NAT reactive are eligible to donate PC. In follow up testing, two of the seven PDs developed HBsAbs. This revealed that their last PC donations might be in the ELISA window period, but failed to be detected. In addition, our retrospective data showed that among three donors with both ELISA (+) and NAT (+), one donor successively gave five PC donations in half a year; however the screening-test results of his previous four donations were ELISA (−) and NAT (−. As we know, the ELISA window period for HBsAg is 59 days and NAT window period for HBV is 34 days. The donor had caused great potential risk to four PC recipients.
Conclusion: To give consideration to both PD's retention and blood safety, we need a new strategy to follow up on PDs with ELISA(−) and NAT reactive results. That is: To create a follow-up program, if NAT confirmatory testing remains positive, the donor will be ineligible to donate for life; If NAT confirmatory test is negative, the donor will only be eligible to donate whole blood; If two whole blood donations pass the screening tests, the donor will regain eligibility as to be a PC donor again. Additionally, it is important that, since China has a large population infected with HBV, the sensitivity for NAT reagents be further improved.

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PREVENTION FROM TRANSFUSION TRANSMISSIVE DISEASES IN THE REGIONAL CENTER FOR TRANSFUSION MEDICINE IN STIP, REPUBLIC OF MACEDONIA FOR THE PERIOD 2009–2010

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Introduction: Blood transfusion is a transplantation of fluid tissue or an introduction of human biological material that needs to survive in the donor organism and to play important biological functions. During the blood and blood products transfusion, it is possible to transmit many transfusion transmissive diseases, which increases the need of securing safe blood transfusion.
Objective: To present the procedures and measures taken in order to prevent the transmission of transfusion transmissive diseases in the blood and blood products donors at the Clinical Hospital in Stip.
Materials and methods: Each blood unit was mandatory tested for HBSAG, anti-HCV, anti-HIV and Treponema pallidum antibodies at the Regional center for transfusion medicine. The testing was done with the ELISA technique by using the Dade Berhing BEP 200 instrument and the tests from Siemens and Ortho for anti-HCV. The confirmation tests were done at the Institute of Transfusion Medicine in the capital Skopje.
Results: In total, 6067 blood samples were tested. The presence of HBAGS was detected in 81 sample (1.33%), anti-HCV in 19 (0.313%), anti-HIV in one (0.016%) and Treponema pallidum antibodies in five samples (0.082%).
Discussion and conclusion: In order to achieve high level of security of the transfusion blood and blood products it is essential to use highly specific and sensitive tests, modern equipment, well trained health personnel and sufficient financial resources allocated specifically for that aim.

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PREVALENCE OF ANTI-HIV (I & II), HBSAG, ANTI-HCV AND SYPHILIS IN BLOOD DONORS

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Background: Transfusion of blood and blood products carries an inherent risk of spread of Transfusion Transmissible Infections (TTI's). Despite careful donor selection and the development of highly specific and sensitive techniques of testing, the achievement of 100% risk free blood still remains an elusive goal.
Aims: The present study was undertaken to analyse the seroprevalence of markers of Transfusion Transmissible Infections viz. Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Syphilis among the donors who donated whole blood at our institute.
Methods: A retrospective analysis of the TTI marker status of 1,85,266 blood donors who donated whole blood at the department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi from 1st of January 2001 to 31st of December 2010, was undertaken. All the donors were carefully selected to exclude paid commercial donors and those with a history of high risk behavior. As is mandatory under the guidelines laid down by the Drugs and Cosmetic Act, the donor samples were examined for various infectious markers i.e. anti-HIV (I & II), Hepatitis B surface antigen (HBsAg) and anti-HCV by Enzyme Linked Immuno-sorbent Assay (ELISA) test. Screening for syphilis was done by employing the Rapid Plasma Reagin (RPR) test. All the donor samples that were found positive by ELISA on initial testing, were repeat tested in duplicate with the same sample. Samples that were found to be repeat reactive were considered positive.
Results: Among the 1,85,266 blood donors, the total number of seroreactive cases was 3987 (2.15%). The number of cases found seroreactive for markers of HIV, HBV, HCV and Syphilis was 451 (0.24%), 2361 (1.27%), 703 (0.38%) and 472 (0.27%) respectively. Out of the total blood donors, 1,74,150 (94%) were males and 11,116 (6%) were females. The seroprevalence of markers of HIV, HBV, HCV and Syphilis in male blood donors was 0.25%, 1.32%, 0.38% and 0.27%. The respective seroprevalence in female blood donors was calculated to be 0.12%, 0.52%, 0.36% and 0.04%.
Conclusions: Among these blood donors, the highest seroprevalence was found to be for HBsAg followed by markers for HCV, syphilis and HIV respectively. The same order of seroprevalence holds true for the male blood donors. However, the rest of the order remaining similar, the seroprevalence of markers for HIV is more than that for syphilis in the female blood donors. Because of the high prevalence of TTIs among the blood donors, our analysis emphasizes the importance of introduction and use of improved testing techniques like Nucleic Acid Testing (NAT) in conjunction with the existing testing methods to further reduce the 'window period' and enhance the safety of blood supply.

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HEPATITIS B CORE ANTIBODY TESTING IN INDIAN BLOOD DONORS: A DOUBLE EDGED SWORD!

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Introduction: Until lately anti-HBc antibodies were considered an effective marker for occult HBV infection and have served their role in improving blood safety. But with the development of advanced tests for HBV DNA detection the role of anti-HBc in this regard stands uncertain.
Aims: This study aims at determing the current role of anti HBc antibody testing in donor screening.
Material and methods: Anti-HBc and HBsAg ELISA and ID-NAT tests were run in parallel on donor blood samples between 1st April 2006 and 31st December 2010 at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. A positive ELISA test was repeated in duplicate, and only repeat reactive samples were considered as 'Positive'. A positive ID-NAT was also repeat tested and a repeat positive ID_NAT was followed by Discriminatory NAT assay to identify HBV, HCV and HIV.
Results: A total of 94,247 samples were tested. The total core positivity rate (IgG + IgM) observed at our centre was 10.22% (9638/94,247), while 1.2% (1134/94,247) of donors were positive for HBsAg. Of the 8660 donors reactive by anti-HBc ELISA but negative for HBsAg, only 0.15% (13/8660) showed the presence of HBV DNA by discriminatory assay, while the remaining 99.85% (8647/8660), were HBV DNA negative. These constitute 9.17% (8647/94,247) of the total donor population. These are the donors who are potentially non-infectious and may be returned to the donor pool.
Conclusion: Addition of anti-HBc to blood donor screening along with HBsAg improves blood safety, however, leads to attrition of donors, especially in India which lies in the intermediate zone of hepatitis B endemicity. The best alternative for improving

blood safety in our country is to add NAT testing to donated blood. It will identify most potentially infectious units of blood including window period donations and sero-negative infections. Therefore, the applicability of anti HBc testing in routine screening of donated blood is doubtful.

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ESTIMATION OF INCIDENCE AND RESIDUAL RISK OF TRANSFUSION TRANSMITTED INFECTION FOR HIV, HCV AND HBV IN HONG KONG: 2007–2009

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Background: Incidence rates and residual risks of transfusion transmitted infections (TTI) are important parameters in the understanding of the current blood safety status and the effectiveness of blood safety related measurements implemented.
Aims: Use mathematical model to estimate the incidence rates and the residual risks of transfusion transmitted HIV, HCV and HBV infections in Hong Kong during 2007–2009.
Methods: During April 2007 to September 2009, all donations to the Hong Kong Red Cross Blood Transfusion Services were tested for anti-HIV-1/2 (replaced by HIV-combo in 2008), anti-HCV and HBsAg using PRISM ChLIA (Abbott, Abbott Park, IL, USA). Repeatedly reactive samples were confirmed by blotting tests and HBsAg neutralization assay respectively. All donations were also screened by Nucleic Acid Testing on individual donor samples (ID-NAT) using HIV-1/HCV/HBV triplex PROCLEIX ULTRIO Assay on automated Tigris platform (Novartis, Emeryville, CA, USA). Reactive samples were retested with discriminatory assays to determine the viral specific reactivity. Discriminatory NAT reactive samples were confirmed by alternative molecular procedures. For estimation of incidence rates, seroconversion and NAT yield among repeat donor population were used. For HBV, HBsAg seroconversion and NAT yield among repeat donor population were adjusted for its in vivo transient nature (multiplied by a factor of 1.2) (Weusten J et al. *Transfusion* 2011; 51:203–215). The incidence rate among first time donors was assumed to be twice the rate of repeat donors. Approximately 82.10% of collections were from repeat donors. The estimated incidence was weighted for the proportion of first-time and repeat donors. Residual risk for each virus was estimated using the model of Residual Risk = Incidence Rate × Window Period (in year). Reported window periods (in days) were applied: HIV-1: 5.5 (3.5–7.9); HCV: 4.9 (4.0–5.8); HBV: 20.6 (12.0–28.0).
Results: Based upon our data and calculation, the estimated incidence rates (per 100,000 person-year) for all donations were: 2.79 for HIV, 0.93 for HCV, and 30.73 for HBV; while the estimated residual risks per donation were: 1 in 2.38 million for HIV, 1 in 8.00 million for HCV and 1 in 58,000 for HBV. The actual risk for transfusion transmitted HBV could be higher since cases of occult hepatitis B infection (OBI) were not addressed in the mathematical model. During the study period, 101 cases of OBI were detected (1 in 5120 donations); 12 in first-time donors and 89 in repeat donors.
Conclusions: In comparison with the estimated residual risks of TTI in 2006–07, the current residual risks were substantially lower on account of the decreasing incidence of HIV and HBV observed in the donor population. Besides, shortening of the infectious window period of each virus after implementation of routine ID-NAT has translated into protection of blood safety and reflected in the lessening residual risks; in particular for HBV where OBI cases were also detected. With better understanding of the dynamics of HBV viraemia, the adjustment factor for the transient nature of HBV markers when assayed by ID-NAT during acute HBV infection, recently proposed Weusten and co-workers, was applied in this study and this has contributed to the lower estimated residual risk.

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THE COBAS® TAQSCREEN MPX TEST, VERSION 2.0 – ENHANCED MULTIPLEX BLOOD SCREENING PERFORMANCE ON THE COBAS S 201 SYSTEM

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Background: The CE Marked cobas® TaqScreen MPX Test, version 2.0 (MPX Test, v2.0) offers automated, real-time PCR viral discrimination of HIV, HBV and HCV with high sensitivity and specificity in a qualitative blood screening test. This test utilizes the fully automated cobas s 201 system. The MPX Test, v2.0 has important changes relative to the CE-IVD and FDA licensed cobas® TaqScreen MPX Test, and improves blood screening laboratory workflow. The system collects data from target-specific fluorescent probes at four independent wavelengths instead of 2, allowing identification of viruses during primary screening, thereby eliminating the need to perform separate viral target discriminatory tests. Extraction and PCR throughput are increased 10% with a new multiplexed HIV-1 Group M, HBV and HCV control. Target primer and probe sequences and reaction chemistry have been modified to improve overall inclusivity.
Aims: Develop and verify performance characteristics of a new multiplex assay to simultaneously detect and identify HIV, HBV and HCV for blood screening, and improve laboratory efficiency by eliminating the additional testing required for viral identification.
Methods: Automated, generic nucleic acid extraction was used for isolation of viral DNA and RNA which were amplified and detected by automated, real-time, TaqMan® PCR. Analytical sensitivity was determined using WHO International Standards of HIV-1 Group M, HBV HCV, and HIV-2. Standard traceable to the CBER Panel was used for HIV-1 Group O. Clinical specificity was assessed by testing 500 individual seronegative specimens from healthy donors. Archived donor specimens (200 for each of HIV-1, HBV and HCV) were tested to determine clinical sensitivity.
Results: The 95% limits of detection by Probit analysis were 46, 2.3, 6.8, 8 IU/ml, and 18 copies/ml for HIV-1 Group M, HBV, HCV, HIV-2, and HIV-1 Group O respectively. Preliminary clinical specificity was 99.8%. No interference was observed in samples with albumin (96 g/l), bilirubin (500 mg/l), hemoglobin (5 g/l), triglycerides (33 g/l), human DNA (4 mg/l) and exogenous medications. Red blood cell contamination up to 2.5% did not interfere with the MPX Test, v2.0. For seropositive specimens, reactivity rates were 98.5%, 100% and 97% for HIV, HBV and HCV respectively. Equivalent sensitivity was observed in EDTA plasma and serum. The MPX Test, v2.0 on neat samples detected HIV-1 Group M, HCV and HBV prior to State of Art serology by an average of 14, 28 and 22 days respectively.
Conclusions: The MPX Test, v2.0 enables automated, high sensitivity screening of blood donations, and provides real-time viral discrimination.
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EVALUATION OF THE ROCHE COBAS® TAQSCREEN MPX TEST V 2.0

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Background: The Roche cobas® TaqScreen MPX test, version 2.0 (MPX v2.0) is a qualitative, in vitro, multiplex test for the detection of HIV-1 groups M and O RNA, HIV-2 RNA, HCV RNA, HBV DNA in human plasma. Plasma may be screened either individually or in pools. The test uses a generic nucleic acid preparation and the extracted nucleic acids are amplified and detected using real-time PCR. The test runs on the Roche automated, blood screening platform, the cobas® s 201 (s 201) system. The use of multi-dye technology to label specific probes, which are detected in specific channels on the instrument, enables the simultaneous detection and identification of HIV, HCV and HBV in a single test. However, the test will not discriminate between HIV-1 group M, HIV-1 group O and HIV-2 since all these targets are detected in the same channel.
Aims: The study was designed to determine the analytical sensitivity of the test for HIV-1, HCV and HBV and the clinical sensitivity and specificity of the test by screening plasma samples from blood donors. In addition, the reproducibility of the test on the s 201 instrument was evaluated.
Methods: Serial dilutions of secondary standards for HIV-1, HBV and HCV, those were directly traceable to the WHO International Standards, were tested on three different days (eight replicates per day). The analytical sensitivities of the test for these three viruses were determined using Probit analysis.
Thirty-six replicates of dilutions of HBV, HCV and HIV-1 containing 30, 50 and 160 IU/ml respectively, were tested individually on four different days (8 replicates/day) in order to evaluate the reproducibility of the test.
The clinical sensitivity and specificity of the test for the Taiwan blood donor population were determined by screening about 6000 random, blood donors in pools of six. Plasma samples from reactive pools were tested individually in order to identify the contaminated donation and the viral contaminant.
Results: The 95% LOD for the test were 4.7, 4.7 and 57.4 IU/ml for HBV, HCV and HIV-1 respectively. In the reproducibility study, all 36 replicates were detected for each virus. For the clinical sensitivity determination, 5951 donors were screened in pools of six and eight donors were NAT-only yield cases. All these donors were positive for HBV. The results are summarized in Table 1 The NAT-only yield rate for this study was 0.13% (1:744).
The overall test failure rate was 1.9%. The failures were due to either instrument errors or failed batches (due to failed batch controls). The IC failure rate for samples from valid batches was 0.5%.

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TRUE POSITIVITY OF ANTI-HCV ELISA REACTIVE BLOOD DONORS – A PROSPECTIVE STUDY DONE IN WESTERN INDIA

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Background: In India, most of the blood centers do test for Hepatitis C virus (HCV) by Enzyme Linked Immunosorbent Assay (ELISA) method. A significant number of safe donations are removed from the blood supply, because of the false reactive anti-HCV screening test results.
Aims: (i) This study aimed to assess if the HCV seropositive donors were confirmed positive by Nucleic Acid Testing (NAT) and Recombinant Immunoblot Assay (RIBA) method. (ii) The study correlated the results of samples reactive by single ELISA kit vs those by two ELISA kits with NAT and RIBA results. (iii) The study correlated sample-to-cut-off (S/CO) ratio of ELISA results with those of NAT and RIBA. (iv) The study also evaluated if there was any association of Alanine Amino Transferase (ALT) levels with that of NAT and RIBA results.
Method: A total of 68,951 blood donors were screened by two anti-HCV ELISA kits over a period of 15 months at three blood centers in Ahmedabad in Western India. A total of 140 anti-HCV ELISA reactive samples were included in the study. These 140 samples were tested by NAT. The NAT negative samples were further tested by RIBA. Analysis of samples reactive in only single ELISA kit vs those reactive by both ELISA kits was done. Correlation of S/CO of the the individual ELISA kit was done with NAT and RIBA result. Correlation of ALT levels with that of NAT and RIBA results was also done.
Results: 1. Out of 140 anti- HCV ELISA reactive samples, a total of 16 (11.43%) were positive by NAT. The RIBA results of 124 NAT negative samples were: 6 (4.84%) positive, 92 (74.19%) negative and 26 (20.97%) indeterminate.
2. Out of 140 anti-HCV ELISA reactive samples, 87 samples were reactive by single ELISA kit only. None of these were positive by NAT or RIBA. Of the 53 samples reactive by both ELISA kits, 16 (30.19%) were NAT positive and 6 (16.22%) were RIBA positive (of the NAT negative samples).
3. Significant number (81.1% in 1st kit and 88.73% in 2nd kit) of NAT negative samples had S/CO ratio <3.8. Significant number (87.5% in 1st kit and 56.25% in 2nd kit) of NAT positive samles had S/O ratio more than or equal to 3.8. Number of NAT positive samples with ELISA S/CO ratio lying on either side of 3.8 varied for the two kits. It was observed that for both the ELISA kits, significant number of RIBA negative samples had S/CO ratio <3.8.
4. Significant correlation of ALT results with NAT results was also observed. For RIBA, correlation of ALT with RIBA negative results was seen but not with RIBA positive results.

Table 1: NAT and RIBA results of 140 ELISA Reactive sample

No. of ELISA Reactive Samples	NAT (n=140)		RIBA (n = 124)		
	Negative	Positive	Negative	Indeterminate	Positive
140	124	16	92	26	06
	88.57%	11.43%	74.9%	20.97%	4.84%
* Confirmed HCV positive by NAT &/ RIBA : 22					

Table 2: NAT/ RIBA results of single vs both ELISA kits

	Samples reactive in single ELISA kit only	Samples reactive in both ELISA kits
Total No. 140	87	53
NAT positive	00	16
RIBA positive	00	06

Conclusion: Only a minority of blood donors with repeatedly reactive anti-HCV screening test are positive by confirmatory testing, but all these blood units are discarded as per existing legal provisions in India. Efforts should be made to retain these donors and also donor units.

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DEVELOPMENT OF THE NEW COBAS® TAQSCREEN MPX TEST, VERSION 2.0 – FOR CADAVERIC DONORS IN BLOOD SCREENING

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Background: The Roche cobas® TaqScreen MPX Test, version 2.0 (MPX Test, v2.0) for cadaveric donors is under development and is designed to offer automated, real-time PCR viral discrimination of HIV, HBV and HCV with high sensitivity and specificity for blood screening. This test utilizes the fully automated cobas s 201 system. The MPX Test, v2.0 has important changes relative to the CE-IVD and FDA licensed cobas® TaqScreen MPX Test and improves blood screening laboratory workflow. The system collects data from target-specific fluorescent probes at four independent wavelengths instead of 2, allowing identification of viruses during primary screening, thereby eliminating the need to perform separate viral target discriminatory tests. Extraction and PCR throughput is increased 10% with a multiplexed HIV-1 Group M, HBV and HCV control.
Aims: Develop and determine the performance characteristics of a test intended for screening plasma and serum of potential organ and tissue donors. Determine suitability for specimens obtained while the donor's heart is still beating, and for blood specimens from cadaveric donors.
Methods: Automated, generic nucleic acid extraction was used to isolate viral DNA and RNA which were amplified and detected by automated, real-time, TaqMan® PCR. Analytical sensitivity in cadaveric specimens was assessed using Standards traceable to WHO International Standards of HIV-1 Group M, HBV and HCV. Standards traceable to the CBER Panels were used for HIV-1 Group O and HIV-2. Clinical specificity was assessed by testing individual cadaveric plasma specimens. Clinical sensitivity and reproducibility were evaluated by testing individual moderately and highly hemolyzed cadaveric EDTA plasma specimens spiked with HIV, HBV, and HCV. Each test sample was diluted 1:5 in cadaveric specimen diluent prior to testing.
Results: The observed lowest level with ≥95% reactive rate in moderately-hemolyzed cadaveric plasma were 250, 30, 60 IU/ml, 120 and 200 copies/ml for HIV-1 Group M, HBV, HCV, HIV-1 Group O, and HIV-2 respectively. Clinical specificity for the moderately and highly hemolyzed cadaveric specimens was 100%. No statistically significant differences were observed in the reactive rates for living and cadaveric specimens when comparing reagent lots and instruments/operators ($P > 0.05$). Analytical sensitivity was statistically equivalent in cadaveric plasma and serum specimens ($P > 0.05$). For the sixty individual positive cadaveric specimens tested the reactivity rates were 98% for HIV-1 Group M and 100% for HBV and HCV.
Conclusions: The MPX Test, v2.0 enables automated, high sensitivity screening of cadaveric donors, and provides real-time viral discrimination.

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COMPARISON OF INDIVIDUAL NUCLEIC ACID TESTING (ID-NAT) AND ELISA FOR DETECTION OF HIV, HBV AND HCV IN BLOOD DONORS AT TERTIARY CARE CENTRE

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Background: Implementation of nucleic acid testing in blood bank setting is a major challenge for a developing nation like ours. Blood safety and cost effectiveness is the prime concern in the current scenario. Looking into the increasing trend of positivity for HIV, HBV and HCV markers, evaluation of whole blood donor testing by ID-NAT for simultaneous detection of HIV, HBV and HCV was undertaken and compared with serological tests for the same.
Aim: To compare ELISA with ID-NAT testing for detection of HIV, HBV and HCV among blood donors at Dr Ram Manohar Lohia hospital for an additional level of blood safety.
Methods: A retrospective study was conducted on 10,130 donors (57.3% voluntary and 42.7% replacement donors) at blood bank in our hospital during the period of July 2010 to May 2011. In addition to the routine mandatory screening tests as per regulatory requirements which include screening for anti-HIV, HBsAg and anti-HCV, all donor samples were subjected to INDIVIDUAL NUCLEIC ACID TESTING (ID-NAT) for the same markers. NAT was done by Transcription Mediated Amplification (TMA) technology using multiplex and discriminatory testing by Procleix Ultrio Assay (Chiron Corp. Emeryville, CA, USA) for simultaneous detection of HIV, HBV and HCV.

Results: A total of 10,130 samples were tested both by ELISA and NAT, out of which 257 (2.53%) were seroreactive and 215 (2.12%) were NAT reactive.87 (0.85%) samples were seroreactive but NAT nonreactive. There were 25 (0.24%) NAT reactive but serononreactive (NAT yield) cases: 1 HIV, 1 HCV and 23 HBV. The NAT coinfection yield cases (seroreactive for one but NAT two reactive markers) was 23; 8 HIV, 5 HCV and 10 HBV whereas only one coinfection was detected by ELISA; 1 HBV/HCV. There were four NAT coyield cases (NAT two reactive but Serononreactive): one HIV-HBV and three HBV-HCV.
Conclusion: This is the pioneer study from a Government tertiary care centre of the region. Although antigen or antibody based screening test has high sensitivity, antigen-antibody seronegative transmission of viral infections can still occur due to window period. The NAT yield rate for each HIV and HCV was 0.1:1000 while for HBV was 2.3:1000 in our centre. Thus NAT together with serology can bring blood safety towards close to zero risk blood supply. ID-NAT is expected to contribute significantly to scientific knowledge on the detection and dynamics of HIV, HBV and HCV infections.

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PREVALENCE OF THE FREQUENCY OF SCREENING POSITIVE RESULTS TO TRANSFUSION TRANSMISSIBLE DISEASES MARKERS AMONG BLOOD DONORS TO NATIONAL CANCER INSTITUTE OF SRI LANKA – FROM 2006 TO 2010

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Background: National Cancer Institute, the only hospital in Sri Lanka totally dedicated to cancer care. It has became the leading centre for catering treatment for cancer patients from all over the country. Transfusion of blood and blood products has been a one of the main stray of treatment specially those who are at marrow suppressive state. Therefore hospital blood bank has demanding requests to meet such a needy environment. Hospital blood bank has its own way of donor recruitment system by conducting mobile donor campaign and inhouse collection. Excluding of HIV, HBsAg, VDRL and HCV infections are extremely important for safe transfusion. Testing of blood units by screening methods for above infections has markedly decreased the incidence of transfusion transmitted infections.
Aims: To determine the prevalence of HIV, HBsAg, VDRL and HCV screening positive donations among voluntary blood donors who donated to our blood donor programme following effective donor counselling.
Methods: A descriptive retrospective study was carried out by analysing all donated test samples from the period of 2006 to 2010. HIV and HBsAg screening tests were carried out by ELISA, Particle agglutination, and Rapid antigen assessment methods. Hep C screening was done by ELISA and Rapid antigen tests. Cardiolipin and the carbon agglutination tests were used to detect VDRL screening positive cases. A details of each screening tests were collected and analysed using SPSS data analysis tool.
Results: During the period of study, there were 79,964 blood donors gave whole blood and it consists of 71,639 (89.58%) voluntary blood donors to the mobiles and 8325 (10.41%) to inhouse collection. Total of screening positive tests detected in HIV test is 0.21% (174), and HBsAg is 0.14%. The number of Hep C screening tests were 263 (0.37%) and total number of VDRL were 236 (0.30%). There is no statistical significant difference between each screening method to detect screening positive cases for a particular test.
Conclusions: There is no significant different in screening test positive results between in house and mobile blood donors. The prevalence of screening positive donors among volunteer donors were not significantly changed over the time and may be due to effective donor selection. The lower prevalence rate of transfusion transmittable infection in our donor population brought safe blood transfusion to the needy patients. Introduction of NAT screening would be helpful to minimize further risks.

P-165
STUDY OF NAT SCREENING IN BLOOD DONOR IN BLOOD CENTER OF ZHEJIANG PROVINCE, CHINA

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Background: Nucleic acid amplification test (NAT) for HIV-1, HCV, and HBV can significantly improve the safety of the blood supply by detecting infectious blood donated during the seronegative window periods. Although NAT has not yet been mandated in China, we are the part of the largest NAT study so far conducted in China.
Aims: To evalute the feasibility of NAT for routine blood screening in blood center, to evaluate the ULTRIO Assay on TIGRIS for its detection capability of seronegative yield cases and clinical specificity for routine blood screening.
Methods: NAT platform (The Procleix Tigris system in individual donation format) for HBV, HCV and HIV-1 testing of blood donor was implemented on August 1st 2010. All samples were also tested by serology using two different EIA assays for each of the three viruses. Samples with discordant results between ULTRIO and serology were further tested with alternative (Alt) NAT assays. Donor follow-up testing for those potential yield cases were further evaluated when possible.
Results: A total of 74,835 donations were included in this study until February 23rd 2011. 208 (0.28%) were ULTRIO reactive; and of these, 105 were also seropositive (14 anti-HIV, 23 anti-HCV, and 68 HBsAg); of the 103 seronegative/NAT reactive samples, 48 were ULTRIO and dHBV discriminatory assay reactive and nine were ULTRIO repeat reactive but nondiscriminated. All them are classified as potential ULTRIO yield cases; the remaining 46 ULTRIO-reactive seronegative samples were non-repeatable and hence considered as false positive by the ULTRIO Assay (6/10,000). Of the 57 potential ULTRIO yield cases, 45 were reactive in Alt NAT, 11 were Alt NAT nonreactive but were ULTRIO or dHBV repeat reactive using a plasma bag specimen, and one FP ULTRIO.
Conclusion: Our data demonstrate that the potential ULTRIO yield rate for HBV was 1:1336 among blood donor populations in Hangzhou China. Implementation of NAT provided a significant increment in safety relative to HBV serologic screening. Our experiences also show that ID-NAT using ULTRIO Assay with TIGRIS platform was feasible for routine blood screening.

P-166
POSSIBILITY OF DETECTION OF ACTIVATED NEUTROPHILS WITH REACTIVE OXYGEN SPECIES PRODUCTION BY USING WBC DIFFERENTIATION SCATTERGRAM ON SYSMEX AUTOMATED HEMATOLOGY ANALYZER

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Background: One of the most important pathogenetic factors for acute lung injury (ALI) including transfusion-related acute lung injury (TRALI) is neutrophil and pulmonary endothelial cell activation induced by transfusion, resulting in increased vascular permeability through the actions of reactive oxygen species (ROS) and other molecules. Additionally, it has been reported that neutrophils change their morphology and composition of cytomembrane by activation.
The leukocyte differentiation function on the Sysmex automated hematology analyzer exposes leukocytes to a special surfactant and fluorescent dye, displays differences in the effects of the resistance to the surfactant and the residual organelles among different types of leukocytes by using WBC differentiation scattergrams (two-dimensional scatter diagrams that show differences in side scatter and fluorescence intensity) with flow cytometry, and thereby performs leukocyte classification.
Aims: In this report, we investigated that the possibility of detecting redundant neutrophil activation, which is one of the most important pathogenetic factors for ALI, development by using WBC-classification scattergrams.
Methods: Peripheral blood from healthy individuals was centrifuged to isolate a polymorphonuclear (PMN) cell fraction. Whole blood or the PMN fraction was activated with phorbol myristate acetate (PMA) or formylmethionylleucylphenylalanine (fMLP) and then analyzed using the Sysmex XE-2100, automated hematology analyzer. PMN fraction was stained with CD16b (a neutrophil specific marker) and treated with APF (a fluorescent reagent for detecting ROS), followed by activation with PMA or fMLP. These samples were morphologically analyzed under a confocal laser scanning microscope (CLSM) and electron microscopes (EM). The samples were subsequently treated with a special reagent for XE-2100 and then analyzed with a general-purpose flowcytometer (FCM).
Results: The PMN fraction was stimulated with PMA or fMLP and analyzed using XE-2100; in the WBC differentiation scattergram obtained in this analysis, a new cluster appeared in the higher of side scatter in addition to neutrophil original cluster. This new cluster was also observed in whole blood samples. FCM analysis of the PMN fraction revealed that the cells in this new cluster were CD16b-positive and APF-positive, thereby indicating that these cells were activated neutrophils with ROS production. CLSM and EM findings revealed that the ROS production occurred in granules and that these activated neutrophils had vacuoles within them.
Conclusions: Activated neutrophils with ROS production were found to have vacuoles in their cytoplasm. This finding suggested that activated neutrophils were detected as another cluster at a location with a higher side scatter than that shown by the ordinary neutrophil cluster on WBC differentiation scattergram probably because these vacuoles increased the side scatter. These results indicated that the Sysmex automated hematology analyzer has the potential to serve as a tool for detecting activated neutrophils, which are the most important pathogenic factors for ALI.

P-167

AUTOMATED DETECTION OF WNV, HIV-1, HIV-2, HCV, AND HBV WITH THE PROCLEIX ULTRIO ELITE AND PROCLEIX WNV ASSAYS ON THE PROCLEIX PANTHER INSTRUMENT

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Background: The Procleix WNV and Procleix Ultrio Elite Assays, qualitative nucleic acid tests (NAT) for blood screening, are being developed for the Procleix PANTHER instrument to test for West Nile virus (WNV), HIV-1/2, HCV, and HBV. The PANTHER instrument is a fully automated NAT testing platform that allows random access of reagents and samples and has a small physical footprint. The system processes 275 tests in 8 h and multiple assays by directly sampling from the blood collection or pool tube. The WNV Assay is licensed in the US and CE-Marked, while Ultrio Elite is currently under development. The PANTHER Instrument is CE-Marked in Europe for diagnostic applications but currently under development for use in blood screening.
Aims: To determine the preliminary performance characteristics of blood screening assays on the PANTHER instrument and to evaluate the instrument's random access capability, panels of HIV-1/2, HCV, HBV, and WNV were tested.
Methods: Panels were made of HIV-1 Group M (subtypes B, G and H), Group O, and Group N, CRF02_AG and CRF01_AE, the WHO HIV-2 RNA International Standard (08/150), HCV (genotypes 1 and 2), and HBV (subtypes A, B, and C) (BBI, NIBSC, DDL, ProMedDx) and tested with Ultrio Elite. Results were analyzed by PROBIT (SAS 9.2) to determine limits of detection. Clinical specimens (n = 101) positive for HIV-2 by serology (Bio-Rad Multispot HIV-1/HIV-2 Rapid test), (Boca Biolistics) were tested undiluted. PANTHER instrument operation and throughput was tested with negative, WNV and HIV-1 positive panels with the WNV and Ultrio Elite Assays.
Results: Predicted 95% detection levels for the HIV-1 subtypes tested in this study ranged from 1.2 to 15.6 copies/ml. The 95% detection levels of HCV-1a and -2b were estimated at 34.0 and 64.1 copies/ml, respectively. Results with HBV subtypes ranged from 26.3 to 88.1 copies/ml. The HIV-2 WHO standard was predicted to have 95% detection at 11.2 IU/ml. Of the 101 HIV-2 positive clinical specimens, valid results were observed in 100 specimens of which 57 (57.0%) were reactive using the Ultrio Elite Assay.
The 80 negative, five WNV positive, and five HIV-1 positive specimens were randomly loaded (not as a batch) onto the PANTHER Instrument to test with WNV and Ultrio Elite Assays. Tests could be added while continuously processing other samples. The instrument automatically took separate aliquots from each specimen without user intervention and performed both assays with 100% agreement to expected results. Testing of the 90 specimens with each of the two assays, and the Ultrio Elite and WNV Assay Calibrators (195 total tests) completed in 6 h, 45 min.
Summary/conclusions: The Ultrio Elite Assay showed sensitive detection of HIV-1, HCV and HBV genetic variants. Detection of HIV-2 RNA in clinical specimens was consistent with performance reported with other commercial assays. The ability to test for WNV, HIV-1/2, HCV, and HBV from a primary specimen tube and the random access capabilities of the platform were demonstrated. The WNV and Ultrio Elite Assays on the PANTHER instrument may provide a flexible and sensitive solution for NAT blood screening.

P-168

DEVELOPMENT OF A COMPLETE AUTOMATED BARCODE CONTROLLED CE-CERTIFIED MINI POOL NAT EXTRACTION SYSTEM BY THE GERMAN RED CROSS BADEN-WUERTTEMBERG – HESSEN NAMED ZELOS ×100

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Background: Recipients are on risk to be infected by transfusion transmitted relevant virus infections, if the donor is still in the diagnostic window period. Therefore a major challenge in transfusion medicine is to develop a new screening technologies to reduce the diagnostic window period to a minimum. The development of real-time nucleic acid testing (NAT) systems represents a hallmark in transfusion medicine to improve blood safety. The German Red Cross Blood Donor Services Baden-Wuerttemberg – Hessen was one of the first who implemented mini pool NAT in 1997 on a voluntary basis for hepatitis B, hepatitis C and HIV-1 for all blood donations. Based on an experience of more than 10 years, a fully-automated system (named Zelos ×100) was developed in 2010 and is used for blood donor screening for hepatitis A, hepatitis B, hepatitis C, HIV-1 and Parvovirus B19 blood donor screening in mini pools of 96 samples per pool.
Aim: The study presents an update after one and a half year of blood donor screening with the automated mini pool NAT system named Zelos ×100. Data performed were used for risk analysis for the automated NAT screening system.
Methods: The new automated extraction system based on a high volume bead extraction process with a 95% level of detection (LOD) of 0.6, 6.8 and 8.9 IU/ml for HBV, HCV and HIV-1, respectively. The new automated NAT screening system was introduced in 2010 and replaced the manual mini pool NAT system which based on an enrichment centrifugation for 1 h at 48,000 *g*. The analytical sensitivity for HBV was improved by one log phase with the new system.
Results: Up to date, 3 million donations were screened with the automated systems. In total 1, 6, and 1 NAT only reactive blood donations were detected by Zelos ×100 for HAV, HCV and HIV-1, respectively. The virus load was in a range of 1.4×10^4–2.5×10^5 IU/ml for HCV, 180 IU/ml for HAV and 3×10^4 IU/ml for HIV-1. Based on window period models, the residual transfusion transmitted infectious risk was calculated to 1:458,082, 1:10.88 million and 1:4.3 million for HBV, HCV and HIV-1, respectively.
Conclusion: The development of a new automated MP-NAT system including a CE-certification was successful. With the Zelos ×100 system, blood safety is improved, by excluding residual risks for mix-up errors, by an enhanced analytical sensitivity for HBV to 0.6 IU/ml and by a reduction of the time schedule for mini pool deconstruction (within one working shift). After 1.5 years blood donor screening with the new NAT system, eight NAT only positive blood donations were detected and transmission of transfusion relevant viruses were prevented. The change from enrichment centrifugation to a bead extraction process represents a state-of-the-art procedure, comparable to other commercially available MP-NAT systems (like Roche s201 or Novatis Tigris system). The new system offers several opportunities for additional improvements in blood safety by reduction of pool sizes to ID-NAT or implementation of new parameters.

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SYPHILIS SEROPOSITIVE DONORS HAVE HIGHER SEROPREVALENCE OF HBV, HCV, HTLV AND HIV COMPARE WITH SYPHILIS SERONEGATIVE DONORS – 5 YEARS EXPERIENCE IN SOUTHERN TAIWAN

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Background: As syphilis, hepatitis B virus (HBV), hepatitis C virus (HCV), human T-lymphotropic virus (HTLV) and human immunodeficiency virus (HIV) share the same transmission route, the patients with syphilis may have higher co-infection rate with these four viral infections compare to the patients without syphilis. Due to the co-positive rate of syphilis serologic marker with these four viral serologic markers of volunteer blood donors in Taiwan still unknown, we conducted this study.
Method: We retrospectively analyzed syphilis serologic marker and these four viral serologic makers in all volunteer blood donors in Kaohsiung Blood Center of Taiwan Blood Services Foundation (TBSF) from January 2006 to December 2010. The serologic marker for syphilis, HBV, HCV, HIV, and HTLV was TPPA, HBsAg, Anti-HCV, Anti-HIV and Anti-HTLV respectively. Chi-square statistics were employed to compare HBV, HCV, HIV and HTLV serologic markers in blood donations that were syphilis seropositive and seronegative.
Result: There were 1,552,813 blood donations from 452,029 donors from January 2006 through December 2010. Of these, 1371 (0.09%) donations were syphilis seropositive and 1,551,442 (99.91%) were syphilis negative. In syphilis seropositive donations, the seropositive rate of HBV, HCV, HIV, and HTLV was 2.6%, 2.0%, 0.6%, and 0.1% respectively. Compared to seronegative syphilis donations, the seropositive rate of HBV, HCV, HIV, and HTLV was 0.4%, 0.1%, 0.1%, and 0.003% respectively. The syphilis seropositive donations were statistically significantly higher seroprevalence of HBV ($P < 0.001$, OR, 7.002; CI, 5.02–9.76), HCV ($P < 0.001$, OR, 16.976; CI, 11.65–24.75), HIV ($P < 0.001$, OR, 5.326; CI, 2.65–10.69), and HTLV ($P < 0.001$, OR, 5.473; CI, 1.36–21.98) than syphilis seronegative donations.
Conclusion: The seropositivity of HBV, HCV, HTLV and HIV was significantly increased among syphilis seropositive donors. In consideration of possible viral window period infection, it is necessary to look back and quarantine of previously collected units of blood and blood components from donors who subsequently become syphilis seropositive.

P-170
SYPHILIS TESTING OF BLOOD DONORS IN SINGAPORE

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Background: Syphilis screening is one of the mandatory tests performed for blood donors in Singapore. In our Blood Services Group, our routine syphilis testing algorithm includes the initial screening using a treponemal test, the treponemal chemiluminescence microparticle enzyme immunosaay (CMIA, ABBOTT ARCHITECT Syphilis TP) and retest positive samples for confirmation by another treponemal test, the Treponemal Pallidum Particle Agglutination (TPPA) test together with a nontreponemal test, the Venereal Disease Research Laboratory (VDRL) test. Donations that have a reactive TPPA and/or VDRL result are not used for transfusion and donors are permanently deferred.
Aim: Our objective is to study the test reactive rates and to review the syphilis testing algorithm and donor deferral strategies.
Methods: Donations tested using CMIA between the period, April 2008 to December 2010 was retrospectively evaluated. The test reactive rate was tabulated and the number of donors deferred using the current syphilis testing algorithm were reviewed and compared to the testing algorithm proposed in the Food and Drug Administration's (FDA) Draft Guidance titled 'Revised Recommendations for Donor and Product Management Based on Screening Tests for Syphilis, June 2003'.
Results: Our current syphilis testing algorithm is similar to the recommended testing algorithm of the FDA Draft Guidance. The only difference is the donor deferral approach. A total of 294,597 donations results were reviewed. Of the 986 (0.33%) CMIA positive donations, 26 (0.009%) donations were tested positive for both TPPA and VDRL method and 76 (0.03%) donations were positive for TPPA only. One hundred and two donors with reactive TPPA and/or VDRL results were deferred permanently according to our testing algorithm. If the FDA Draft Guidance's testing algorithm is followed, 26 donors will be deferred for a minimum time period of 12 months and 76 donors may potentially re-enter the donor pool with documentation of successful treatment for syphilis.
Conclusions: The adoption of an alternative testing algorithm as proposed in the FDA Draft Guidance may allow more donors to re-enter the donor pool. However, the requirements of the documentations of successful treatment for syphilis must be clearly defined.

P-171
ANALYTICAL AND OPERATIONAL PERFORMANCE VALIDATION OF PROCLEIX TIGRIS SYSTEM AT BLOOD SCREENING LABORATORY

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Background: Performance Validation of a new testing system used in blood screening laboratory is a part of quality assurance and the most important quality activity in BTS. Understanding the performance qualification of new equipment will help operator to use new equipment correctly and efficiently and ensure smooth blood screening process.
Aim: Analytic and operational performance of Procleix TIGRIS System was validated before Nucleic Acid Testing (NAT) is implemented at blood screening laboratory, to confirm Procleix TIGRIS System's feasibility and ensure Procleix TIGRIS System running efficiently and testing process in control.
Method: The WHO International Standards for HIV-1 RNA, HCV RNA, HBV DNA were used to determine the analytic sensitivity of the Procleix TIGRIS System. Severe hemolytic and midrange lipaemic blood samples with low-load HIV, HCV and HBV virus respectively were used to evaluate the Procleix TIGRIS System in the presence of endogenous, interfering substances. High positive samples containing HBV (>106 IU/ml) were used to evaluate the robustness of Procleix TIGRIS System against cross-contamination.
Result: The 95% limits of detection (LODs) for HIV-1, HCV, HBV were 18.1 IU/ml (range from 12.2 to 36.9 IU/ml), 7.4 IU/ml (range from 4.7 to 15.2 IU/ml), 11.7 IU/ml (range from 7.6 to 22.5 IU/ml) respectively, for Procleix Ultrio Assay on TIGRIS System. It is in coincidence with the analytic sensitivity claim in PI of ULTRIO. No significant difference was observed between normal and severe hemolytic, midrange lipaemic blood samples with HIV (40 IU/ml), HCV (10 IU/ml), HBV (20 IU/ml) respectively testing on Procleix TIGRIS System. When Procleix TIGRIS System was challenged with <10% positive samples (>106 IU/ml HBV) in a run, no cross-contamination was observed in negative samples. However, false-positive result owing to the aerosol could be found when the positive samples ratio increased to 20% or 50% in a run, but negative samples weren't truely contaminated by the aerosol at all.
Conclusion: Procleix TIGRIS System was a fully automated NAT system with high sensitivity. The results of analytic performance validation of Procleix TIGRIS System before NAT implementation demonstrated our lab's capability on NAT. We also confirm that hemolytic, lipaemic blood samples will not interfere the NAT results significantly on Procleix TIGRIS System. And that there was no cross-contamination with no more than 10% positve samples in a run, which made NAT available to routine samples in our laboratory.

P-172
SEROCONVERSION SENSITIVITY OF ELISA SCREENING ASSAYS FOR HCV, HIV AND HBV ANTIGENS AND/OR ANTIBODIES

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Background: The main benefit of introducing nucleic acid amplification methods (NAT) has been the detection of highly infectious window period (WP) donations, but the cost effectiveness of this intervention is questioned and blood transfusion in many parts of the world still relies on serologic screening for HBV, HIV and HCV antigens and/or antibodies as the sole markers to prevent transfusion transmitted viral infection. Moreover, it is in this context that the use of combo ELISAs as an alternative method to reduce transfusion risk of WP donations, particularly in developing countries, can be recommended, as it has been already done by the World Health Organization expert group in their Recommendations for screening for TTIs. Therefore the use of highly sensitive HIV, HBV and HCV screening assays is crucial for efficiently avoiding transfusion transmitted infections and seroconversion panels are an useful tool to evaluate their clinical sensitivity and also compare sensitivity of different commercially available kits.
Aims: The performance in terms of clinical sensitivity of Murex HBsAg v3, Murex HIV Ag/Ab Combination, Murex HCV Ag/Ab Combination ELISA assays were evaluated, and compared with other well established blood screening assays on the market.
Methods: The Murex HBsAg v3 is a 4th generation ELISA assay for the determination of Hepatitis B surface Antigen. The Murex HIV Ag/Ab Combination Assay is a microplate based assay for the simultaneous detection of antibodies to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O), detection of anti-HIV-2 antibodies and HIV core antigen in the same well. Murex HCV Ag/Ab Combination is a 4th generation ELISA assay for the simultaneous detection of hepatitis C (HCV) core antigen and anti-HCV antibodies.
A set of 58, 39 and 75 seroconversion panels commercially available for HBV, HIV, and HCV respectively were evaluated.
Results: On 58 panels, Murex HBsAg assay detected an additional five and 10 seroconversion panel members compared to the Architect HBsAg qualitative and quantitative assays respectively. On 28 and 29 panels Murex HBsAg assay showed an higher sensitivity (135 and 118 positive bleeds) than Vidas HBsAg (108 positive bleeds) and Roche HBsAg (103 positive bleeds) respectively. Analysis of 39 panels tested showed that the Murex HIV Ag/Ab (140 bleeds positive) had excellent sensitivity compared to PRISM and Architect (134, 141 positive bleeds). Comparison to the p24 Ag only assays from Coulter and Murex using NAT positive/Antibody negative samples showed the Murex HIV Ag/Ab assay to be broadly equivalent (Mean delay v NAT 6.03 days, Coulter HIV Ag 6.67 days, Murex HIV Ag 7.77 days). Analysis of 75 HCV panels tested showed that the Murex HCV Ag/Ab had a superior sensitivity compared to Ab only HCV assays (on average 177 bleeds earlier) and a better specificity.
Conclusions: The Murex ELISA assays demonstrated to be a state of the art immunoassays suitable for the screening of serum and plasma samples, with comparable performance with chemiluminescence immunoassays. The enhanced sensitivity of combination antigen could represents a viable alternatives for countries with limited financial resources.

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This abstract has been withdrawn.

P-174
ANALYSIS OF SYPHILIS SCREENING RESULTS ON BLOOD DONORS IN QINGDAO AREA DURING 2008–2010

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Background: The incidence of syphilis rises year by year in Qingdao. syphilis antibodies (anti-TP) response is the second factor of blood rejection.

Aims: To investigate the syphilis antibodies (anti-TP) response rate among voluntary blood donation in Qingdao area, to decrease blood scrap which is caused by syphilis antibodies response. and to reduce blood waste.
Methods: For the period 2008–2010, Detected syphilis antibodies (anti-TP) from 298,234 blood donors during 2008–2010 with Enzyme-linked immunosorbent assay (ELISA), made statistical analysis on the results according the level of education, occupational distribution, and the season for blood donation.
Results: The anti-TP response rate was 0.33% among blood donors in Qingdao, according to educational level the rate of anti-TP was contrary to the response rate, that is to say, the lower level of education was with a higher anti-TP reaction rate; Antibody response rate in ascending order was student, soldier, staff, individuals and unemployed; In the third season of each year, the response rate increased significantly and achieved the highest level of 1 year.
Conclusions: We should make intervention and education to the blood donors according their different education and different occupation;We should make preliminary blood screening in the high response rate season from July to September, so that the blood scrap rate will be reduced, thereby reducing the waste of blood resources.

P–175
THE ALERT SYSTEM FOR TESTING TRANSFUSION-RELATED VIRAL INFECTION ON THE ELECTRIC MEDICAL RECORD

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Background: Testing for viral infection (HBV, HCV, HIV) in patients who have received transfusion is a recommended procedure in Japan. To detect transfusion related viral infection, a blood test is performed 2–3 months after blood transfusion. Our institute uses two ways to provide the clinical physician concerned with information about the patient's transfusion history for further post-transfusion testing. The first is using the record of the patient concerned. The second is using the alert system on the electronic medical record.
Aim: To examine the efficacy of these two ways
Method: Patients transfused between September 2008 and May 2010 were studied (3537 patients). The period was divided into three terms. Period 1 is the period before patient records were sent (September 2008–December 2008). During this period, the blood, transfusion division did not send patient information to the clinical physician. Period 2 is the period during which patient records were transmitted (January 2009–February 2010). The patient data from around 2 months after the blood transfusion were sent to the clinical physician every month. Period 3: The period when the electronic alert system was used (March 2010–present). The alert system is part of the electronic medical record. The patient who received blood transfusion 60–150 days previously is picked up by this alert system, and an alert signal is shown on a panel of the electronic medical record. The rate of testing viral infection after blood transfusion is then calculated. The test items are HBV: HBs-Ag or HBV-DNA (NAT); HCV: HCV Core-Ag or HCV-RNA (NAT); HIV: HIV-Ag/Ab.
Results: The rates of post-transfusion testing for viral infection are shown in the Table 1 below.

Table 1: Result

	Period 1	Period 2	Period 3
Group 1: Test of HBV+HCV (%)	21.3	22.0	32.0
Group2: Test of HBV+HCV+HIV (%)	7.9	8.1	20.9

Conclusion: The alert system on electronic medical records is more effective for performing transfusion-related viral infection tests.

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This abstract has been withdrawn.

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4.2 Hepatitis B (HBV)

P–178
SEROLOGICAL CHARACTERISTICS OF HEPATITIS B SURFACE ANTIGEN POSITIVE HBV DNA NEGATIVE DONATIONS IN HONG KONG CHINESE

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Background: The population in Hong Kong has a high prevalence of hepatitis B virus (HBV) infection, mostly acquired vertically during early childhood. To enhance blood safety, the Hong Kong Red Cross Blood Transfusion Service implemented nucleic acid test (NAT) screening for HBV DNA on individual donor samples in April 2007. A subset of donations with HBsAg positive HBV DNA negative assay results were identified.
Aims: To characterize the serological profile of these HBsAg+ HBV DNA- samples from Hong Kong Chinese population.
Methods: Routine blood screening for HBsAg was performed by the most sensitive serologic assay using Abbott PRISM by ChLIA. Repeatedly reactive samples were confirmed by neutralization. All donations were screened by NAT on individual samples (ID-NAT) using the HIV-1/HCV/HBV triplex Procleix ULTRIO Assay on TIGRIS platform (Novartis Diagnostics) (detection limit: 10.4 IU/ml; 95% probability: 9.2–12.2 IU/ml). NAT reactive samples were retested with discriminatory assays to determine viral specific reactivity. Available archive samples with discordant results (HBsAg+ HBV DNA–) were further tested with triplex Procleix ULTRIO PLUS Assay and discriminatory tests by ID-NAT (detection limit: 2.1 IU/ml; 95% probability: 1.7–3.0 IU/ml) and for other HBV serologic markers, i.e. repeat HBsAg and anti-HBs (Architect; Abbott), IgM and total anti-HBc (Vitros; Ortho Clinical-Diagnostics), HBeAg (Murex) and anti-HBe (Monolisa, Bio-Rad).
Results: From April 2007 to September 2009, 517,072 donations were collected and tested; 1815 (0.35%) of which were confirmed positive for HBsAg; of these, 166 (0.03% of all donations, 9.15% of all HBsAg+ cases) were HBsAg+ HBV DNA– by ULTRIO Assay. With regard to demographic data, 77 (46.4%) were from males and 89 (53.6%) females; 143 (86.1%) from new donors and 23 (12.9%) repeat donors; mean age and SD were 28.7 and 11.5 years old respectively. For the other serological markers: (i) repeat HBsAg: positive (n = 48/49, 98.0%), negative (n = 1/49, 2.0%), (ii) anti-HBs: <10 mIU/ml (n = 53/54, 98.1%), 17 mIU/ml (n = 1/54, 1.9%), (iii) IgM anti-HBc: negative (n = 55/55, 100%), (iv) total anti-HBc: positive (n = 53/53, 100%), (v) HBeAg: negative (n = 52/52, 100%), (vi) anti-HBe: positive (49/49, 100%). The ratios of sample reading to cut-off value (S/CO) generated from PRISM HBsAg ChLIA for the two groups of sample (HBsAg+, ULTRIO–, ULTRIO PLUS– group and HBsAg+, ULTRIO–, ULTRIO PLUS+ dHBV+ group) were significantly different (P = 0.028). The former group (n = 39) had a median S/CO of 17.29 (Range: 1.20–444.00) and the latter group (n = 31) median S/CO of 87.55 (Range: 1.30–621.90).
Conclusions: In Hong Kong, where HBV prevalence is high, when donations were screened by a highly sensitive ID-NAT, 9.1% of all confirmed HBsAg+ donations had HBV DNA levels undetectable. The serological pattern of these donations was overwhelmingly homogeneous: HBsAg+, anti-HBs < 10 mIU/ml, IgM anti-HBc–, total anti-HBc+, HBeAg–, anti-HBe+, consistent with chronic infections. As the proportion of HBsAg+ HBV DNA– samples was close to 10% of all HBsAg+ donations, HBsAg assay is still pertinent in donation screening and cannot be replaced by NAT alone. HBsAg S/CO values were higher in the ULTRIO PLUS+ group when compared with the ULTRIO PLUS– group, but a bright-line S/CO value could not be defined to predict ULTRIO PLUS results.

P–179
SIGNIFICANTLY DECREASED LEVEL OF SOLUBLE FAS IN BLOOD DONORS WITH OCCULT HEPATITIS B VIRUS INFECTION

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Background: Chronic HBV infection is diagnosed by the detection of hepatitis B surface antigen (HBsAg) and HBV DNA, while occult HBV infection (OBI) is defined by the detection of HBV DNA and the lack of detectable HBsAg in serum. The cause of OBI remains unclear. Recently, several cytokines have been shown to effectively suppress HBV gene expression and replication. Moreover, soluble Fas (sFas), an inhibitor of Fas-mediated apoptotic pathway, is suggested to be associated with the viral persistence.
Aims: In this study, we analyzed the plasma levels of IL-6, IFN-γ, TNF-α, and sFas in OBIs, asymptomatic HBsAg carriers and HBV uninfected donors to assess the possible factors correlated with OBI.

Methods: Sixty-nine donors were enrolled in this study: 23 OBI donors, 23 asymptomatic HBsAg carriers, and 23 HBV uninfected donors. Plasma levels of IL-6, IFN-γ, TNF-α were measured by MILLIPLEX MAP Cytokine Kit. The concentration of sFas in plasma samples were analyzed by Quantikine sFas Immunoassay. HBV DNA was quantified with Roche COBAS® TaqMan® HBV Test. The differences in cytokines and sFas between OBIs and uninfected donors or HBsAg carriers were assessed by Wilcoxon rank-sum tests. Spearman rank tests were utilized to evaluate correlations between sFas and clinical parameters including age, ALT, HBV viral load.
Results: It was observed that the levels of IL-6, IFN-γ and TNF-α in OBI were not significantly different from those of asymptomatic HBsAg carriers or HBV uninfected donors. Noticeably, the level of sFas showed statistically decreased in OBI compared to either asymptomatic HBsAg carriers or HBV uninfected donors. (2225 vs 3021 pg/ml, $P < 0.05$ and 2225 vs 4342 pg/ml; $P < 0.0001$, respectively). The correlations of sFas to HBV viral load, ALT, age were also examined. No significant correlation was found between sFas and clinical parameters.
Conclusions: Our results indicated that the level of sFas was significantly decreased in OBI. It was known that sFas inhibited Fas-mediated apoptosis of HBV-infected cells. Therefore, decreased apoptotic inhibition might improve clearance of HBsAg and partially explain the low levels of HBV DNA in occult HBV infection.

P-180
THE EPIDEMIOLOGICAL CHARACTERISTICS OF HBV INFECTION IN HIV INFECTED DONORS AND PATIENTS IN SICHUAN, CHINA

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Background: Hepatitis B viruse (HBV) and human immunodeficiency virus (HIV) have similar routes of transmission, enabling co-infection of the two viruses a common event. However, information on the characteristics of HBV infection in HIV infected blood donors in China is limited.
Aims: To study the epidemiological characteristics of HBV infection in HIV infected donors and patients in Sichuan, China.
Methods: Six hundred and fourteen blood samples from HIV infected blood donors (417) and patients (197) in Sichuan were collected from 2007 to 2010. All the samples were detected for HBsAg, anti-HBs, anti-HBc, anti-HBe and HBeAg (KEHUA, China) as well as viral loads of HBV (Qiagen, German). For the HBsAg positive samples, the neutralization confirmatory testing were implemented by LIZHU HBsAg Neutralization. For the HBV positive samples determined by HBsAg confirmatory testing or viral loads detection, the viral DNA were extracted, the S region of HBV was amplified and HBV genotype was determined by direct sequencing and phylogenetic analysis or by multiplex-PCR.
Results: A total of 79 samples out of 614 (12.9%) were detected as HBV positive with 42 of 417 (10.1%) in blood donors and 37 of 197 (18.8%) in patients which suggested that the HBV prevalence in HIV infected patients was significantly higher than that in HIV infected blood donors. In addition, the HBV positive rate was found significantly higher in the young aged group (≤45 years old) HIV infected patients than in the old aged group (>45 years old). In the 79 HBV positive samples, three were HBsAg negative but nucleic acid testing positive while one sample was HBsAg positive with no HBV nucleic acid detected. For the 67 successfully genotyped samples, genotypes B, A, C and D accounted for 56.7%, 26.9%, 10.4% and 4.5% of all samples, respectively, and one sample was detected as mixture of genotype B and D.
Conclusions: There is high prevalence of HBV in HIV infected blood donors and patients in Sichuan, China and genotype B and A are the main genotypes of HBV.

P-181
HBV CORE ANTIBODY AS A MARKER FOR HBV DNA REACTIVE DONORS' REENTRY

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Background: Two pilot NAT studies with the Chiron PROCLEIX ULTRIO assay and the Roche cobas MPX assay were held in Taiwan and confirmed 23 occult HBV infection (OBI) donors. The total yield rates and analysis of the OBI donors were beneficial to estimate the implementation of NAT screening and to evaluate pool size. It was known that the OBI donors were characterized by the present of HBV DNA without detectable hepatitis B surface antigen (HBsAg) outside the window period. The levels of HBV DNA were fluctuating. Follow-up samples from OBI donors with low and fluctuated viral load might be negative for routine HBV NAT assays. Long-term following up the OBI donors was help to propose an optimal algorithm for confirmation of future yield cases and reinstatement of HBV NAT reactive donors. Study design and methods:
Twenty-three OBI donors were enrolled in this study. Besides routine HBV NAT and HBsAg EIA, the additional serological assays such as HBV core antibody (anti-HBc), HBV surface antibody (anti-HBs) were performed for index donation and 1-year follow-up samples. HBV DNA results were confirmed by triplicating the HBV NAT tests, alternative assays with higher sensitivity, or with the extraction of HBV DNA from larger plasma volume. For the long-term follow-up samples, we only tested HBsAg EIA, routine HBV NAT, and quantification of viral load.
Results: For index donation and 1-year follow-up samples of 23 OBI donors, the presence of virus in plasma was confirmed. Anti-HBc remained positive for 23 OBI donors. Only eight donors remained Anti-HBs positive, besides two were turned into positive and two were turned into negative in follow-up samples. All samples were tested negative for HBsAg. For long-term follow-up samples, the results showed the fluctuation of HBV DNA in OBI donors. Only one donor had higher level of HBV DNA, while other donors had HBV DNA level slightly higher than the detection limit of quantification assay or turned into target-not-detected. The HBsAg remained negative for all samples.
Conclusion: Analyzing the data, we considered anti-HBc the marker for reentry of HBV DNA-reactive donors. In the proposed algorithm, for donors with HBsAg negative and HBV DNA-reactive results, the units were discarded and donors were suggested follow-up testing 6 months after this donation. The follow-up samples were tested for HBsAg and HBV NAT. The donors were permanent deferral with either reactive/positive results. If the results were both negative/nonreactive for HBsAg and HBV NAT, samples were tested with anti-HBc. The donors were permitted reentry when anti-HBc came out negative. If anti-HBc were positive, donors were permanent deferral. For decades, anti-HBc was considered not suitable for screening donors in an HBV endemic area like Taiwan. But for occult HBV infection, the anti-HBs was not all positive and HBV DNA level was sometimes lower than detection limit of NAT assays. Anti-HBc was the marker that remained positive after infection. The improvement of blood safety and donor management will be evaluated after implementing this proposed algorithm.

P-182
SENSITIVITY OF FIVE NEW HEPATITIS B SURFACE ANTIGEN ASSAYS

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Background: HBV surface antigen (HBsAg) is the established serological marker routinely used for the diagnosis of acute or chronic HBV infection and screening of blood or organ donors. Moreover, HBsAg quantitative measurements may play a role in staging disease and monitoring efficacy of treatment. Natural variation and mutations in the HBV S gene can induce HBsAg conformational changes which may affect the performance of HBsAg assays. In this study, we analysed the analytical sensitivity and capability for HBsAg mutant detection of five new HBsAg assays.
Study design: The HBsAg assays in this study were Architect HBs Ag Qualitative II (Abbott); ADVIA Centaur HBsAg II (Siemens); Elecsys HBsAg II (Roche) and its quantitative version, Elecsys HBsAg II quant as well as another quantitative assay, LIAISON XL Murex HBsAg Quant (Dia Sorin). In total, 273 samples were tested consisting of the following panels
Panel 1: 2nd WHO international standard HBsAg (00/588) in six dilutions from 0 to 0.30 IU/ml in duplicate (n = 12);
Panel 2: Samples of genotype A–H in singlet (n = 94) or three serial dilutions (14, n = 42)
Panel 3: 34 native mutants at two different dilutions (n = 68); three samples in single (n = 3);
Panel 4: 18 recombinant mutants at three different dilutions (N = 54).
Results: The following table shows the results.

Table 1

	Architect HBsAg Qualitative II	ADVIA Centaur HBsAg II	Elecsys HBsAg II	Elecsys HBsAg II quant	LIAISON XL Murex HbsAg Quant
Limit of detection					
WHO 2nd Int standard (IU/ml)	0.020	0.029	0.032	0.051	0.032
Number positive					
Genotype (n=136)	136	133	134	128	135
Native mutants (n=71)	71	71	69	51	70
Recombinant mutants (n=54)	38	35	21	16	41
Total number positive	245	239	224	195	246
Score (%)	93.87	91.57	85.82	74.71	94.25

Architect HBsAg had the best analytical sensitivity and together with LIAISON XL and Centaur achieved the best score for mutant detection even at lower concentration whereas both versions of the Roche assays missed a couple of recombinant mutants even at the highest concentration studied.
Conclusion: All assays showed excellent analytical sensitivity using the current HBsAg WHO standard. Moreover, the use of a several monoclonal and/or polyclonal antibodies allows in general a broad mutant detection. However, differences exist regarding the concentration at which certain mutants are detected and analytical sensitivity on wildtype is not predictive for mutant detection at the lowest level.

P-183
PREVENTION OF ACUTE AND OCCULT HEPATITIS B VIRUS INFECTION IN LOW AND HIGH EPIDEMIC REGIONS – 5 YEAR EXPERIENCE OF ANTI-HBC BLOOD DONOR SCREENING IN GERMANY

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Background: The German Red Cross Blood Donor Service Baden-Wuerttemberg – Hessen introduced mini-pool nucleic acid testing (MP-NAT) for hepatitis B virus on a voluntary basis in 1997. All blood donors were screened in addition for HBsAG. Although all blood donors were screened by MP-NAT and HBsAG, some cases of transfusion transmitted hepatitis B infections were reported based on low viremic occult hepatitis B infected blood donors. Therefore the German authority introduced anti-HBc blood donor screening in 2006 for all blood donations.
Aim: The current study reports about the experience with anti-HBc blood donor screening. Based on these data risk analysis calculations as well as developments of new screening strategies for low and high epidemic regions were performed.
Methods: From 2006 to 2010 approximately seven million blood donations were screened for HBsAG, MP-NAT (96 samples per pool) and anti-HBc. Blood donors, repeat reactive for anti-HBc, and negative for HBsAG and HBV MP-NAT, were screened in addition for anti-HBs and HBV ID-NAT. Re-entry of anti-HBc repeat reactive donors was allowed, if anti-HBs titer was higher than 100 IU/l and HBV ID-NAT was negative.
Results: Prevalence of anti-HBc reactive donations was 2% for donors in North, Middle and South of Germany, respectively in 2006. Over the study period of 5 years, it was stable for first time donors but reduced for repeat donors to 0.2%. In total 16 lock-back examinations of repeat donors positive for HBV DNA and anti-HBc were investigated. Only one recipient out of 16 was anti-HBc reactive. The transfusion transmitted infectious pathway was not confirmed, because the recipient was HBV DNA negative. In a case/control study 118 recipients transfused with anti-HBc confirmed reactive blood components (anti-HBc and anti-HBe reactive components) were compared with 120 recipients transfused with anti-HBc negative blood components. The percentage of anti-HBc reactivity in both recipients groups were not significant different.
Conclusions: The introduction of anti-HBc into blood donor screening was able to improve blood safety regarding to low viremic occult hepatitis B infected blood donors. No hepatitis B transfusion transmitted infections were observed after introduction of anti-HBc into blood donor screening in 2006. Donor re-entry with anti-HBs antibodies (titer higher than 100 IU/l) is a safe procedure. Nevertheless based on our data, the most important test for donor re-entry is HBV ID-NAT for anti-HBc repeat reactive donations. Therefore we conclude that regions with low epidemic HBV prevalence can improve blood safety by introduction of anti-HBc blood donor screening in addition with a re-entry procedure, if HBV ID-NAT is negative and anti-HBs is reactive with a titer higher than 100 IU/l. In HBV high epidemic regions, anti-HBc blood donor screening can be introduced with a re-entry strategy of all blood donations that are negative by HBV ID-NAT. This modified screening strategy, enables a detection of infectious occult hepatitis B donations without loosing a high number of non infectious anti-HBc only reactive blood donors.

P-184
COULD BLOOD DONOR SCREENING BY HBV MINI POOL NUCLEIC ACID TESTING AND ANTI-HBC REPLACE SCREENING FOR HBSAG?

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Background: In the 1970ies blood donor screening for HBsAG has been implemented to reduce the residual risk of transfusion associated hepatitis B virus infections. In 1997 the German Red Cross Blood Donor Services Baden-Wuerttemberg – Hessen introduced hepatitis B virus mini pool nucleic acid testing system for all blood donations on a voluntary basis with a maximum pool size of 96 samples per pool. Because of transfusion transmitted hepatitis B infections, caused by HBV chronic infected blood donors (occult hepatitis B infections with low virus concentration), anti-HBc screening for all blood donation was introduced by the German authority, the Paul-Ehrlich-Institute, in 2006. Therefore all blood donations were currently screened in our blood donor service for HBsAG, HBV MP-NAT and anti-HBc.
Aim: We analysed data between 1998 and 2010 for HBsAG, HBV MP-NAT, HBV ID-NAT and anti-HBc, for the impact of each screening test to blood safety.
Methods: Between 1998 and 2010 3,475,605 blood donations were screened for HBsAG, HBV MP-NAT, and anti-HBc. HBsAG as well as anti-HBc were screened by ABBOTT PRISM technology. HBV DNA MP-NAT was performed by the German Red Cross NAT system with an analytical sensitivity of 5.7 IU/ml.
Results: In total 697 PRISM HBsAG reactive donations have been identified among the 3,475,605 donations. 687/697 (98.6%) would also have been detected by anti-HBc screening and 540/697 donations (77.4%) were positive by MP-NAT screening. If a more sensitive ID NAT was applied, 612/697 (87.9%) were ID-NAT positive. Notably, no HBsAG only reactive donation (negative for anti-HBc and HBV ID NAT) was identified during the observed time period. In contrast HBV MP-NAT identified one HBsAG and anti-HBc negative donation in the early infectious window period.
Conclusion: Currently blood donor screening for HBV is performed at the German Red Cross Blood Donor Services Baden-Wuerttemberg – Hessen by testing for HBsAG, HBV MP-NAT and anti-HBc. Retrospective analysis of data from HBsAG reactive donations revealed that all these donations would have also been identified either by testing for HBV MP-NAT or by anti-HBc detection. Therefore testing for HBsAG seems to be redundant following the introduction of HBV MP-NAT and anti-HBc testing. Discontinuation of HBsAG-testing may therefore be considered to reduce the cost associated with HBV-testing without compromising blood safety.

P-185
BLOOD DONOR SCREENING FOR HBV DNA USING NAT UNDER ROUTINE CONDITION IN QINGDAO, CHINA, 2010–2011

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Background: In Chinese Mainland, NAT screening of blood donations for HBV DNA, HCV RNA, and HIV RNA was carried out as a pilot project in 15 blood banks in 2010. Qingdao blood centre as the first pilot blood collecting and supplying organizations carry on the project in 1st June, 2010.
Objectives: To evaluate the presence of HBV-DNA in blood donors based on serologically negative units, who donated blood in the period from June 2010 to January 2011, in Qingdao, China.
Materials and methods: The HBV NAT yield was examined by testing about 65,800 voluntary, serologically negative blood donor samples on the cobas TaqScreen multiplex (cobas MPX) test in pools of six with the cobas s 201. Samples positive for HBV DNA and negative for HBsAg were confirmed by a second molecular test, the viral DNA was quantified, and serological tests evaluating hepatitis B s antigen (HBs-Ag), anti-hepatitis B core antigen (HBc-Ab), anti-hepatitis B e antigen (HBe-Ab), hepatitis B e antigen (HBe-Ag) by Electro Chemiluminescence Immunoassay (ECLIA).
Results: MN-pool NAT identified 80 reactive pools. The ID-Pool NAT identified 58 reactive pool samples. HBV-DNA quantitative determination identified 38 positive ones. The overall HBV-DNA positive rate was 0.8‰. HBV-DNA viral load, which can be detected, range from <12 IU/ml to about 30,000 IU/ml. Among the 58 reactive pool samples, HBV-DNA positive/anti-HBc positive ones is 41 samples, HBV-DNA positive/anti-HBc negative ones is 12 samples.
Conclusions: Our data indicate that blood donor screening for HBV DNA using NAT could detect more HBV infection in blood donors who are HBs Ag negative, which is important for the prevention of transfusion-transmitted HBV infection.

P-186
RATE OF HBV-NAT DETECTION IN REPEAT DONORS PREVIOUSLY NEGATIVE FOR HBV DNA BY NAT AT SIRIRAJ HOSPITAL, THAILAND

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Background: Screening of HBV infection in donated blood by nucleic acid technology (NAT) testing was implemented at Siriraj Hospital in 2007. Samples are screened in pools of six donations with the Roche cobas® MPX test, a multiplex PCR test for HIV-1 (groups M and O), HIV-2, HBV and HCV on the Roche cobas® s 201 platform, an automated system for blood screening. However, despite routine screening of donors with a very sensitive HBV NAT test, a few repeat donors subsequently became HBV

NAT reactive. This study was designed to calculate the rate of HBV NAT reactivity in repeat donors and to classify these infections as either window period or occult infections.
Material and methods: The donor database and records of the infectious screening laboratory were retrospectively reviewed from April 2007 to December 2010. The number of repeat donors was indentified and the repeat donors who subsequently became HBV NAT reactive were examined further. The HBV viral load and HBV serological profile were used to classify the infections as either window period or occult. The total HBV NAT yield rate, as well as the rates for window period and occult infections, was calculated.
Results: From April 2007 to December 2010, a total of 179,858 donations were collected at Siriraj Hospital. There were 112,169 donations from 30,369 repeat donors who donated during this period. Of these repeat donors, 24 donors (17 males and seven females) were reactive for HBV NAT on the last donation. The HBV NAT yield rate is 1:1265 (1:1079 in males and 1:1717 in females). Based on HBV viral load and HBV serological profile, nine donors had window period infections and 15 donors had an occult HBV infection. The viral load in all the donors with an occult HBV infection was below the level of detection of the test used (20 IU/ml). Thus the rate of occult infections in repeat donors at Siriraj Hospital is 1:2024.
Conclusions: HBV DNA was detected in repeat donors at Siriraj Hospital. The majority of these donors had an occult HBV infection with very low viral loads and it is possible that HBV DNA detection in some of these donors could have been missed in previous donations. These cases will be the subject of a look-back study for transfusion-transmitted HBV.

P-187
HBV NAT REACTIVE IN NORTHERN THAI BLOOD DONORS

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Background: The high prevalence of HBV infection is the problem of the country in Southeast Asian region. False negatives still persist, and HBV transmissions still occur, especially in northern Thailand. However the new technologies, including nucleic acid technology (NAT) testing, have affected the rates of detection in blood donors.
Aims: The aim of this study was to evaluate the performance of two commercial multiplex NAT tests, the Chiron eSAS Procleix Ultrio test (Ultrio test) and the Roche Cobas® s201 automated platform and the Cobas TaqScreen MPX test (MPX test) for screening blood donations in northern Thailand.
Methods: Two commercial NAT assay systems were used: the Chiron PROCLEIX eSAS platform with PROCLEIX ULTRIO test and The Roche Cobas s201 platform with the Cobas TaqScreen MPX test for screening norhern Thai blood donors for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Eighty four HBV NAT-reactive/hepatitis B surface antigen (HBsAg)-negative donors were tested for HBV serologic markers. The reactive samples were tested on both NAT system and donors were followed up.
Results: The majority of the HBV reactive donors had an occult HBV infection (65.5%), followed by donors with acute infection (34.5%). Fourty eight (57.1%) donors were followed. Based on the follow-up results, 14.5% were in the window period.
Conclusions: The blood donors with occult HBV were detected by both tests. However, the MPX test seems to have any increased rate of detection for HBV. However, NAT should be used in conjunction with serological testing to identify low-level HBV infections as well as infections at the ends of the window periods of detection.

P-188
TRANSFUSION-TRANSMISSION OF HEPATITIS B VIRUS (HBV) FROM A NAT-NEGATIVE OCCULT HEPATITIS B VIRUS CARRIER

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Background: Transfusion-transmission of HBV (TT-HBV) has become rare since the implementation of the donor screening by nucleic acid amplification test (NAT) in 1999 in Japan. However, the risk of TT-HBV still persists. We experienced a case of TT-HBV from an occult HBV carrier with negative individual NAT.
Case report: A 20 years old male patient suffering from multiple injuries following a road traffic accident was conveyed to the emergency room of our hospital and received blood transfusion including, platelet concentrates, red cell concentrates and fresh frozen plasma during the period between September 2010 and October 2010. In January 2011, about 4 months after the last transfusion, post-transfusion viral test was carried out for him according to 'The Guideline for Transfusion Practice (Ministry of Health, Labor and Welfare)'. He was positive for HBV DNA, negative for HCV core antigen and negative for HIV antigen and antibody, although he had no symptom of hepatitis. The status of his pre-transfusion HBV-related markers was tested with his pre-transfusion blood sample, which had been stored in a deep freezer at Asahikawa Medical University Hospital. His stored samples showed no positive result for HBV markers before receiving a blood transfusion. The 22 blood donors were involved in his transfusion and the look-back study with the stored blood samples of the 22 donor's were then carried out. The individual HBV-NAT of the 22 samples was all negative. In February 2011, one of the 22 donors donated blood again and turned out to be positive for HBV DNA. HBV DNA sequences of the patient and the causative donor samples showed 99.2% homology between the 2. The screening results of the causative donor at first-time blood donation were weak positive anti-HBc antibody (8.7C.O.I), weak positive anti-HBs antibody (29.6 mIU/ml) and negative HBsAg. His plasma was transfused to the patient.
The results indicated that donated blood of the occult HBV carrier with negative individual NAT caused the post-transfusion HBV infection. Now in Japan, blood products from some of the anti-HBc-positive donors can be used for transfusion, if their anti-HBc activities are weak (<12COI), HBsAg-negative and HBV pool NAT-negative, even if anti-HBs is <200 mIU/ml.
Discussion: Although the post-transfusion HBV infection became rare, the risk of HBV transmission still exists. To minimize the HBV transmission risk, we are now trying to lower the cut off value of anti-HBc antibody for blood product. Also, to rescue patients suffering from viral transmission, we should routinely carry out post-transfusion viral test according to the national guideline.

P-189
ANTI-HBC SCREENING IN BLOOD DONORS FOR PRESENCE OF OCCULT HBV INFECTIVITY

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Background: Despite mandatory screening of donor blood for HBsAg, transfusion-associated HBV (TAHBV) continues to be a major problem in India, more so in patients receiving repeated transfusions.
Aims: The present study was undertaken to assess the prevalence of anti-hepatitis B core antigen (anti-HBc) positivity, prevalence of antiHBs in blood donors who are anti-HBc positive and presence of HBV-DNA in serum sample of healthy blood donors negative for HBsAg. Since anti-HBc detection is not mandatory in India, this study was aimed to evaluate whether anti-HBc detection could be adopted in India as a screening assay for HBV in addition to HBsAg to improve further the safety of blood transfusion.
Methods: Seventeen hundred serum samples negative for HBsAg collected from healthy blood donors were tested for the presence of anti-HBc antibody. All samples positive for anti-HBc antibody were then investigated for determination of anti-HBs and for liver function tests (LFTs). One hundred serum samples reactive for anti-HBc with and without anti-HBs were tested for HBV DNA by PCR method.
Results: Of the 1700 samples tested, 142 (8.4%) blood samples were found to be positive for anti-HBc. It was lower in voluntary (6.9%) as compared to replacement donors (10.4%) (P = 0.011). Seventy two (50.7%) anti-HBc reactive samples were also reactive for anti-HBs with levels >10 mIU/ml and 70 (49.2%) samples were non-reactive fore anti-HBs, these units were labeled as anti-HBc-only. These 142 anti-HBc reactive units were also tested for LFTs (serum bilirubin, AST, ALT). All the samples had normal serum bilirubin levels, 25 (18%) samples showed enzyme elevation. HBV DNA was detected in one out 100 samples tested, this sample also contained anti-HBs levels >150 mIU/ml, LFTs for this sample were within normal limits.
Conclusions: Keeping in view that 8–18% of blood donor population in India is anti-HBc reactive (as reported in various studies from India), inclusion of anti-HBc testing will lead to high discard rate. Anti-HBs as proposed previously does not seem to predict clearance of the virus as the single donor who tested reactive for HBV DNA in our study had high anti-HBs titers. Cost effectiveness of introducing universal anti-HBc screening and discarding large number of blood units vs considering ID NAT (Individual Donor nuclic acid testing) needs to be assessed. Awareness and education of donors is required regarding minor modes of HBV transmission, modification of the donor questionnaire to eliminate all donors with a history of jaundice in adult life and more stringent one-to-one donor screening to elicit such information should be implemented.
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P-190
EVALUATION OF THE ROLE OF NUCLEIC ACID AMPLIFICATION TESTS (NAT) AMONG BLOOD DONORS IN SOUTH CHINA

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Background: Nucleic acid amplification test (NAT) for HIV-1, HCV, and HBV can significantly improve the safety of the blood supply by detecting infectious blood donated during the seronegative window periods. Although NAT has been routine used for blood screening in many countries since 1999, it is yet to be implemented in China. We decided to conduct a study in Xiamen, south China to evaluate the necessity of NAT among blood donors in China.
Aims: To evaluate the necessity of nucleic acid amplification tests (NAT) among blood donors in south of Fujian, China.
Methods: Blood donor plasma specimens were collected at Xiamen Blood Service, Fujian Province, China, between June 2010 and September 2010. All samples were tested for serology using two different EIA assays for HIV, HCV and HBV. EIA negative samples were selected for NAT. Roche COBAS® TaqScreen MPX Test on the cobas s 201 system (MPX Test) was used to simultaneously detect HIV, HCV and HBV, which is a single assay, multiplex blood screening NAT that may be performed on individual specimens, but is designed to run in pools of six. NAT reactive samples were further tested with the discriminatory assays using COBAS AmpliScreen Tests for the three viruses. Donor follow-up testing for those potential yield cases were conducted if possible.
Results: A total number of eight EIA-/NAT+(0.078%, 1/1286) cases were detected; All were HBV cases. All these eight cases had a viral load <20 IU/ml except one (91.9 IU/ml). Of these eight cases, seven were positive for anti-HBc (OBI) and one were anti-HBc negative (WP cases). Follow up testing was possible on four donors, including one WP and three OBI cases. The WP donor showed HBsAb sero-conversion after 4 months.
Conclusions: Our data demonstrate that, after EIA screening, there is still a rate of 0.078% (one in 1286) HBV yield cases among blood donor populations in south China and implementation of NAT can significantly improve the safety of the blood safety.

P-191
STUDY OF IMMUNOREACTIVITY OF EXPRESSED HBX PROTEIN IN HEPG2 CELL VIA ANTIBODY TO HBX ANTIGEN

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Background: Chronic hepatitis B virus (HBV) infection is one of the main causes of hepatocellular carcinoma (HCC) worldwide. The HBV- X (HBX) protein, a small regulatory protein that is required for the establishment of viral infection, is believed to contribute to the development of HCC.
Aims: Expression of HBX protein in HepG2 cell to study presence of antibody to hepatitis B virus X antigen (anti- HBX) in HBV Infected Patients.
Methods: The HBX gene was amplified from the whole HBV genome using polymerase chain reaction (PCR). Next, the HBX gene was cloned into the eukaryotic expression vector pcDNA3 and constructed mammalian expression vector pcDNA3- HBX encoding the HBX gene. This expression vector was then transfected into the HepG2 cells by FuGENE 6 Transfection kit. In the end, the complete HBX gene of hepatitis B was expressed in HepG2 cells and confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and western blotting.
Results: The PCR products were separated by electrophoresis on 1.2% agarose gel, A band with 465 bp size was detected that showed HBX gene was amplified successfully using PCR. recombinant plasmid pcDNA3-HBX was confirmed by restriction endonucleases digestion and colony-PCR and sequencing, presence of 465 bp fragment showed that recombinant plasmid was made. Expression of recombinant plasmid HBX was confirmed by SDS-Page and western blotting and a band of 17 KD was detected.
Conclusions: The results of this study showed that the recombinant plasmid pcDNA3-HBX was made successfully. Finally the results of western blotting showed that HBX is a immunoreactive protein and can be used for subsequent studies.

4.3 Hepatitis C (HCV)

P-192
DISTRIBUTION OF HCV GENOTYPES AMONG VOLUNTEER BLOOD DONORS IN CHINA

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To determine hepatitis C virus (HCV) genotype distribution in China, a total of 507 HCV TNA positive serum samples were collected from 17 geographic areas in China and subjected to RT-PCR followed by direct DNA sequencing in both directions and phylogenetic and geographic analysis of E1 and NS5B regions. E1 and NS5B sequences were amplified from 461 and 453, respectively. According to the E1/NS5B phylogenetic trees, subtypes including 1a, 1b, 2a, 3a, 3b, 6a, 6n and 6v are identified among the 17 areas of China. The major prevalent subtype is 1b, accounting for 48.93%. Subtype 2a is the second prevalent subtype, accounting for 18.88%. HCV patterns differed between the donors from southern China and northern China, especially in subtypes 6a ($P < 0.000$, $\chi^2 = 45.175$) in southern China, 1b ($P < 0.000$, $\chi^2 = 14.260$) and 2a ($P < 0.000$, $\chi^2 = 25.410$) more frequent in the northern group. The 30 subtype 3a isolates formed three clusters, designed cluster I, II and III. In cluster I, all Kunming and Fujian strains are dispersed among the strains from southwestern China (Yunnan), Cluster II contained isolates only from GuangDong and cluster III contained isolates from northern China. These HCV-3a samples indicate a northern China clade from Russia and a Guangdong clade from Hong Kong. The distribution of HCV genotypes among volunteer blood donors in china indicated that epidemiological modes may be changed in some subtypes, with Hunan and Beijing playing a crucial role in HCV-3a transmission, just as they are important in population migration between different regions in China.

P-193
This abstract has been withdrawn.

P-194
EVALUATION AND COMPARISON OF THREE ANTI-HEPATITIS C VIRUS ANTIBODY ASSAY SYSTEMS FOR DIAGNOSIS OF HEPATITIS C INFECTION BLOOD DONORS IN TAIWAN

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Background: HCV EIA 2.0 or HCV Version 3.0 ELISA has been evaluated in the immunocompetent populations with anti-HCV prevalence <10% (e.g. volunteer blood donors). The proportion of false-positive averages approximately 35% (range: 15–60%). To minimize the possible of false-positive anti-HCV results, the Taiwan Blood Services Foundation have increased that all anti-HCV positive results are confirmed by second and third anti-HCV assay.
Aims: The aim of this study was to evaluate and compare three anti-HCV tests: Murex anti-HCV (version 4.0), Abbott AxSYM HCV 3.0 and VITROS Anti-HCV had been used for screening blood donor samples in Taiwan.
Methods: There were 438 blood donors included in this study from August to December 2010. All samples were analysed using three different commercially available anti-HCV test systems. They were the Murex anti-HCV (version 4.0) (Abbott Laboratories, Diagnostics Division), the Abbott AxSYM HCV 3.0 (Abbott Laboratories, Diagnostics Division) and the VITROS Anti-HCV (Ortho-Clinical Diagnostics).
Results: The concordance rates were 70.3% (308/438) among Murex anti-HCV (version 4.0), Abbott AxSYM HCV 3.0 and VITROS Anti-HCV. The concordance rates of Murex anti-HCV with VITROS Anti-HCV and Abbott AxSYM HCV 3.0 were 75%(331/438) and 73%(324/438). In addition, the concordance rates were 91% (399/438) between AxSYM HCV 3.0 and VITROS Anti-HCV. This was significant higher than other rates.
Conclusion: The findings of our study suggest that two immunoassays for anti-HCV presented a high concordance, the concordance rates of AxSYM HCV 3.0 with VITROS Anti-HCV was higher than the concordance rates of Murex anti-HCV with AxSYM HCV 3.0 or VITROS Anti-HCV. Therefore, the results should be explained carefully, because there were still significant differences between assay methods.

P-195

EVALUATION OF TWO HCV COMBINED AG-AB ASSAYS IN A BLOOD BANK CONTEXT

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Background: Early detection of HCV infection is crucial to prevent transfusion transmitted infection in blood banks. The use of combined assays which detect simultaneously HCV antigen and antibody reduce dramatically the window period thanks to the antigen detection. However, the antibody detection and the specificity remain crucial features in a blood bank context to avoid false negative and false positive results.
Aims: To date, two HCV Ag – Ab combined assays (CE-IVD) are available: Monolisa™ HCV Ag-Ab ULTRA (Bio-Rad) and Murex HCV Ag-Ab Combination (Diasorin).
The aim of this study, performed at the CTSA (Blood center of the French Army), is to compare the performance of both assays especially the anti-HCV sensitivity and specificity on blood bank routine samples.
Methods: A full validation of each HCV Combined assay has been performed before starting the evaluation on the Summit microplate processor (Ortho). According to the CTSA Quality Management System, the following parameters have been tested: contamination, edge effect, repeatability, reproducibility and sensitivity on an anti-HCV qualification panel (QHV711). The specificity has been evaluated on negative blood bank samples (n = 425 samples). Then, both HCV combined assays have been tested with an anti-HCV positive panel from donors constituted during the blood bank routine activity (n = 29 samples) and with seven commercial seroconversions (n = 72 samples).
Results: No contamination around strong positive sample and no edge effect were found with both assays during their validation on the microplate processor. The repeatability was calculated on negative samples, weak positive samples and strong positive samples for both assays with equivalent results (CV < 10%). The reproducibility was calculated with 13 QC for Monolisa HCV Ab-Ab ULTRA (CV = 10.3%), and calculated with eight QC for Murex HCV Ag-Ab Combination (CV = 28%). The specificities were calculated at 100% (425/425) with Monolisa HCV Ag-Ab ULTRA and 99% (421/425) with Murex HCV Ag-Ab Combination.
All PCR positive samples (13/29) from the CTSA routine panel were found positive with both HCV combined assay. Among the 16 samples PCR negative, six immuno-reactive samples were discrepant between the two combined assays: four were positive with the Bio-Rad assay and two were positive with the Diasorin assay. Moreover Monolisa has detected 38 positive samples and Murex 34 out of 72 seroconversions samples tested.
Conclusion: This study suggests a better sensitivity of Monolisa HCV Ag-Ab ULTRA assay on HCV positive antibody samples and highlights its very good specificity vs Murex. Monolisa HCV Ag/Ab ULTRA can be used instead of an HCV Ab assay with no risk of decreasing sensitivity for HCV Ab detection while reducing the window period by detecting HCV antigen.

P-196

INTEREST OF CONFIRMATION TESTS IN THE DIAGNOSIS OF HEPATITIS C VIRUS TO BLOOD DONORS IN ABIDJAN, COTE D'IVOIRE

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Introduction: The RIBA test anti-HCV (Recombinant Immuno Blot Assay) permit to verify the presence in the serum, to antibodies HCV detected by ELISA. In the developing countries, including Côte d'Ivoire, screening for hepatitis C is limited by the immunoassay because of the high cost of RIBA test. To reduce the number of exclusion of blood donors who are positive to HCV in ELISA test, we conducted this study whose objective was to demonstrate the interest of the RIBA test confirmatory diagnosis of hepatitis C virus among blood donors in côte d'Ivoire.
Materials and methods: Our study took place from 2 to 23February, 2008 in the laboratory of the National Blood Transfusion Center (CNTS) in Abidjan, Côte d'Ivoire, examined 200 serum from blood donors anti-HCV positive (ELISA Murex anti-HCV version 4.0) selected according to the ratio Elisa: low (1.01–5), medium (5.01–8) and high (>8). The confirmatory test DECISCAN HCV PLUS BIORAD was used to test 200 HCV positive samples. The software Epi Info 6.04 fr was used for data entry and statistical analysis.
Results: Our overall results showed that the 200 samples positive by HCV EIA, 49% (98/200) were confirmed positive. The RIBA was indeterminate in 40% of cases (80/200) and 11% of cases (22/200) positive signals observed with the EIA screening tests are proven false positive (ie negative). The analysis of samples tested RIBA has allowed us to note that 96 samples had a low ratio ELISA with 21% (20/96) were negative in RIBA, and 79% (76/96) were indeterminate. RIBA-positive samples (98/200) had a ratio high in 82% (80/98). Among the samples positive RIBA, there was the presence of NS3 (C33) and NS4 (C100) in 100% of (98/98). The C2 was present in 37% (36/98) of cases and C1 in 18% of cases (18/98). Among RIBA indeterminate is noted especially the presence of NS3 in 98% of cases (78/80) and also the presence of NS4 in 30% of cases (24/80).
Discussion: The proteins C1, C2 and NS4 are required to confirm the diagnosis of viral hepatitis C by RIBA.
Conclusion: These results reflect the lower specificity of enzyme immunoassays (ELISA), hence the interest in the use of RIBA confirmation tests. We conclude that a significant number of donors are excluded from blood donation in Cote d'Ivoire on the basis of false obtained positive by ELISA.

4.4 HIV

P-197

HIV/HUMAN PARVOVIRUS B19 CO-INFECTION IN BLOOD DONORS AND CLINICAL PATIENTS IN SICHUAN, CHINA

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Background: Human parvovirus B19 is a common human pathogen which causes a variety of diseases. Several studies have demonstrated that the presence of a B19 persistent infection with low-level viraemia beyond 6 month post acute infection is associated with the degree of host immunodeficiency caused by HIV infection; however, the existence, loading, evolution and distribution of B19 virus in Chinese HIV positive individuals have not been determined.
Aims: To investigate the prevalence of parvovirus B19 infection in HIV-infected individuals from blood donors and clinical patients in Sichuan, China, and to reveal the evolutionary status of human parvovirus B19 distribution in this area during last two decades.
Methods: Five hundred and seventy-three HIV serological positive individuals from blood donors and clinic patients were investigated in Sichuan, China in last two decades. DNA extracts were used for detection and quantification of viral genome by Real time PCR. The Nested PCRs were used to confirm the existence of B19 genome and the sequences were subjected for phylogeny and haplotype analysis. Seventy-nine HIV positive individuals and 92 HIV negative individuals comprised of blood donors were tested for B19 virus specific antibodies.
Results: Twenty-six out of 573 of HIV positive individuals tested positive for B19 genome DNA, putting overall prevalence of B19 genome DNA at 4.5% (95% CI: 2.8–6.2%). The B19 genome DNA prevalence in HIV positive population is significantly higher than that of normal blood donors. The quantitative DNA levels ranged from 5.3×102 to 1.1×105 copies/ml. The characteristic of these B19 genome DNA positive samples was also studied. The seroprevalence of IgG in HIV positive samples was significantly lower than that of HIV negative blood donors (32.6%, 95% CI, 23.0–42.2%, $P < 0.05$) indicating the deficiency of producing B19 specific IgG. Genome sequencing, phylogeny and haplotype analysis of the isolates from B19/HIV co-infected samples were studied. The B19 sequences from this study were placed in Genotype 1 subtype B19-1A which formed a monophyletic group; seven distinct haplotypes were discovered with 60% of the B19/HIV co-infected samples sharing one central haplotype.
Summary: Human parvovirus B19 in HIV positive patients in Sichuan, China has been investigated. This study not only elucidates the importance infection demographic information, the existence, loading, virus evolution and distribution of B19 in Sichuan, Chinese HIV positive patients, but also shed lights on the diagnosis and therapy of B19/HIV co-infection patients.

P-198

HIGH HIV-1 GENOTYPE DIVERSITY AMONG BLOOD DONORS IN SICHUAN, CHINA

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Background: The increasingly HIV-1 diversity challenges the highly active antiretroviral therapy treatment and vaccine development. HIV-1 has been becoming leading cause of death among infectious diseases in China, in which Sichuan province is one of the high prevalence areas. Although there have been several studies examining the genetic diversity in HIV-1 infected individuals from high risk populations in China, there are very limited data from HIV-1 infected volunteer blood donors.
Aim: Molecular epidemiological method was applied to determine genotype diversity in Sichuan province among the HIV-1 infected blood donors.
Method: HIV-1 confirmed reactive serum samples were collected from HIV-1 confirmatory laboratory, Institute of Blood Transfusion, Chinese Academy of Medical Sci-

ences during 2007–2010. HIV-1 Pol including whole protease and partial reverse transcriptase (RT) genes was amplified, sequenced, and analyzed for the subtype determination.
Result: Ninety-nine amplified sequences had the following genotype characteristics: G (1/99, 1.0%), F1 (1/99, 1.0%), circulating recombinant form (CRF) 08_BC (2/99, 2.0%), CRF01_AE (44/99, 41.4%), and CRF07_BC (54/99, 54.6%). Five subtypes or CRFs of HIV-1 were identified in Sichuan province from 2007 to 2010, and the main prevalence strains were CRF01_AE and CRF07_BC.
Conclusion: HIV-1 infection in Sichuan blood donors reflects highly genetic diversity. This is the first report on HIV-1 genotype diversity in Sichuan province among infected blood donors. Our further study will link the epidemiology characteristic results to monitor the HIV-1 genotypes evolutionary trends in Sichuan blood donors as a part of an overall China HIV control project.

P-199
SURVEY OF HEPATITIS B VIRUS (HBSAG), HEPATITIS C VIRUS (HCV-AB) AND SYPHILIS COINFECTION AMONG HIV POSITIVE BLOOD DONORS BETWEEN 2006 AND 2010

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Background: HIV, HBV and HCV are major public health concerns. Because of shared routes of transmission, HIV-HCV coinfection, HIV-HBV coinfection and HIV-syphilis coinfection are common. HIV-positive individuals are at risk of coinfection with HBV and HCV infections. The prevalence rates of coinfection with HBV and HCV in HIV-blood donors have been variable worldwide depending on the geographic regions, and the type of exposure.
Aim: This study aimed to retrospecticly examine HBV, HCV and Syphilis coinfection serologically and determine the shared and significant factors in the coinfection of HIV-positive blood donors.
Methods: This descriptive, cross-sectional study was carried out on 351 HIV-positive blood donors including 333 males and 18 females in Taiwan, to survey coinfection with Syphilis, HBsAg and anti-HCV. The retrospective demographic data of the subjects was collected and the donors' serums were analyzed by TPHA/ELISA kits including Syphilis, HBsAg and anti-HCV. The collected data was analyzed with MINITAB software and Chi-square. Fisher's exact test with 5% error intervals was used to measure the correlation of variables prevalence rates.
Results: The results of the study indicated that the prevalence of coinfection in HIV-positive blood donors with syphilis was 13.1% (46 in 351), hepatitis viruses was 5.98% (21 in 351), out of whom 14 (3.99%) cases were anti-HCV positive, 7 (2.0%) cases were HBsAg positive, and 3 (0.9%) cases were both HBsAg and anti-HCV positive.

Table 1: Prevalence of Syphilic, HCV and HBV positivity among HIV blood donors

Variables	N (Percent)	Syphilis+ N	anti-HCV+ N	HBsAg+ N	anti-HCV+/HBsAg+ N
Total HIV	351 (0.006%)	46 (13.1%)	14 (3.99%)	7 (2.0 %)	3 (0.9%)
All Donors	5472,868	8,753 (0.16%)	10,714 (0.19%)	37,429 (0.68%)	*
Sex					
Male	333 (94.9%)	45 (13.5%)	11 (3.3 %)	7 (2.0 %)	3 (0.9%)
Female	18 (5.1%)	1 (5.6%)	3 (16.7 %)	0 (0.0 %)	0 (0.0%)
Age					
≦30	255 (72.6%)	32 (12.5%)	13 (5.1 %)	6 (2.4 %)	3 (1.2%)
31-50	81 (23.1%)	12 (14.8%)	1 (1.2 %)	0 (0.0 %)	0 (0.0%)
≧50	15 (4.3%)	2 (13.3%)	0 (0.0 %)	1 (6.7 %)	0 (0.0%)

*not applicable

Conclusion: There was a significant correlation between coinfection with syphilis, HCV and HBV and/or both among HIV-positive patients depending on different variables including sex, age status exposured to risk factors.

P-200
CURRENT INCIDENCE AND ESTIMATED RESIDUAL RISK OF TRANSFUSION-TRANSMITTED HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN QINGDAO, CHINA

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Background: Accurate estimation of the risk of human immunodeficiency virus (HIV) infection through transfusion is essential for monitoring blood safety. The risk, however, is so low that it can only be estimated by mathematical modeling.
Aims: This study evaluates the HIV antibody screening strategy of duplicate enzyme-linked immunosorbent assay (ELISA) in QingDao Blood Center and therefore estimates current incidence and the residual risk of transfusion-transmitted HIV infection.
Methods: Data from QingDao Blood Center between January 2006 and May 2011 were used. During the study period 312,394 donations from voluntary blood donors, including new donors and regular ones. The data of screening and confirmatory test results for this study were collected from the electronic data files with the software of Tangshan standardized management system for modern blood centers, containing information on donor profiles. We estimated the risk of HIV transmission caused by transfusion on the basis of the window period associated with the use of current, sensitive enzyme immunosorbent assays and recent data on HIV incidence among blood donors.
Results: A total of 312,394 donations from voluntary blood donors in QingDao Blood Center were screened for HIV antibody, including 118,715 (38%) regular donors and 193,679 (62%) new ones. There were 18 HIV-positive donations (nine in regular donors and nine in new ones) which occurred predominantly in males. By calculating, the incidence rates of HIV-positive was 7.58 and 4.65 donors per 100,000 person-years in repeated donors and new ones respectively, and the new frequency of infecting HIV was 0.116 and 7.10 donors per 100,000 person-years. The mean interdonation interval in years was 239 days that was obtained by dividing the number of donations by the number of donors and by multiplying this ratio by the duration of the study period. The length of the preseroconversion window period for anti-HIV was derived from published data: 22 days. Using serological methods, the residual risk using a statistical model was 1:186,512. Otherwise, we tested 81,989 donors' samples by NAT pilot project between July 2010 and May 2011, and the residual risk was 1:208,975.
Conclusions: The estimates of the transfusion risk of HIV infection in each country is important, both to assess the impact of current preventative strategies and contribute datas to policy decisions to reinforce transfusion safety. The major factor contributing to the differences in risk between QingDao and other areas with similar testing regimes is the lower prevalence of HIV in the source populations. Except for adorpting NAT, we recommend further improvements to more efficient selection of blood donors for high incidence HIV-positive in regular donors. The greater challenge is to identify, recruit, and retain a pool of donors with lower prevalence of the target diseases.

4.5 Bacteria

P-201
THE PRELIMINARY STUDY OF PHOTOSENSITIZER YWW007 ON PLASMA INACTIVATION OF BACTERIAS

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Objective: To investigate the effects of different concentrations of YWW007 phenothiazines photochemical to different kinds of bacterias in blood plasma.
Methods: Using *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *E. coli* as model bacterias, the bacterias were seeded into 5 ml plasma, a new synthesized phenothiazine derivative YWW007 was tested for its ability of bacterias inactivation with different concentration in 4, 8, 12 and 16 μM, 2.33 $\pm$ 0.17 mW/cm^2 intensity of absorption wavelength of 600–700 nm red light was used to irradiate for 30 min.
Results: The ability of inactivating bacterias was as follow: *Bacillus cereus* > *Staphylococcus aureus* > *Staphylococcus epidermidis* > *E. coli* > *Pseudomonas aeruginosa*. During the growth of bacterials, when the value of the OD is about 0.6, the inactivation of bacterias is better than other values. In addition, when the concentration of the YWW007 is only 4 μM, the Gram-positive bacterias, such as *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were inactivated. Furthermore, the ability of inactivating bacterias was weaker in the Gram-negative bacterias, for instance, *E. coli* as well as *Pseudomonas aeruginosa*, especially when the value of the OD is more than 1.
Conclusion: YWW007 in the procedure of Gram-positive bacterias inactivation is better than Gram-negative bacterias inactivation. Moreover, the ability of inactivating bacterias is much better when the bacterias in the logarithmic growth stage.

P-202
This abstract has been withdrawn.

4.6 Parasites

P-203

SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION AMONG HEALTHY BLOOD DONORS IN TAIWAN

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Background: Toxoplasmosis is one of important zoonotic diseases worldwide, which is caused by *Toxoplasma gondii*. The seroprevalences of toxoplasmosis varied in different countries from 20% to 40% in UK, 50–60% in USA to 80–90% in France. However, little is known about seroprevalence among Taiwanese.
Methods: Serological and molecular biological methods were used for the toxoplasmosis confirmation in CDC. Taiwan Blood Services Foundation was collaborated in this study, and a total of 1600 blood samples of healthy blood donors were collected from six branch blood service centers.
Results: The preliminary results indicated that the IgG positive rate in Hualien, Taipei, Hsinchu, Taichung, Tainan and Kaohsiung were 14%, 9%, 8%, 8%, 9% and 7%, respectively, and total positive rate was 9.8%. Two IgM positive cases were detected. But their IgG avidity was high and real-time PCR was all negative. Altogether, the preliminary results indicated there was no *Toxoplasma gondii* contamination in the blood bank so far. IgG positive was more prevalent among blood donors who used not-boiled underground water (OR = 1.07, $P < 0.05$), low education status (OR = 0.76, $P < 0.05$) and ate raw shellfish such as scallop and Taiwanese abalone (OR = 1.98, $P < 0.05$).
Conclusion: These findings highlighted the prevalent *T. gondii* in healthy blood donors in Taiwan, and risk factors should be addressed for disease control measures.

4.7 Newly Emerging Pathogens and Other Transfusion Related Pathogens

P-204

HUMAN HERPESVIRUS TYPE 8 IN END-STAGE RENAL DISEASE PATIENTS WITH BLOOD TRANSFUDION

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Background: Human herpesvirus type 8 (HHV-8) is the etiologic agent of Kaposi's sarcoma (KS). The incidence of KS in renal transplant patients is much higher than in healthy controls and the risk of KS is higher among recipients who were anti-HHV-8-positive before transplantation. Patients with end-stage renal disease (ESRD) are candidates for renal transplantation and usually receive blood transfusion, but the prevalence of HHV-8 infection in ESRD patients with or without blood transfusion has not been well documented.
Aims: To compare the prevalence of HHV-8 infection in ESRD patients with or without blood transfusion and age-matched healthy controls.
Methods: Blood samples from 97 ESRD patients and 97 age- and sex-matched healthy controls were collected and analyzed for lymphocyte and monocyte counts, HHV-8 antibody and DNA, and anti-HIV.
Results: ESRD patients had significantly lower lymphocyte counts, higher monocyte counts, and higher prevalence of lymphopenia and monocytosis than did healthy controls ($P < 0.0001$, each). The seropositive rate and titers for HHV-8 antibodies in ESRD patients were significantly greater than those in healthy controls ($P < 0.002$, both). The mean age of the seropositive male patients (58.7 years) was significantly younger than that of females (67.4 years) ($P = 0.0176$). The high seropositive rate in ESRD patients was not associated with lymphopenia, monocytosis, diabetes, dialysis duration, or history of blood transfusion. All subjects were negative for anti-HIV. One diabetic ESRD patient was positive for both HHV-8 antibody and HHV-8 DNA (18,720 copies/ml).
Conclusions: ESRD patients have high seropositive rates for HHV-8. ESRD patients, particularly those positive for HHV-8 DNA, should be closely monitored for HHV-8-associated clinical manifestations if they are to receive renal transplants.

P-205

ESTABLISHMENT OF AN IN VITRO CULTURE SYSTEM AND THE METHODOLOGY FOR EVALUATING AN INFECTIOUS TITER OF HEPATITIS E VIRUS (HEV) USING HEV-RNA-POSITIVE PLASMA OBTAINED FROM BLOOD DONORS IN JAPAN

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Background: The hepatitis E virus (HEV) was found to be transmitted by blood transfusion and to occasionally cause severe hepatitis, although it has been generally considered that hepatitis E is a water-borne disease and mostly found in developing countries. Recently, HEV has been recognized to be spreading widely throughout the world including industrialized nations. Thus, the possibility of HEV infection via transfusion is a major concern globally. It is necessary to establish an evaluation system for HEV infectivity, which will be applicable to evaluating strategies for ensuring blood product safety.
Aims: It has recently been reported that the virus obtained from plasma has a different composition in the envelope-like structure from that obtained from feces. Hence, it is necessary to establish a culture system using HEV-RNA-positive plasma obtained from blood donors and cell lines, and to expand this system to a methodology for evaluating an HEV infectious titer referred to as the tissue culture infectious dose (TCID). Moreover, the numerical relationship between the copies of HEV RNA and HEV TCID was investigated.
Methods: Fourteen plasma specimens containing HEV of genotype 3 were employed, which were either positive or negative for specific IgM and IgG. The cell lines of human hepatoma cells (PLC/PRF/5) and human lung adenocarcinoma cells (A549) were inoculated with viral specimens for 2 h at 37°C. The cells infected with HEV were incubated in a maintenance medium containing 30 mM Mg^{2+} and 2% FCS. This medium was collected and replaced every week. HEV RNA copies were determined by real-time RT-PCR analysis. The establishment of HEV infectivity was confirmed by the detection of viral progenies in recovered media after 3 weeks of incubation. High-load HEV particles were collected by ultracentrifugation (82,700 *g*), and limiting dilution assay was carried out to confirm the TCID of HEV.
Results: Viral progenies were detected in recovered media when the HEV JRC-HE3 strain [IgG(–), IgM (+)] or HEV UA1 strain [IgG(+), IgM(+)] was used, indicating that HEV-positive plasma can express infectivity on cells. No cytopathic effect was observed in any case. The viral progeny concentration reached approximately 10^8copies/ml as a result of a longer incubation of HEV-infected cell lines. Moreover, $10^{9.72}$copies/ml of the JRC-HE3 strain was obtained by ultracentrifugation. Limiting dilution assay showed that at a 10^4-fold dilution of this concentrated JRC-HE3, the infectivity of the strain was expressed. These findings lead us to the conclusion that 1TCID in this situation corresponded to $10^{5.42}$copies of HEV.
Summary/conclusion: We were able to establish an in vitro culture system using HEV-RNA-positive plasma obtained from blood donors in Japan. The methodology for the titration of HEV infectivity may be applied to evaluating strategies for ensuring blood product safety, e.g. pathogen inactivation.

P-206

PARVOVIRUS B19 CONTAMINATION IN CHINESE PLASMA POOLS AND PLASMA DERIVATIVES

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Background: Human parvovirus B19, a member of the genus Erythrovirus within the subfamily Parvovirinae, is a common human pathogen which causes a variety of diseases, such as erythema infectiosum in children, aplastic crisis, chronic pure red cell aplasia, foetal hydrops, foetal death, and so on. The B19 virus can be transmitted via blood/blood products and its resistance to common viral inactivation/removal methods raises the importance of B19-related blood safety. Because of known risks in individuals with chronic anaemia and those who are pregnant or immunocompromised, European Pharmacopoeia and FDA impose a limit of ≤104 IU/ml for all plasma-derived products. However, the prevalence and levels of B19 in Chinese blood products have not been investigated.
Aims: The aim of this study was to determine the prevalence and the levels of B19 DNA in plasma pools destined for fractionation and in a wide variety of plasma derivatives made by Chinese manufacturers.
Methods: Two hundred lots of blood products and 90 lots of plasma pool samples made during 1993–1995 and 2009–2011 from four Chinese blood product manufacturers were analyzed for B19 viral DNA content using an in-house developed Q-PCR assay which detects all three genotypes of the human erythrovirus DNA simultaneously. Results were confirmed by nested-PCR.

Results: The prevalence of B19 DNA in plasma pools were 16.3% (13 out of 80 lots) and 10% (two out of 20 lots) from two manufacturers, and the levels of B19 DNA varied ranging from 2.0 × 103 to 5.85 × 107 geq/ml. The resolving processes are ongoing. The prevalence of B19 DNA were 30% with median level of 2.74 × 103 and 11.1% with median level of 1.24 × 106 in albumn and IVIG, respectively made during 1993–1995. The prevalence of B19 DNA were 5.6% with median level of 5.83 × 103, 25% with median level of 1.12 × 105 and 50% with median level of 3.32 × 104, respectively among IVIG, factor VIII and Fibrinogen made during 2009–2011; however the prevalence of B19 DNA was not detected in albumin made in the same period. In comparison, the prevalence and level in factor VIII and Fibrinogen are relative high.
Summary/conclusions: B19 DNA was detectable in 20–30% of Chinese plasma pools and blood derivatives and the levels of B19 DNA varied among different products. The high prevalence of B19 DNA in Chinese plasms pools and blood products poses high risk to public health, and Chinese SFDA needs to consider to require B19V testing in plasma pools and plasma derivatives.

P-207
DENGUE FEVER VIRAL EXPOSURE RATES AMONG AUSTRALIAN BLOOD DONORS DURING LOCAL OUTBREAKS

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Background: Dengue is not endemic in Australia; rather in North Queensland, outbreaks occur seasonally. One of the largest epidemics in the last 50 years took place in 2008/2009, affecting a significant geographical area of North Queensland, with separate outbreaks in Cairns (and surrounding regions; DENV-2,3,4 08–09) and Townsville (DENV-1,3 09). Collectively, in these outbreaks there were more than 1000 confirmed clinical cases, with the majority of cases occurring in the Cairns region. Given the absence of an approved screening test, the strategy utilised by the Australian Red Cross Blood Service (Blood Service) for managing the risk of transfusion-transmitted dengue was exclusion of at risk donors. During this epidemic, supplementary questioning for all donors was implemented to determine exposure risk, and fresh components were not manufactured from at risk donors.
Aims: This study aimed to estimate dengue fever viral exposure rates among Australian blood donors during this large epidemic.
Methods: Samples were collected from blood donors during the 2008/2009 epidemic and 3 months after the last confirmed cased. Selected samples were tested for the presence of the dengue NS1 antigen with commercially available ELISA-based assay kits from PanBio.
Results: Nineteen of 1020 donations collected in Cairns during the epidemic and selected for testing showed repeat reactivity towards the NS1 antigen, and one of 67 donations collected in Townsville during the epidemic and selected for testing showed repeat reactivity towards the NS1 antigen. Viral RNA was not detected in any of these NS1 reactive donations.
Summary/conclusions: This study suggests recent dengue exposure in a self-declared asymptomatic population, and provides an understanding of the rate and dynamics of asymptomatic dengue infection in North Queensland during these recent outbreaks. Discordant results between NS1 and viral RNA detection needs further evaluation. Collectively, this study justifies the use of DENV management strategy during a DENV outbreak in north Queensland.

P-208
A DUPLEX TRANSCRIPTION-MEDIATED AMPLIFICATION ASSAY FOR THE SIMULTANEOUS QUANTITATION OF PARVOVIRUS B19 DNA AND QUALITATIVE DETECTION OF HEPATITIS A VIRUS RNA ON A FULLY AUTOMATED INSTRUMENT SYSTEM

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Background: To reduce the risk of contamination of plasma derived products with parvovirus B19 (B19) and hepatitis A virus (HAV), nucleic acid testing (NAT) is performed as an in-process test for both Source Plasma and recovered plasma. The US FDA recommends and European regulations require (for certain plasma products) B19 testing to ensure that the viral load of B19 DNA in manufacturing pools does not exceed 104 IU/ml. In process testing for HAV is also commonly performed to achieve an increased margin of safety but its implementation is not universal.
Aims: To develop and assess the performance of a duplex transcription-mediated amplification (TMA) assay for the simultaneous quantitation of B19 DNA and the qualitative detection of HAV RNA on the fully automated TIGRIS System.
Study design/methods: We evaluated precision of B19 quantitation and linearity (quantitative range) from 300 to 175,000 IU/ml using standard panels and diluted parvovirus clinical specimens. To assess the accuracy of quantitation of parvovirus genotypes 1, 2, and 3, the 1st WHO International Reference Panel (NIBSC code: 09/110) was diluted and tested at 104 IU/ml. The limit of detection (LOD) for HAV was determined using the WHO First International Standard for HAV (NIBSC code: 00/560) and was also evaluated in the presence B19 concentrations up to 109 IU/ml.
Results/findings: A quantitative range of >2.5 logs was demonstrated, indicating suitability for screening for high titers of B19 DNA in plasma pool sizes ranging from 16 to 512 donations. Reproducible quantitation of diluted parvovirus clinical specimens was observed; intra-sample variation ranged from 0.02 to 0.09 log IU/ml standard deviation (SD) over the range of concentrations tested (approximately 600–37,500 IU/ml). Genotypes 2 and 3 were quantified nearly equivalently to genotype 1, with <0.2 log IU/ml difference. The 95% LOD for HAV RNA was determined to be 0.84 IU/ml (95% fiducial limits: 0.59–1.48). Hgh titers of B19 had little effect on HAV analytical sensitivity, as 96% detection of HAV at 1 IU/ml was observed in the presence of 109 IU/ml of B19.
Conclusion: These results showed the feasibility of a duplex assay on the fully automated TIGRIS System for quantifying B19 DNA over a quantitative range that would allow screening for high titer B19 samples in commonly used pool sizes. Additionally, we showed sensitive detection of HAV RNA, even in the presence of high titers of B19. Overall, these results demonstrated that this assay may be useful for in process testing of Source and recovered plasma to further improve the safety of plasma-derived products.

P-209
HTLV-1 AND -2 SEROPREVALENCE AMONG UNITED STATES BLOOD DONORS, 2000–2009

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Background: HTLV-1 and -2 infection is prevalent at low levels in the United States, and monitoring of HTLV prevalence among large numbers of blood donors screened at donation may provide useful data for public health surveillance.
Aims: To measure contemporary HTLV-1 and -2 seroprevalence and demographic associations among US blood donors.
Methods: Computerized data on all first-time blood donors in a large network of United States blood centers was examined during the period 2000–2009. HTLV-1/2 antibody was measured by enzyme immunoassay (EIA) screening with reactive samples retested with an EIA from an alternate manufacturer (ALT EIA). From 2006 to 2009, confirmatory data was available from either the California Dept of Health or the Innolia recombinant immunoblot. Prevalence rates were calculated, and odds ratios (OR) and 95% confidence intervals (CI) for associations with demographic characteristics were assessed using multivariate logistic regression.
Results: Among 1,904,155 first-time blood donors, HTLV-1/2 ALT EIA reactivity decreased from 10 per 10,000 in 2000 to 5 per 10,000 in 2009 (P trend < 0.0001). During the latter years with confirmatory testing data, about one quarter of ALT EIA reactives were confirmed, yielding a prevalence of 1.43 per 10,000 (95% CI 1.19–1.72 per 10,000). HTLV-1 was half as common as HTLV-2 (prevalence 0.40 per 10,000 vs 0.87 per 10,000; HTLV positive but untypable prevalence was 0.25 per 10,000). Prevalence increased with age through middle age and then decreased in older age. HTLV-1/2 infection was associated with female sex (OR = 2.28, 95% CI 1.43–3.65), age 50–59 (OR = 15.58, 95% CI 6.01–40.38), Black (OR = 9.16, 95% CI 4.75–17.67) and Asian/Other (OR = 9.24, 95% CI 4.63–18.44) race/ethnicity, and inversely with university education (OR = 0.51, 95% CI 0.27–0.96). There was minimal geographic variation, although donors from the southwestern US had the highest prevalence.
Conclusions: HTLV-1/2 prevalence is decreasing slowly among US blood donors, both over the 10 years of this study and compared to published studies from the 1990's. This is consistent with a birth cohort effect whereby donors born in the 1950s and 60s with the highest HTLV prevalence are now less likely to be first time donors. HTLV associations with female sex, nonwhite race and lower educational achievement are consistent with those from previous studies. With 3.2 million first-time donors annually, US blood banks still detect almost 500 HTLV infections per year which require counseling and medical followup.

P-210
HTLV-1 INFECTION AMONG BLOOD DONORS IN SOUTH CHINA

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Background: HTLV-1 is causatively associated with adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). It is known that transmission of these viruses may occurs through blood transfusions. No effective treatment drugs and preventive vaccines have been successfully developed yet for

HTLV-1 infection. Therefore, the effective method for disease control is to take measures to block the transmission routes of the virus.
Aims: To assess the distribution of HTLV-1 in blood donors and particularly the risk of infected HTLV-1 during the blood transfusion.
Methods: Blood samples were obtained from unpaid blood donors (n = 131,823) from the Xiamen Blood Services from February 1st, 2004, to March 31st, 2009. Primary screening for HTLV-1/2 antibody was performed using the anti-HTLV (1 + 2) Antibody ELISA Kit. Positive samples first screened by ELISA were reconfirmed by western blotting (WB) and/or real-time fluorescence PCR (Taqman-MGB probe).
Results: Twenty-four (0.02%) cases were identified as positive for HTLV-1. Sixteen of them were identified as the HTLV-1 A subtype (Cosmopolitan), 15 strains belonged to the Transcontinental Subtype, and one strain belonged to the Japanese Subtype.
Conclusions: HTLV virus-infected individuals occur in Xiamen blood donor and mainly concentrated in the southeast coastal areas. To prevent HTLV transmission via blood transfusions and to improve blood safety, it is necessary to detect the virus in a timely manner in areas with high incidence and central cities with high population mobility. More systematic studies need to be performed on the molecular epidemiology of HTLV in Xiamen and surrounding areas.

5. Immune Haematology
5.1 Red Cell Immunology: Serology

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This abstract has been withdrawn.

P-212
THE SELECTION OF SCREENING CELLS FOR THE DETECTION OF ANTI-DIA ANTIBODY
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Background: The selection of screening cells is very important because it may directly affect the antibody detection rate. Prior to 1984, there was no difference in selecting screening cells between the white and Taiwanese. From 1984 to 1988, we had only identified 11 cases of Anti-Mia antibody. Since the Mia-positive screen cells became available in 1990, the most common alloantibody now in Taiwan had switched to anti-Mia which relegating anti-E and anti-c to the second and third places. As currently known to us, Mia antigen are positive in 7.3% of Taiwanese and 88% of Amis aboriginal population. Similarly for anti-Dia antibody, it rarely occurred in Taiwan and we encountered only nine cases before the year of 2000. Since then, its detection rate gradually increased after Dia-positive cells added in the screen panel. Here we reported the recent status of anti-Dia antibody, another emerging alloantibody in Taiwan.
Patients and methods: We collected the pretransfusion testing data of our hospital patients from January 2001 to December 2010. Three-cell antibody screening kits (O type, DAT negative cells) were provided by Taiwan blood donation center and Formosa Biomedical Technology Corporation. Both manual polybrene (MP) method and LISS indirect antiglobulin test (LIAT) method were used.
Results: Among all patients received the pretransfusion testing, there were 3666 cases positive for identifiable alloantibodies. The average detection rate of anti-Dia antibody was 1.4% (51/3666).

Table 1

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Alloantibody identified	274	201	219	269	441	369	364	456	539	534	3666
Anti-Dia	0	3	3	3	7	8	9	9	6	3	51

Conclusion: At 37°C anti-Dia is a clinically significant IgG antibody which may cause newborn hemolytic diseases and hemolytic transfusion reaction. We prefer to use the MP method due to its easier performance for issuing blood, especially with 96.8% Dia-negative rate in Taiwan. The LIAT method is more sensitive but time-consuming as compared to MP method. Antibody screening is indispensable in the compatibility test for issuing blood. Professor Marie Lin recommends screening cells must include all antigens with 3% or higher incidence in Taiwan, namely Mia and Dia in addition to frequent antigens of the white people. In order to enhance the antibody detection rate for transfusion safety, she considers the antigen distribution and dosage response. For the former, separate distribution of Mia, Dia, and E, c antigens in three screening cells is suggested. For the latter, it is better to choose homozygous cells than heterozygous cells.

P-213
DETECTION OF ALLOANTIBODIES IN PATIENTS WITH WARM AUTOANTIBODY
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Background: Patient with hematological disease especially autoimmune hemolytic anemia (AIHA) was usually thought to have higher chance to carry red cell alloantibody. In our previous study in early 1980s we reported one case with alloantibody (anti-M) out of nine cases of AIHA (11%), and in 1997–1998 we reported two cases with alloantibodies (anti-E, anti-E+'Mia') out of eight cases AIHA (25%). We reviewed serological study records of warm autoantibody during 22 months period (2005 January–2006 October) at Blood Bank, Mackay Memorial Hospital.
Materials and methods: A total number of 34 cases with warm autoantibody were studied (serological investigation and brief clinical record review). Serological study include antibody identification by manual Polybrene method and LIAT; adsorption and elution of autoantibody.
Results and discussion: Of 34 patients with warm autoantibody, 16 patients were with hematological disease (five cases ITP, two SLE, two AIHA, two MDS, each one case of acute leukemia, lymphoma, anemia, hypereosinophilic syndrome and hemophagocytic syndrome) -first group. The other 18 cases with warm autoantibody of non hematological diseases including pneumonia, liver cirrhosis, etc -second group. The alloantibody associated with first group patients was each one case of anti-'Mia', anti-E+c, anti-C+e and anti-Wra, with the alloantibody frequency of 25% (4/16). Alloantibodies associated with second group were three cases anti-E, three anti-'Mia', each one case of anti-D, anti-E+c, anti-E+Jka and anti-E+Jka+Fya, with alloantibody frequency of 55% (10/18).
The alloantibody frequency associated with hematological disease and AIHA was kept around 11–25% since 1980s in our hospital, however, it is surprisingly to find that the alloantibody was more frequently (55%) associated with patients of non hematological disease who carry warm autoantibody. The reason for these difference is probably due to the patients with hematological diseases are usually under immunosuppressive treatments.
Conclusion: Difference from previous assumption that patients with AIHA have more chance to carry alloantibody in addition to warm autoantibody, while in this study we found that the patients of non hematological disease with warm autoantibody on the contrary have more chance to carry alloantibody (55%) than patients with hematological disease (25%) including AIHA. This probably due to patients with hematological disease are usually undergoing immunosuppressive treatments.

P-214
ANTI-LEA IN LE(A−B+) INDIVIDUALS
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Background: There are differences in Lewis phenotype as well as phenotype distribution between Taiwanese and Caucasians. In Taiwan except for Taiwan Aborigines (2% of total population) having Le(a + b−) phenotype, the majority Taiwanese are 0% Le(a + b−), 25% Le(a + b+), 67% Le(a−b+) and 8% Le(a−b−). During studying the Le(a + b+) phenotype we observed various different strength of Lea and Leb antigens in this phenotype. In our hospital transfusion service we also encountered different pattern of Lewis antibody production among our patients, especially surprisingly to find anti-Lea among Le(a−b+) patients.
Materials and methods: During 5 years period (2005–2010), Blood Bank of Mackay Memorial Hospital issued 188,625 units of red cells, detected 130 cases of Lewis antibodies during antibody screening and pretransfusion testing. The routine procedure of antibody screening and cross matching are by manual Polybrene method. The antibody was further evaluated by LIAT with or without prewarmed technique. Clinical history of 35 patients including 10 patients with anti-Lea, four with anti-Lea + Leb, and nine with anti-Leb in Le(a−b−) phenotype; 10 with anti-Lea in Le(a−b+) phenotype; and two with anti-Leb in Le(a + b−) phenotype were studied.
Results: The clinical history of 35 cases studied showed no significant difference between patients producing Lewis antibodies in various phenotypes. Among 27 cases (7 + 20) with anti-Leb, only one case were group O, so almost all of them were anti-LebL.

Table 1: The Lewis phenotype of 130 cases with Lewis antibody

Lewis phenotype	Lewis antibody	No. of cases
Le(a-b-)	anti-Le^a	91
	anti-Le^a+Le^b	7
	anti-Le^b	20
Le(a-b+)	anti-Le^a	10
Le(a+b-)	anti-Le^b	2

Discussion: It was generally believed that the individuals with Le(a−b+) phenotype should not carry Lewis antibody since their serum contain Lea and Leb. However, in this study we found 10 patients carried anti-Leb (although six of them had recent transfusion). In the previous serological study of Le(a + b+) phenotype, we found not only Leb were weak in some cases, but Lea could also be weak in some other cases. This makes us considered the molecular basis of Le(a−b+) could also be possible to be due to variant FUT3 in our population (to explain carrying anti-Lea). Molecular genetic study of Asian Lewis blood group system warrant further study.

P-215
MICROPLATE POLYBRENE METHOD FOR BLOOD BANK AUTOMATION

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Background: Application of manual Polybrene method (MP) in pretransfusion testing has been widely used in Asian countries especially Taiwan and China. This method was reported by Lalezari and Jiang (*Transfusion* 20: 206–2011) in 1980. We introduced MP to Taiwan in1983 and to China in 1990s. MP soon proved to be the most suitable method for Taiwan. It is a sensitive, simple, rapid and inexpensive method for compatibility testing to standardized pretransfusion testing procedures. Within a few years, MP was incorporated successfully into routine pretransfusion testing procedures throughout the whole of Taiwan. In the current trend of automation in laboratory medicine, however, blood bank is the only exceptional area in Taiwan to be automated. Although several tertiary hospital blood banks owned Western automation machines, however, since the relative shortage of blood bank staffs in Taiwan as compare to Western country, the efficacy of machine was not fully operated (MP is a rapid method). Another reason for not fully used is probably due to inexpensiveness of MP method. Therefore we initially try to automate the MP method by using microplate (microplate Polybrene method, MPP).
Materials and methods (modifying MP method forMPP): Microplate: Nunc 96 well, U-shaped bottom, nontreated microplate.
Reagents: properly modifying the concentration, constituents and volume of LIM, Polybrene and resuspending solutions used in MP for MPP.
Procedure: modifying the reacting temperature and duration for MPP.
Results and discussions: Since the volume of microplate well is small (300 μl). Therefore we modified the reagent volume and procedures (duration and temperature) for MPP method to get proper sensitivity and specificity. We also add procedure to avoid picking up too many nonspecific cold agglutinins as MP do. The initial results are shown in the following table. The results of MPP correlated well with Cellbind test. The initial results were promising.

Table 1

Antibody	Manual Polybrene		Microplate Polybrene		LIAT		Gel column Cellbind (Sanquin)		No. of cases
	+	-	+	-	+	-	+	-	
+	152	**0**	136	**18***	115	37	137	**17****	152
-	0	100	0	100	0	100	0	100	100

*18 cases were 10 cases of weak nonspecific cold agglutinins, 2 weak anti-E, 3 weak anti-Mia, 2 weak anti-Lea and 1 weak anti-Leb. **17 cases were 10 cases of weak nonspecific cold agglutinins, 2 weak BSA (autoantibody of broad specificity), 2 weak anti-E and 3 weak anti-Mia

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This abstract has been withdrawn.

P-217
THE COMPUTER CROSSMATCH: THE 5 YEARS EXPERIENCE IN THE CZECH REPUBLIC

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Background: The computer (electronic) cross match (CCM) is a method for blood compatibility screening. It is based on correctly collected donor/recipient data, and its safe input to the information (computing) system. Consequently, the software, while using the pre-set constant pre-requisites, determines whether the chosen erythrocytic preparative is acceptable for the blood recipient's transfusion, or whether serological cross match needs to take place.
Method: The computer cross match is an additional module for laboratory information (computing) system in a blood bank lab. It provides electronic control over fulfillment of the conditions for blood administration, with no need for serological cross matching.
Conditions which must be met for the CCM:

1. The electronic (computer) request only,
2. Patient is suitable for the CCM and his/her request is conforming; the unsuitable cases must be clearly defined,
3. Compatibility of patient and donor blood group,
4. Negative red cell antibodies (examination is not older than 42, or 14 days in case of a pregnant woman),
5. Laboratory testing are preferentially provided by a fully automated system; if provided manually, the minimum of two identical results must be delivered,
6. The computer system, as well as the CCM, must be validated.

Unacceptable scenarios for the CCM:

1. Patient was evaluated to be unsuitable for the CCM in the past,
2. Blood grouping was not verified,
3. Presence of red cell antibodies, now or in patient's history,
4. Red cell antibodies screening was done 43+ days ago,
5. Last blood is more than 72 h old and was not put through the new antibody screening,
6. Attributes of haemolysis are present.

Practice in the Czech Republic:
The CCM has been in use routinely since August 1, 2005 in Central Military Hospital Prague. The fully automated system Galileo ImmucorGamma is used for blood grouping serology and antibody screening. The CCM is used in 82% cases (Tab No 1). The CCM brings the following benefits: (i) it saves time (especially with massive transfusions), (ii) it saves costs, (iii) it cuts staff.

Table 1: Statistics of CCMs in CMH Prague

	Total number of cross-matches	Number of CCM	% CCM from total N of cross matches
2005 (since August)	4 798	3 315	69 %
2006	13 167	11 029	84%
2007	13 252	11 174	84%
2008	14 266	12 020	84%
2009	13 807	11 377	82%
2010	13 326	10 455	79%
Total 2006-2010	67 818	56 055	82,6%

Conclusion: The CCM is a save, economical and fast solution, suitable for all types of hospitals.

P-218
AMOXICILLIN/CLAVULANATE-INDUCED HEMOLYTIC ANEMIA: A CASE STUDY

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Background: Amoxicillin/clavulanate is known to cause hemolytic anemia (HA). A 3-years-old child with bronchitis was treated with amoxicillin/clavulanate days before. The patient revealed pale, yellowish skin, tachycardia and hypotension on admission. Her hemoglobin (Hb) dropped from 11.7 to 3.0 g/dl in 5 days. Serum lactate dehydrogenase (LDH), haptoglobin and urine all showed evidences of hemolysis. No glucose-6-phosphate dehydrogenase defeciency was found.

Aims: Our aim was to prove patient's hemolytic crisis was caused by amoxicillin/clavulanate. Non-immunologic protein adsorption (NIPA) onto red blood cells (RBCs) was thought to be the amoxicillin/clavulanate's hemolytic mechanism.
Methods: Our investigations included direct antiglobulin tests (DATs), indirect antiglobulin tests (IATs). The antibody screen and identification was verified by LISS (low ionic strength solution), manual polybrene (MP) tests. Serum and eluate from the patient were tested against amoxicillin/clavulanate treated RBCs. Patient's serum was also tested against untreated RBCs in presence of amoxicillin/clavulanate. We also compared the patient's and normal serum samples (non-diluted and diluted one in 20) for proving NIPA.
Results: The DAT result was negative before administration of amoxicillin/clavulanate. After applying amoxicillin/clavulanate, patient's RBCs showed strongly positive DAT (anti-IgG negative, anti-C34+), but the eluate was nonreactive with commercial panel cells. The autologous control was positive either IAT or MP. However, patient's serum showed positive by IAT, but negative by MP. The patient's sera did not react with RBCs in presence of amoxicillin/clavulanate, but the patient's sera and normal sera reacted with amoxicillin/clavulanate -treated RBCs. We discontinued amoxicillin/clavulanate use and patient received two units of RBCs transfusion. Prednisolone was also used at the same time. Patient's Hb and LDH levels returned to near normal values 6 days later. There was no direct clinical infection evidence contributing to hemolytic event.
Conclusions: Combined with patient's history, clinical data and laboratory results, we strongly suspected the HA was caused by amoxicillin/clavulanate. More specialized laboratory examination may be required to confirm this definite diagnosis. The MP test may not be sensitive enough for survey drug related HA.

P-219
PREVALENCE OF RH PHENOTYPES AND PROBABLE GENOTYPES IN RH (D) NEGATIVE BLOOD DONORS AT SPS APOLLO HOSPITALS, LUDHIANA, PUNJAB, INDIA

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Background: The Rh (Rhesus) blood group system is clinically the most important blood group system after ABO system. The Rh blood group system currently consists of about 50 defined blood group antigens, amongst which the five antigens, D, C, c, E and e are the most important ones. These antigens are encoded by two adjacent gene loci, the RHD gene and the RHCE gene. The Rh phenotypes are readily identified by the presence or absence of Rh surface antigens. The exact genotype of any individual can only be identified by DNA analysis. The Rh phenotype is clinically significant and ensures that the patient is not exposed to an antigen, he is likely to develop antibodies against.
Aims: To study the prevalence of Rh antigens (phenotype) in Rh (D) Negative blood donors and to classify them in the probable genotype categories.
Methods: The study was conducted at Department of Transfusion Medicine at SPS Apollo Hospitals, Ludhiana, India from January 2006 to December 2010. All blood donors during this period were initially typed for Rh D antigen, using anti-D monoclonal antisera (ERYCLONE) manufactured by Tulip Diagnostics (P) Ltd. The blood donors testing Rh D Negative were then tested for other Rh antigens using monoclonal anti-C, anti-c, anti-E and anti-e, manufactured by Tulip Diagnostics (P) Ltd.
Results: A total of 17,616 blood donors were tested from January 2006 to December 2010. Out of which 1539 (8.73%) were Rh D Negative. The prevalence of Rh antigens in Rh D Negative blood donors was antigen C 13.2%, E 7.2%, c 99.9% and e 90.8%. The probable genotypes amongst these blood donors was r/r 7.6%, r′/r 0.52% and r″/r 0.63%.
Conclusion: The Rh antigens C, c, E and e are highly immunogenic. It is recommended to test all blood donors for these antigens so that antigen negative blood could be transfused to the patients for increased blood transfusion safety. It is also recommended to blood centres to have a database of all the blood donors, which can be utilised for patients especially Rh D Negative patients.

P-220
ANTI-A, B ANTIBODY FOUND IN A BLOOD GROUP A RECIPIENT FOLLOWING LIVER TRANSPLANTATION

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Background: Owing to limited sources ABO minor-mismatched organs were often used for clinical transplantation. 'Passenger'lymphocytes producing antibodies against recipient's RBCs may provoke immune-mediated hemolysis following organ transplantation, known as 'passenger lymphocyte syndrome (PLS)'.
Aims: To work out a patient suspicious for PLS.
Methods and results: A 22-year-old, blood group A female patient experienced hemolysis 10 days after receiving liver transplantation from a group O donor. Her Hb fell from 14.2 g/dl before and down to 8.5 g/dl 10 days after transplantation. Serum typing demonstrated anti-A and anti-B antibodies. The result of serum irregular antibody screening was negative. DAT was positive for IgG and anti-A, B was eluted from her RBCs. PLS was diagnosed. She was successfully treated with combination of group O RBC transfusion, steroid, and immunosuppressants.
Summary: PLS is a complication of ABO minor-mismatched organ transplantation, particularly occurring in a blood group A or B recipient from a group O donor. It usually develops within 1–3 weeks following transplantation and is a self-limited process. Transfusion of compatible RBC or plasma component, plasmapheresis, and anti-CD20 monoclonal antibody are most considered therapeutic strategies.

P-221
A NEW *RHCE* ALLELE WITH 358G>C (ALA120PRO) MUTATION AFFECTING THE EXPRESSION OF RH3 AND RH4

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Background: The Rh antigens arise from polymorphism in two highly homologous and closely linked genes, *RHD* and *RHCE*. The *RHD* gene encodes the D polypeptide, whereas *RHCE* encodes the polypeptide carrying C (RH2) or c (RH4) together with either E (RH3) or e (RH5) antigens. Numerous variant *RHCE* alleles with reduced expression of C, c, E, and e antigens have been described in several populations, especially in individuals of African origin and Caucasian.
Aims: We report the molecular genetic analysis of two Japanese individuals with weak expression of c and E antigens.
Methods: Standard serological tests were performed using polyclonal and monoclonal antibodies. Expression of Rh antigens on the RBCs was measured by a flow cytometer using FITC-labeled anti-human IgG. Relative fluorescence intensity was calculated using R1R2 RBCs as the control. Genome DNA was isolated from whole blood cells and cDNA was synthesized by RT-PCR using mRNA extracted from reticulocyte. *RHD*, *RHCE*, and *RHAG* genes were amplified by PCR and nucleotide sequences were analyzed. Expression study of RhCE antigens on CHO-K1 cells was performed using the constructs of *RHCE*-expression vector.
Results: Both Japanese individuals were typed as D + C + c + E + e+ but weakened reactivity was noticed for c and E antigens. Flow cytometric analysis revealed that the relative fluorescence intensities of c and E were 5–6% and 7%, respectively as compared with those of the R1R2 RBCs. Nucleotide sequence analysis revealed that both individuals had common *RHD* and *RHAG*, but had heterozygous G/C at position 358 in exon 3 of the *RHCE*. By cloning, the 358G>C (Ala120Pro) mutation was observed in *RHcE*. When expression study using the constructs of common and mutant *RHc*E was performed, no c and E antigens were observed on the transfectant of the mutant *RHcE*.
Conclusion: We identified a new *RHcE* allele having 358G>C (Ala120Pro) mutation from Japanese individuals with weak expression of c and E antigens. This mutation is presumed to be positioned at transmembrane domain of the RhCE polypeptide. Expression study of the mutant *RHcE* was correlated well with the serological results.

P-222
AN EFFICIENT MASSIVE 'MI$^{A'}$ SCREENING METHOD BY AUTOMATIC BLOOD GROUPING SYSTEM

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Background: In Taiwan, the frequency of 'Mi$^{a'}$ is 7.3% and the indigenous people have higher frequency, Amis 88.4%. The area of aboriginal distribution stretches along in eastern Taiwan; it causes higher risk of transfusion reaction with 'Mi$^{a'}$. The Hualien Blood Center in eastern Taiwan offer specific antigen-negative RBC to patients in hospitals. Considering the high frequency of 'Mi$^{a'}$ in Hualien areas, screening 'Mi$^{a'}$ antigen of donors in the eastern Taiwan by traditional manual method will spend time and cost.
Aims: The aims of this study are to prevent higher risk of transfusion reaction with 'Mi$^{a'}$, design a protocol to screening 'Mi$^{a'}$ antigen of donors by PK7300 automated blood analyzer and confirming 'Mi$^{a'}$ antigen-positive by manual method.
Methods: Donor samples from Hualien blood center in Taiwan were screened from May to October in 2010. The anti-'Mi$^{a'}$ were prepared by donor's plasma with anti-'Mi$^{a'}$ and twice diluted with normal saline. Twenty-five microlitre 1.7% red blood cells suspension and 25 μl anti-'Mi$^{a'}$ were transferred to the microplates in PK7300. The microplates were incubated at 30°C for 1 h. The results were interpreted automatically by machines according to their optimized threshold of SPC, P/C and LIA. To validation of method, we prepare 'Mi$^{a'}$ antigen-negative samples test with donor's plasma with anti-'Mia' in PK7300 for specificity test, and 'Mi$^{a'}$ antigen-positive samples for sensitivity test. Those samples were performed parallel test with Manual Polybrene method. QC control was

performed with 5 'Mi$^{a'}$ antigen-positive samples and 5 'Mi$^{a'}$ antigen-negative samples in each batch. Refer to the donor's plasma cannot detect all of the Miltenberger antigens, only the 'Mi$^{a'}$ antigen-positive screening result samples were confirmed with the other donor's plasma with anti-'Mia' by Manual Polybrene method. Those 'Mi$^{a'}$ antigen-positive results were recorded into the blood management system.

Results: We prepare two lots of anti-'Mi$^{a'}$ from two different AB blood type donor's plasma. Test with 10 and 5 'Mi$^{a'}$ antigen-negative samples for two lots of anti-'Mi$^{a'}$ are all negative for specificity test, respectively. Test with 10 and 5 'Mi$^{a'}$ antigen-positive samples for two lots of anti-'Mi$^{a'}$ are all positive for sensitivity test, respectively. The 40 random samples were parallel test by PK7300 and Manual Polybrene method with two lots of anti-'Mi$^{a'}$ and present expected result. In this study, 800 'Mi$^{a'}$ antigen-positive donors were identified from 10,365 blood donors within 6 months. The frequency of 'Mi$^{a'}$ in this study is 7.72%.

Conclusions: In this study, we provide an economic and efficient method of screening 'Mi$^{a'}$ blood phenotype from large number of blood donors by automatic blood grouping instruments. Those 'Mi$^{a'}$ antigen-positive results were recorded into the blood management system. It can be reduce 'Mi$^{a'}$ antigen-positive blood providing to the patients who have anti-'Mi$^{a'}$ to prevent higher risk of transfusion reaction with 'Mi$^{a'}$ in eastern Taiwan.

P-223
PREVALENCE OF RED CELL ALLOANTIBODIES IN HEMODIALYSIS PATIENTS IN SOUTHERN TAIWAN

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Background: Red blood cell (RBC) alloantibodies may be formed following repeated RBC transfusions. However, RBC transfusions are frequently used in the management of patients with dialysis-related anemia. Consequently, they are subject to all hazards associated with RBC alloimmunization. The objective of this study was to compare the prevalence of RBC alloantibodies between hemodialyisis and general non-hemodialysis hospital-based patients at the Blood Transfusion Unit of E-DA Hospital, Kaohsiung, Taiwan.

Materials and methods: A retrospective study was performed utilizing data of antibody screening tests for transfusion from 2005 to 2010. The transfusion records of a total number of 30,071 hospital-based patients were reviewed for the frequency of alloimmunization. Among them, 965 patients were from the unit of hemodialysis of the Nephrology ward at E-DA Hospital. The RBC antibody screening and identification were performed by manual polybrene method and indirect antiglobulin tests.

Results: The overall prevalence of alloimmunization after blood transfusion was 0.82% (253/30,071). There was a significantly higher RBC alloimmunization rate of 4.24% (41/965) in hemodialysis patients ($P < 0.001$). The majority of these patients (81%) had a single alloantibody, whereas the remaining 19.0% had multiple antibodies. The mean number of transfusion received by the immunized hemodialysis patients was 25.7 units, while all immunized patients received an average of 9.5 units. The difference was significant ($P < 0.001$). The anti-Mia antibody was the most common alloantibody encountered (46.3%) followed by the anti-E antibody (36.6%) and the anti-c antibody (7.3%).

Conclusions: The rate of RBC alloimmunization after blood transfusion was significantly higher in hemodialysis patients. Our study confirms the significance of post-transfusional alloimmunization as a complication in hemodialysis patients and they had a higher risk of red cell alloimmunization than other general hospital-based population. The majority of alloantibodies demonstrated were anti-Mia, anti-E and anti-c. Since it is generally not difficult to find donor red cells negative for these antigens, the study also emphasizes the necessity to perform immunohematology studies for Mia and E antigens prior to blood transfusion, particularly in cases that require multiple transfusions for a long period of time such as in hemodialysis patients.

P-224
DEVELOPMENT OF A DOUBLE VERIFICATION BLOOD TYPING SYSTEM IN BLOOD BANK USING HEALTHCARE FAILURE MODE EFFECTIVENESS ANALYSIS

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Background: To eliminate ABO testing error in the blood bank we develop a double verification blood typing system (DVBTS) using both manual test tube method and automatic gel test and based on healthcare failure mode and effect analysis (HFMEA) in Kaohsiung Medical University Hospital.

Method and material: A proactive thorough analysis of transfusion process in the blood bank using HFMEA was performed to determine the critical process in ABO testing. Before implementation of DVBTS, a total of 165,000 transfusion episodes were reviewed from July 2007 to June 2010. Among these episodes, ABO testing was done in 14,184 out of 61,077 blood samples. There were 30 ABO mismatched transfusion including wronged sampling 20 (1/3054), blood typing error 3 (1/4728), and wronged patient identification 7 (1/23,571). A Double verification blood typing system was implemented.

Result: The preliminary report showed the feasibility of DVBTS in clinical blood banking. After implementation of DVBTS in July 1st 2010, we did the pilot performance evaluation the performance. A total of 3268 out of 31,610 transfusion episodes were asked for ABO typing test from July 1st, 2010 to January 31, 2011. Only two mismatched typing between the tech and AutoVue were identified. Also we can find the specific ABO red blood cell typing in DVBTS. So DVBTS can eliminate the potential risk of ABO testing error caused by the tech in the blood bank.

Inclusion: DVBTS with HFMEA is a useful tool to reduce the potential serious transfusion fatalities due to ABO testing caused by the tech in the blood bank. Further study is warranted.

P-225
A RARELY ANTIBODY OF JK3 IN BLOOD BANK OF TAIWAN

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Background: The patient is a 74-year-old female with diabetes mellitus (DM) and hemodialysis history, due to discovery of liver abscess with slightl fever requiring surgical operation for teatment. The pathology results pre op were Hb: 7.5 g/dl; Hct:23.8%. Four RBC units and six FFP units were requested to cover the surgery. We discovered that irregular antibody screening cell has 1+ responses, Another technique, the MP, AHG phase method of panel cell also has the same response. Taken together, these results demonstated the presence of an alloantibody. After inquiring the about the patients medical history, it was found that the patient had hemodialysis and blood transfusion records in other hospitals; No blood transfusion response.

Aim: When irregular antibodies are found, the antibody must be identified as irregular antibodies may cause hemolytic transfusion reactions, which may lead to mortality.

Method: Direct antiglobulin tests (DAT) performed using antihuman globulin (polyspecific), panel cell (Sanquin), enzyme-treated panel cell (papain) by MP, AHG the phase method also has 1+ responses proving the existence of irregular antibodies.

Results: The patient's serum with panel cell has positive (1+) response, using enzyme-treated panel cell increase to enhanced response, (2+), direct antiglobulin tests (DAT) was positive (±), patient's auto-control was negative. Using anti-serum tested the patient's phenotype is Jk (a−b−). (Table.1)

Table 1: Panel results

Table. 1.

	Rh-Hr				Duffy		Kidd		Lewis		MNS			MP	IS	37℃	AHG	enzyme
	C	D	E	c	e	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s					
1	+	+	0	0	+	0	+	0	+	0	+	+	0	+	0	1+	R	a	1+	2+
2	+	+	0	+	+	+	+	0	+	0	+	+	0	+	0	1+	R	a	1+	2+
3	0	+	+	+	0	+	+	0	+	+	0	0	+	+	+	1+	R	a	1+	2+
4	+	0	+	+	+	0	+	+	+	0	+	+	+	+	+	1+	R	a	1+	2+
5	+	0	0	+	+	+	+	+	+	0	+	0	0	+	+	1+	R	a	1+	2+
6	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	1+	R	a	1+	2+
7	0	0	+	+	+	+	+	+	0	+	+	0	+	+	+	1+	R	a	1+	
8	0	0	0	+	+	+	+	0	+	+	+	+	0	+	+	1+	R	a	1+	
9	0	0	0	+	+	+	+	+	0	+	+	+	0	+	+	1+	R	a	1+	
10	0	0	0	+	+	+	+	+	0	+	+	+	0	+	+	1+	R	a	1+	
11	+	+	+	0	+	0	+	+	+	0	+	+	+	0	+	1+	R	a	1+	
Phenotype	3+	4+	1+	1+	3+	2+	R	W	+	0	R	4+								

Conclusions: JK (a−b−) is rare in most population of the world. These individuals with the blood type detected after has immunized to Kidd antigens during transfusion or pregnancy. The Kidd system antibodies will cause the delayed hemolytic transfusion reaction. Our hospital only found two Anti-Jk3 cases from 2000 to 2010. At present, the blood center has set up the files for rare blood-type blood donors, and makes the blood to frozen, thawed deglycerolized red blood cell (can be preserved for 10 years) to supply to the rare blood-group patients for emergency purpose.

P-226
THE PREVALENCE OF 'HIGH TITER' ANTI-A OR ANTI-B IN GROUP O SINGLE- DONOR APHERESIS PLATELETS IN TAIWAN

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Background: Owing to low availability of type-specific single-donor aphersis platelets (SDPs), transfusion services sometimes issue ABO-mismatched platelets. However, hemolytic transfusion reactions are rare but potentially severe complication in minor ABO-mismatched platelet transfusions. Group O SDPs are most commonly impliced due to the presence of unusually high titers of anti-A or anti-B antibodies in the large volume plasma. The specific aim of this study was to determine the prevalence of 'high-titer' anti-A or anti-B in group O single- donor apheresis platelets in Taiwan.

Materials and methods: Anti-A and anti-B titers were determined in plasma samples of one hundred group O SDPs by using tube methods of indirect agglutination. The reciprocal of the highest dilution giving macroscopic agglutination was considered as the agglutinin titer. Results:
Titers of at least 16 and/or 256 from either buffered (generally reflective of IgM antibodies) or AHG phase, respectively. Significantly high IgM titers of anti-A and anti-B were found in 35% and 42%, whereas, high IgG titers of anti-A and anti-B were found in 29% and 33%, respectively. There are 17 samples with both IgG high-titer of anti-A and anti-B. Of these 17 samples, IgM high-titer of both anti-A and anti-B were found in eight samples.
Conclusion: The prevalence of high-titer anti-A or anti-B in group O SDPs is relatively high in Taiwan. Thus, the risk of minor ABO-mismatch and potential intravascular hemolysis during group O SDPs transfusion may occur. Therefore, the anti-A and anti-B of group O SDPs should be determined, prior to out-of-group platelet transfusion.

P-227
EVALUATION OF EIGHT-COLUMN GEL CARD BASED ON THE COLUMN AGGLUTINATION TECHNIQUE (DG GEL®)

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Background: There is an increase in the number of laboratories that replace conventional tube tests with column tests for pre-transfusion purposes. The type of microtube gel column used in Japan is a 6-column format. However, there are also the DG Gel® cards (Diagnostic Grifols S.A., Barcelona, Spain) consisting of a plastic support of eight microtubes. The column tests offer many advantages over traditional methods: improved sensitivity and specificity, no-wash antiglobulin procedure, standardized procedures, improved turnaround time and enhanced regulatory compliance.
Aims: The aim of the present study was to evaluate the estimated diagnostic accuracy of the new Grifols DG Gel® system and to compare the data with the well-established DiaMed-ID® system and the conventional tube test in Japan.
Methods: ABO/D grouping test, antibody screening test, antibody identification and DAT (direct anti-globulin test) were compared using samples in routine tests performed in two different facilities. Grifols DG Gel® cards and DiaMed-ID® cards were examined in one facility. In addition, conventional tube tests were compared in another facility. Specific reagents were used for card tests and tube tests.
Results: Of the 432 analyses performed for the ABO/D grouping on DG Gel® cards and ID-cards, 431 (99.8%) were concordant. One discrepant result was a false-positive on an ID-card for an unexpected antibody. Of the 421 analyses performed for the ABO/D grouping on DG Gel® cards, ID-cards and tube tests (three methods), 420 (99.8%) were concordant. The discrepant result (weak-positive on tube test) was a false-negative on DG Gel® cards and ID-cards for reverse grouping test. Of the 445 antibody screening and identification performed on DG Gel® cards and ID-cards, discordant results were observed in five samples (1.1%). Three samples were cases of an antibody detected by DG Gel® cards only and two samples were cases of an antibody detected by ID-cards only. Of the 426 antibody screening and identification tests performed using all three methods, discordant results were observed in seven samples (1.6%). Of the seven discrepancies, one was nonspecific positive result on ID-cards, one was a case of an antibody detected by DG Gel® cards only, two were cases of antibodies detected by DG Gel® cards and tube tests, and three were cases of negative or undetected reactions in the three methods. Of the 31 DAT performed in the three methods, discordant results were observed in four samples (12.9%) that showed negative in the tube test. Of the four discrepancies, one was a case of an autoantibody and three were nonspecific positive results on DG Gel® cards and ID-cards.
Conclusions: Grifols DG Gel® system is a valid method comparable to the DiaMed-ID® system and conventional tube tests. The 8-column card of the DG Gel® system might represent an advantage since it allows higher numbers of tests per cycle. Patient safety is expected to improve based on sensitivity, specificity, time saving and cost-effectiveness in the laboratory.

P-228
SCREENING FOR RED CELL ANTIBODIES IN BLOOD DONORS AND RECIPIENTS IN SAURASHTRA REGION OF GUJARAT, INDIA

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Introduction: Antibody screening for donors and patients is performed at very few centres in our region. Many patients currently receive only saline cross-matched blood due to lack of proper Antibody screening protocols.
Aim: To determine the extent of red cell antibodies in donor and patient population in Saurashtra region.
Materials and methods: Thirty-four thousand eight hundred and twenty-seven blood donors and 14,209 patients blood samples were screened for presence of red cell alloantibodies using a two cell panel with the (Galileo) SPRCA technology. All positive cases were further tested for antibody identification with the 11 cell panel (Diamed ID Diapanel)
Results: 0.12% (43 of 34,827) donors and 0.73% (104 of 14,209) patients showed presence of red cell alloantibodies. The occurance of antibodies in female donors (0.35%) significantly higher than in males (0.11%). 16.2% donors had a positive DAT. Positive antibody screening results were observed in 0.87% of female patients and 0.64% of male patients. Thalassaemia (21%), non hemolytic Anemia (12.5%) and AIHA were the most common clinical conditions (10.5%) associated with irregular red cell antibodies. The most common antibodies identified were anti -e, anti -E, Anti- D and Anti- K.
Conclusion: Presence of red cell alloantibodies is detected in significant number of patient population especially those with hemolytical disorders. Screening protocols for red cell allo- antibodies shall remain a challenging task for future.

P-229
PREVALENCE OF ALLOANTIBODIES TO GP.MUR AND DIEGO PHENOTYPES IN SOUTH KOREA DETERMINED BY THE USE OF SCREENING CELLS CONTAINING MUT/MUR KODECYTES

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Background: The hybrid glycophorin GP.Mur is commonly seen in Chinese and South East Asian populations but little is known of its incidence in the South Korean population and whether or not alloantibodies to GP.Mur are seen. Commercial screening cells that are internationally compliant and which also have the ability to detect antibodies to variant glycophorins and Dia have not previously been available to diagnostic laboratories.
Aims: To antibody screen a minimum of 4000 hospital in-patients using screening cells expressing MUT, Mur and Dia antigens. To phenotype 100 random patients for the presence of the GP.Mur phenotype using a monoclonal anti-Mur antibody.
Results: A total of 4830 antibody screens were conducted using DiaMed Column Agglutination Technology at three major South Korean public hospital laboratories: ASAN Medical Centre, Chonnam Hwasun National University Hospital and Pusan National University Hospital. Positive samples were shipped to CSL Limited, Parkville, Australia for antibody identification. Clinically significant antibodies were identified in 0.7% of patients. As expected, antibodies to Dia were detected in significant numbers but antibodies to hybrid glycophorins were only seen in one patient. No GP.Mur phenotypes were detected in the 100 samples tested.
Conclusion: Alloantibodies to hybrid glycophorins were not commonly detected in this population and the incidence of the GP.Mur phenotype appears to be <1%, much lower than that seen in China and South East Asia. Antibodies to Dia are present in significant numbers.

P-230
EVIDENCE FOR AN INFLUENCE OF THE SECRETOR STATUS ON THE LEVELS OF THE ABO ISOANTIBODIES

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Background: ABO-iso-antibodies are naturally occurring antibodies and are regularly present if corresponding antigen is absent. The antibodies are developed during early childhood of 3 months and remains throughout the adult life. The levels of antibodies tend to vary from one individual to another under the influencing factors like age, sex, infections, vaccination etc.
Aims: The present study was undertaken to find an influential role of the secretor status on the levels of the ABO-antibodies among the Omani healthy youths.
Methods: The students, the age group of 17 years and above at the Institute of Health Science, Muscat were participated in this prospective study. Standard serological methods by tube technique were employed. Secretor status on saliva samples of the participants was determined by haemaggltination inhibition of anti-A, anti-B and/or anti-H reagents. Level of the antibodies in serum of the participants was determined by titration against the reagent red cells of appropriate ABO phenotype. An individual having a titer value of 128 or more was defined as having high titer while the one with the titer of 1:64 or less was considered as having a low titer of the antibody. Statistical analysis was performed on the data using a 2×2 contingency table with the Fisher's exact test.
Results: Eighty four of 130 participants in the study showed a high titer (≥128) antibodies while 46 individuals displayed a low titer (≤64) for the antibodies. Inter-

estingly, as many as 77 persons showed a high titer values among 95 subjects classified as 'Secretor'. On the other hand, 28 individuals of 35 'non-secretor' had a low level of the antibodies. Statistical analysis showed the association of secretors status with the levels of the ABO-isoantibodies was extremely significant (the two-tailed P-value being <0.0001).

Conclusion: Individuals classified as 'secretors' often have a high-titer ABO iso-antibodies.

P-231

RED CELL ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSAEMIA PATIENT'S IN SAURASHTRA REGION OF GUJARAT, INDIA

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Background: Thalassaemia Major is a common hemoglobinopathy in Saurashtra region of Gujarat, India. However, very little is known about the frequency of red cell alloimmunization in this transfusion dependent Patient group.

Aim: To Study the Red cell alloimmunization in Transfusion dependent Thalassaemia Patients in our region.

Materials and methods: Retrospective analysis of data was done and a total of 160 Thalassaemia Major patients data was found to be complete and available for analysis. Patient's clinical; immunohematological and biochemistry reports were reviewed. The age at which RBC allo/auto antibodies developed and the complete transfusion history was noted.

Results: Sixteen out of 160 (10%) Thalassaemia Major Patients developed RBC allo-antibodies. The auto control and DAT was positive in six cases of which four (25%) Patient's had an allo-antibody underlying the autoantibody. Antibodies against the antigens in Rh system (25%) and Kell (18.75%) were the most common clinically significant allo-antibodies identified. Anti-K developed in 3 (18.75%) Patient's, Anti-E in 3 (18.75%) Patient's, Anti-D in 2 (12.5%) Patient's and Anti-Kpa in 3 (18.75%) Patient's.

All allo-antibodies developed between the age of 1–24 years, earliest alloimmunization to RBC transfusions was observed in a Patient only after receiving three blood transfusions. Only 5% of Thallasaemia Patients were on leucodepleted blood component therapy.

Conclusion: The various contributory factors leading to alloimmunization in our Thalassaemia Major Patients need to be identified. Lack of completely matched blood (many centers still provide only blood compatible in saline phase of cross-matching) and non-leucodepleted blood components could be major contributory factors to transfusion complications in Thalassaemia Major Patients.

Provision of pre storage leucodepleted blood and phenotyped matched RBC transfusion can prevent of delay alloimmunization in Thalassaemia Major Patients.

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This abstract has been withdrawn.

P-233

EVALUATION OF THREE ANTIBODY DETECTION METHODS IN PRETRANSFUSION SCREENING FOR ANTI-MI^{A} AND ANTI-E WITH AND WITHOUT DITHIOTHREITOL TREATEMENT

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Background: The manual polybrene method (MP) is recommended and adopted as routine in most blood banks for antibody detection in pretransfusion testing in Taiwan. Although MP is time-saving with high sensitivity, it can not differentiate cold active or clinically insignificant IgM from IgG types of alloantibodies which was characterized in a previous study (Lo et al, Vox Sanguinis 2002). Moreover, as increasing use of automation in pretransfusion testing, the performance of automated detection of anti-Mi^{a} and anti-E, the two most prevalent alloantibodies in Taiwan, remains unclear when considering it as a substitute for MP in antibody detection in terms of antibody characteristics.

Aims: The present study was to compare MP, indirect antiglobulin test using low ionic strength solution (LISS-IAT) and automated column agglutination test (CAT) for detection of anti-Mi^{a} and anti-E with and without Dithiothreitol (DTT) treatment.

Methods: Serum or plasma sample submitted to pretransfusion testing and finally confirmed to have either anti-Mi^{a} only or anti-E only were prospectively recruited. MP was used as routine to detect antibodies. All samples were subject to LISS-IAT and CAT for antibody detection as comparison. LISS-IAT was performed according to the AABB guideline. The anti-human globulins used in LISS-IAT were polyspecific (Immuncor Gamma, Norcross GA, USA). CAT was performed on a fully automated AutoVue system using BioVue Polyspecific Anti-Human Globulin cassettes (Ortho-Clinical Diagnostics, Raritan, NJ, USA). A repeated testing was performed using samples treated with DTT (Amresco, OH, USA) according to the AABB guideline. All screening red cells used in this study were 3% MediPro reagent RBCs (Formosa Biomedical Technology, Taiwan) which was the only available and commercial in-vitro diagnostic product able to detect antibodies including anti-Mi^{a} and anti-E in Taiwan.

Results: Twenty-seven and 30 samples containing anti-Mi^{a} only and anti-E only, respectively, were recruited. Before DTT treatment, the detection sensitivity of anti-Mi^{a} was 85.2% (23/27) for LISS-IAT and 81.5% (22/27) for CAT, whereas the detection sensitivity of anti-E was 83.3% (25/30) for LISS-IAT and 76.7% (23/30) for CAT. With DTT treatment, 10 and 12 positive samples became negative for anti-Mi^{a} and anti-E, respectively, when tested by MP. When interpreted along with the reaction of DTT control, only 18.5% (5/27) and 13.3% (4/30) of them were respectively confirmed to have anti-Mi^{a} and anti-E of IgM type only. Among these IgM-positive samples detected by MP, only 20% and 0% of samples were respectively positive (before treatment) for LISS-IAT and CAT for anti-Mi^{a} detection, whereas only 20% of them were positive for both LISS-IAT and CAT for anti-E detection. After exclusion of IgM-positive samples, the adjusted rates for anti-Mi^{a} detectoin were both 90.9% (20/22) for LISS-IAT and CAT, whereas, for anti-E detection, they were 88.5% (23/26) and 80.8% (21/26) for LISS-IAT and CAT, respectively.

Summary and Discussion: Without consideration of immunoglobulin classes, the antibody detection rates of LISS-IAT and CAT for anti-Mi^{a} and anti-E were inferior to MP. However, thsee two methods were less sensitive to antibodies of IgM class, when encountering these clinically insignificant antibodies which happen in most situations.

P-234

PREVALENCE OF RED CELL ALLOANTIBODIES IN ANTENATAL WOMEN IN SRI LANKA

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Background: In Sri Lanka, antenatal antibody screening is specifically focused on Rh-D negative women, in order to prevent hemolytic disease of fetus and newborn (HDFN) due to most immunogenic anti-D. Due to the scarcity of resources, antenatal antibody screenings of Rh-D positive women is not being routinely carried out. It is necessary to determine the prevalence of clinically significant antibodies with respective to HDFN in the population of antenatal women to expand the antenatal antibody screening in Sri Lanka.

Aim: 1. To determine the prevalence of red cell alloantibodies among antenatal women.

2. To assess the prevalence of clinically significant alloantibodies in them with respective to HDFN.

Methodology: A descriptive cross sectional study was done at the Immunohematology Reference Laboratory, where all antibody screening positive samples are being sent from 87 regional blood banks in the country, for antibody identification.

All positive antenatal antibody results recorded at the Immunohematology Reference Laboratory during 01/06/2010–31/12/2010 period were analyzed.

Results: Out of all 291 antenatal women, 135 (46.4%) had anti- Le-b, 26 (8.9%) of them had anti-Le a and in 37 (12.7%) both anti-Le a and Le b were present. Anti D was present in 66 (22.7%). Multiple Rh antibodies were identified in some women. Out of the sample anti-D and anti-C were in 03 (1.0%) and anti-D and anti-E were in 02 (0.6%). Anti-E and anti-c were present in 01 (0.3%) woman. In 13 (4.5%) anti-Mur was positive. In 03 (1.0%) anti-P1, which was room temperature reactive was present.

One woman each (0.3% each) had anti- Le b with anti-MUT, anti-D with anti Le-a, anti Le-b with anti-Kell and anti-D with anti Le-b.

Out of the 291 antenatal women 89 (29.4%) had alloantibodies which could have caused HDFN. From these 89 women, who had clinically significant alloantibodies (with respective to HDFN), 66 (74.16%) had anti-D and 23 (25.84%) had antibodies other than anti-D.

By applying z-test it was found that the prevalence of clinically significant antibodies other than anti-D was significantly less ($P < 0.001$) from the population of antenatal women with clinically significant antibodies (with respective to HDFN).

Conclusion: The most prevalent antibody among antenatal women is anti Le-b followed by anti-D. Anti-Le a and anti-Le-b occurs together, as the third. According to this study, other Rh antibodies never appear to occur alone, but as multiple Rh antibodies.

Apart from anti-D prevalence of other antibodies which could have caused HDFN were statistically insignificant, but the clinical significance is yet to be assessed by the outcome of these pregnancies in the local setup. Though this is a statistically insignificant prevalence to this study population, which includes mainly Rh-D negative women, which is the minority in respective to RH-D status, these results may not be applicable to Rh-D positive antenatal women.

Therefore, furthure studies have to be carried out to determine the prevalence of clinically significant antibodies among Rh-D positive antenatal women.

P-235
FREQUANCY OF ALLO ANTIBODIES TO RH SYSTEM IN TRANSFUSION DEPENDANT PATIENTS, IMMUNOHAEMATOLOGY REFERENCE LABORATORY, NATIONAL BLOOD CENTER, SRI LANKA

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Background: Rh blood group system is one of the most immunogenic system with 49 Rh antigens. The most important of there is D and then C, c, E, and e. Anti-D is highly immunogenic and known to cause severe immediate or delayed haemolytic transfusion reactions and it is the most common cause of severe haemolytic disease of the fetus and newborn (HDFN). Clinically anti-c is the most important Rh antigen after anti-D and may cause severe HDFN.
Aim: This study aims to demonstrate the frequency of Rh allo antibodies in transfusion dependant patients, Immunohaematology reference laboratory, National Blood Centre, Sri Lanka.
Method: Retrospective analysis was conducted for the period of 6 months from July 2010 to December 2010, based on transfusion request forms received by Immunohaematology reference laboratory, National Blood Center, Sri Lanka. The study include transfusion dependant patients such as Thalassaemia, Aplastic anaemia, Leukamia, Renal failure, Anaemia of chronic disease and medical and obstetric emergencies.
Results: There were total number of 914 samples received by the Immunohaematology reference laboratory during the mentioned period, out of that 326 (35.66%) were males and 588 (64.34%) were females. Out of this, total number of 107 (11.7%) patients developed Rh alloantibodies. Development of one allo antibody was detected among 75 (70.1%) of patients, 28 (26.15%) developed two alloantibodies and 4 (3.75%) patients developed more than two alloantibodies. The most frequent alloantibody found was Anti-D (35.53%) followed by Anti-E (31.78%), Anti-C (1.86%) and Anti-c (0.93%). When considering two alloantibodies, most common combination was Anti-E+c (14.95%) followed by Anti-D+C (6.55%), Anti-D+E (1.86%). Anti-C+E, Anti-D+Le a, Anti-E+kell showed similer rate of incidence with the value of 0.93%.
Conclusion: The frequency of alloimmunization to red cell antigens is high among transfusion dependant patients. Majority of patients developed Anti D antibodies. Rh phenotype match blood is recommended for transfusion dependant patients.

P-236
ANTIBODY CONTROL IN THE DELIVERY TERM, YES OR NO?

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Introduction: According to the up to date recommendations of the developed countries, the first antibody screening regardless the RhD blood group is performed from the 12 till the 16th gestation week. RhD status is checked in the 28th gestation week (in duplicate), when antibody screening is also performed and which is repeated once more in the 36th gestation week.
Objective: Presentation of the frequency of anti erythrocyte antibodies, both in RhD positive and RhD negative pregnant women, in the delivery term (from 37 to 42 gestation week), as well as the evaluation of the clinical justification of performed testing.
Material and methods: At the Prenatal Protection Laboratory of the Clinical Center Dr Dragiša Mišović-Dedinje, from January 1st till September 15th 2010, 600 pregnant women were tested during the delivery term. Tube method with the use of Biotest reagents was applied for the determination of blood groups, while screening and anti erythrocyte antibody identification were performed using tube gel method with test erythrocytes and panel manufactured by DiaMed and the Blood Transfusion Institute of Serbia.
Results: From January 1st till September 15th 2010, 600 analyses of ABO system blood groups and RhD status, as well as the antibody screening were performed: 476 (79.3%) pregnant women were RhD positive and 124 (20.66%) were RhD negative. Among 476 RhD positive, combination of two antibodies was detected in one pregnant woman (0.21%): Fy^a i M. Out of 124 RhD negative investigated pregnant women, IgG class autoantibody was found only in one (0.80%).
Conclusion: Based on our preliminary results, antibody screening in the delivery term in RhD positive pregnant women is both clinically and cost effectively unjustified, as well as the repeated antibody screening (after the 36th gestation week) in RhD negative pregnant women.

P-237
PREVALENCE AND SPECIFICITY OF ALLOANTIBODIES IN KOREAN POPULATION

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Background: Alloantibody testing has been used for prenatal and pretransfusion study. The prevalence and specificity of antibodies are different among population and ethnic groups.
Aim: We investigated the frequency of alloantibodies in Korean population and compared with the previously reported experience.
Methods: We showed the prevalence of alloantibodies on 62,595 specimens from all over Korea from July 2009 to May 2011. We used DiaMed, Diacell, and DiaPanel reagents (Cressier, Switzerland) for alloantibody screening and identification. Results: The overall prevalence of alloantibodies was 0.5% (337/62,595). The specificity of alloantibodies was as follows: anti-E (46.0%), anti-Lea (17.5%), anti-M (9.6%), anti-c (9.6%), anti-D (6.6%), anti-Leb (6.6%), anit-e (6.0%), anti-C (5.3%), anti-Jka (2.3%), anti-Fyb (1.7%), anti-P1 (1.0%), anti-S (0.7%), anti-K (0.3%), and anti-N (0.3%). Among positive antibody screening sera, the majority had single alloantibody (78.1%), whereas remaining 21.9% had multiple alloantibodies.
Conclusions: The prevalence of alloantibodies was in agreement with the previous comparative study whereas the specificity of frequent alloantibodies were somewhat different.

P-238
ANTI-JS^B COMPLICATING THREE CONSECUTIVE PREGNANCIES CULMINATED IN FETAL LOSSES TO AN OMANI WOMAN: A CASE STUDY

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Background: Feto-maternal incompatibility of the Kell blood groups yields a serious out come of the pregnancy as the antigens of this system are well developed during the early fetal life. If the mother has antibodies to the high frequency antigens of this system, it certainly pause a greater risk of the HDFN and pose a challenge to every one of the stake-holders including blood bank.
Aim: The present report deals with an investigation on a case of maternal anti-Js^b giving rise to the fetal losses in three pregnancies.
Methods: Standard serological methods and investigative protocol were used in the study.
Results (case report): HAQ, age 27 years was followed up at the Central Blood Bank, Bosher, Oman for the antibody work up during the three pregnancies in 2007, 2009 and 2010. She was grouped as O, Rh (D) positive with antibody screening test positive. The atypical antibody reacted with all the red cells of the commercial cell panel indicating towards the specificity to the high frequency antigen. Since the patient's red cell reacted with anti-H, a possibility of her being 'Bombay' (Oh) phenotype was ruled out. So also, her red cells were typed positive for some high frequency antigens like I, k (small), Kp^b and Lu^b ruled out a possibility of specificity towards these antigens. Since antibody displayed a strong agglutination with the red cells pre-treated with proteolytic enzyme, though it was abolished against red cells pre-treated with AET reagent, hence a possibility of anti-In^b was also ruled out. Negative results on DAT and auto-control test on the patient indicated that the antibody involved was an allo-antibody reacting to a high frequency antigen. Her serum was referred to the International Blood Group Reference Laboratory, Bristol, U.K. where the antibody was identified as anti-Js^b.
Conclusion: Anti-Js^b associated fetal losses in three consecutive pregnancies reflect the nature of an antibody to Kell blood group system.

P-239
COMPARISON OF THE FOUR METHODS REGULARLY USED FOR PRE-TRANSFUSION ANTIBODY SCREENING

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Background: The goal of this study is to compare four methods commonly used in serology, including manual polybrene (MP), Polyethylene glycol antiglobulin (PEG), Low ionic strength saline (LISS) and Sanquin Cellbind immunofixation column, which were used to screen antibodies in clinical tests. The study tested the sensitivity of different antigen-antibodies respectively and compares the specificity of four methods. Also compared the reactions of four methods with known positive specimens and compare their specificity with unknown specimens.
Methods: Commercial human based blood grouping anti-sera, screening panel 123 cells (Sanquin Netherlands), 100 detected and frozen known patient antibodies and 187

routine pre-transfusion specimens were used for (i) To compare the sensitivity and specificity of four methods by rare blood grouping anti-sera (C, E, D, c, e, S, s, K, k, Fya, Fyb, Jka, Jkb) and screening panel 123 cells. (ii) To test the known positive specimens with screening panel 123 cells and compare the specificity of different methods. (iii) To identify the antibodies of routine pre-ransfusion specimens by screening panel 123 (the blind test).

Results: Cellbind column could detect anti-D, anti-C, anti-E, anti-e and anti-c. All of them had the titer 1:1024. There were eight among the 13 antibodies had the highest titer. The results of retesting 100 frozen known positive specimens by four methods, we found the positive rate were 91%, 66%, 81% and 79%, respectively. The highest was MP, followed by PEG and Cellbind and the lowest was LISS. In the blind test, there were six positive reactions by MP and Cellbind (6/187) and four positive reactions by LISS and PEG (4/187). This result showed the sensitivity of MP and Cellbind was higher in clinical tests.

Discussion: According to the results tested by serial dilution of commercial anti-sera, Cellbind and PEG had same higher sensitivity (8/13) and then MP and LISS. Since the MP is the routine antibody detection method in Taiwan, the 100 known frozen specimens have been tested once in the hospital. It could be the reason that MP showed the better reproducibility in comparison with other three methods by those specimens. The other method shows its advantage to some antibodies, such as PEG to antibody M and Cellbind to antibody E, Lea or Mia. In the blind test, MP and Cellbind could detect six positive results higher than LISS and PEG detect four antibodies. It indicated Cellbind was able to be used regularly in clinical test as MP.

Conclusion: By the comparison, we demonstrated the advantage of Cellbind is high sensitivity and equal detection rate with conventional methods. It could replace the time-consuming PEG and less sensitive LISS to be a regular examination. Moreover, it could be used for confirmation test when the result interpretation by MP test was not clear and definite.

P-240
CLINICAL AND LABORATORY PRESENTATIONS OF PAROXYSMAL COLD HEMOGLOBINURIA IN A PEDIATRIC PATIENT

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Background: Paroxysmal cold hemoglobinuria (PCH), also known as Donath-Landsteiner syndrome, is an autoimmune hemolytic disease caused by polyclonal cold-acting IgG autoantibodies. PCH primarily affects children but is rarely reported in Chinese. Clinical features and laboratory findings of a pediatric patient are herein presented.

Case report: A 5-year-old boy, native of Taiwan, was admitted with a chief complaint of fever for 2 days. One day before admission, abdominal pain occurred but was relived by enema. Dark urine was also noted three times later. The past history and physical examinations were not remarkable. The results of routine chemistry tests were within normal limits except a high serum level of C-reactive protein (9.25 mg/dl). Blood hemoglobin concentration was 13.0 g/dl. Urinalysis showed hemoglobinuria in the absence of microhematuria. Three days after admission, blood hemoglobin concentration decreased to 7.0 g/dl. Meanwhile, laboratory workup revealed mild hyperbilirubinemia (total bilirubin 1.7 mg/dl; direct bilirubin 0.33 mg/dl) and severe deficiency of serum haptoglobin (<10 mg/dl). Intravascular hemolysis probably associated with infection was thus impressed. Serological tests demonstrated positive direct antiglobulin test (anti-C3d) and negative indirect antiglobulin test. Antibody detection performed on the eluate was negative. However, a positive Donath-Landsteiner test suggested the presence of anti-P biphasic hemolysin in the sera of patient. Autoimmune hemolytic anemia consistent with paroxysmal cold hemoglobinuria was therefore confirmed. After empirical antibiotic treatment, vital signs and blood hemoglobin concentrations were gradually improved without any sequela.

P-241
RHD PARTIAL DBT TYPE 2 SEROLOGY

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Backgraund: The DBT type 2 is frequently miss-typed in routine blood typing. Characteristically the subjects carrying this RHD phenotype are classified as RhD positive. The problem arises when the same subjects, due to pregnancies or blood transfusion, produces anti-D antibodies.

Aim: Elucidate the serology of the DBT type 2 with different anti-D reagents.

Methods: We have found a DBT type 2 in a blood donor. Blood typing was performed with the Galileo (Immucor) and with the Autovue Innova (Ortho Clinical Diagnostics). The IgM MoAbs were tested with column agglutination technology using the NaCl Cards (Diamed ID microtyping system); 50 μl of the 0.8 RBC suspension and 25 μl of each MoAb were added to each microtube of the ID cards, incubated for 15 min at room temperature, centrifuged, and read according to the manufacturer's instructions. The IgG MoAbs were test with the same method but using the Liss Coombs Cards (Diamed ID microtyping system). The IgM MoAbs used were: RUM-1 (Ep 6.1), and TH28+ MS26 (Immucor); MS201 (Sanquin); BS232 (Ep 6.4), BS226 (Ep 6.4), BS232+ BS221 [IgG] + H4111B7 [IgG] (Biotest, Dreieich, Germany); 175-2 (Ep6.1) (Diamed); HM10 (Ep 6.6), P3x61 (Ep 6.4), P3x21211F1 (Ep 8.2), and P3x21223B10 (Ep 9.1) (Diagast, Loos, France); and D7B8 (Ep 6.4) + 1121G6+ LORIFA (Ortho Clinical Diagnostics, Raritan, NJ). The IgG MoAbs tests were: P3x35 (Ep 5.4), P3x290 (Ep 3.1), P3x249 (Ep 2.1), P3x241 (Ep 5.4), and HM16 (Ep 6.4) (Diagast). Partial RHD genotyping was performed using a commercial PCR kit with SSP Kit Partial D-TYPE (BAGene).

Results: The double RhD typing with the Galileo (Clone RUM-1 and TH28/MS26) resulted positive (pos.) 4+. The RhD typing with the Autovue Innova (Clone D7B8) resulted pos. 4+. The screening of irregular antibodies was pos. 4+, the characterization of the antibody gave: anti-D titer 1/128 plus anti-KELL titer 1/32_. The IgM MoAbs in gel technology saline gave the following results: Rum-1 pos. 4+, 175-2 pos. 4+, BS232 pos. 4+, BS226 pos. 4+, HM10 pos. 4+, P3x61 pos. 4+, P3x21211F1 pos. 4+, P3x21223B10 pos. 2+, CAZ negative, MS 201 pos. 2+. The IgG MoAbs gave the following results: P3x35 negative, P3x290 negative, P3x249 negative, P3x241 negative, and HM16 pos. 4+. Blended MoAbs in saline gave the following results: H1121G6+ LORIFA + D7B8 pos. 4+, MS26+ TH28 pos. 4+, BS221+ H4111B7+ BS232 pos. 4+. From Partial RHD genotyping resulted a DBT Type 2.

Discussion: The DBT phenotype is strongly reactive with most of the IgM MoAbs commercially available for routine blood typing. This is a problem because this phenotype was found in women typed as RhD positive, which produced anti-D antibodies. Between the IgM antibodies tested in saline, all except the CAZ were positive. Between those that were positive two MoAbs, the MS 201 and the P3x21223B10 were weakly positive 2+. So it seems that these three anti-D reagents are capable of indicating the presence of the DBT type 2 phenotype.

P-242
AUTOMATION TECHNIQUE IN RED CELL ANTIBODY SCREENING: EXPERIENCE OF A TEACHING HOSPITAL IN NORTHEASTERN MALAYSIA

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Background: Alloimmunization to foreign red cell antigens is a major complication in routine blood transfusion services. Automation of immunohaematological tests is advantageous. The adaptation of this automated instrument for antibody screening resulted in detection of widest possible range of antibodies with substantial time and cost savings.

Aims: The aim of this study was to determine the prevalence positive antibody screening among patients in Hospital Universiti Sains Malaysia using automation technique in 2010. The results were compared with data in 2009 using semi-automated technique.

Methods: This is a retrospective study over a period of 9 months (January–September 2010) involving 20,590 samples. All samples requested for Group Screen and Hold (GSH) underwent antibody screening using a indirect agglutination (IAT) method on a Diamed ID screening cell using Techno Twin Station (Diamed, Switzerland). Results were compared with antibody screening performed in January to September 2009 using semi-automated technique.

Results: A total 20,590 samples were screen for antibody within 9 months period in 2010. One hundred and sixty (0.78%) samples were found to be positive antibody screening. Red cell alloantibodies were identified in 75% (120/160) of samples. In 25% (40/160) of the samples, no specifities could be identified. Anti Mia (32/120) was the most common antibody detected followed by anti E (24/120).

In 2009 a total of 22,019 samples were send for antibody screen. Positive antibody screening were seen in 111 (0.50%) samples. Ninety-eight (88.2%) samples had specificity and 13 (11.8%) samples showed no specificity. The most common antibody identified was anti E (19/98), followed by a combination of anti Lea and Leb (13/98).

Conclusions: Rate of detection of red cell antibodies were increased with greater sensitivity by automated techniques than semi automated techniques. The studies are the basis for introduction of the automated technique into routine use for antibodies in blood donors and recipients.

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P-243

DETECTION OF DEL IN AN AUTOMATED SEROLOGICAL WEAK D TEST

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Background: It is standard practice to perform an antiglobulin weak D test on donor samples that react negative with monoclonal Anti-D reagents in direct agglutination, to minimize risk of blood from donors that are weak D or D variants such as DVI being mislabelled as D Negative, as red cells with these antigens may cause alloimmunization when transfused to a D Negative recipient.

During the last year, there have been several reports of donors that test positive in the weak D test automated on the Galileo or NEO instruments (Immucor, Roedermark, Germany) have negative results indirect antiglobulin (weak D) testing with other methods.

Methods: Donor samples were initially tested on Galileo or NEO instruments using Novaclone (Dominion Biologicals, Dartmouth, Canada) (clone D-175) and ImmuClone Anti-D Rapid (Immucor, Roedermark, Germany), (clone RUM1) reagents in direct agglutination.

Samples reacting negative in these tests were reflexed to the automated indirect antiglobulin 'weak D' test on the same instrument using Capture R Select and Novaclone Anti-D (containing IgG clone D415). Samples reacting positive in the weak D test were confirmed by antiglobulin tests in column agglutination (Bio-rad, France; Biovue, Ortho Clinical Diagnostics, Raritan NJ, USA) or tube tests (Anti-D Novaclone and Transclone Anti-RH1(D) Fast M (Bio-rad, France). Samples that were positive in the automated weak D test, but other methods were investigated using additional methods including absorption/elution (Gamma Elu-kit II, Immucor) and DNA-based methods (BAGene RHType kit, BAG Health Care GmbH, Lich, Germany), Bloodchipv2.0 (Progenika, Derio, Spain) and RHD Beadchip (BioArray Solutions, Warren, NJ, USA).

Results: Between three laboratories, a total of 35 samples were found that reacted negative for D in direct agglutination, but positive in automated solid phase weak D test, but negative in tube or column agglutination tests for D.

Some of these samples were tested by absorption-elution and found to be D positive. DNA testing was performed on 30 of the samples, and all were found to have the nucleotide change 885G>T (amino acid change M295I).

Summary/conclusions: The term DEL is used to describe cases where a very low level of RhD antigen is detectable only by absorption/elution method which is not suitable for routine use. There have been a small number of documented cases where DEL blood has caused alloimmunization. For this reason, there have been suggestions that DNA methods should be implemented to eliminate DEL donors from the D Negative donor pool.

The M295I mutation, in combination with phenotype Ccee (which the majority of these samples had) is characteristic of DEL. This mutation causes weak D type 11 when combined with with CCee phenotype.

Detection of these samples that were negative in other weak D methods indicates that the automated NEO/CaptureR Select/Novaclone Anti-D weak D test is markedly more sensitive than other routine serological methods for D antigen detection. The sensitivity of this method provides higher safety for detection of the weakest variants of D and its use reduces the attractiveness of DNA testing of D negative donor samples.

5.2 Red Cell Immunology: Molecular

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JK(A−B−) PHENOTYPE SCREENING AND GENOTYPING ANALYSIS IN CHINESE POPULATION

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Background: The distribution of Jk(a−b−) phenotype is very rare in all populations except for Polynesians (0.1–1.4%) and Fins (0.03%). After transfusion or pregnancy, the people with this rare phenotype can be immunized and produce anti-JK3. In East Asia, Japanese, Taiwanese, and Chinese Han populations from northern and middle regions have been tested and show a rare distribution of the Jk(a−b−) phenotype of <1/10,000, whereas a comparatively higher frequency of 0.025% was found in Thai donors. In this study, Chinese donors living in the East-southern region of China were screened to detect the frequency and genetic basis for Jk(a−b−).

Aims: To detect the frequency and genetic basis of Jk(a−b−) in the Han Chinese population in East-southern region of China.

Methods: Totally, 100,000 donors from East-southern region of China were screened using the 2 M urea lysis method. The Jk(a−b−) phenotype was confirmed using routine IAT methods. DNA samples from Jk(a−b−) probands were isolated. The coding regions (from exon 4 to exon 11) and the adjacent parts of intron of the JK gene were PCR amplified and sequenced. The JK gene of the available family members was also sequenced.

Results: Nineteen Jk(a−b−) donors were obtained. So, the frequency of Jk(a−b−) among the Chinese Han population living in East-southern region was found to be nearly 0.02% (19/100,000). DNA from 15 of the donors was available. In addition, one patient with anti-JK3 was genetically characterized. Only two (already described) mutant alleles derived from wild-type JKb sequence were detected. These were 1) the IVS5-1 G>A, previously found in all reported rare Polynesians and result in skipping of exon 6 and 2) c.896G>A (p.299Gly>Glu) (n = 3) only reported in Taiwanese. A recessive hereditary pattern of Jk(a−b) was obtained based on sequencing results from the available family members.

Conclusions: A relatively high frequency of Jk(a−b−) (1/5000) was found among the Chinese Han population in the East-southern region in China. The two common JKnull mutations should be involved in high-throughput genotyping, aimed to identify rare blood donors in East Asian population.

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NOVEL ALLELES AT THE KELL BLOOD GROUP LOCUS THAT LEAD TO THE KELL VARIANTS PHENOTYPE

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Background: Sanquin Blood Supply determines the K antigen expression in all blood donors. Last year, an exercise was started to identify serologically k-negative donors among the K-positive ones. Subsequently, the K+k− phenotype is confirmed by molecular testing. If in these donors a K2 allele is detected, follow-up serological analysis (absorption and elution technique to detect a low level of k expression) and sequencing of the KEL alleles is performed.

Aims: To investigate KEL genotypes that lead to Kmod and Knull phenotype in the Dutch population.

Methods: Three individuals with the K/kmod phenotype, three with the K/knull phenotype, one with a K+/k− phenotype, and two with Knull phenotype were identified and phenotypes were determined by standard serological methods. Allelic discrimination (TaqMan technology based) was used for K1/K2 genotyping. Then, the 19 exons and the intron-exon regions of the KEL gene were directly sequenced and compared with KEL wildtype. Expression of Kell antigens were determined by flowcytometry using anti-CD238 (recognizing all Kell antigens), anti-K and anti-k monoclonal antibodies (tested in six available samples).

Results: Three novel mutations were identified in each individual with K/kmod (Q362K), K+/k− (R700X), and Knull (R492X) phenotypes, respectively. The five reported variations, (R516X)null, (R406X)null, (G573G)el, (IVS3 + 1G>A)null, and (R516X)null, were also detected in seven donors. Flowcytometry showed an overall decreased expression of Kell antigens and an increased K expression compared to that of heterozygous K+/k+ controls for five samples with normal K expression and weak or absent k expression using standard serological methods.

Conclusions: Three novel molecular alterations at the Kell blood group locus were defined in the Dutch population. The increased K expression in individuals with K/kmod or K/knull phenotypes suggests that the XK protein is the rate-limiting factor for surface expression of Kell antigens.

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THE RH-MLPA ASSAY ALLOWS THE RAPID RECOGNITION OF PHENOTYPICALLY RHD-NEGATIVE CHINESE INDIVIDUALS WHO DO NOT NEED RHD-NEGATIVE BLOOD

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Background: In the Asian population the frequency of RhD-negativity is very low (<1%). Consequently, in the Asian transfusion practice the availability of RhD-negative blood is limited. Not all Asian persons that are serologically typed as RhD-negative have a deletion of the RHD gene. Many of these persons carry an RHD-variant, called D-elution (DEL), which is expressed at such a low density that it can only be detected upon the absorption and elution technique. Persons who have a DEL variant can safely receive RhD-positive blood. However, at the moment there is no simple method to distinguish patients carrying a DEL variant from truly RhD-negative patients. Hence patients with a DEL variant unnecessarily receive RhD-negative blood.
Aims: To evaluate the use of the RH Multiplex Ligation-dependent Probe Amplification (MLPA) genotyping assay for the identification of RH-DEL in a Chinese phenotypically RhD-negative donor population.
Methods: In total, 200 Chinese donors with RhD-negative phenotype were recruited from Guangdong province in China. Standard serological methods were used to determine the RhD and RhCE status in these donors. The MLPA RHD/CE assay, which can discriminate between 44 RhD and RhCE variants, was used to genotype the RHD and RHCE status in these donors. Furthermore, this assay can determine RHD copy number variation (zygosity). When the MLPA was not able to assign a variant, direct sequencing of all exons of the RHD gene was performed.
Results: Using the MLPA assay in 197 of the 200 Chinese RhD-negative donors, loss of the RHD gene and/or aberrant RHD genes could be determined. In 127 individuals (63.5%) the absence of all RHD exons was confirmed. Asian type DEL (G1227A) was detected hemizygously in 38 donors and homozygously in four donors (21% of the phenotyped RhD-negative donors). In 11.5% of the donors, the hybrid allele RHD (1–2)-CE (3–9)-D (10) (19 hemizygously and four homozygously) was detected. Three donors were identified with two different RHD alleles [hybrid allele RHD (1–2)-CE (3–9)-D (10) and DEL (G1227A)]. The DFR-2 variant and weak D type 15 was hemizygously present in two donors separately. The MLPA could not assign a variant in three donors. Two donors carried the RhD negative variant hemizygously (c.711delC) by sequencing of the RHD gene. One donor with two RHD alleles, one of them is hybrid allele RHD (1–2)-CE (3–9)-D (10) detected by MLPA and the other allele show a new RHD variant (c.1154G>T, p.385Gly>Val) by sequencing.
Conclusions: The MLPA assay is a fast, easy and robust method to genotype RhD-negative donors in the Chinese populations. This assay can be used to genotype the serologically RhD-negative patients, to determine which patients have a DEL variant. These patients can then receive RhD-positive blood during transfusion, restricting the use of the rare RhD-negative blood.

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This abstract has been withdrawn.

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A NOVEL AM ALLELE WITH 595C>T MUTATION IDENTIFIED IN TWO UNRELATED CHINESE INDIVIDUALS WITH THE AM PHENOTYPE

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Background: The Am phenotype is a rare subgroup of ABO blood group system. Am red cells are not agglutinated, or agglutinated only very weakly, by anti-A and anti-AB, and Am serum doesn't contain anti-A.
Aims: This study analyzes the molecular genetic basis of two Am individuals, and increases genetic information about the Am phenotype.
Methods: In two unrelated Chinese individuals who were typed as Am subgroup by serological tests, complete exon 6 and 7 in the ABO genes were amplified by PCR. The PCR products were directly sequenced and cloning sequenced to identify their genotype.
Results: A novel allele was identified in the both two Am Chinese individuals. Compared to ABO*A102 allele, one missense mutation was detected in the Am allele, in exon 7 at position 595, where C was replaced by T.
Conclusion: The mutation at nucleotide 595, which alter amino acid 199 from Arginine to Cysteine, resulted in the great reduction of activity of the A glycosyltransferase. It is indicated that the amino acid 199 may reflect the importance of this region for the ABO transferase efficiency.

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MOLECULAR ANALYSIS OF B_V PHENOTYPE OF THE ABO BLOOD GROUP SYSTEM IN HONG KONG CHINESE

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Background: B_v phenotype, being recognized as a distinct category of B subgroups, was first reported in 1964 and is characterized by that B_v red cells do not react with polyclonal anti-B adsorbed with rabbit cells. It was considered that the B_v determinant corresponded to the portion of the B antigen present on rabbit red cells. In a survey carried out in 1984–1989, among 567,210 Chinese blood donors in Hong Kong, 46 cases of B_v (one in 12,400) and eight cases of AB_v (one in 70,900) were found.
Aims: The study was undertaken to determine the molecular basis of B_v phenotype in blood donors in Hong Kong.
Methods: Eighteen blood samples were collected from blood donors with B_v (n = 17) and AB_v (n = 1) phenotypes. The ABO phenotypes were determined by standard serologic techniques. Commercial polyclonal anti-B (DiaMed AG, Cressier, Switzerland) was adsorbed at least twice with equal volumes of washed and packed rabbit red cells at 4°C for 2 h. The adsorbed anti-B was used to confirm the B_v phenotype which gave negative reaction with routine ABO grouping technique. The ABO genotype was determined by an established multiplex polymerase chain reaction (PCR) followed by single-strand conformation polymorphism. DNA sequencing and PCR cloning were used to characterize the mutations underlying the B_v phenotype. Restriction digestion methods using *Ssi*I and *Pvu*II were then designed to genotype all samples. Separate digestion was also performed on 20 randomly selected individuals of normal group B and 22 subjects possessing weak B antigens including B_3 (n = 9), AB_3 (n = 4), B_w (n = 6) and AB_w (n = 3), where B_w refers to weak B antigens for which the specific B subgroup status could not be assigned unambiguously.
Results: All 17 samples phenotyped as B_v were heterozygous for a *B* allele. Two mutations were separately identified on a typical *B101* background: non-synonymous substitutions 721C>T (Arg241Trp) and 695T>C (Leu232Pro) defining alleles *Bw03* and *Bw11* respectively, according to the registration of alleles in the Blood Group Antigen Gene Mutation Database. *Bw11* was found in heterozygous state in 16 out of 17 samples (94%) studied by digestion with restriction enzyme *Ssi*I. *Bw03* was found heterozygous in only one sample (6%) by digestion with *Pvu*II. None of these two mutations were detected in the normal B and the weak B samples. No mutation was found in all the exons of the variant *B* allele of the AB_v sample.
Conclusions: This is the largest series of molecular studies of B_v phenotype published in the literature. Two B_v alleles were identified: *Bw03* and *Bw11*; the latter being the commonest cause of the B_v phenotype in the Chinese population in Hong Kong. These two mutations have been found in individuals with B subgroups, but have never been described as associating with B_v phenotype expression. Simplified molecular methods have been devised to enable genotyping of the two B_v-associated mutations for routine analysis in service laboratories.

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AX03 AND *AEL02* ARE HIGH FREQUENCY ALLELES IN GROUP O JAPANESE WITH WEAK SERUM ANTI-A AND THOSE ACCOUNT FOR B(A) PHENOTYPE

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Background: Red cells of B(A) phenotype react with murine monoclonal anti-A regents but not with human polyclonal anti-A. The generation of B(A) is caused by the overlapping specificity of B-transferase that creates a few amount of A antigen. Especially, highly active B-transferase and bifunctional B-transferases having amino acid substitutions, such as Ser235Gly and Pro234Ala, are responsible for B(A) phenotype.
Aim: In the present study, we describe two different *A* alleles account for B(A) phenotypes.
Methods: Twenty-four B(A) blood samples, and 42 blood samples with weak serum anti-A selected from 132,220 group O donors were examined. Serological tests were performed using automated blood grouping system (PK7300) and manual tube method including adsorption-elution tests. Genome DNAs were extracted from whole blood and ABO genotype was determined by PCR-SSP and nucleotide sequencing.

Results: RBCs of 24 donors were strongly agglutinated with anti-B and weakly agglutinated with murine monoclonal anti-A, but not with human polyclonal anti-A. Adsorption-elution test was also negative with polyclonal anti-A and thus those were typed as B(A). PCR-SSP and nucleotide sequencing revealed that 17 of the 24 B(A) individuals had the genotype *Ax03* (*R102*)/*B* and other seven individuals had *Ael02* (*A110*)/*B*. *Ax03* had 646T>A (Phe216Ile), 681G>A, 771C>T, and 829G>A (Val277Met) mutations as compared with common *A* allele. *Ael02* had 467C>T (Pro156Leu), 646T>A (Phe216Ile), and 681G>A mutations. When 42 group O individuals with weak serum anti-A were examined for DNA analysis, 20 of them had *Ael02*/*O* and six had *Ax03*/*O*. No A antigen was detected on their RBCs by adsorption-elution test using both monoclonal anti-A and polyclonal anti-A. We identified one *Ael02*/*O* and one *Ax03*/*O* from 2015 Japanese with common group O.
Conclusion: Two different known alleles, *Ax03* and *Ael02*, are responsible for both B(A) and group O when those are heterozygote with *B* allele and *O* allele, respectively. Because RBCs of the individuals having *Ael02* or *Ax03* were not reacted with polyclonal anti-A, it should be given some attention in transfusion practice and DNA based ABO grouping.

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USE OF CELL STUDY MODELS TO CONFIRM THE WEAK ABO PHENOTYPES CAUSED BY POINT MUTATIONS AMONG TAIWANESE

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Background: Numerous phenotypes with a weak expression of the A or B antigens, including A3, Ax, Ael, cis-AB, B3, Bx, Bel, and B(A), have been found on the red blood cells (RBCs) and are usually defined by the serological characteristics. Some of these minor alleles have mutation(s) in the coding sequence of the ABO gene, and most of the mutations are single-nucleotide substitution leading to an amino acid alteration.
Aim: However, whether these mutated ABO alleles lead to the serological subtypes are not well defined and is investigated in this study.
Methods: We used site directed mutagenesis to generate expression constructs with point mutation corresponding to the ABO subtypes found in Taiwan.
Results: These subtypes included A2 (A207; 539 G to C), A3 (A303; 838 C to T), weak A (Ael07; 829 G to A), and Bel (Bel03; 502 C to T). The constructs were transfected into the cells followed by flow cytometry analysis to compare antigen-expressing intensity on the cell surface for each ABO subtype. These percentage of the A antigen-expressing cells decreased by 75.07%, 53.23%, 62.33% for A2, A3, and weak A1, respectively. Similarly, the B antigen-expressing cells decreased to 57.45% for Bel.
Conclusion: These data demonstrate that the point mutation occurred in various ABO subtypes is the key to weaken ABO gene expression and plays an important role in the regulation of ABO gene expression.

P-252
CHIMERISM OF ABO BLOOD GROUP IN TAIWAN

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Background: Chimeras are individuals that have more than one genetically-distinct population of cells. This phenomenon is recognized in skin and blood. The chimerism was confirmed by the analysis of the red blood cell antigens, which revealed the presence of two different populations. For example, if two populations of red cells were group A and group O, the serology resulted in mixed filed agglutination. Unlike chimerism, mutations in antigen-related genes resulted in subgroups which had only one genetic population. Genotyping the ABO blood group of chimeras showed ambiguous or inconsistent results. This report described two chimerism donors. Donor A, who is blood group A with mixed field agglutination; Donor B, who is blood group AB and the mixed filed agglutinations were observed.
Study design/methods: Serologically, donors' red cells were tested with antisera by tube method and gel-based micro typing system. Both donors' genotypes were initially determined by detecting SNPs in ABO gene. When the partial digestion patterns were observed in PCR-RFLP, further ABO allele was determined by allele-specific PCR analysis. Microsatellite genotyping confirmed the genetics bases of donors' blood cells. Family study of donor B was performed.
Results: Donor A's red cells showed mixed filed agglutinations with anti-A and anti-A1. This donor was suspected to be an A3 subgroup. The PCR-RFLP showed partial digestion pattern, which indicated a very weak A allele. The sequence analysis of two exons showed only O allele. We analyzed 16 microsatellite markers, no tri-allele or tetra-allele was shown. Further allele-specific PCR was performed to selectively amplify A allele. The result proved donor A had an A allele. Donor B's red cells showed mixed field agglutinations with anti-A, Anti-B and anti-A, B. This donor was A3B3 phenotype. Her spouse was common group O, and her two offsprings were common group A and group B. The PCR-RFLP of donor B's DNA showed only A and O alleles. The initial genotyping result was inconsistent with phenotyping data. Direct sequencing of exon 7 showed a mixture of A, B and O alleles. Six loci of microsatellite markers demonstrated tri-allelic patterns and the marker AMEL indicated a Y chromosome. The chromosome analysis of donor B's white blood cells revealed the 46XX/46XY chimerism. Analyzing 40 cells, 27 were 46XY, and 13 were 46XX. Allele-specific PCR confirmed A, B and O alleles.
Conclusion: In this study we reported two chimerism cases. Donor A was an A/O chimera. This donor had two populations of cells which had large difference in the number. The minor population was shown by partial digestion pattern of PCR-RFLP and allele-specific PCR. Female donor B was speculated to be an AB/O chimera. Six loci of microsatellite markers demonstrated tri-allele results. Y chromosome was detected. For both donors; it will take further family studies tracing back to their parents and more sensitive assays to clarify the paternal or maternal DNA contribution. Discrepancies between blood group genotype and RBC phenotype do occur. More DNA-based genotyping techniques will be implemented to study this complicated genetic phenomenon.

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ABO GENOTYPING BY MINISEQUENCING IN KAOHSIUNG BLOOD CENTER

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Background: ABO discrepancy is a common problem in serological blood grouping. To determine correct blood group, many genotyping methods are applied to identify ABO alleles. The PCR-RFLP is used to detect ABO alleles in our laboratory for many years. However, it still has some limitations. Here we perform a minisequencing method to type ABO alleles and evaluate the suitability to clarify mismatched ABO typing result or subgroups encountered in routine work.
Aims: The aim of the present study is to evaluate the applicability of minisequencing method for genotyping ABO alleles.
Methods: About 60 samples included both ABO subgroups and mismatched typing result were selected and analyzed by PCR-RFLP. The DNA fragments of exon 6 and exon 7 were amplified by duplex PCR and minisequencing reaction were performed with SNaPshot kit and single base extension primers. The products were analyzed by DNA genetic analyzer and GeneScan software.
Results: In many subgroup cases, five common alleles (ABO*A101, ABO*A102, ABO*B101, ABO*O01, ABO*O02) can be identified. The 14 samples exhibited an O phenotype with weak reaction of reverse typing can be clearly genotyped and assigned according to the reference table. Only few samples are ambiguous results.
Conclusion: Our results have revealed that minisequencing analysis enables simple and rapid multiplex genotyping of ABO alleles. It can provide an accurate genotyping result than PCR-RFLP.

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MOLECULAR BASIS OF NOVEL ALLELE AEL08 OF ABO BLOOD GROUP

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Objective: To analyze the molecular genetics basis for Ael08 allele of ABO blood group. Methods:
The ABO group antigens on red cells of the proband were identified by monoclonal antibodies and the ABO antibody in serum was detected by the standard A, B, O cells. The weak antigen on RBCs was detected by absorption-elution test. The exon 5 to exon 7 coding region of ABO gene was amplified by polymerase chain reaction (PCR) and the PCR product was sequenced directly for exon 6 and 7 after the double enzymes digestion. The amplified product for exon 5–7 was also cloned by TOPO TA cloning sequencing kit to split the two alleles apart and chosen colonies were sequencing bidirectionally for exon 5–7 of ABO gene.
Results: Weak A antigen was found on red cells of the proband which can only be identified by the absorption-elution test, there was also anti-B and weak anti-A antibody in his serum. There was 261G deletion heterozygote and 297A/G in exon 6 and showed 467C/T, 46T/A, 681G/A, 771C/T, 829G/A, 804insG/G heterozygotes in exon 7 by directly DNA sequencing. After cloning and sequencing, it was obtained one common allele O02 and a novel ABO allele which was nominated as Ael08 by dRBC NCBI. The sequence of Ael08 has one nucleotide changes at 804 position G insertion (as

previously described by Olsson) compared with that of A10, which results in read frameshift and the encoded product is 37 amino acids longer than the A1 transferase.
Conclusions: A novel allele Ael08 was identified and insertion G at 804 position of *N*-acetylgalactosaminyltransferase gene (A gene) can result in Ael phenotype.

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TWO MAJOR GYP (B-A) ALLELES IN JAPANESE WITH STONES ANTIGEN (MNS15)

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Background: The MNS blood group system is second only to the Rh blood group system in its complexity. The MNS antigens are carried on glycophorin A (GPA), glycophorin B (GPB), or hybrids of GPA and GPB. The low incidence MNS antigen St^a is most commonly associated with a *GYP(B-A)* hybrid but also can be associated with *GYP(A-B-A)* or *GYP(A-E-A)* hybrid gene. St^a antigen is specified by a hybrid junction formed between the exon 2 of *GYPB* (or *GYPA*) and exon 4 of *GYPA* upon transcript processing. It occurs with relatively high frequency among Asians, but its incidence is rare in Caucasians and Africans. GP.Sch phenotype is encoded by a *GYP(B-A)*, which represent distinct genetic isoforms (type A, type B and type C) in the location of crossing-over sites[1)].
Aims: We report here the molecular background of St^a gene identified in unrelated St^a heterozygote from Japanese population.
Methods: Screening with monoclonal anti-M and anti-N against bromelin-treated RBC was performed using automated blood grouping system (PK7300). When positive reaction with anti-M or anti-N was observed, St^a, M, N, S and s antigens were examined by tube method. DNA samples of 115 St(a+) individuals were extracted from whole blood. DNA fragment of the *GYP(B-A)*, including from pseudoexon 3 of *GYPB* to exon 4 of *GYPA*, was amplified by PCR and nucleotide sequence was analyzed.
Results: Serological tests revealed that 1003 in 24,292 (4.1%) Japanese individuals were typed as St(a+). MNSs typing on St(a+) red cells showed that 36% of the St(a+) individuals had S+. This frequency was significantly higher than that of the randomly selected Japanese (S+ was approximately 10%). PCR amplification and nucleotide sequence analysis revealed that all 115 St(a+) individuals had *GYP(B-A)* hybrid allele. These alleles were classified into seven groups on the bases of intron 3 sequence. Two of them were major alleles with frequencies of 63% (72 in 115) and 30% (35 in 115), and the *GYP(B-A)* crossing-over sites in intron 3 were 356–488 and 722–762, respectively. These alleles were identical to the previously described St^a type B and type C genes, respectively. Interestingly, 50% (36 in 72) individuals with St^a type B were S+, whereas only 6% (two in 35) individuals with type C were S+.
Conclusion: In Japanese, St(a+) frequency was 4.1%. The St^a type B allele was most frequent in St(a+) Japanese and 50% of them appeared to be associated with *GYPB*S*.
Reference:
1. Haung CH, Blumenfeld OO (1991) J Biol Chem 266: 23306–23314.

P-256
GENETIC ANALYSIS OF 16 PARA-BOMBAY PHENOTYPE INDIVIDUALS – A NOVEL NON-FUNCTIONAL FUT1 ALLELE AND AN NEW EVIDENCE OF GENETIC LINKAGE WITHIN FUT1 AND FUT2 GENE

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Background: The mutation of FUT1 gene results in decreased synthesis of H antigen, will lead to the para-Bombay phenotype. Relatively high frequency (one in 8500) of para-Bombay phenotype in Fujian province and a potential h2-Se357,716 heplotype had been reported in our previous study. In this study, another 16 individuals with para-Bombay phenotype have been analyzed.
Aims: The goals of this study were to analyze the genetic characteristics of the para-Bombay individuals in Fujian province, to found new h alleles, and to prove the hypothesis of genetic linkage within FUT1 and FUT2 by more experimental data.
Methods: The subject of para-Bombay phenotype were collected and identified from the sample with ABO discrepancy in blood screening, and were confirmed by serological analysis. The sequences of FUT1 and FUT2 gene of para-Bombay individuals were analyzed with direct sequencing of PCR products and gene cloning products.
Result: Four different alleles (h1, nt547–552Δag; h2, nt880–882Δtt; h3, nt658ct; h9, nt424ct) were observed in this study. The FUT1 genotypes of these para-Bombay individuals were h1/h1 (nine individuals), h1/h2 (four individuals), and h3/h2 (one individual), h2/h2 (one individual), h1/h9 (one individual), respectively. Two prevalent h alleles, h1 and h2, with the frequency of 68.75% and 21.87%, are predominated in h alleles leading to H deficiency. A mutation of nt357ct was detected in all FUT2 gene, another mutation of nt716ga was detected in the individuals with h2 gene, no null FUT2 gene was detected.
Conclusion: A novel h alleles (h9) with a nucleotide 424C>T point mutation was discovered. This study confirms the previous observations that h1 and h2 alleles are predominate in Fujian H deficient individuals. The fact that all Se357,716 only have found in individuals with h2 gene and more haplotype of h2-Se357,716 observed in this study, confirms the previous hypothesis of genetic linkage within the FUT1 and FUT2.

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APPLICATIONS OF NOVEL MOLECULAR TYPING TECHNIQUES IN THE AUSTRALIAN RED CROSS BLOOD SERVICE

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Background: To overcome the limitations of conventional immuno-haematological methods, molecular techniques are emerging as important investigation tools. In the Australian Red Cross Blood Service, certain problematic samples have been referred for external investigation by Progenika Biopharma (Spain), where samples are analyzed in a two step process: firstly with BLOODchip® Reference (a blood group genotyping DNA array platform) and if required, by other molecular techniques such as sequencing, to further investigate the problem.
Aim: The aim of this study was to determine the extent to which samples referred during 2009–2011 for SNP typing and, if appropriate, sequencing, were resolved after molecular testing.
Methods: DNA was extracted using the Qiagen EZ1 DNA blood kit method. DNA was checked for concentration and purity and then sent to Progenika Biopharma (Derio, Spain). The Progenika protocol commences with SNP analyses with the BLOODchip® Reference database. This genotyping platform detects 128 SNPs in 10 red blood cell and 12 human platelet antigen systems, including 72 SNPs that render 101 variants of RhD group (10 Del, 47 Partial D, 21 Weak D and 23 D negative). This analysis is complemented with other techniques such as RhD zygosity, RhD exon scanning or coding region sequencing, if the problem is not resolved with the previous test.
Results: A total of 52 samples were sent for analysis, comprising 32 patients, seven donors and 13 maternity samples.
Review of the reasons for referral showed 40 (76.9%) were due to serologic inconsistencies in the RHD group, and 8 (15.4%) in other groups, including ABO (3), MNS (2), Duffy (1), Colton (1) and RhCE (1). Four cases (7.7%) were from patients who had received multiple transfusions and had auto-antibodies, leading to difficulties when undertaking extended antigen typings by regular serological methods.
Testing with BLOODchip® Reference analysis resolved 78.8% (41) of all cases and a further 7.7% (4) were resolved by sequencing. One sample (1.9%) was tested for zygosity. In 5.8% (3) cases, one for ABO, one for MNS and one for RHD, the group of interest was confirmed by genotyping and therefore classified as resolved although it was noted antibodies of unexplained specificity were present. Finally a further 5.8% (3) cases remained partially unresolved.
Summary/conclusions: In 94.2% of cases that were unresolved with serology, molecular genetic techniques contributed to a resolution.
This study shows that genotyping is particularly valuable in resolving serological inconsistencies in the RhD group (including donors and patients such as pregnant women) and for typing in multi-transfused patients.
In conclusion the application of novel molecular typing techniques has demonstrated considerable utility to complement serology in the resolution of problematic samples in these investigations.
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P-258
THE SINGAPORE RARE DONOR PROGRAM – THE SEARCH FOR DIB (DI2) NEGATIVE DONORS

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The primary role of any blood transfusion service is to provide patients needing transfusion with compatible blood. This provision is at times complicated by the presence of clinically significant alloantibodies. Transfusion in such cases is made compatible only with blood lacking the antigen against the offending alloantibody.
The Dib blood group antigen is prevalent in most populations. Transfusing patients immunized with anti-Dib will pose tremendous challenge to most transfusion services due to the rarity of the Dib-negative phenotype. It is therefore unsurprising that Dib-negative phenotype is categorized as a rare phenotype among other international rare

donor programs such as the American Rare Donor Program (ARDP) and the World Health Organization International Donor Panel (WHO IDP).
In efforts to support the need for such phenotype and to further develop the Singapore Rare Donor Program, the Blood Services Group undertook a project to screen and identify Dib-negative donors. A two-stage screening strategy incorporating both serological and molecular methods was necessary due to the unavailability of commercial anti-Dib phenotyping reagent.
Three hundred and eighty-seven EDTA-anticoagulated random blood donor samples collected between June and August 2010 were screened for the antithetical Dia antigen (DI1) using serological methods. Dia screening was performed with anti-Dia (Diamed, Switzerland) using indirect antiglobulin test (IAT) method on an automated column agglutination technique system (Techno Twinstation; Diamed, Switzerland). Identified Dia-positive samples were subjected to a secondary molecular screening using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) to determine zygosity of Diego antigens. Gene amplification was targeted towards SLC4A1 gene, while RFLP was performed using MspI restriction enzyme, a method previously described by Baleotti et al., 2003. The genotype and predicted phenotype of these samples were determined based of the fragment sizes of the PCR product. PCR-RFLP sample that demonstrated DI1/DI1 genotype which encode for Dib-negative phenotype was sequenced for confirmation.
Of the 387 samples screened, 18 (4.7%) were found to be phenotypically Dia-positive while the remaining 369 (95.3%) were Dia-negative. PCR-RFLP on the 18 Dia-positive samples produced expected fragment sizes of 363, 275 and 88 bp. Seventeen (4.4%) of these samples demonstrated all three fragment sizes, indicative of a heterozygous Dia-positive and Dib-positive phenotype: while an undigested PCR product was demonstrated for 1 (0.3%) sample. DNA sequencing of the undigested PCR product confirmed the DI1/DI1 genotype for the expected rare Dib-negative phenotype.
The need to screen blood donors for rare blood types, such as Dib-negative phenotype, remains a struggle for many blood centres. The process remains extremely resource-intensive but yet necessary, if compatible blood for patients exhibiting antibodies toward these high incident blood group antigens is needed. The lack of licensed blood grouping reagents and unfavourable geographical distribution of rare blood types make the task even more daunting.
Although the Singapore Rare Donor Program is at its infancy stage and has seen some successes at incorporating some of our unique screening strategies with our existing donor recruitment and freezing programs, the need to synergize with other like-minded blood centres remains imperative.

Table 1: Screening results for Dib-negative donors

Phenotype	Serological Test	PCR-RFLP	Genotype
Di^a/Di^a	18 (4.7%)	1 (0.3%)	*DI1/DI1*
Di^a/Di^b		17 (4.4%)	*DI1/DI2*
Di^b/Di^b	369 (95.3%)	-	*DI2/DI2*
Total	387 (100%)	-	-

P-259
MOLECULAR GENETIC ANALYSIS FOR THE VARIANT PHENOTYPES OF THE ABO BLOOD GROUP SYSTEM

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Background: The ABO blood group system is the most clinically significant system in transfusion and transplantation medicine. In addition to common ABO blood group, many variant phenotypes that are the so-called ABO subgroup have been found. To understand the molecular genetic basis of this polymorphic system, we have analyzed genomic DNAs obtained from Chinese individuals possessing variant ABO phenotypes including A2, Ax, Ael, cis-AB, Bw, and Bel.
Methods: Serologic investigations were performed with standard methods. DNA sequences from exon 6 to exon 7 were analyzed using genomic DNA by polymerase chain reaction and direct DNA sequencing or sequencing after gene cloning.
Results: We identified nine different alleles which contained two novel A allele. One novel A allele being different from the allele A101 by 467C>T, 721C>T missense mutation in exon 7 responsible for the Ax phenotype, the other being different from the allele A101 by 467C>T, 607G>A missense mutation in exon 7 responsible for the A2 phenotype. Correspondingly resulting in the amino acid changes Pro156 Leu, Arg 241 Trp and Pro156 Leu, Glu 203 Lys in the A glycosyltransferases. The mutations were not found in 50 randomly selected samples. Seven allele were previously reported ABO alleles.
Conclusion: Two novel A allele responsible for Ax and A2 subgroup were firstly reported. Amino acid substitutions resulted from novel mutations 721C>T and 607G>A on ABO gene may change conserved regions of the enzyme and reduce the activity of the glycosyltransferases, leading to the variant phenotype. Our datas still indicate that different alleles could cause the same ABO variant phenotypes, molecular bases for the variant phenotypes in Chinese population have highly polymorphism.

P-260
VALIDATION OF HRM PROCEDURES FOR GP.MUR AND DIA GENOTYPING

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Background: Antibodies to the Dia polymorphism and to neoantigens (Mut, Mur, and Hil) found on GP.Mur can cause haemolytic transfusion reactions and haemolytic disease of the foetus and newborn. Dia and GP.Mur are found in 5–8% in East Asian populations. The aim of the study was to establish and apply high resolution melt (HRM) genotyping procedures to type these polymorphisms. For Gp.Mur the intronic crossover site was targeted for development of a novel typing procedure.
Aim: To apply molecular genetic techniques to type and investigate the levels of these polymorphisms found in Asian ethnic groups in donor cohorts Australia.
Methods: Standard serological procedures were performed to detect Dia and GP.Mur pos donors. Using primers from published methods and novel primers for the intronic crossover in GpMur, HRM genetic typing was performed using an HRM kit (Qiagen). DNA from donors that were serology-positive and serology-negative were investigated to validate published methods for GPMUR typing and a novel HRM genotyping procedure. A similar characterisation was carried out for the published HRM typing procedure for the Dia SNP.
Results: HRM genotyping showed 100% concordance with serology. GP.Mur was detected in 3% of selected donor samples by both serology and genotyping. For GP.Mur, the full profile of neoantigens and the expected intronic crossover were detected, no variant types were found. Dia/Dib HRM typing revealed 1 Dia homozygote (1.8%) and 7 Dia/Dib heterozygotes (12.8%) in a preliminary study.
Summary and Conclusions: HRM genotyping reliably genotyped the Dia SNP and GPMUR. In this selected study cohort, the antigen frequency for GP.Mur was 3% and for Dia a surprising 14.6%. Currently, 8% of the Australian population is of Asian ethnicity and in some donor cohorts, the frequency of these ethnic-associated blood group types is considerably higher than expected in Australia.

P-261
BLOOD GROUP GENOTYPING IN CHRONICALLY TRANSFUSED PATIENTS: COMPARISON WITH THE ERYTHROCYTE PHENOTYPING METHOD

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Background: Chronically transfused patients such as thalassemia major and sickle cell anemia carry in their circulation the donor derived erythrocytes from previous transfusion. This prevents the occurate phenotyping of erythrocytes. Discordance between the real phenotype of the patients and the phenotype of the transfused red blood cell (RBC) units results in alloimmunization. This is associated with hemolytic transfusion reactions, shortening of the erythrocyte life span and increased frequency of transfusion. Although strategies to provide phenotype-matched RBC units by many blood banks in the world have partially controlled the high rate of alloimmunization among this population, the risk still remains.
Aim: To perform blood group genotyping of chronically transfused patients in a single center and compare the results with the results of erythrocyte phenotyping.
Method: Genotyping was performed by using sequence specific primer based PCR for RhD, RhCcEe ve Kell (KEL1, KEL2), Kidd (Jka, Jkb), Duffy (Fya, Fyb) antigens in 38 patients (age range: 1–67) (34 beta thalassemia major, two sickle cell anemia/Sβtal, one congenital dyserythropoietic anemia, one chronic anemia) receiving their RBC units from a single blood center. Phenotyping was performed by gel agglutination method.
Results: Among the 36 patients who had been phenotyped by gel agglutination method and compared with the genotyping results, 19 had discordance between

phenotype and genotype in a total of 25 antigens. The most frequent haplotypes were RHD*+ (97%), RHCE*Ce (61%) and RHCE*ee (68%), KEL*2/KEL*2 (95%), JK*A/JK*B (58%) and FY*A/FY*B (43%). Twelve patients had genotype-phenotype mismatch which had the risk of alloimmunization in case of phenotype matched transfusion. One patient had weak D, three patients had FY*null haplotype. The alloimmunization ratio was 7% (3/38). Patients who were alloimmunized were adult patients. None of the thalassemia patients had allo-antibodies.
Conclusion: This study supports the use of molecular genotyping in chronically transfused patients to determine blood group antigens occurately and provide genotype-matched RBC units to prevent alloimmunization. In spite of the high genotype-phenotype mismatch, the alloimmunization ratio was low, the causes of which should be further investigated. If the phenotyping can not be done before the first transfusion, erythrocyte genotyping should be performed to prevent false phenotyping after multiple transfusions.

P-262
GENOTYPING ANALYSIS OF KIDD, KELL, DUFFY, SCIANNA AND RHCE BLOOD GROUP ANTIGENS POLYMORPHISMS BY PCR-SSP IN SOUTHEAST CHINESE BLOOD DONORS

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Background: Molecular testing is more precise compared to serology and has been widely used in genotyping blood group antigens. Single nucleotide polymorphisms (SNPs) of blood group antigens can be determined by the PCR-SSP assay. Commercial platforms can be expensive and are not approved in China. The genotype frequency of Kidd, Kell, Duffy, Scianna, RhCE blood group antigens in Jiangsu province were unknown.
Aims: To detect the genotype frequency of Kidd, Kell, Duffy, Scianna, RhCE antigens in Jiangsu using molecular methods with laboratory developed tests.
Methods: DNA was extracted from EDTA-anticoagulated blood samples of 146 voluntary blood donors collected randomly within 1 month. Standard serologic assay for red blood cell antigens were also performed except the Scianna blood group antigens. PCR-SSP was designed to work under one PCR program to identify the following SNPs: Jka/Jkb, K/k, Fya/Fyb, Sc1/Sc2, C/c and E/e.
Results: Serologic antigen results were identical to the phenotypes that were predicted from genotyping results. The gene frequencies for *Jk*A* and *Jk*B* were 0.51 and 0.49, respectively; for *Fy*A* and *Fy*B* 0.94 and 0.06; for *RHCE*C* and *RHCE*c* 0.68 and 0.32; and for *RHCE*E* and *RHCE*e* 0.28 and 0.72. Among 146 blood donors, all were *k/k* and *Sc1/Sc1*, indicating allele frequencies for *KEL*2* and *SC*1* close to 1.00.
Summary: The use of PCR-SSP working under the same condition for testing multiple antigens at the same time is practical. This approach can be effective and cost-efficient for small-scale laboratories and in developing counties. These molecular tests can also be used for identifying rare blood types.

5.3 Platelet Immunology

P-263
GENE MUTATION RELATED TO CD36 DEFICIENCY BY PCR-SSP IN CHINESE CANTONESE

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Background: CD36 deficiency is proved to be important in some clinical situation, but the related mutation frequency in China is not known.
Objective: In this study, we used sequence-specific primer-PCR to detect the frequency of CD36 gene mutation in GuangDong district of China.
Methods: A total of 470 blood samples from unrelated health donors were involved and the PCR-SSP method was used.
Results: We detected 30 samples from these donors having CD36 gene mutation, the mutation types and frequencies are as follows: three for C268T (0.64%); 14 for 329–330del AC (2.98%); two for C380T (0.43%); two for C760T (0.43%); eight for A1237C (1.70%); and only one combined mutation for C268T and 560T ins (0.21%).
Conclusion: Here, we first used the PCR-SSP method to detect the mutation frequencies of CD36 in China. The 329–330del AC appears to be the major mutation type of CD36 and the A1237C comes to be the secondary in GuangDong. Our results are somehow different from previous studies which have reported that the major types of CD36 gene mutation were: C268T, 329–330del AC and 949in A.

P-264
ANALYZING THE BCL-XL LEVEL IN PLATELETS OF IDIOPATHIC THROMBOCYTOPENIC PATIENTS

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Background: Thrombocytopenia could result from decreased thrombocytogenesis in bone marrow or increased destruction in situations of infection, hemorrhage, autoimmune, splenomegaly etc. However, idiopathic thrombocytopenia is quite often observed in clinics. Life span of platelet in blood is about 7–10 days. The 'multiple hits' model, whereby damage by external hits and clearance of older platelet by the reticuloendothelial system, is believed to regulate life span and number of platelets eventually. Another recently emerged 'internal timer' model demonstrates an apoptotic pathway in platelets that dominated their survival in vivo. In the mouse model, mutations in the anti-apoptotic protein Bcl-xl, the Bcl-2 family protein, were shown to reduce platelet life span and cause thrombocytopenia. Bcl-xl could be the key component of apoptosis pathway in platelets, and might function as a timer of their life span. A decline in Bcl-xl levels would trigger Bak activation and initiate apoptosis
Aim: The aim of this study is to evaluate the Bcl-xl content of platelets collected from idiopathic thrombocytopenic patients.
Methods: Whole blood specimens were collected from 15 patients who have lower platelet count (between 100,000 and 150,000/μl) with most thrombocytopenic etiology excluded. Platelets from these patients were separated by two-step centrifugation and the Bcl-xl content of platelets was analyzed by Western blotting.
Results: The Bcl-xl amount was estimated by normalizing with the GAPDH protein level. We found these patients' platelets have no consistent lower amount of Bcl-xl protein. Only two of 15 patients' platelets contain less Bcl-xl protein. The ratio range is from 0.80× to 1.94× compared to the normal patients. However, simply analyzing the centrifuged intact platelets may not actually represent the instant degradation of Bcl-xl. The apoptotic pathway may proceed quickly in platelets right after Bcl-xl degradation, so it is hard to detect low level amount of Bcl-xl in platelets which were still alive.
Summary: Here we demonstrated that there is no significant difference of Bcl-xl protein level between idiopathic thrombocytopenia patients and normal controls. Additional studies such as investigation of Bcl-xl stability or evaluation of upstream proteins of apoptotic pathway need to be done to demonstrate the relation of platelet apoptosis and idiopathic thrombocytopenia in human.

P-265
EXPRESSION OF ANTI-PLATELET GLYCOPROTEIN SPECIFIC AND ANTI-HLA ANTIBODIES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA PATIENTS

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In order to investigate the expression of the anti-platelet glycoprotein specific antibodies and anti-HLA antibodies in idiopathic thrombocytopenic purpura (ITP), 45 patients with ITP were seleceted. An easy PCR-SSP assay was used to detect single-nucleotide polymorphisms or deletion in HPA and HLA systems. The anti-platelet glycoprotein specific antibodies and anti-HLA antibodies in plasma or platelet eluate were tested with a solidphase ELISA. There were 45 patients expressed anti-platelet glycoprotein specific antibodies in plasma or platelet eluate, among which anti-GPII b/IIIa/and anti-GpIb/IX are most common. There were 11 patients expressing both the anti-platelet glycoprotein specific antibodies and anti-HLA antibodies in plasma. Pedigree study was used in two patients. In conclusion, the results suggested that detection of the anti-platelet glycoprotein specific antibodies and anti-HLA antibodies in plasma or platelet eluate is significant in diagnosis for idiopathic thrombocytopenic purpura.

Table 1: The expression of the anti-platelet specif

	Type	Number	Percentage(%)
Plasma	GP·II b/IIIa	22	48.9
	GPIb/IX	13	28.9
	GPIa/IIa	7	15.6
	GP·IV	7	15.6
Eluate	GP·II b/IIIa	17	37.8
	GPIb/IX	15	33.3
	GPIa/IIa	14	31.1

Table 2: Co-expression of the anti-platelet specif

Antidodies		HLA-Antidodies	Plasma				Eluate		
			GP II b/IIIa	GPIb/IX	GPIa/IIa	GP IV	GP II b/IIIa	GPIb/IX	GPIa/IIa
Number		11	7	3	6	5	2	2	2
Case ID	35	+	-	-					
	36	+	-	-					
	37	+	-		-	-			
	38	+	-	-	-	-			
	39	+	-		-	-			
	40	+	-		-	-			
	41	+							
	42	+	-		-	-			
	43	+	-		-	-			
	44	+			-		-	-	-
	45	+					-	-	-

P-266

FVIII GENE VARIATIONS IN HEMOPHILIA A PATIENTS OF SOUTHWEST CHINA

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Background: Hemophilia A (HA) represents the most common and severe inherited hemorrhagic disorder in southwest part of China. It is caused by variations in the FVIII gene, which leads to a deficiency or dysfunctional factor VIII protein, resulting in frequent spontaneous bleeding in joints, muscles and internal organs.
Aims: This study aimed to detect FVIII gene variations in HA patients of southwest part of China, and to establish a diagnostic strategy for HA genetic diagnosis in this area.
Methods: Long-distance PCR (LD-PCR) with three primers was used to detect the inversion of intron 22. In order to increase the sensitivity and accuracy of the method, the original one-tube reaction was splited into two separate reactions. Each of them contained two of the three primers. One reaction could detect the non-inversion form of intron 22. Meanwhile, another reaction could demonstrate the inversion form with different length of PCR products compared to the former one. For the detection of intron 1 inversion, method of two multiplex PCR reactions was adopted. Each reaction contained three of the total four primers, forming different length of the PCR products when there was inversion or not. As to strengthen the reliability of mutation scanning in the whole FVIII exonal regions, PCR amplification followed by the direct sequencing was used to identify the mutational points.
Results: The results of detailed detection were presented among the patients with HA in southwest part of China. There were six families and five individuals identified with inversion of intron 22, and one families recognized as inheritance of intron 1 inversion. By direct sequencing, there were two families and eight individuals were identified with exonal mutations, although in different points. There was no exonal deletion or duplication event detected in this study. The overall mutation detection rate of HA was 100%.
Conclusions: A diagnostic strategy for HA genetic diagnosis in southwest part of China was proposed in this study. Both of the PCR methods used to detect the inversions were reliable, and sequencing method was also a powerful tool in the identification of FVIII gene exonal mutations.
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P-267

INITIAL ESTABLISHMENT FOR ASSAY METHOD OF HEPARIN IN ANTITHROMBIN? CONCENTRATES

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Background: Antithrombin III (AT-III) concentrates are employed as prophylaxis or treatment of thromboembolic disorders in patients with congenital or acquired deficiencies of AT-III, but so far there has been no mature product in China. Nowadays most of available therapeutic AT-III concentrates are obtained by affinity chromatography to heparin, so heparin content is an important indicator for AT-III concentrates quality. Heparin can bind with AT-III to form the antithrombin-heparin complex which inactivates thrombin, factor IXa, factor Xa and etc, so the role of AT-III should be taken into account in heparin assay. In the coagulation method for assay of heparin content in Chinese Pharmacopoeia (2010 Edition), interference of AT-III in the tested samples is not eliminated, therefore this method is not suitable for heparin detect in AT-III concentrates.
Aims: To establish a efficient method for assay of heparin content in AT-III concentrates.
Methods: The standard heparin was proportionally diluted with reaction buffer to obtain the gradient concentrations, which were 0.12, 0.1, 0.05, 0.025, 0.0125, 0 IU/ml. Then 40 μl of diluted standard heparin was mixed with AT-III standard material in 96 well microplates. After incubation at 37°C for 3 min, 40 μl of prewarmed factor Xa was added, and lasting for another 2 min, and then add 40 μl of chromogenic substrate S-2765 for 2 min in 37°C. The whole reaction was terminated by adding 50% acetic acid. Measure the absorbance at 405 nm, and draw the calibration curve of heparin, then heparin was analyzed in the tested AT-III concentrates according to the calibration curve of heparin.
Results: When 0.5, 1.0 and 1.5 IU/ml of AT-III standard material, the activity unit of the factor Xa is 21 nkat/ml, at 0–0.05 IU/ml of heparin content the correlation coefficients of the three heparin calibration curves are all >0.99, however at 0–0.12 IU/ml of heparin content the correlation coefficients are all <0.90. At 0.5 or 1.0 IU/ml of AT-III standards material and 21 nkat/ml of factor Xa, there is no evident discrepancy in the heparin content of tested AT-III concentrates and good repeatability is achieved.
Conclusions: We have initially established the assay method of heparin in AT-III concentrates. Under the conditions of 0.5 or 1.0 IU/ml AT-III standards material and 21 nkat/ml factor Xa, the heparin content ranging from 0 to 0.05 IU/ml can efficiently be determined in AT-III concentrates.

P-268

A CASE REPORT ON NEONATAL ALLOIMMUNE THROMBOCYTOPENIA DUE TO ANTI-CD36 (NAKA) IN TAIWAN

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Background: Naka antigen (CD36) was first reported by Dr. Ikeda et al in 1989. Naka is an isoantigen expressed on platelets and monocytes. Some Naka (–) individuals do not present CD36 on platelets and monocytes, or sometime is only absent on platelets (referred as Type I and Type II CD36 deficiency, respectively). It has been reported that Type I CD36 deficient mothers produce isoantibodies during pregnancy and give birth to babies with thrombocytopenia.
Case Report: A full-term baby was delivered by caesarian section from a healthy mother (gravida 2, para 1). The Taiwanese boy, now 17 years old, was 3500 g at birth who presented fever and jaundice after 4 days. His Hb and Ht were normal but the baby developed severe thrombocytopenia (<40,000/μl). Neonatal alloimmune thrombocytopenia (NAITP) was suspected and the baby was treated with a single dose of intravenous immune globulin (IVIgG) (1 g/kg) at day 7. The maternal blood was collected for further analysis.
Methods: The maternal serum was first screened for platelet antibodies with her husband and a panel of 108 random donors using a solid phase red cell adherence assay (SPRCA). CD36 phenotype on the mother platelets and monocytes was confirmed by flow cytometry with monoclonal antibodies. Specificity of alloantibodies was identified using monoclonal antibody immobilization of platelets assay (MAIPA) and a commercial kit (PakLx; GTI diagnostics) based on Luminex bead technique. Lastly, the genomic mutation of CD36 gene was determined by sequencing after amplification of 12 coding domain sequences (CDS).
Results: The maternal serum showed positive reaction with 105 of 109 donors' platelets (96.3%). This outcome corresponds to the frequency of Naka antigen in the Taiwanese population. Further phenotyping revealed that father and baby carried Naka antigen and that mother was Naka (–) phenotype for platelets using SPRCA. In addition, the mother was confirmed as a type I CD36 deficiency, as both her platelets and monocytes lacked reactivity with monoclonal anti-CD36 by flow cytometry. It agrees with the presence of anti-Naka in the maternal serum. Recently, the mother serum was retrospectively tested by MAIPA and PakLx kit. It showed that her antibodies reacted with isolated CD36 molecules.
Sequencing further identified two mutations in the mother CD36 gene. One was a known dinucleotide deletion on the third CDS (referred as CD36:c.329_330delCA, previously named as 539–540del) leading to frame-shift. The other was not previously reported and was a 6 bp deletion starting on nucleotide position 6 after the end of CDS 11 (referred as CD36: c.1254 + 6 delTATTTG). However, this deletion cannot be used to predict any interaction on the splicing process.
Conclusion: This is the first NAITP case caused by anti-Naka, which has been reported at transaction of Taiwan society of blood transfusion in 1995. But the underlying molecular mechanism leading to CD36 deficiency is still not clearly understood and need further study.

P-269
CLINICAL FEATURES AND DETECTION OF ANTIPLATELET ANTIBODIES IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: Primary immune thrombocytopenia (ITP) is one of the most common acquired bleeding disorders of childhood. It's well known that pediatric ITP is most commonly associated with a preceding viral infection. Furthermore antiplatelet antibodies react against platelet glycoproteins and cause autoimmune thrombocytopenia purpura.
Aim: This study was to evaluate the relationship between clinical presentations, antiplatelet antibodies and antecedent of preceding infection in children with ITP.
Methods: Twenty-five children diagnosed with ITP were identified following a retrospective review of medical records at two medical centers in north Taiwan. We investigated and evaluated the demographics, clinical and laboratory features, as well as previous infection and vaccination history of 25 children with ITP. Detection of plasma platelet antibodies by ELISA (PakPlus GTI, Waukesha, WI, USA) was used. Statistical analyses were performed using the R statistical software. In univariate analysis, the means of continuous variables were compared with two-sample *t* test, whereas the associations between categorical variables were analyzed with Fisher's exact test.
Results: Among the 25 children, 10 were males and 15 were females. The mean age at onset was 5.7 years, with 9 (36%) patients <2 years, 10 (40%) patients between 2 and 10 years, and 6 (24%) patients >10 years of age at the onset of ITP. There was no apparent proclivity of season of onset for the occurrence of ITP. The mean platelet count at diagnosis was 12.1×10^9/l (range, 2×10^9/l–43×10^9/l), with 20 patients (80%) with initial platelet count $<20 \times 10^9$/l. Eighteen (72%) patients had antecedent of preceding infection (API), while 7 (28%) patients did not. There were no significant differences between patients with and without antiplatelet antibodies regarding the gender, age and season of onset, initial platelet count, API. Analyzing antiplatelet antibodies, 15 (60%) were positive and 10 (40%) were negative. Antibodies reactive with GPIIb/IIIa, GPIa/IIa, GPIb/IX, and GPIV were detected in 47%, 20%, 6.6%, and 6.6% patients, respectively. Three patients (19.8%) had antibodies against more than one glycoprotein ($P < 0.001$). Further analysis of demographic and clinical parameters by the type of antiplatelet antibodies revealed that patients without API were more likely to have anti-GPIa/IIa than those with API (42.9% vs 5.5%, $P = 0.048$). There were no significant differences in patients with or without anti-GPIIb/IIIa, and in patients with or without anti-GPIb/IX with respect to the gender, age and season of onset, initial platelet count, API.
Conclusions: In our study, antiplatelet antibodies were detected in 60% patients with ITP and anti-GPIIb/IIIa was the most common type which compatible with the previous studies. Detection of the specific platelet antibodies was helpful selected matched platelet. In addition, API was associated with absence of anti-GPIa/IIa antibody. On the other hand, patients without API perhaps showed multiple types of platelet antibodies.

5.4 Granulocyte Immunology

P-270
MATERNAL ALLOIMMUNIZATION TO GRANULOCYTE-SPECIFIC ANTIGENS AND NEONATAL ALLOIMMUNE NEUTROPENIA

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Background: Placental transfer of maternal allo- and auto-antibodies can cause the destruction of neutrophils in fetus and/or infant after birth and cause the immune neonatal neutropenia. There are no data available about the granulocyte antibody appearance among pregnant women in Russia. The aim of our study was to estimate the granulocyte antibody appearance among pregnant women.
Methods: We've tested sera from 94 pregnant women and nine women having neutropenic newborn infants. The gel agglutination assay (ID-microtyping system DiaMed) and granulocyte agglutination test were used for granulocyte antibody detection. All sera were tested with five donor granulocyte samples. Sera of nine women having newborn infants with neutropenia were tested with father's granulocyte samples.
Results: Sera of 94 women were tested. No granulocyte-specific IgG-antibodies were found among studied pregnant women, but six women (6.2%) had IgM-antibodies found by granulocyte agglutination test. Six of 94 pregnant women (6.2%) had lymphocyte-specific IgG-antibodies. Also we've tested sera of nine women having newborn infants with neutropenia. Three of these women had granulocyte-specific IgG-antibodies. One woman had granulocyte autoantibodies, and another one had both granulocyte-specific alloantibodies and autoantibodies. We didn't detect the granulocyte-specific allo- and autoantibodies in sera of four women having newborn infants with neutropenia.
Conclusion: The study confirmed neonatal immune neutropenia, caused by the granulocyte antibodies, in five cases. However, there were no antibodies detected in sera of 94 pregnant women.

P-271
DISTRIBUTION PROFILE OF HUMAN NEUTROPHIL ANTIGEN-3A AND -3B AMONG TAIWANESE

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Background: Transfusion-related acute lung injury (TRALI) is rare, but most frequently causes mortality and morbidity after blood transfusion. It has been reported that alloantibodies against human neutrophil antigen HNA -3a is associated with some severe or fatal TRALI cases. The molecular mechanism of HNA-3a/3b has been uncovered in 2010. It showed that HNA-3 is a biallelic system resulting from a single nucleotide exchange (G461A or Arg154Gln) in the choline transporter-like protein 2 gene (SLC44A2).
Method: An in house polymerase chain reaction with sequence-specific primers (PCR-SSP) was designed and assessed in our laboratory. To evaluate the risk of developing HNA-3a alloantibodies in Taiwanese, HNA-3a/3b genotyping was firstly performed among unrelated healthy individuals.
Results: In our preliminary genotyping, 79 (40.9%) Taiwanese individuals among 193 were homozygous for HNA-3a, 24 individuals (12.4%) were homozygous for HNA-3b and 90 individuals (46.6%) were heterozygous for HNA-3a/3b. Accordingly, the gene frequencies of HNA-3a and -3b were 0.6425 and 0.3575, respectively. Test for Hardy-Weinberg equilibrium did not deviate from expectation.
Conclusion: In this preliminary screening, the frequency for HNA-3b homozygote is two time greater among Taiwanese (12.4%) than in German (5.5%). This implies that Taiwanese may be more potent in inducing HNA-3a antibodies. The rate of immunization among German was reported to be 7% against HNA-3a and 0.5% against HNA-3b. The immunogenicity against HNA-3 among Taiwanese is still undefined and will needs to be determined in future studies to evaluate if the risk of inducing HNA-3a antibodies is greater or lesser in Asia.
Female donors homozygous HNA-3b are more likely to develop anti-HNA-3a antibodies during pregnancy, and therefore more likely to induce a risk in platelet transfusion. Genotyping the HNA-3 system among female platelets donors would be helpful in identifying blood donors involving a transfusion risk and in reducing TRALI caused by HNA-3a alloantibodies.

P-272
THE DISTRIBUTION OF HUMAN NEUTROPHIL ALLOANIGENS IN THE CHINESE HAN POPULATION OF SHANGHAI

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Background: The antibodies for human neutrophil antigens were thought as a key role in the pathogenesis of TRALI. It is not completely clear about the distribution of HNA antigens in Chinese population, and the current family plan policy may lessen the possible incidence of HNA antibodies.
Aims: To establish a method to evaluate the frequencies of HNA antigens in Chinese and provide related information for potential TRALI diagnosis.
Methods: Three hundred and twenty-one unrelated healthy and 28 pregnant were studied, all of them were from Shanghai. A universal PCR-SSP genotyping method for HNA-1 (−a, b, c), HNA-3 (−a, −b), HNA-4a (+,−) and HNA-5a (+,−) was set up, PCR-RFLP and PCR-sequencing were then utilized as double check methods. HNA-2 antigen was analyzed by Flow-cytometry in 76 unrelated healthy in parallel with 28 pregnant individuals.
Results: The results of PCR-SSP were confirmed by PCR-RFLP or PCR-sequencing. The gene frequencies of HNA-1a, HNA-1b, and HNA-1c were 0.667, 0.333 and 0 respectively. HNA-3a and HNA-3b were 0.650 and 0.350. The frequency of HNA-3b in Shanghai was higher than Caucasian as well Guangdong Han population, while other systems were similar with those in Guangdong Han population. HNA-4a was 1 and HNA-5a was 0.897. The HNA-2 expression presented variation among different persons, we can classify results into five groups both in the healthy and the pregmant, (i) negative (CD177+ <10% neutrophil cells); (ii) most negative (10–20%); (iii) partial negative while partial positive (20–75%); (iv) most positive (75–90%). (v) positive (>90%). The percentage of listed five types in healthy were 0%, 7.9%, 65.79%, 21.05% and 5.3% respectively, but the results in the pregnant were 10.71%, 1.32%, 35.71%, 32.14% and 14.29% respectively. And the reaction strength varied among different individuals too.

Conclusions: The universal PCR-SSP can be used for genotyping of HNA-1, 3, 4, 5 systems. Human neutrophil antigens in the Han population of Shanghai have distinguishable polymorphism. HNA-1c and HNA-4a negative were not found in this study. HNA-3b had a higher frequency which possibly accompanies corresponding antibodies which were supposed to be a role in TRALI in Han. Both of the positive CD177 expression and the negative expression are more in the pregnant than that in the healthy, the clinical significance of the difference may deserve further investigation in more pregnant population.

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TOXIN OF STREPTOCOCCUS PNEUMONIA BACTERIA INDUCES NB1 GENE TRANSCRIPTION IN HNA-2A NEGATIVE NEUTROPHIL SUBPOPULATION

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The recruitment of neutrophil to infection site is the first step of host defence system against invasive organisms. Molecules accelerating neutrophils diapedesis toward infection site play therefore an important role. Recently, our group has demonstrated that human neutrophil antigen-2a (HNA-2a, NB1) interacts with PECAM-1 on endothelial cells and mediated thereby neutrophil diapedesis. HNA-2a shows bimodal expression pattern (HNA-2a positive and HNA-2a negative populations) on neutrophil surface, which can be upregulated during bacterial infections. However, the mechanism arranged behind is not yet known. In this study, we investigate the impact of pneumolysin (PLY), the most potent bacterial toxin of Streptococcus pneumonia, on the regulation of HNA-2a cell surface expression.

Neutrophil treated with PLY migrated faster through endothelial cells in comparison to untreated neutrophils in the flow chamber experiment. Interestingly, flow cytometry analysis with different mabs against HNA-2a showed significant upregulation of NB1 on HNA-2a (–), but not on HNA-2a (+) neutrophil subpopulations. This result could be confirmed by the use of cell sorted neutrophils. Treatment of HNA-2a (–) sorted neutrophils with PLY caused increase of total NB1 protein as well as NB1 surface expression as shown by immunoblot and flow cytometry, respectively. In the control experiment, neutrophils derived from HNA-2a defective individuals (NB null) did not show any NB1 production after PLY treatment.

Our results indicate that bacterial toxin is able to activate neutrophil signaling pathway, which initiates the gene transcription in HNA-2a negative neutrophil subpopulation. This mechanism is probably the most important step for the NB1 mediated neutrophil diapedesis during bacterial infections.

P-274
NEUTROPHIL ACTIVATION BY HNA-2A ANTIBODIES IS MEDIATED BY MAC-1 INTEGRIN

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Antibodies against human specific neutrophil antigen-2a (HNA-2a; NB1) play a role in immune mediated neutropenia and transfusion-related acute lung injury (TRALI). In our previous studies, we found NB1 dependent neutrophil activation and superoxide production in neutrophils after exposure with anti-HNA-2 antibodies. Since NB1 is a GPI-linked protein lacking a cytoplasmic domain, signal transduction could not be directly ascribed.

In this study, we searched for integral membrane protein on neutrophils, which may act as NB1 co-receptor for the transduction of outside-in signalling.

Digitonin lysates of intact neutrophil membrane were immunoprecipitated by anti-NB1 and analysed by MALDI-TOP analysis. A major protein, Mac-1 integrin, which forms a complex with NB1 protein, could be identified by this approach. This result was confirmed by immunoblotting analysis using a panel of mabs against NB1 and Mac-1 integrin as well as by real-time analysis of protein-protein interaction by surface plasmon resonance technology (SPR). Furthermore, the involvement of NB1/Mac-1 complex in signalling process could be identified by lipid raft analysis. Inhibition studies using selected mab against Mac-1 blocked neutrophil activation and superoxide production induced by HNA-2a antibodies. This study demonstrated for the first time the mechanism of NB1-antibody mediated activation in neutrophils, which may add our understanding in the pathomechanism of TRALI.

P-275
THE FREQUENCIES OF HUMAN NEUTROPHIL ANTIGENS (HNA) AMONG THE JAPANESE POPULATION

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Background: Antibodies against human neutrophil antigens (HNA) are involved in the pathogenesis of a variety of clinical conditions, such as neonatal immune neutropenia (NIN), refractoriness to granulocyte transfusions, febrile transfusion reactions. Recently, their involvement in the pathogenesis of transfusion-related acute lung injury (TRALI), a severe complication of blood transfusion, was confirmed. Five HNA systems, namely HNA-1 to -5 have been characterized. HNA-1, -2, -4 and -5 antigens are attributed to polymorphisms or deletion on CD16, CD177, CD11b and CD11a, respectively. The HNA-3 antigen was recently determined to be dependent on a single amino acid substitution on choline transporter-like protein-2. HNA-1, -2, and -3a alloantibodies have been implicated in the pathogenesis of TRALI, and in especial HNA-3a alloantibody has been found in the severe cases requiring artificial ventilation or with fatal reactions. The detection of anti-HNA antibodies is essential for the diagnosis of the pathological conditions in which these antibodies are involved, as well as for their prevention. Therefore the determination of the distribution of HNA in a population is important for the prediction of the risk of alloimmunization to HNA. The HNA frequency distributions have been reported among different populations in US, Europe, Asia and Africa. Also, reports from Japan have described the frequency of some, but not all, HNA.

Aim: In the present study, we aimed to investigate the frequency distribution of all HNA among the Japanese population.

Methods: Healthy volunteer donors were genotyped for the HNA-1 (92), HNA-3 (93), HNA-4 (122), and HNA-5 (111), using the Luminex (PCR-rSSOP, WAKFlow-HNA; Wakunaga pharmaceutical Co., Hiroshima, Japan) or PCR-SSP. Also, HNA-2a phenotype in 96 donors was determined by GIFT, using specific antisera and monoclonal antibodies.

Results: The gene/antigen frequencies were as follows: HNA-1a: 79 (85.9%), -1b: 62 (67.4%), -1c:0 (0%); HNA-2a: 94 (97.9%); HNA-3a: 83 (89.2%), -3b: 56 (60.2%); HNA-4a: 122 (100%), -4b: 0 (0%); HNA-5a: 109 (98.2%), -5b: 30 (27.0%). The gene frequencies were as follows. HNA-1a (0.573), -1b (0.427) -1c (0); HNA-3a (0.645), -3b (0.355); HNA-4a (1.000), -4b (0.000); HNA-5a (0.856), -5b (0.144).

Conclusion: We describe the frequencies of HNA among Japanese population. This study will be helpful in the future for the prediction of the risk of alloimmunization to HNA, especially to determine the risk of HNA alloantibody production by transfusion of HNA incompatible blood and feto-maternal incompatiblity.

P-276
LENTINAN INDUCES CYTOTOXIC T-CELL RESPONSES DEPENDANT ACTIVATION OF HUMAN MONOCYTES CELL LINE (THP-1) CELLS VIA DECTIN-1

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Background: Lentinan, a β-1, 3/β-1, 6-glucan isolated form *Lentinula edode*, has been used as an adjuvant for tumor immunotherapy in Japan and China. Early studies mainly focused on anti-tumor activities of innate immune cells induced by Lentinan. Dectin-1 is the key pattern recognition receptor for β-glucans. Activation of dectin-1 signaling on dendritic cells (DCs) can trigger adaptive T cell responses (Th1 and Th17).

Aims: Our study was to investigate whether dectin-1 on monocytes/macrophages stimulated by Lentinan could prime the anti-tumor cytotoxic T-cell responses.

Methods: The mRNA level of IL-12 and dectin-1 were detected by real-time RT-PCR. Western blotting was used to analyze the protein expression of dectin-1. The concentration of IL-12p70 was determined by ELISA. Allogeneic mixed lymphocyte reaction between THP-1 cells stimulated with Lentinan and T cells was performed. LDH release test was used to evaluate cytotoxicity of CTL.

Results: Exposure of THP-1 cells to Lentinan led to increased gene expression of IL-12p35, IL-12p40, dectin-1 and secretion of IL-12p70 and dectin-1. Lentinan stimulated THP-1 cells promoted the expansion and differentiation of cytotoxic T lymphocyte (CTL) which could kill the colorectal cancer cells Sw480, Caco-2 in vitro. Anti-IL-12p70 neutralizing antibody and dectin-1 inhibitor blocked the tumor cytotoxicity of CTLs.

Conclusions: These data suggest Lentinan primed the anti-tumor cytotoxic T-cell responses dependent the activation of dectin-1 on monocytes/macrophages. Transfusion of CTL induced by Lentinan may be a promising treatment for tumor patients.

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HUMAN NEUTROPHIL ANTIGEN-1 GENE FREQUENCY IN THAI BLOOD DONORS

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HNA-1 antigens are located on the human neutrophil Fc gamma-receptor IIIb (Fcγ RIIIb). These antigens encoded by the FCGR3B gene. Alloimmunization against these polymorphisms can result in immune neutropenia and transfusion-related acute lung injury (TRALI). The aim of this study was to determine the gene frequencies of the HNA-1a, -1b and -1c alleles in 175 unrelated healthy Thais from blood donors using sequence-specific primers. All participating individuals gave the informed consent. The result showed that the HNA-1a and HNA-1b gene frequencies in Thais (0.72 and 0.28, respectively) were statistically significant different ($P < 0.001$) from Asian Indians, North Americans, Africans and Tunisians. In contrast, the present data showed that the HNA-1 allele frequencies in Thais are similar to Asians, Chinese, Japanese and Korean populations, respectively. The HNA-1c antigen was not present in this study. This information may be helpful in the future for HNA gene and disease association studies.

5.5 Fetal Maternal Immunology

P-278

HEMOLYTIC DISEASE OF NEWBORN DUE TO MATERNAL ANTIBODY OF KIDD BLOOD GROUP SYSTEM AT MACKAY MEMORIAL HOSPITAL

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Background: The hemolytic disease of newborn (HDN) is caused by the destruction of red cells due to the incompatibility between the mother and her infant blood. The infant red cells become sensitized and coated with the IgG alloantibody of maternal origin. The infant IgG-coated RBC may undergo accelerated destruction both before and after birth. Jaundice, pallor, an enlarged spleen or even hydrops may be present in severe cases. Kidd sensitization is an uncommon cause of HDN. From 1984 to 2011, there were only five cases of HDN with Kidd antibodies at Mackay Memorial Hospital, in which four with anti-Jkb and one with anti-E + c + Jka.

Report and Review of Cases: Here we reported a recent case and summarized all cases in the following table. In April 2011, a male baby weighing 3276 g was born to a G2P2A0 mother via normal spontaneous delivery at Mackay Memorial Hospital. His condition was stable with good activity and appetite. He developed jaundice on the fourth day after birth. The yellowish skin discoloration and icteric sclera were noted. The laboratory data showed total bilirubin 17.7 mg/dl, Hb 11.3 g/dl, reticulocyte 5.7% and a normal level of G6PD level. He was then admitted under the diagnosis of hyprebilirubinemia for further care. The blood bank examination demonstrated positive results (1+) for both direct and indirect Coombs tests. The presence of anti-Jkb antibody was detected in both maternal and infant serum by manual polybrene method. The anti-Jkb antibody was also eluted from the infant RBC by LIAT method. No other antibody was identified. The mother was group A, Jkb negative and the infant was group A, Jkb positive. There was no maternal history of blood transfusion. Thus, the etiology of this infant's HDN was maternal anti-Jkb who had a Jkb positive infant. The infant received phototherapy and the level of bilirubin gradually recovered to normal.

Table 1

Year	Case	ABO	Rh	Jk^a	Jk^b	DAT	IAT	Antibody	Treatment
2011	Infant	A	nt	+	+	1+	1+	anti-Jk^b	Phototherapy
	Mother	A	nt	+	–	nt	1+	anti-Jk^b	
2010	Infant	O	nt	+	+	±	±	anti-Jk^b	Phototherapy
	Mother	O	nt	+	–	nt	±	anti-Jk^b	
1995	Infant	O	R_1R_2	+	+	4+	2+	anti-E+c+Jk^a	Exchange blood
	Mother	O	R_1R_1	–	+	nt	3+	anti-E+c+Jk^a	
1984	Twin A	O	nt	+	+	4+	1+	anti-Jk^b	Exchange blood
	Twin B	O	nt	+	+	4+	1+	anti-Jk^b	
	Mother	B	nt	+	–	nt	2+	anti-Jk^b	

Conclusion: Neonatal hyperbilirubinemia (bilirubin > 15 mg/dl) in Taiwan is mainly due to ABO maternal-fetal incompatibility, erythrocyte G6PD deficiency, and low birth weight. However, some cases remain unknown in the etiology of HDN. Other than ABO blood group system, maternal irregular antibodies against Rh, MNS, Kidd, or Duffy systems have been reported. The antibody of Kidd system responsible for HDN was first reported in 1953 (anti-Jkb) and in 1959 (anti-Jka). The Jk antibody is clinically significant since it can cause acute and delayed transfusion reaction as well as HDN. Fortunately, most cases had mild hemolysis and the babies required no treatment. Since we encountered only five cases in the past 27 years, antibody against the Kidd system must be very rare in Taiwan and all our cases of immunization are resulted from previous pregnancies. For your reference of interest, the phenotype frequency of Kidd system in Taiwan are 28.3% in Jk (a+b–), 35% in Jk (a–b+), and 36.7% in Jk (a+b+), as observed in our previous study on 60 healthy individuals.

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STUDY OF IMMUNE ANTI-D IN PREGNANT WOMEN IN SRI LANKA

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Background: Immune anti-D is the most common cause of haemolytic disease of fetus & newborn in any population. In Sri Lanka the Rh D negative population is 5%. Routine antibody screening for unexpected antibodies are carried out at booking visit & 28 weeks of gestation.

In Sri Lanka routine administration of Anti-D Prophylaxis (RAADP) has not been universally advocated to Rh D negative pregnant women in the recent past. Therefore Rh D HDFN is not uncommon in Sri Lanka.

Aim: The study was planned to find the significance of age, parity, anti-D titer & the significance of RAADP to the development of HDFN in Sri Lankan population.

Method: Data were collected retrospectively for the study from January 2010 to December 2010.

All 85 immune anti-D cases reported to immunoheamatology Laboratory of National Blood Center of Sri Lanka were selected.

Their age, parity, length of pregnancy of the detection of immune antibody & antibody titer were collected.

Antibody titer testing is done only at the immunoheamatology laboratory in Sri Lanka, by titration method using freshly prepared pooled O R_1r red cells from donors. Titer of 32 & above was considered as significant high titer.

Parity of the 85 cases analyzed were categorized into Primipara (P1), 2nd pregnancy (P2), multipara (P3 + P4), & grandmultipara(more than five pregnancies).

Results: Of the 85 reported cases 4 were in 15–20 year, eight were in 21–25 year, 26 were in 26–30 year, 30 were in 31–35 year, 15 were in 36–40 year & two were in 41–45 year age groups.

Of the 85 reported cases of immune anti-D, 10.6% were detected in the 1st pregnancy, 29.5% were detected in the 2nd pregnancy, 50.6% were detected in maltipara in subsequent pregnancies & 9.4% were detected in grand paltiparous women for the 1st time. RAADP was not routinely administered, nor were there proper data available regarding prophylaxis anti-D to cover sensitizing events, but 500–1500 IU of anti-D was administered to all Rh D negative women following delivery of Rh D positive fetus & quantification tests were not preformed in any of the cases following a sensitizing event.

Of the 85 cases, 30.58% of immune anti-D were detected at the booking visit. 46.15% of them had high titer of immune anti-D & 16.6% of them were primi mothers, 8.3% were in their 2nd pregnancy, 66.66% were multipara & 8% were grandmultipara.

Of the 85 cases, immune anti-D was detected for the 1st time while screening for unexpexted antibodies at 28 weeks of gestation in 69.42%, 52.54% had high titer, 19.35% were primi mothers, 22.58% were in their 2nd pregnancy, 48.38% were multipara & 9.6% were grandmultipara.

Conclusion:

1. Most of immune anti-D positive mothers were in 26–35 year age group.
2. Multiparous women develop immune anti-D more frequently than primipara.
3. Majority of cases immune anti-D was detected in antibody screening at 28 weeks of gestation.
4. Multiparous women were more prone to develop high titer of immune anti-D levels than primipara & women in 2nd pregnancy.

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This abstract has been withdrawn.

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HAEMOLYTIC DISEASE OF NEWBORN BABIES IN SRI LANKA

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Introduction: Haemolytic disease of new born is a condition in which the red cells of the foetus or newborn are destroyed due to maternal antibodies which passage across the placenta. The production of these antibodies is due to incompatibility of blood group between mother and foetus.
Rh HDN develops when a Rh negative mother previously sensitized with Rh D Antigen due to previous pregnancy or past Rh D positive cellular component transfusion.
During pregnancy commonly they get sensitization with feto maternal haemorrage at the last trimester and at delivery. Also there can be other sensitizing events during pregnancy.
HDN due to ABO incompatibility is mild and more common than Rh HDN. Unlike Rh HDN ABO HDN does not present in utero and does not cause hydrops fetalis.
It usually occur when mother is blood group O & fetus is blood group A or B and having maternal high titer of IgG anti A & anti B (>1:64). Less commonly it occurs with blood group A or B Mother having fetus B or A respectively.
Aim: The objective of this study is to see the frequency of HDN in new born babies and to find the most common causes for HDN in Sri Lanka.
Meterial and Methods: A retrospective study was done for 3 months duration in a teaching hospital in Sri Lanka. The new born babies who were suspected for Rh, ABO HDN and Jaundice were followed up. The dirct anti globulin test (DAT), blood group & Rh, serum bilirubin and Hb were done in all of them.
Results: The two cases of ABO HDN both mothers were Blood Group O Rh positive and babies were Blood Group A positive. These two babies were given double phototharpy and exchange transfusion was not done.

Table 1

	February	March	April	Total
Number of deliveries	355	488	466	1309
Number of DAT	183	240	241	664
Number of DAT positive	0	0	0	0
Number of DAT negative	183	240	241	664
Number of cases with clinical jaundice	1	1	1	3
Causes for clinical jaundice total no %				
Jaundice due to ABO HDN	2	0.15		
Jaundice due to septicemia	1	0.08		

Conclusion: For 1309 deliveries there were only three cases of clinical jaundice and two cases were due to ABO incompatability and one case due to septicemia. There were no cases of Rh system or Kell blood group.
The above results show HDN is rare in Sri Lanka and the more common cause for HDN is ABO incompatibility.
Also these results show DAT is of little value in newborn babies as a screening test for HDN.
In Sri Lanka RhD HDN is rare because all Rh D negative mothers with antibody screening negative are given anti D prophylaxis during pregnancy and after delivery for prevention of HDN.

P-282
CLINICAL STUDY ON THE RELATIONSHIP BETWEEN TITERS AND MATERNAL BLOOD GROUP ANTIBODIES AND THE INCIDENCE OR SEVERITY OF HDN

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Background and Objectives: Haemolytic disease of the newborn (HDN) is a clinical condition in which foetal red blood cells are destroyed by maternal alloantibodies directed against red cells antigens acquired from the father. ABO-HDN occurs almost exclusively in infants of blood group A or B who are born to group O mothers because IgG anti-A or -B occurs more commonly in group O than in group A or B individuals. The placenta is relatively impermeable to naturally occurring IgM anti-A/anti-B antibodies. However, immune anti-A and anti-B of the IgG type will cross the placenta and may thus cause ABO-HDN. The aim of the study was to evaluate the relationship between the titers and sorts of maternal IgG type blood group antibodies and the incidence or severity of HDN, and to provide the basis in diagnosing, predicting, and preventing of HDN.
Methods: The titers and types of IgG blood group antibodies of 80 antenatal O group mothers (who had non O group husbands) were determined during prenatal diagnosis using the blood group serology method. The bilirubin and hemoglobin level of newborn infants were tested with routine methods.
Results: The percentage of the 80 cases whose titers of blood group antibody levels were equal or more than 1:64 in group O mother to group A father(O-A), group O mother to group B father (O-B), and group O mother to group AB father (O-AB) was 68%, 64%, and 71%, respectively, $P > 0.05$. With increasing of titers of anti-A/(B) antibodies of IgG type (higher than 1:512 or more), the incidence or severity of HDN was higher, $P < 0.01$. The percentage of HLA antibodies in serum of mothers who had newborns suffered from mild or severe HDN was 85% and 47%, respectively, There was a significant difference between the two groups, $P < 0.01$.
Conclusion: The incidence or the severity of HDN was related to the types and titers of prenatal antibodies of IgG type, with the increasing of the titers of blood group antibodies of IgG type, the incidence or severity was higher. The incidence of HLA antibodies in serum of pregnant women against their husbands was related to severity of HDN.

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RELATIONSHIP BETWEEN THE MIDDLE CEREBRAL ARTERY PEAK SYSTOLIC VELOCITY AND ANTIBODY TITER IN ISOIMMUNIZED PREGNANCIES

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Background: Antibodies titration is still a cornerstone in the management of isoimmunised pregnancies. However often antibody titration did not strictly correlated with the fetus anemia condition. The introduction of middle cerebral artery peak systolic velocity (MCA-PSV) permitted the non invasive detection of fetus anemia.
Aims: Detect the relationship between antibody titration and the MCA-PSV to permit a better management of pregnancies.
Methods: We studied the relationship between 259 antibodies titers in 72 pregnancies and the MCA-PSV values expressed as the multiples of the median (MoM), considering two screening values values 1.5 and 1.29. For antibodies titrations we performed serial twofold dilutions of the serum using the gel technology (Biorad). The antibodies were: 61 pregnancies (232 titrations) with anti-D, titer from 1 to 8192; 11 pregnancies with non-RhD antibodies, titer from 1 to 4096.
Results: The association between antibody titer ≥32 and MoM ≥1.3 was significant odds ratio 3.496 (CI 95% 1.504–8.123), $P = 0.004$; the association between antibody titer ≥64 and MoM ≥1.3 was significant odds ratio 3.556 (CI 95% 1.660–7.614), $P = 0.001$; the association between antibody titer ≥128 and MoM ≥1.3 was significant odds ratio 4.578 (CI 95% 2.417–8.669), $P = 0.000$; the association between antibody titer ≥32 and MoM ≥1.5 was significant odds ratio 10.639 (CI 95% 1.420–79.684), $P = 0.02$; the association between antibody titer ≥64 and MoM ≥1.5 was significant odds ratio 6.827 (CI 95% 1.588–29.335), $P = 0.01$; the association between antibody titer ≥128 and MoM ≥1.5 was significant, odds ratio 10.015 (CI 95% 2.966–33.819) $P = 0.000$. The association between antibody titer ≥16 and MoM ≥1.3 was not significant, odds ratio 2.371 (CI 95% 0.879–6.394), $P = 0.088$; the association between antibody titer ≥16 and MoM ≥1.5 was not significant, odds ratio 5.301 (CI 95% 0.699–40.168), $P = 0.107$.
Discussion: In our study we quantified the relationship between the antibody titration and the MoM of MCA-PSV at 2 values ≥1.3 and ≥1.5 in isoimmunized pregnancies. This permitted us to detect the critical value of antibody titers significantly related with the MCA-PSV. At antibody titer 32 both the MCA-PSV MoM 1.3 and 1.5 begin to be statistically associated. Until now, the antibody titer importance was evaluated empirically. We think that the measurement of the relationship between these two non-invasive tests can be useful for the management of isoimmunized pregnancies.
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P-284
CASE REPORT OF TESTING & MANAGEMENT OF BOMBAY (OH) RH D NEGATIVE PREGNANCY

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Background: The Bombay phenotype (Oh) is a rare red cell type that is characterized by the deficiency of H antigen on the surface of the red cell & the presence of anti-H in their serum. Inheritance of Rh phenotype is independent to the inheritance of Bombay phenotype. This case report illustrates the clinical course & management of a Bombay Oh Rh D negative pregnant mother.
In Sri Lanka all Rh D negative pregnant women are screened for unexpected antibodies in early pregnancy as there is a possibility of developing Anti-D & the consequence of sever heamolytic disease of the fetus & newborn when the partner is Rh D positive.
To detect any unexpected antibodies, Anti-H needs to be absorbed in the Bombay O Rh D Negative pregnant mother. Cells selected for absorption of anti-H should absorb only

anti-H antibodies & not any other clinically significant alloantibodies which could be present during pregnancy.
Aim: To describe the antenatal serological testing & management of Bombay Oh, Rh D negative pregnancy with the available facilities in Sri Lanka.
Method: The following steps were taken to absorb anti-H antibodies.
Her extended red cell phenotyping was done using commercially prepared antisera following manufacturer's guidelines. A regular voluntary nonremunerated donor with a red cell phenotype similar to that of the mother (Rh, Kell & Kidd matched) was selected & the cells treated with papain & used for absorption of anti-H antibodies at 40°C, room temperature & 370°C.
Following absorption, antibody screening was performed at 370°C IAT phase to detect for any clinically significant unexpected antibodies.
Results: Antibody screening was negative following absorption.
Conclusion: Following absorption antibody screening was negative for unexpected antibodies. Thus this mother is a candidate for routine administration of anti-D prophylaxis (RAADP) at 28 & 34 weeks of gestation. Cord blood grouping & Rh phenotyping & DAT should be performed at delivery of baby & if cord blood is positive for Rh D phenotype, fetomaternal hemorrhage should be estimated & adequate anti-D prophylaxis should be administered to mother to prevent sensitization against Rh D.

P-285
PREVALENCE OF UNEXPECTED ANTIBODIES AMONG PREGNANT WOMEN

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Background: In pregnant women it is essential to do blood group and unexpected antibody screening at booking visit. Then depending on the results there is an algorithm to follow which is given in BCSH (British Committee for Standards in Hematology) guidelines. In Sri Lanka the current practice is to perform ABO and Rh D grouping in all pregnant women at the booking visit and then to perform antibody screening in Rh D negative women only. Blood bank General Hospital Kaluthara follows the same practice for many years. Additionally, blood bank G H Kaluthara recently started to perform antibody screening in pre transfusion compatibility testing for pregnant women at the time of delivery to provide blood.
Aim: To study the prevalence of unexpected antibodies among pregnant women and then to compare the prevalence between Rh D positive and Rh D negative women.
Results: The total number of antibody screening tests carried out from January to June 2011 was 520. There were nine anti Le b antibodies, one anti Le a antibody and one anti E antibody among these pregnant women. In this group there were 461 Rh D positive pregnant women and seven had anti Le b antibodies, one anti Le a antibody and one anti E antibody. Out of 59 Rh D negative females in the study group only two Le b antibodies were identified.
Conclusion: Antibody prevalence among all pregnant females was 2%. While the unexpected antibody prevalence was 2% in Rh D positive females the higher prevalence of 3% was observed among Rh D negative cases.

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THE FREQUENCY OF GENETIC DIFFERENCES ON HUMAN PLATELET ANTIGENS OF PREGNANT WOMEN AND THEIR NEWBORNS, A STUDY CONDUCTED IN A COMMUNITY HOSPITAL IN NORTHERN TAIWAN

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Neonatal alloimmune thrombocytopenia (NAIT) is a clinical syndrome that resembles hemolytic disease of newborn. The cause of NAIT is that human platelet antigens (HPA) in the neonatal are attacked by antibodies from mothers. The aim is to investigate the genetic difference of HPA between mothers and their newborns, and to assess the relationship between the difference and frequency of antibody production. ELISA kits were used for detection of HPA antibody in the plasma of the mothers. Thirty pairs of mothers and newborns were genotyped by molecular methods using DNA extracted from their whole blood or umbilical cord blood. The result indicates that HPA-1a, -4a, -5a, and HPA-6a were expressed in all mothers and newborns. HPA-5b and -6b are rare while HPA-1b and -4b are absent. The frequency of HPA-2a in mothers and newborns is 93.3% and 96.7% while that of HPA-2b is 3.3% and 6.7%, respectively. The frequency of HPA2a is 93.3–96.7 and that of HPA-2b is 3.3–6.7%. The frequency of HPA-3a in mothers and newborns is 66.7% and 63.3% while that of HPA-3b is 33.3% and 36.7%. The frequency of HPA-15a is 44.3% both in mothers and newborns but that of HPA-15b is 51.7%. The discrepant rate of antigen between mothers and newborns is 83.3%. The most discrepant is HPA-3b which is different in 40.0% of the mothers and newborns. The highest ratio of the antigen is HPA-3b with 20.0% that the mother is homozygous and the newborn is heterozygous. The plasma samples of the mothers are absent of anti-HPA antibody. Only anti-HLA was found in two mothers. Discrepancy of HPA in mothers and newborns is not the main cause of anti-HPA antibody.

6. Clinical Transfusion
6.1 Neonatal and Pediatric Transfusion

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RATIONAL USE OF FFP IN NEONATES BY COMPARATIVE ASSESSMENT BETWEEN ITS TRANSFUSION BEFORE AND AFTER APPLICATION OF ACUB TRAINING FOR PEDIATRICIAN IN SHEBIN EL KOM TEACHING HOSPITAL

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Background: Blood is a limited resource which should be conserved and use properly. Therefore it's essential to look in to the existing blood transfusion practices and collect background information about the type of existing blood transfusion practices and modify theses practices for appropriate utilization of blood.
FFP transfusion may be a life saving in certain circumstances but it can also cause adverse effects such as anaphylactic reactions, TTIS, TRALI and venous thrombo-embolism. Thus it should be used only when there's a clear clinical indication for its use.
Last year a study was carried out to assess rational use of FFP in neonates treated in neonatology department in Shebin El Kom teaching hospital and the study concludes that this hospital was not rational in prescribing FFP for neonates and we interpreted this behavior due to lack of awareness of pediatricians about guidelines on clinical use of FFP.
We were able to establish training for pediatricians on ACUB. This was started from January 2011 by helding workshops and giving lectures.
The present study has therefore looked at the appropriate use of FFP on the basis of transfusion triggers against standard guidelines.
A comparison between FFP Transfusion before and after application of ACUB program had given an idea about current patterns of FFP transfusion and will help to develop strategies to optimize transfusion practices.
Aim: A study was carried out to assess the rational use of FFP products in neonates treated in neonatology department in Shebin El Kom teaching hospital by comparative assessment between its transfusion before and after application of ACUB training for pediatricians.
Materials & Methods: Blood issuing Registers.
Statistics.
Blood request form.
Neonatology department Registers.
Result: Analysis of consumption of FFP showed that from 1 October 2009 to 30 September 2010 a total of (226) Pedi pack unites (50 ml) for (56) patient were administered and from 1 October 2011 to 30 June 2011 a total of (110) for (57) patient were administered in neonatology department in Shebin El Kom teaching hospital.
Indication of FFP Transfusion in neonates before and after application of ACUB training are shown in the illustrated tables.

Table 1: Decision to transfusion Afetr ACUB training

Month	No. of patient admitted to neonatology Dep.	No. of patient Received FFP transfusion	Appropriate		In appropriate	
			No. of patient	%	No. of patient	%
January 2011	24	12	9	75 %	3	25 %
February 2011	26	12	8	66.7 %	4	33.3 %
March 2011	29	4	4	100 %	0	0 %
April 2011	18	12	10	83.4 %	2	16.6 %
May 2011	22	9	7	77.8 %	2	22.2 %
June 2011	20	8	6	75 %	2	25 %
Total	**139**	**57**	**44**	**77.2 %**	**13**	**22.8 %**

Table 2: Indications of pedi pack plasma transfusion in neonates from 1 October 2009 to 30 September 2010

Indications	Symptoms & Diseases	Shebin El Kom Teaching Hospital	
		No. of patient	%
I. Appropriate	1. Bleeding tendencies é ↑ pt & ptt .	2	3.6
	2. Coag. Defects .	2	3.6
	3. Exchange transfusion .	10	17.8
	4. DIC.	1	1.8
	5. LC Hge .	2	3.6
	Total	17	30.4 %
II. Inappropriate	• Nutritional edema on ventilators .	31	55.4
	• Septicemia .	3	5.3
	• As a Source of lgs.	5	8.9
	Total	39	69.6 %
Total No. Of Patients		56	

Conclusion: The study concludes that ACUB implementation succeeded in tremendously minimizing the inappropriate use of FFP showing increase for appropriate use of FFP for neonates after implementing ACUB program than before its implementation (77.2%/30.4%).

This study highlights: 1. The importance of continuous medical education & training for pediatricians who prescribe blood components.

2. More attached to the blood bank, prior to issuing to avoid unnecessary issues.

3. Re – auditing the usage of blood after education sessions will also help to minimize inappropriate use.

(a) This idea is not new but it was applied in this place for the first time.

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This abstract has been withdrawn.

6.2 Therapeutic Apheresis

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PLASMA EXCHANGE AS RESCUE THERAPY FOR CRITICAL SYSTEMIC LUPUS ERYTHEMATOSUS: ONE CENTER EXPERIENCE

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Objectives: To analyze the role of plasma exchange as a rescuing therapy for critical systemic lupus erythematosus (SLE) patients.

Methods: A retrospective review was conducted to evaluate the patients with SLE undergoing rescuing plasma exchange (RPE) due to critical manifestations such as diffuse alveolar hemorrhage (DAH), neuropsychiatric syndromes of SLE (NPSLE), catatrophic antiphopholipid syndrome (CAPS), hemophagocytosis syndrome, thrombotic thrombocytopenic purpura (TTP), and symptomatic cryoglobulinemia between February 1985 and January 2010. The primary outcome detection contained all cause mortality and SLE disease activity index (SLEDAI) scores one month after RPE. The secondary outcome measurement included complications such as infection and hemolysis one month after RPE.

Results: The study population comprised 33 patients with SLE. The mean time for the duration of the disease was 68.4 months, from the diagnosis of SLE to the first RPE (range from 1 week to 22 years). The mean age at first RPE was 38.7 years. The 23 events of DAH attacked in 20 patients, while eight patients for NPSLE, three patients for CAPS, three patients for pancytopenia, one patient for TTP, and one patient for symptomatic cryoglobulinemia were evaluated. The overall sessions of PE performed were 232 times, with a median for each patient of six sessions per course (range 1–33). There were two patients died due to septicemia, one patient for DAH with acute respiratory failure, one patient for CAPS, one patient for TTP and one patient for cardiac thrombus with systemic thromboembolsim. The overall survival rate of all patients was 83.8%, while 87% in DAH, 75% in NPSLE. One case had experienced anaphylaxis, while cytomegalovirus viremia occurred following PE in two patients. There were 51.4 percents of patients receiving low dose prednisolone therapy before the events of RPE. The patients received pulse methylprednisolone therapy (750–6000 mg) simultaneously or separately with RPE. In total courses, the mean SLEDAI scores were 24.6 and 8.0 before and three weeks after the RPE, respectively. Nevertheless, only one patient received cyclophosphamide (CY) pulse therapy. The health care system disbursed all expenses for PE.

Conclusions: Transient RPE combine with following methylprednisolone pulse therapy instead of pulse CY therapy successfully saved the patients with life threatening complications. Clinicians could cautiously prescribe early RPE and pulse steroid therapy in critical circumstance to prevent laborious complications.

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This abstract has been withdrawn.

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COMPARISON OF 'DOUBLE RBC' PHLEBOTOMY AND WHOLE BLOOD PHLEBOTOMY IN THE TREATMENT OF ERYTHROCYTOSIS

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Background: Whole blood phlebotomy has long been a primary method of treatment for erythrocytosis patients. In this study, we have employed ALYX (Fenwal, Lake Zurich, IL, USA) component collection system as a modified RBC phlebotomy in erythrocytosis patients. This system has been used for collecting double red blood cell (RBC) units from donors but not as a treatment modality for erythrocytosis patients.

Aims: The aim of this study was to evaluate the effectiveness and the safety of 'double RBC' phlebotomy (DRP) using ALYX in erythrocytosis patients and to compare the result with conventional whole blood phlebotomy (WBP).

Methods: We retrospectively reviewed electronic medical records of 110 erythrocytosis patients who visited Asan Medical Center in Seoul, Korea from June 2008 to May 2010 and received either DRP (n = 11) or WBP (n = 99), and we compared the effectiveness of both treatment methods. To rule out possible inter-individual differences, we also evaluated the changes of hematologic results in 19 erythrocytosis patients who received both methods in the course of their treatment. The removed volume was 360–420 ml of RBCs in DRP and 400–600 ml of whole blood in WBP. None of the patients received other medications or medical interventions as the treatment of erythrocytosis. Before each phlebotomy procedure, vital signs were checked to exclude any unstable patients. Hematologic parameters including hemoglobin and hematocrit as well as WBCs and platelets were measured before and after phlebotomies. The Mann–Whitney Test was used for statistical analysis of the differences in changes of WBC, hematocrit, hemoglobin, and platelet between DRP and WBP methods. Paired-*t* test was used for the comparison of the test parameters of DRP and WBP within the same patient. Statistics was done using SPSS v. 17.0 (SPSS, Chicago, IL, USA).

Results: The mean decrease in hematocrit for each phlebotomy method was 6.3 ± 2.2% in DRP and 3.1 ± 2.2% in WBP, and the difference was statistically significant ($P < 0.001$). The mean decrease in hemoglobin for each method was 2.3 ± 0.7 g/dl in DRP and 1.1 ± 1.6 g/dl in WBP and this difference was also statistically significant ($P < 0.001$). There were no statistical differences in changes of WBCs and platelets between the two methods ($P > 0.05$). In patients who received both types of treatment, mean decrease in hematocrit was 7.6 ± 2.0% by DRP, but only 2.8 ± 2.0% by WBP, and this difference was also statistically significant ($P < 0.001$). Hemoglobin was also decreased more by DRP than by WBP ($P < 0.001$) in the same group of patients but differences in changes of WBCs and platelets were not significant ($P > 0.05$). Adverse reactions occurred in 1/3 of patients during DRP but were limited to mild citrate toxicity.

Conclusion: DRP was superior to WBP in terms of decreasing more hematocrit and hemoglobin in erythrocytosis patients. DRP method could help reduce the frequency of hospital visit by the patients. DRP using ALYX system was a safe and more effective technique for the treatment of erythrocytosis than WBP.

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EARLY RELAPSE OF THROMBOTIC THROMBOCYTOPENIC PURPURA(TTP)/HEMOLYTIC UREMIC SYNDROME (HUS) REFRACTORY TO PLASMA EXCHANGE ASSOCIATED WITH CATHETER RELATED ACINETOBACTER BAUMANNII BACTEREMIA

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Abstract: Thrombotic thrombocytopenic purpura (TTP)/Hemolytic-uremic syndrome (HUS) is a syndrome characterized by thrombocytopenia, microangiopathic hemolytic anemia, fever, neurologic manifestation and renal failure. Most of the cases are idiopathic. Plasma exchange is one of the standard treatment for TTP/HUS. However, secondary TTP/HUS associated with bacterial, viral and mycobacterial infections, drugs, autoimmune disease, pregnancy, solid tumors and bone marrow transplantation

have been described. Early relapse associated with catheter-related bacteremia is a rare occurrence. The patient we report had a classic presentation of TTP/HUS that responded to plasma exchange but relapsed earlier as reflected by the increased schistocytosis, decreased hematocrit, decreased platelet counts and increased lactate dehydrogenase. This relapse may be attributed to *Acinetobacter baumannii* bacteremia, secondary to chemo-port infection. After removal of the chemo-port, the syndromes of TTP/HUS had improved without additional plasma exchange. The importance of identifying the possible bacterial colonization of an indwelling catheter is thus emphasized.

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THERAPEUTIC LEUKAPHERESIS IN SARDJITO HOSPITAL YOGYAKARTA INDONESIA: A START STEP

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Background: Leukemia is one of the hematological malignancies, and the incidens tends to increase in recent years. Leukemic patient with leukocyte counts >100 × 10^9/l are at risk for leukostasis, especially patient having an acute myeloid leukemia. Leukostasis might cause multi-organ and tissue failure or death attributed to the circulation of high number of leukemic blast cells. Pulmonary and neurologic complications are extremely serious and most common causes of morbidity and mortality in leukostasis. It has been shown that in hyperleukocytosis, leukapheresis can reduce the chance in early mortality, therefore, the leukocyte count should be reduced as soon as possible. Induction chemotherapy could not be used for this purpose, so therapeutic leukapheresis can be applied besides immediate start with cytoreductive agents.
Aim: To see whether leukapheresis in the system of the Sardjito Hospital, Yogyakarta, Indonesia could be of help in fast reduction of the leukocyte count in leukemic patients at risk of leukostasis.
Methods: Data were collected from leukemic patients who were treated with therapeutic leukapheresis procedures in the Sardjito Hospital in the period of February 2010 until June 2011. The procedures were performed using MCS+ applying the TLR protocol. The patient's pre- and post-procedure leukocyte count were determined before and 1 h after the procedures respectively.
Results: A total of 22 procedures in 19 patients were performed during the period. Eighteen patients were diagnosed having an acute myeloid leukemia, and one patient having an acute lymphoid leukemia. The age of the 11 male and eight female patients was ranged from 22 until 78 years. The median pre-procedure leukocyte count was 394 × 10^9/l (65 × 10^9/l–517 × 10^9/l). The mean decrease of leukocyte count after the leukoreduction procedure was 21%(6–46%).
Conclusions: The therapeutic leukapheresis procedures could reduce leukocyte count quite efficaciously. In our hands, this procedure should be considered for decreasing risks of leukostasis in patients with leukemia.

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RETROSPECTIVE ANALYSIS OF INDICATIONS FOR THERAPEUTIC PLASMA EXCHANGE FOR 18 MONTHS AT NATIONAL HOSPITAL OF SRI LANKA

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Introduction: Therapeutic plasmaperasis, which was recognized modality of management, in wide range of immune and non immune mediated clinical conditions, had gained increased popularity in the recent past in this part of the world. Newly established specialist unit of the existing blood bank facility of National Hospital of Sri Lanka, had recently encountered many requests for therapeutic plasmaperasis for wide range of indications some of which are not documented but are acceptable given the complexity of the conditions and the lack of knowledge of the outcome.
Therefore this study is aimed at identifying and categorizing the conditions on which therapeutic plasmaperasis were requested. Moreover this is done as a preliminary step in the process identifying the categories of the conditions, especially non conventional indications, and the clinical outcome of therapeutic plasma exchange (TPE) as a treatment modality in those conditions.
Objectives: List the indications and the frequencies of TPE procedures for 18 months starting from 1st January 2010 and categorize them according to the basis of evidence, (i) Evidences supported by randomized controlled trials, (ii)Consensus of help in treatment, (iii) Suggestion of the help in treatment, (iv) Refuted by randomized controlled trials, and (v) Indications on which no data available with regard to the plasmapherasis and analyze them to identify the pattern of indications.
Methodology: TPE form and the Register maintained in this respect were used for the data source from which indications were gathered. Those indications were matched against indication categories for TPE and any undocumented indications, if any, were recorded in the category of unrecognized indications.
Analysis and Discussion: Most requesters were fallen in to the category of definite indications (299 out of the total of 446) and the percentage was 67.4 and the category of indications with consensus of helpfulness had 38 procedures (8.5%).
There were no procedures for the third category of indications and the forth category of illnesses for which therapeutic plasma exchange was refuted as a treatment modality had five procedures done (1.12%).
Most significant finding of the study was 104 procedures of therapeutic plasma exchange were done for indications for which plasmaperasis was not previously recognized as a therapeutic modality.
Therefore based on the results of this study, it is nessecery to identify the conditions for which TPE was used as a treatment modality and the outcome of the conditions in these instances.

6.3 Evidence Based Transfusion Medicine Practice

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A STUDY ON GYNAECOLOGICAL & OBSTETRIC TRANSFUSIONS IN COLOMBO SOUTH TEACHING HOSPITAL IN SRI LANKA

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Background: The health service in Sri Lank has a well established nationally coordinated Blood Transfusion Service which serves the entire country through the action of 85 hospital based blood banks and the entire system is managed by the National Blood Transfusion Centre and 16 Regional Blood Transfusion Centers. In this study, the hospital based blood bank situated at the Colombo South Teaching Hospital in the western province was taken as the study focal point. Colombo South Teaching Hospital is one of the main tertiary care institutions in the district of Colombo (bed strength 1105).
Aim: Information on the use of red cells and the characteristics of transfusion recipients is limited in Sri Lanka. Collection of such data may improve the understanding of fluctuations in demand, help to predict future trends in red cell use, and define the potential value of blood sparing techniques.
Method: This study is mainly focused on the blood requests sent from Gynaecology & Obstetric units in the Colombo South teaching hospital.
Patient information abstracted and relevant to the current study included patient indication for transfusion, date of birth, date of hospital admission, ward at which the patient was seen, nadir hemoglobin level, total number of transfusions administered and indication of transfusion. We collected information from hospital blood bank using a one page preprinted report form.
Following records and registers were used as sources of data extraction.
1. Blood Bank red cell request form (Health – 137).
2. Patient's BHT.
3. Anesthesia record sheets.
4. Patient's other investigation reports. (Hb %, Hct, Blood picture reports attached to BHT),
5. Red cell issue registers in the Blood Bank.

Results: Pattern of blood requests were mainly in two ways, routine requests and urgent requests, according to the sample analysis majority (70.5%) were routine requests while 28.5% were urgent requests. Results are shown in Tables 1 and 2.

Table 1: Analysis of gynaecological & obstetric transfusion

Indication	Requests	Issues	% Usage
LSCS	1478	52	7.2%
PPH/APH	98	86	11.8%
Bleeding PV	239	208	28.7%
TAH	259	18	2.4%
VH&R	112	12	1.6%
Myomectomy	81	32	4.4%
Laparotomy	52	25	3.5%
D & E	346	105	14.5%
Low Hb and Pallor	258	138	19.1%
Radical Hysterectomy	38	20	2.7%
Others	199	25	3.5%

Table 2: Pretransfusion hemoglobin concentration among tran

Haemoglobin level g/dl	Percent (%)
<7	9.6%
7 - 10	28%
>10	57.7%
Not done	4.7%
Total	100%

Conclusions: The decision to transfused blood depends mainly on pre operative haemoglobin level, blood loss, and clinical condition of patients. Though guidelines and instructions are available to aid the clinicians for taking decisions on administration of red blood cells, for most of the patients the decision to transfuse may have been entirely based on a clinical observation or condition that was not recorded accurately.

There is no universal 'trigger' for red cell transfusions, i.e. a given concentration of haemoglobin at which transfusion of red cells is appropriate for all patients. Clinical judgement plays a vital role in the decision to transfuse red cells or not. The findings of this study should be presented to the hospital transfusion commitee, and strategies to improve transfusion-guideline compliance and transfusion documentation should be considered. The initial recommendations include increasing education of clinicians.via informative conferences as well as the use of more informative letters.

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DISCREPENCY BETWEEN SEROLOGIC AND GENOTYPE TESTING RESULTS OF 'MIA' BLOOD ANTIGEN AND ANTISERUM IN TAIWAN

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Background: The Miltenberger subsystem (Mi) series includes a group of antigens formed from hybrid glycophorin genes. Mi.III (GP.Mur) is reported to have a mean frequency of 7.3% among Taiwanese. Since the specificities of Mi.III (GP.Mur) antisera are not further specified, such antisera and the cells identified by them have been applied a collective term as anti-'Mia' and 'Mia'(+) cells in Taiwan. Anti-'Mia' antibodies are the most common clinically important antibodies detected in Taiwan. Taiwan Society of Blood Transfusion have issued a guideline of including a 'Mia'(+) cell in the antibody screening panel. However, the sensitivity and specificity of this detection strategy have never been validated.

Aims: To evaluate the current practice of using 'Mia'(+) screening cells to detect anti-'Mia' antibodies in comparing with glycophorin genotyping method.

Methods: Blood samples were obtained from randomly selected patients. Antisera containing anti-'Mia' specificity were collected routinely in our blood bank. Manual polybrene method without a supplementary antiglobulin phase was used for serologic test. Each blood sample was tested with anti-'Mia' from five different individuals. Genomic DNA was extracted from the whole blood using QIAamp DNA Mini Kit. The primers used were referenced to a previous study conducted by Palacajornsuk, et al. The results of serologic test were compared with PCR-sequencing data. The study was approved by our Institutional Review Board.

Results: Among 389 individuals tested by PCR, 25 samples were positive. All of them belonged to GP.Mur genotype, which was confirmed by sequencing. The prevalence of Mi.? (GP.Mur) is 6.4% (25/389). Among 1945 serologic tests for these 389 samples, 210 tests showed a positive reaction (1+ or more). Among them, only 84 positive results belonged to PCR(+) samples. The sensitivity and specificity of our routine practice using 'Mia'(+) screening cells to detect anti-'Mia' antibodies are thus estimated to 67.2% (84/125) and 93.1% (1694/1820), respectively. The positive predictive value is 40% (84/210).

Summary/Conclusions: The 'Mia'(+) screening cells used in Taiwan blood bank routine were harvested from blood donors tested with anti-'Mia' antisera. This practice is based on an assumption that Mi.III (GP.Mur) is the overwhelming glycophorin variant in Taiwan. Our results showed that the Mi.III (GP.Mur) has a prevalence of 6.4% in Taiwan, and no GP.Hut, GP.Hop, GP.Bun, or GP.HF were detected. However, our study also showed that our screening system for detecting anti-'Mia' has a low sensitivity of 67.2% with a specificity of 93.1%. Since a previous study suggested the presence of other glycophorin variants in Taiwan, the results suggested the existence of other (Mi) phenotypes and the current detecting method may not be sufficient to identify antibodies other than anti-GP.Mur. A larger series is needed to discover the significance of other (Mi) phenotypes and the potential value of adding them to the screening system. The low sensitivity of 'Mia'(+) cells for screening anti-'Mia' is another issue warrant further study.

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AUDIT OF BLOOD USAGE FOR CANCER PATIENTS IN SURGICAL WARDS AT NATIONAL CANCER INSTITUTE, SRI LANKA

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Background: The current practice of National Cancer Institute, Sri-Lanka is to send blood samples for grouping, cross matching and reservation of blood prior to most elective surgical procedures. The cross matched blood for surgical patients are reserved for 5 days. It is observed that many units of blood ordered for elective surgeries were never being utilized. Non-utilization of reserved blood is a waste of resources, where blood is used infrequently and out-dating of blood due to unnecessary storage affects badly on maintaining stocks.

Adapting to a local maximum surgical blood order schedule reduces unnecessary cross matching for most surgical procedures. It includes full ABO and Rh D grouping and antibody screening for all samples. If unexpected alloantibodies were not detected in the patient's serum, blood of same ABO and Rh D group can be issued after cross matching by the appropriate rapid procedure. Otherwise antibody negative blood should be reserved prior to surgeries.

Aims: To analyze the cross matching and transfusion practice for patients in surgical wards at the National Cancer Institute of Sri Lanka and to see the possibility of implementing a maximum surgical blood order schedule for elective cancer surgeries.

Method: A retrospective analysis of blood usage was conducted for one year duration from January to December 2010. Data were collected from blood bank records and bloods transfusion request forms sent from all surgical wards including general surgery, orthopedic, oral maxillofacial and gynaecology & obstetrics units. Indications for requests, number of units cross matched and transfused for all requests were recorded and cross match to transfusion (CT) ratio and transfusion index (Ti) were calculated for each surgical procedure. CT ratio of 2–3:1 has been held to be acceptable for a hospital which corresponds to a blood usage of between 30% and 50%. Ti value of 0.5 or above is considered indicative of significant blood utilization.

Results: Out of total 1997 requests, 1585 have been sent for grouping and reserving of blood prior to elective surgeries, for which, 3153 units were cross matched and 596 packs were transfused (18.9%). The overall CT ratio was 5.3:1 and Ti value was 0.37. Seventy 5% (30 for 40 listed cases) of procedures in this study had CT ratio above 3:1 and 65% of procedures had Ti value was <0.5 (26 out of 40 listed cases).

Economical CT ratio and Ti were observed in oesophagectomy, gastrectomy, abdominoperinial resection, anterior resection, pelvic exenteration, nephrectomy, colectomy, mandibulectomy and hemimandibulectomy. High CT ratio and low Ti values were seen in total mastectomy, total thyroidectomy, cervical and inguinal block dissection, vaginal hysterectomy, radical hysterectomy, orchidectomy, penil amputation, maxillectomy, laryngectomy, parotidectomy and wide local excision of malignant lesions.

Conclusions: Eighty percent of blood cross matched for elective cancer surgeries were not transfused for these patients. The grouping and antibody screening policy can be recommended for many surgical procedures and introduction of maximum surgical blood order schedule could be attempted for National Cancer Institute of Sri Lanka.

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ORGANIZATION FACTORS AFFECTING RED BLOOD CELL TRANSFUSION IN A MEDICAL CENTER

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Aim: A computerized transfusion decision support system (CTDSS) has been used in Kaohsiung Medical University Hospital since September 2004. In this study, the factors affecting red blood cell (RBC) transfusion were investigated.

Materials and Methods: Totally 20,551 RBC-transfusion episodes between January and December 2008 were reviewed. The nearest hemoglobin concentrate before transfusion is defined as the transfusion trigger. The physician compliance, the factors associated with the transfusion triggers and post-transfusion hemoglobin increment were investigated.

Results: The physician compliance is 83.1%. The transfusion trigger of all RBC transfusion episodes is 8.32 ± 1.84 (mean ± standard deviation) g/dl. The transfusion

triggers are statistically significant in terms of both different order sources and disease types ($P < 0.05$). The post-transfusion hemoglobin level increased in two-thirds of the episodes. The percentages of hemoglobin increments after transfusion are dependent on the transfusion triggers.
Conclusions: A transfusion episode with a lower pre-transfusion hemoglobin level would have a higher possibility of hemoglobin increment after transfusion. CTDSS should be more powerful and effective to intervene in the appropriateness of transfusion practice.

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TO DETERMINE THE MEAN HEMOGLOBIN DROP PER MONTH (30 DAYS) IN? THALASSAEMIA MAJOR MALE PATIENTS (12–16 YEARS) BEING MANAGED IN NATIONAL THALASSAMIA CENTER, SRI LANKA

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National Thalassamia center is the main center in Sri Lanka where 810 β Thalassemia major patients are being managed, and is attached to the Teaching Hospital Kurunegala (Bed Strength1318). The center consists of inward fascilities, theater facilities and laboratory facilities directly funded both by the government of Sri Lanka and by the world health organization as well. These patients are registered since the first day of the diagnosis and are being managed whole throughout.
Aim: To determine the mean hemoglobin drop per month (30 days) in β thalassaemia major male patients(12–16 years age)who are being managed in National thalassaemia center, Sri Lanka. Above group was selected as the study group to exclude discrepancies due to the level of physical activities and gender. As they used to visit clinics monthly for assessment it wasn't been a burden for the patients to participate for the study.
Method: The data collected from January 1st 2010 to December 31st 2010. Both pre and post transfusion hemoglobin levels were recorded calculated the drop using previous months records. No exclusion criterias considered othere than gender and age.
Post transfusion Hb – Pretransfusion Hb = Hb drop in 1 month.
Last visit this visit Hematology analyzer was used to detect Hb levels.
All the patients were transfused different amounts (450–325 ml/visit) of packed leuco reduced red cells until they reach the Hb level of 14 g/dl approximately.
The patients were not informed about the study as a control group was not selected.
Collected Data and Analysis
Total no of patients (Beta thalassamia major) attached = 810
Males 12–16 years old = 76
Exclusions = 734
Mean Hemoglobin drop in 1 month = 5.24 g/dl
Discussion: As the participants were not informed about the study few possible exclusion criteria's may have biased the study as being ill [viral fever, gastroenteritis (3DOTS) etc], participating in competitive sports, attending house hold work, part time jobs,..etc.
Conclusion: The mean hemoglobin drop for a month of 30 days were detected as 5.24 g/dl in β thalassemia major male patients in age group of 12–16 years who are being flowered up in National Thalassaemia Center, Sri Lanka.

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IMPORTANCE OF THE EXPECTED ELEVATION VALUE OF SERUM ALBUMIN BEFORE REPLACEMENT THERAPY: COMPARISON BETWEEN BROMCRESOL GREEN- AND BROMCRESOL PURPLE- MEASURED ALBUMIN

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Background: Pharmaceutical albumin has been used excessively in Japan. Japan consumed one-third of the world supply in the 1980s. While some projects to decrease dependence on albumin based treatments, Japanese consumption of pharmaceutical albumin was 57,541 kg in 1999, which was 1.6 and 6.2 times more than that of the USA and UK, respectively.
Aims: Since then, the target elevation value of serum albumin was adopted in the 'Japanese Guideline for Blood Transfusion, updated in 2005, to determine the necessary dosage of pharmaceutical albumin for patients. The optimum dosage is calculated by the formula; the expected necessary dosage (g) = the expected elevation value (g/dl) × amounts of whole plasma (dl)/0.4. It contributed considerably to decrease consumption to 0.64 in a past decade (2009). Recently, the measurement method of serum albumin is shifting from bromcresol green (BCG) assay to bromcresol purple (BCP) assay because the latter has less contamination than pure albumin. In this study, the clinical importance of the target value of serum albumin was reevaluated for the BCP assay.
Methods: We analyzed the serum albumin value by BCG and BCP analyses in 251 cases, and examined the correlation between them.
Results: AlbBCP = 1.1 AlbBCG – 0.5 served as the standard curve and total correlation of coefficient value (r) was 1.0. We changed the target elevation value in our institute according to the standard curve from 2.5 to 2.2 g/dl in chronic diseases and 3.0 to 2.7 g/dl in acute diseases. However, the entire hospital consumption of pharmaceutical albumin was 3476 g/month, increased 23%. Extensive examination revealed the poorer correlation between them 6 months later. We analyzed a further 28 cases whose serum albumin levels were <3.0 g/dl with BCG assay. AlbBCP = 1.0 AlbBCG – 0.3 served as the new standard curve and the correlation of coefficient value (r) was 0.9. After we modified the target elevation value to 2.0 g/dl for chronic disease and 2.5 g/dl for acute disease, the consumption of pharmaceutical albumin was 2860 g/month, returned to the previous level of 6 month ago. The coefficient value (r) was significantly poorer in lower serum albumin levels, and the serum albumin value did not elevate more than expected after pharmaceutical albumin treatment in such cases ($P < 0.05$).
Conclusion: The indication of pharmaceutical albumin varies and is complex especially in cases whose serum albumin level is lower. Target elevation value of serum albumin might be continuously effective in reducing the consumption of albumin. However, we have to understand the difficulty in estimating the required clinical dosage of pharmaceutical albumin from laboratory data even if we adopted more advanced assay. Simultaneously, we need more information about efficacy on each background disease accompanying hypoalbuminemia.

P-301
BLOOD TRANSFUSION IN TOTAL HIP ARTHROPLASTY

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Total hip arthroplasty (THA) is associated with a relatively large bleeding and a need for perioperative transfusion. Postoperative anemia may delay recovery, but transfusion increases the well-known risks, such as transmission of infection or transfusion infections. The objective of this study is comparing the difference between patients who underwent total hip arthroplasty with or without allogenic blood transfusion.
Materials and Methods: We reviewed 354 patients underwent total hip arthoplasty from January 1, 2010 to March 3, 2011. Blood transfusion data, blood indicators before and after surgery were obtained from laboratory information system (LIS), patients characteristics, length of hospital stay, infection status were obtained from hospital information system (HIS). We then compared the indicators between the two groups.
Results: Two hundred and fifty-seven (72.6%) patients received blood transfusion. Female patients had higher probability of allogenic blood transfusion than male patients(87.6% vs 54.3%). 77.2% patients over 55 years old received blood transfusion, whereas only 56.6% patient under 55 years old received blood transfusion. Patients receiving blood transfusion had longer length of hospitalization (14.32 ± 7.35, 12.84 ± 11.38, $P < 0.05$) than patients not transfused. Preoperative Hct was independent indicator for blood transfusion in THA surgery, other indicators, Hb, Plt, total protein, albumin, PT, APTT had no relationship with blood transfusion in surgery.
Conclusions: Age, gender and Hct were correlated with blood transfusion in THA surgery. Patients who received perioperative blood transfusion had longer hospital stay than whose not transfused.

P-302
NEW AUSTRALIAN PATIENT BLOOD MANAGEMENT GUIDELINES – THE PERIOPERATIVE MODULE & THE LIKELY NEED FOR SYSTEM CHANGES

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Background: Currently, in Australia, a suite of clinical scenario based Patient Blood Management Guidelines (PBMGs) are being developed to replace the 2001 National Health Medical Research Council (NHMRC)/Australasian Society of Blood Transfusion (now ANZSBT) Appropriate Blood Use Guidelines, which were blood product based. These PBMGs are being developed under the auspices of the NHMRC by the National Blood Authority – Australia (NBA) and the Australian and New Zealand Society of Blood Transfusion, and involve a range of relevant medical and other experts. The second module of this suite focuses on Perioperative care.
Aims: To produce an expert Guideline for Perioperative Patient Blood Management, including pre-operative haemoglobin optimization, intra-operative and post-operative interventions to diminish blood loss, improve outcomes and also reduce the likely need to transfusions.
Methods: A major systematic literature review was undertaken. The Patient, Intervention, Comparison, Outcome (PICO) schema was used in this review. NHMRC defined

processes were used to form an evidence matrix for each question. Following this, formal Evidence Statements were developed for each research question. Arising from these, formal Recommendations and Practice Points were crafted to guide clinical practice in the pre-operative, intra-operative and post-operative phases of care.
Results: Specific Recommendations and Practice Points were developed in a wide range of areas of Perioperative practice, including: Establishing multidisciplinary multimodal perioperative PBM programs, pre-operative anaemia assessments and treatments, iron therapy and the use of erythropoietin stimulating agents, the timing of cessation of platelet antagonists, intra-operative haemodilution procedures, the utility of thromboelastography to guide transfusion support, the use of fibrinolytic inhibitors, post-operative cell salvage measures, transfusion triggers and use of recombinant Factor VIIa in prophylaxis.
Conclusions: Systematic detailed literature review and our guideline development has demonstrated there is a significant range of perioperative interventions that may improve haemoglobin, diminish blood loss and reduce the need for transfusion support. However, in the current Australian or New Zealand hospital settings, there are likely significant logistic and resource implications to the full implementation of the recommendations in these new Perioperative Patient Blood Management Guidelines. These Guidelines are scheduled for release in October 2011.
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P-303
A RANDOMIZED COMPARISON OF HEMOGLOBIN CONTENT-BASED VS STANDARD (UNIT-BASED) RBC TRANSFUSION POLICY EFFICIENCIES

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Background: Although the volume of the RBCs has been identified according to the standards, the Hb content of them are all different. In practice, the RBC transfusions are based on the number of units. No study has yet been carried out to compare an Hb content-based RBC transfusion with a unit-based one.
Aims: In this study, we aimed to compare the efficiencies of these two different RBC transfusion methods.
Methods: Eighty-nine patients were enrolled for the study. The median age was 46. Male:Female ratio was 55:34 and diganoses were; 44 acute leukemia, 17 lymphoproliferative disorder, 11 plasma cell disorders, 15 bone marrow defects, one sickle cell disease and one solid tumor. Ninety-two of the 178 transfusion episodes were randomly allocated to the study group and 86 of them to the control group. The calculations were done by Hemosoft Blood Banking Management & Information System. The Hb level of the units was calculated with respect to the donors' Hb concentrations. Within the study group, the required Hb amount was calculated based on the patients' height, actual body weight and their actual and targeted Hb. The Hb levels of the RBCs within the inventory were checked and if an available RBC with the required Hb amount was found, used in order to reduce the number of RBCs transfused. The number of units as ordered was used in cases where there were no available matching RBC and within the control group. Posttransfusion Hb was checked 2 h after transfusion. Characteristics of the patients, within the study group and the control group, were similar. Fifty-one patients had only one transfusion episode and 38 patients had two or more (range 1–7).
Results: Two units were ordered for 84 of the 92 episodes within the study group and 3 units were ordered for eight of them. A suitable matching unit was found for 38 of the episodes. (41.3%; 38/92). Two-unit orders were reduced to one within 30 episodes and three unit orders were reduced to two within eight episodes (19,8%; 38/192). The possibility of finding a suitable unit was higher within low weight-short patients and within a bigger size of inventory. The ratio of achieving the target Hb within the study group and the control group ($P = 0.1$), within the matched and unmatched groups ($P = 0.3$) and within the control group and the unmatched group ($P = 0.4$) were similar. The relation between the Hb content of the RBC and the rate of achieving the target Hb was found to be significant ($P = 0.01$). The relation between the shelf life of the RBC and the rate of achieving the target Hb was found to be insignificant.
Conclusions: We demonstrated that the efficiencies of Hb content-based and unit-based RBCs transfusion policies were similar. Less number of RBCs were used by Hb-content based transfusion policy. We also demonstrated that the possibility of finding a suitable Hb content match was directly proportinal to the size of the inventory and inversely proportional to the weight and height of the patient.
E-mail: erdenatilla@gmail.com PICO

P-304
PREPERATION OF AMAXIMUM BLOOD ODERSCHEDULE IN A GENERAL HOSPITAL IN SRI LANKA

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Background: General Hospital, Kalutara is a tertiary care hospital situated in the western province of Sri Lanka, which has major specialties namely, Medical, Surgical, Gynecology & Obstetrics and Peadiatrics units. In one study carried out recently, found that the cross match, transfusion (C:T) ratios were 1:1, 2:1, 4:1 and 9:1 among Peadiatrics, Medical, Surgical and Gynecology & Obstetrics units, respectively. This study paved the way for us to prepare a Maximum Surgical Blood Order Schedule(MSBOS) and to implement it in surgical units. MSBOS is a table of elective uncomplicated surgical procedures where surgeries are grouped into two categories. Those that are catered by group and antibody screen (G&S) only and those for which blood is cross matched according to an agreed schedule.
Aim: To find out the C:T ratios and Transfusion Index (TI) for common surgeries that are performed at General Hospital, Kalutara.
1. To group surgeries which have a C:T ratio of over 3:1 and/or TI of <40% in G&S category, and to group others into the category that need blood to be cross matched.
Results: Laparoscopic cholecystectomy, laparotomy, haemorrhoidectomy, herniotomy, tracheostomy, mastectomy, thyroidectomy, pyelolithotomy, ureterolithotomy, Internal Fixation of fractures, vaginal hysterectomy abdominal hysterectomy and myomectomy have C:T ratio of over 3:1. Leg Amputation has 3:1 C:T ratio. All the above surgeries except laparotomy & myomectomy have a TI of <40%.
Conclusion: Laparoscopic cholecystectomy, laparotomy, haemorrhoidectomy, herniotomy, tracheostomy, mastectomy, thyroidectomy, pyelolithotomy, ureterolithotomy, Internal Fixation of fractures, vaginal hysterectomy abdominal hysterectomy are grouped into G&S category.
Myomectomy, Laparotomy & leg amputation are grouped into surgeries that need one blood unit to be cross matched.

P-305
ACTIVITIES REPORT TO SUPPORT BLOOD OF MAJOR SICKLE CELLS PATIENTS TO THE NATIONAL BLOOD TRANSFUSION CENTER (CNTS) IN ABIDJAN, CÔTE D'IVOIRE: JANUARY–AUGUST 2010

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Introduction: Sickle cell disease is a condition that requires monitoring and a specific treatment because of complications related to anemia, vascular occlusives crises and infections. If multiples drugs have been proposed in order to correct the insolubility of sickle cell hemoglobin, transfusion therapy retains its usefulness to improve tissue oxygenation and reduce the severity of the problem vascular occlusive by cleaning hemoglobin S. However, handled carelessly, the transfusion may be more harmful than helpful. In order to ensure better blood safety, to improve both life expectancy and quality of life for sickle cell disease patients, to guarantee the availability of blood products, we decided to set up a support unit of blood for sickle cell disease in the National Blood Center Transfusion (CNTS) of Abidjan. The objective is to ensure better management of transfusion for the patients with sickle cell disease.
Patients and Methods: We conducted a prospective study from January to August 2010, to the CNTS, in Abidjan, Côte d'Ivoire. It covered 37 major sickle cell disease patients, followed in this structure and with a serious complications of the disease requiring either transfusion or bleeding due to hyperviscosity.
Results: From an epidemiological and clinical: the average age of patients was 21.6 years. The vascular occlusives crisis alone accounted for 49% of the reasons for consultation. The average annual crisis was 5. The homozygous form (SSFA2) predominated. 91.9% of our patients have been previously transfused. The heart anemic led the anemic complications. Pneumonia predominated table infectious. The acute chest syndrome (STA) had a majority in ischemic complications.
In terms of support transfusion: the rate of pre-transfusion Hb average of 7.54 g/dl. The average percentage of the S fraction is 93.6%. Seven patients had a previous alloimmunization anti-erythrocyte. Thirty-four patients have benefited from transfusion of which 25 have used a manual exchange transfusion, three patients were regularly bled and one patient was removed from the program because of an impasse and is currently on erythropoietin therapy.
In evolutionary terms: changes in transfusion program was marked by improvement of complications, a lower percentage of Hb S, an improvement in hemoglobin.

Conclusion: The transfusion management of patients with severe sickle cell at CNTS in Abidjan found that alloimmunization post transfusion is a problem in our working conditions. The transfusion program is necessary for the improvement of the quality of life in sickle cell disease severe. Erythropoietin is an alternative for patients with alloimmunization.

6.4 Haemorrhage and Massive Transfusion

P-306

This abstract has been withdrawn.

P-307

ANALYSES OF ACQUIRED HYPOFIBRINOGINEMIA INDUCED BY PERIOPERATIVE MASSIVE BLEEDING TREATED WITH CRYOPRECIPITATE

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Background: Cryoprecipitate, prepared from frozen plasma in hospitals, is effective for treatment of acquired hypofibrinoginemia induced by perioperative massive bleeding. According to USA guidelines, it may be indicated when plasma fibrinogen is considerably low, although there is no clear recommendation for its use. The indication of the infusion of cryoprecipitate should be based on the bleeding tendency, bleeding volume and coagulation data.

Aims: We analyzed our six experiences of its use to understand the appropriate indication and benefit of cryoprecipitate.

Methods and Results: Cryoprecipitate was prepared from fresh frozen plasma in our hospital. It included 703 mg/dl (625 mg/dl, 440–1208 mg/dl) fibrinogen and 150 ml (150 ml, 120–180 ml) was generally used per case. (Case 1) A 83-year-old woman, whose fibrinogen level decreased to 50 mg/dl during an aortic valve regurgitation operation, bled 3699 ml. Cryoprecipitate was infused, increasing the fibrinogen level to 136 mg/dl (272%), and the bleeding stopped significantly. (Case 2) A 36-year-old woman, whose fibrinogen level decreased to 83 mg/dl during an operation for intra-uterine fetal death, bled 2947 ml. Cryoprecipitate was infused, increasing the fibrinogen level to 115 mg/dl (139%), and her massive bleeding was immediately stopped. (Case 3) A 43-year-old man, whose fibrinogen level decreased to 132 mg/dl in a thoratic aortic aneurysm operation, bled 9703 ml. Cryoprecipitate increased the fibrinogen level to 155 mg/dl (117%) and his massive bleeding was stopped. (Case 4) A 14-year-old boy, whose fibrinogen level decreased to 57 mg/dl during an operation for scoliosis, bled 5100 ml. Cryoprecipitate increased the fibrinogen level to 160 mg/dl (281%) and stopped his bleeding tendency. (Case 5) A 73-year-old woman, whose fibrinogen level decreased to 109 mg/dl during a thoratic aortic aneurysm operation, bled 6210 ml. Cryoprecipitate increased his fibrinogen level to 133 mg/dl (122%) and the bleeding dramatically stopped. (Case 6) A 37-year-old woman, whose fibrinogen level decreased to 97 mg/dl during a placenta previa operation, bled 4290 ml. Her bleeding stopped after cryoprecipitate increased her fibrinogen level to 185 mg/dl (191%).

Conclusions: In all these cases, a rapid and considerable increase in fibrinogen level and significant improvement in bleeding tendency were observed after treatment with cryoprecipitate. In our experience, bleeding tendencies were not amplified in cases where the fibrinogen level was >100 mg/dl; in such cases, infusion of cryoprecipitate might be rarely indicated. Therefore, fibrinogen levels should be closely monitored in treatment with cryoprecipitate. The staff of the transfusion unit should continuously provide such data to anesthesiologists and surgeons, as well as information on its proper usage, to improve the perioperative management of blood transfusions and adjuvant therapies.

P-308

RED CELL AND ALBUMIN RESUSCITATION FOLLOWING ACUTE MASSIVE HAEMORRHAGE ALTERS HAEMOSTASIS

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Background & Aim: Blood transfusion is a critical component of acute haemorrhage management. However, there is limited prospective controlled data on alterations to haemostasis following acute haemorrhage and the effects of transfusion. Our aim was to characterise haemostasis in an in vivo ovine model after acute haemorrhage and the subsequent resuscitation with (i) 4% albumin (Alb) or (ii) fresh (<5 do) homologous ovine packed red blood cells (oPRBC) or (iii) aged (>30 do) homologous oPRBC. The ovine model was selected as sheep have been found to be a suitable species for translational coagulation studies (Siller-Matula et al, Thromb Haemost. 2008;100(3):397–404).

Method: Healthy male sheep were anaesthetised then bled of 30% of their blood volume. Thirty minutes later they were resuscitated with replacement volumes of either Alb alone, or 2 units of fresh oPRBC topped up with Alb to replacement volume, or 2 units aged oPRBC topped up with Alb. After which they were monitored for four hours. Blood samples were collected pre-haemorrhage (baseline), post-haemorrhage and every hour post-transfusion. Samples were analysed using the rotational thromboelastometry device, ROTEM®. The contribution of the extrinsic coagulation factors, fibrinogen and platelets to clot formation was assessed by EXTEM, with the specific contribution of fibrinogen and fibrin polymerisation to clot formation by FIBTEM.

Results: Relative to baseline levels, the acute massive haemorrhage did not significantly alter the clotting time (CT), which measures how fast the formation of fibrin starts, maximum clot firmness (MCF) which reflects clot quality or clot formation time (CFT) which describes the kinetics of the formation of a stable clot through activated platelets and fibrin.

Platelet counts were also not significantly altered (data not shown).

All three forms of resuscitation decreased the MCF and alpha angle of EXTEM for up to 4 h post-resuscitation.

The EXTEM, CFT was also significantly increased ($P < 0.01$) with all forms of resuscitation.

Table 1: Tracked changes to haemostasis

		n	Clotting Time (secs) EXTEM	Clotting Time (secs) FIBTEM	Max. Clot Firmness (mm) EXTEM	Max. Clot Firmness (mm) FIBTEM	Alpha Angle EXTEM	Alpha Angle FIBTEM	Clot Formation Time (sec) EXTEM
Baseline		16-18	73.3	71.9	71.4	25.1	77.6	79.4	103.7
Haemorrhage		16-18	70.7	65.8	69.1	22.2	76.0	78.9	119.2
Alb	0 hr	4	77.5	73.0	64.0	16**	64.8 ***, ###	66.8 ***, ###	182.8 ***, ##
	2 hr	4	80.0	73.5	65.8	17.5*	65.8 **, ##	70.5 **, ##	172**, #
	4 hr	4	80.5	69.5	65.3	16.75*	67 **, ##	71.75 *, #	168.8**, #
Fresh	0 hr	5-6	88.3*	82.5*, #	61.2	14.2***, #	59.7 ***, ###	63.33 ***, ###	181***, ##
	2 hr	5-6	80.2	78.4	62.3	16.8*	67 *	69.6	176.4**, #
	4 hr	5-6	74.3	69.3	63.7	17.17*	66.8 *	68.3 *, #	165.4**
Aged	0 hr	6	62.7	57.0	60.2	15.33***, #	65***, ##	66.8 ***, ###	169.2**, #
	2 hr	6	63.5	60.2	64.2	17.5**	70.7	76.3	162.2**
	4 hr	6	68.2	66.0	63.8	17.67**	70.0	75.0	176.8***, ##

Relative to baseline (B0): * is P= 0.01- 0.05; ** is P= 0.001 - 0.01; *** is P <0.001

Relative to haemorrhage (H0): # is P= 0.01- 0.05; ## is P= 0.001 - 0.01; ### is P <0.001

Discussion & Conclusion: In this model, acute massive haemorrhage did not significantly alter haemostasis, as determined by rotational thromboelastometry, but resuscitation did. Decreases in EXTEM MCF and EXTEM alpha angle, and increases in EXTEM CFT following resuscitation suggest that these changes are associated with alterations to plasma clotting factors, specifically functional fibrinogen and/or factor XIII, rather than platelet number. Further detailed investigation into these plasma factors is required to clarify this.

P-309

TRANSFUSION OF FRESH OR AGED RED CELLS AFTER HAEMORRHAGIC SHOCK REDUCES SELENIUM AND GLUTATHIONE PEROXIDASE

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Background: Packed red blood cell (PRBC) transfusions are a routine part of acute haemorrhage and anaemia management. However, little is known about the effect of transfusion per se on the levels of antioxidant trace elements and the associated antioxidant response. Reductions in certain trace elements such as selenium, can reduce the antioxidant response and increase susceptibility to oxidative stress. Oxidative stress is a significant factor in the development of tissue/organ injury especially after trauma and cardiac surgery, both situations where transfusion is common.

Our aim was to investigate if and how transfusion of packed red cells (PRBC) altered the host's antioxidant trace element levels and their antioxidant response. This was performed in an in vivo ovine model of haemorrhagic shock and transfusion.

Methods: Ovine blood (400 ml) was collected from healthy male marino sheep, and processed into ovine PRBC (oPRBC), then stored at 4°C for up to 42 days. Twelve healthy male merino sheep were anaesthetised and haemorrhaged 30% of their blood volume. Haemorrhagic shock (determined by cardiac output and mean arterial pressure) was maintained for 30 min. Sheep were assigned into two transfusion groups; (i)

2 units of fresh [2–5 d.o. (mean 3 d.o.) n = 6] or (ii) aged PRBC [32–32 d.o. (mean 37 d.o.) n = 6]. Only cross-match compatible oPRBC's were transfused, and both cohorts were topped up with 4% albumin to meet full volume replacement. Blood samples were collected pre-haemorrhage, post-haemorrhage and 4 h after transfusion. These blood samples as well as samples from the transfused oPRBC, were tested for trace elements: selenium, copper, zinc and antioxidant response: glutathione peroxidise and superoxide dismutase.

Results: Haemorrhagic shock did not significantly alter trace element levels or antioxidant response of the host.

The transfusion of oPRBC (irrespective of age) significantly reduced all three trace element levels and glutathione peroxidise response in the host.

Both fresh and aged oPRBC contained very low levels of selenium and copper but high levels of zinc.

Table 1: Glutathione peroxidase (GPx), superoxide dismutase (SOD), ovine packed red blood cells (oPRBC). Values expressed as mean ± SD. Asterix * indicates P < 0.05 with respect to baseline by unpaired *t*-test

			4hr Post Transfusion of		Transfused Product	
	Pre-haemorrhage	Post-Haemorrhage	Fresh oPRBC	Aged oPRBC	Fresh oPRBC	Aged oPRBC
n	12	12	6	6	6	6
Selenium (umol/L)	1.35 ± 0.29	1.26 ± 0.2	1.02 ± 0.2*	1.08 ± 0.1*	0.21 ± 0.1	0.15 ± 0.12
Copper (umol/L)	17.50 ± 3.2	16.1 ± 2.9	13.0 ± 3.6*	12.2 ± 1.6*	3.0 ± 2.4	2.8 ± 1.6
Zinc (umol/L)	12.7 ± 2.2	11.8 ± 1.9	6.8 ± 1.9*	6.7 ± 1.6*	24.7 ± 2.3	26.3 ± 6.4
GPx (nmol/min/ml)	2.24 ± 0.88	2.8 ± 1.3	1.29 ± 0.8*	1.17 ± 0.7*		
SOD (U/ml)	10.2 ± 7.0	9.8 ± 5.1	10.1 ± 5.5	14.0 ± 8.3		

Table 1: Glutathione peroxidase (GPx), superoxide dismutase (SOD), ovine packed red blood cells (oPRBC). Values expressed as mean ± SD. Asterix * indicates P<0.05 with respect to baseline by unpaired t test.

Conclusion: In this in vivo ovine model, haemorrhagic shock did not alter the host's antioxidant response. But volume replacement transfusion did reduce selenium, copper and zinc levels, and glutathione peroxidise response in the host. As glutathione peroxidase response is associated with circulating selenium levels, the very low selenium levels in transfused oPRBC may have contributed to the observed reduced antioxidant response. Such a reduced antioxidant response could contribute to further tissue damage in transfusion recipients with increased reactive oxygen species generation. Further studies are required to determine if there is an association between the transfusion of PRBC and a reduced antioxidant response in human patients.

P-310
HAEMORRHAGE, MASSIVE TRANSFUSION AND NOVOSEVEN IN PATIENT WITH ABRUPTIO PLACENTAE

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Aim: To present a case of abruption placentae with massive haemorrhagie treated with massive transfusion and Novoseven.

Introduction: An abruptio placenta is one of the important causes of ante partum hemorrhage and cause for massive postpartum hemorrhage (MPPH) that might be fatal. Excessive uterine bleeding seen vaginally or concealed placental abruption with large clot formation may lead to consumptive coagulopathy and disseminated intravascular coagulation (DIC)

Case Presentation: In our depat ment came request for 5 units of blood (deplasmated eritrocites in SAG-M) for patient NN, 39 years, Dg. abruption placentae treated with cesarean section who present a massive haemorrhagie during the operation. Immidiately we started with monitoring of coagulation-PT, APTT and TT, and follow the number of platelets and fibrinogen. Eight units of Eritrocites, 5 units of FFP and 2000 UI of crioprecipitat was given.

Close monitoring of PT, APTT, TT and platletes number was done during the treatment. Neawborn suspected for ARDS was sent at the Clinical hospital in Skopje, while the mother was treated in our hospital in Prilep. number of Platelets was low around 45,000)and vaginal bleeding was still present. We decided to give Amp Novoseven in dose of range 90 μg/kg.

Bleeding was stoped and because of low number of platletlets, stabilized patient was sent in Clinical hospital Skopje for further observation, with recomandation to start with LMH treatment. Stabilisation of all parametars was achieved From the next day LMWH was given and continued during 1 month with monitoring of PT, APTT and TT and Platelets. All results was normal.

Conclusion: In the patient with abruption placentae we should be prepared for massive haemorrhagie. Correction of hypovolemia, coagulopathy and bleeding should be done promptly. Novoseven is a drug of choice to stop bleeding in such cases.

6.5 Adverse Events, including TRALI

P-311
DISTRIBUTION OF HAPTOGLOBIN GENE DELETION IN THE TAIWANESE POPULATION

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Haptoglobin (HP) is a hemoglobin-binding protein of the plasma. Haptoglobin-deficient patients were first reported in Japan to present anaphylactic shock after blood transfusion and their HP gene was completely deleted. This gene deletion is now referred as the HPdel allele. The HPdel allele is restricted to East and Sourtheast Asian populations, it is not found in South Asians (India), Europeans or Africans, and the HPdel allele frequency ranges from 1.1% to 3.8% throughout Asia.

A simple polymerase chain reaction (PCR) protocol, used to detect the allele deletion, had been established. This method was used in the Mackay Memorial Hospital laboratory to assess and therefore to prevent anaphylactic transfusion reactions associated to the deletion.

In this study, DNA samples were obtained from 193 healthy random individuals. Our preliminary screening showed nine individuals heterozygous for the HPdel allele which should corresponds to an HPdel allele frequency of 2.33%. Accordingly, the prevalence of haptoglobin-deficient phenotype with homozygous deletion can be estimated to be approximately 0.054% in the Taiwan population.

This study indicates that the potential risk of anaphylactic shock caused by haptoglobin deficiency in Taiwan is alarmingly high and identical to previous findings in other Asian groups. Regrettably, no institution so far has ever initiated a routine preventive testing. This simple PCR method is helpful in clarifying if HPdel allele is the causing factor in patients suffering from a non-hemolytic transfusion reaction, such as allergy or anaphylactic shock. To provide matched blood to haptoglobin-deficient patients, we propose that a registry program of plateletpheresis donors, like the red cell rare donor database, should be established in Taiwan's blood centers.

P-312
IDENTIFICATION OF IGA DEFICIENCY AMONG JAPANESE PATIENTS WITH NONHEMOLYTIC TRANSFUSION REACTIONS AND BLOOD DONORS BY SIMPLE ELISA

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Background: Anaphylactic transfusion reactions (TRs) are reported among IgA-deficient patients with anti-IgA antibodies. Identification of IgA deficiency is important for patients with nonhemolytic transfusion reactions (NHTRs) to determine the cause of TRs and to prevent TRs in subsequent transfusions. For donors, it would also be important to prevent their possible TRs in the future and to be able to donate blood for IgA-deficient patients as registered members of an IgA-deficient donor pool. We already have identified haptoglobin deficiency among Japanese patients with NHTRs and normal blood donors by simple sandwich ELISA.

Aims: The aim of this study is to identify IgA-deficient individuals among Japanese patients with NHTRs and normal blood donors by simple ELISA and to determine its prevalence.

Materials and Methods: Testing for serum IgA by simple ELISA. Twenty-five microliters of a serum sample was added to a microplate precoated with anti-human IgA rabbit IgG and incubated for 10 min at RT. After washing the microplate, 50 μl of diluted HRP-conjugated-anti human IgA goat anti-serum was added to the microplate and incubated for 20 min. Enzyme activity was determined using the TMB substrate after washing the microplate. Three standard IgAs (3, 30 and 300 μg/dl) and positive and negative controls were assayed simultaneously. A test sample was identified as IgA-deficient if its optical density was lower than that of the standard IgA of 3 μg/dl. Identification of IgA-deficient individuals among Japanese patients and donors A total of 16,181 patients who had experienced NHTRs were reported by hospitals to the Japanese Red Cross Society between 1995 and 2010, among which 2487 showed anaphylactic TRs. They were tested for serum IgA by this ELISA when their serum IgA concentrations were lower than 10 mg/dl, as measured by peak-rate nephelometry. Blood donors (n = 733,802) who visited the Japanese Red Cross Tokyo Blood Center between 2009 and 2010 were also tested for serum IgA if they showed low IgA concentrations as measured by nephelometry. Anti-IgA antibodies produced in IgA-deficient individuals were determined by ELISA and western blot analysis.

Results: IgA-deficient individuals whose serum IgA concentrations were <3 μg/dl could be identified within 1 h by our simple ELISA. Six patients were identified as having IgA deficiency. All of them produced high titers of anti-IgA (IgG class). Three of them had developed severe anaphylactic shock. Seventy-two blood donors were identified as being IgA-deficient. Anti-IgA antibody was detected in 17/72 (23.6%).
Conclusions: IgA-deficient patients with high-titer anti-IgA were identified among Japanese patients with NHTRs [6/16,181(0.038%)] or among those who experienced anaphylactic TRs [3/2,487 (0.12%)]. IgA-deficient individuals were also identified among Japanese blood donors with a prevalence of 72/733,802 (0.01%), which is about one-tenth of that in European and North American countries. Most of the cases of anaphylactic TRs developed in patients without IgA deficiency. However, the higher prevalence of IgA deficiency among the patients with NHTRs, particularly among those with anaphylactic TRs compared with the normal donors, suggests a higher risk of such TRs in IgA-deficient patients.

P-313
A SENSITIVE HOMOGENEOUS IMMUNOASSAY FOR SERUM IGA

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Background: IgA-deficient individuals are at risk of anaphylactic transfusion reactions. Precise measurement for low levels of serum IgA is necessary to identify IgA-deficient individuals without an overestimation of such individuals and to prevent the transfusion reactions. Luminescent oxygen channeling immunoassay (LOCI) is a sensitive and homogenous assay consisting of two steps: (i) the formation of immune complexes using two different types of beads and (ii) the measurement of luminescence induced by singlet oxygen molecules.
Aims: The aim of this study is to develop a new sensitive immunoassay for the measurement of low levels of serum IgA using AlphaLISA technology, a type of LOCI.
Materials and Methods: Reagents and equipment IgA purified from pooled human plasma was purchased from Athens Research Co. and used as a standard. The unconjugated acceptor beads, streptavidin-conjugated donor beads, and 384-well microplates used in this study were supplied by Perkin Elmer Co. A rabbit anti-human IgA antibody (DAKO #A0262) was immobilized onto unconjugated acceptor beads using sodium cyanoborohydride to prepare anti-IgA-antibody-conjugated acceptor beads. A biotin-conjugated anti-IgA antibody was prepared using a rabbit anti-human IgA antibody and water soluble NHS-biotin (Vector Lab. Co.). The intensity of luminescent signals was measured using an EnVision plate reader in the AlphaScreen mode (Perkin Elmer Co.) Measurement of IgA concentration Twenty microliters of a mixture containing anti-IgA-antibody-conjugated-acceptor beads, a biotinylated anti-IgA and a test sample was dispensed to each well of a 384-well microplate and incubated for 1 h in the dark at RT. Streptavidin-conjugated donor bead solution (5 μl) was added to the mixture and incubated for 30 min in the dark at RT. The IgA concentration of the test sample was calculated from the measured intensity of the signal. After the optimal conditions of the reaction were determined, the sensitivity, specificity and reproducibility of the assay method, and the effects of coexisting materials were analyzed.
Results: At the optimized concentration of each reagent and using 5 μl of a sample, the detection limit of this assay was 50 IgA ng/dl. The analytical range expanded from 50 to 100,000 ng/dl. The detection limit decreased to <5 ng when the sequence of the addition of the reagents was improved. A luminescent signal was generated by normal human serum but was not generated by IgA-deficient serum. There was no effect of bilirubin (up to at least 10 mg/ml) or chyle (up to at least 4700 folmazine turbidity units) on signal intensity. However, a high concentration of hemoglobin (more than 50 mg/ml) or neat serum/plasma reduced the intensity of signals. Serum/plasma samples were suitable for testing at more than 10-fold dilution. The intra/inter-day coefficients of validations were 2–10%.
Conclusions: This newly developed immunoassay is highly sensitive and easy to perform using a simple mix-and-measure protocol, requiring small amounts of serum/plasma samples. This technique is considered to be useful for the identification of IgA-deficient patients and/or blood donors distinguishing true IgA deficiency from those with low levels of serum IgA.

P-314
DETECTION OF ANTI-PLASMA PROTEINS BY LUMINEX SYSTEM

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Background: Anti-plasma proteins especially anti-IgA and anti-haptoglobin are reported to cause anaphylactic transfusion reactions. Anti-IgA or anti-haptoglobin are occasionally produced in the IgA- or haptoglobin-deficient patients who are infused with blood products. Besides anti-HLA or anti-HNA, detection of anti-plasma proteins is important to analyze the factors of transfusion-related adverse reaction.
Aim: Screening of anti-plasma proteins is carried out by ELISA in our laboratory, however, few positive sera was detected in cases of transfusion-related adverse reaction. To detect anti-plasma proteins more effectively, we designed and evaluated more sensitive and specific immunoassay using Luminex system.
Methods: Purified human plasma proteins (IgA, IgA1, IgA2, haptoglobin, α2-macroglobulin, celluroplasmin, C4, C9 or albumin) were covalently coupled with different-colored carboxylated microspheres by standard EDC/NHS protocol, respectively. Sample serum was incubated with the mixture of protein-coupled microspheres and stained with PE conjugated anti-human IgG, followed by Luminex system assay. Absorption test was performed simultaneously with sample serum which was pre-incubated with pooled human plasma. Conventional ELISA was performed by standard protocol with a microtiter plate coupled to each specific plasma protein. Specific antibody was detected by HRP conjugated anti-human IgG and TMB substrate.
Results: In sensitivity test using two fold diluted anti-IgA and anti-haptoglobin serum, Luminex assay showed 64 times higher sensitivity than ELISA. In screening of 86 sera derived from healthy donors, 13 sera were positive for anti-IgA, anti-IgA1 or anti-IgA2 by Luminex assay. Only 1 of the 13 positive sera was confirmed as anti-IgA2 specifici by absorption test. Other 12 sera were regarded as nonspecific reactions since their reactivities were not absorbed by pooled human plasma. All of 13 Luminex positive sera were negative for anti-IgA by ELISA. Simultaneous test of Luminex and ELISA with 39 sera derived from transfusion-related adverse reaction cases, 1 serum was positive for anti-haptoglobin only by Luminex. This patient was confirmed as haptoglobin gene deletion (Hpdel/Hpdel) by PCR. No other anti-plasma proteins were detected either by Luminex or by ELISA.
Conclusion: Screening of anti-plasma proteins using Luminex showed higher sensitivity than conventional ELISA with shortened assay time up to 2 h including absorption test. Although high sensitive immunoassay often showed nonspecific reactions, simultaneous absorption test could improve specificity of Luminex assay. Therefore, our Luminex assay is suitable for anti-plasma proteins detection.

P-315
HAEMOVIGILANCE: INVESTIGATION OF ADVERSE REACTIONS FOLLOWING BLOOD TRANSFUSION IN REGIONAL BLOOD CENTER NORTH CENTRAL PROVINCE SRI LANKA

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Background: Recipient haemovigilance consists of the detection, collection and analysis of information regarding untoward and unexpected effects related to the blood transfusion. All adverse reactions related to blood transfusion s are documented, investigated and analyzed by local haemovingillance units in each blood centers and HBBB. The results are reported to the main haemovigilance unit in National Blood Centre (NBC) Colombo monthly. After that annual report of haemovigilance published by NBC.
We report the summary of adverse events during the last 3 years (2008–2010) in RBC – N.C province Sri Lanka.
Material and Methods: During 36 months, 84,815 units of red blood cells including leucodepleted and filtered blood, 29,917 plasma units and 17,887 platelets units including single donor aphaeresis units were given to the patients. (Total blood transfusion units – 132,619).
Post transfusion reaction should be documented investigated and reported according to the GMP.
Results: A total of 219 adverse reactions (0.16%) were reported as minor adverse transfusion reactions consists of FNHTR (55%) and allergic reactions (45%). No major reactions including ABO incompatibility and TRALI were announced. No near miss events during 2008–2010.
Conclusion: According to the gender, 62% of female patients had reactions to blood and blood products and 38% of men. No near miss events were reported during these study periods due to clinical staff awareness regarding better blood transfusion practices programmes conducted by NBTS yearly.
The rate of post transfusion reactions is very low, underreporting is still probable. The feedback of haemovigilance system is reported each year.

P-316
ANTI-WEBB ANTIBODY CAUSING ACUTE HAEMOLYTIC TRANSFUSION REACTION

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Background: Webb (Wb) antigen of the human erythroid cells is expressed on glycophorin C and is part of the Gerbich (Ge) antigen family. It occurs at a low prevalence. Antibodies against some of the Gerbich antigens, such as anti-Ge2 and anti-Ge3 are known to cause haemolytic transfusion reactions. Anti-Wb antibody was first described in 1963 by the Blood Transfusion Service in Brisbane, Australia, and was thought to exhibit the characteristics of a 'natural' antibody rather than of immune in nature. We report the first known case of an immediate haemolytic transfusion reaction to an anti-Wb antibody.
Case Report: A 73 year-old woman of Greek ancestry presents with symptomatic anaemia in the setting of progressive chronic lymphocytic leukaemia, previously treated with fludarabine-based chemotherapy 16 months earlier. Her blood group was Group O, Rhesus D positive, and no antibodies were detected on 3 cell screening panel (CSL Abtectcell™ III). Red blood cells were crossmatched electronically and issued for transfusion for symptomatic anaemia.
During the red cell transfusion, the patient developed an acute haemolytic transfusion reaction with rigors, hypoxia, fever and acute renal failure. Laboratory confirmation of haemolysis included hyperbilirubinaemia, elevated lactate dehydrogenase and spherocytosis on the blood film. The patient recovered with conservative management and received further red cell units following crossmatching by indirect anti-globulin testing (IAT) without incident.
The implicated red cell unit was returned to the laboratory as part of routine investigations for blood transfusion reactions. No clerical error was identified. Repeat grouping and manual crossmatching of pre- and post-transfusion specimens revealed a non-compatible crossmatch by IAT and no evidence of ABO incompatibility. The offending antibody was not able to be identified locally and the plasma was referred to the Reference Red Cell Serology Laboratory of the Australian Red Cross Blood Service in Melbourne, Australia.
A strongly reactive Anti-Wb was found in both pre- and post-transfusion specimens, in the absence of other antibodies, and phenotyping of the implicated red cell unit revealed the presence of Wb antigen. These findings were confirmed by the International Blood Group Reference Laboratory in Bristol, United Kingdom.
Conclusion: The clinical features and laboratory findings of the transfusion reaction are consistent with an anti-Wb antibody causing a clinically significant acute haemolytic transfusion reaction.

P-317
THE SURVEY OF NON INFECTIOUS COMPLICATION OF TRANSFUSION IN PEDIATRIC HOSPITALS AND THALASSEMIC CENTERS IN TEHRAN

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About 10% of all transfusion recipients may experience a transfusion complication that could be acute or chronic. If the clinician has knowledge about these complications prevention measures may be used This study evaluated the prevalence of acute noninfectious complication in Tehran over a one year period.
In a 1 year period for each transfusion the vital signs of the recipient and every sign or symptom of complication were recorded carefully.
The survey compiled findings on more than 152,757 transfusions in Tehran pediatric hospitals and thalassemic centers and recorded about 217 cases of complication. One hundred and twenty-three of them were diagnosed by the attending physician, including hemolytic reaction in seven cases, FNHTR in 33 cases,four cases of anaphylactic reaction,72 cases of allergic reaction and three cases of TAGVHD, and five cases of concurrent allergic reaction and FNHTR. Three cases of TAGVHD died and the diagnosis was confirmed by liver biopsy.
The prevalence of transfusion complication was higher in patients who received more than one component. The most recipient diagnoses were AML, ALL and thalassemia.
After confirmation of three cases of TAGVHD the irradiation blood component center was established in the Tehran Blood Transfusion Centre.

6.6 Haemovigilance and Transfusion Safety

P-318
CORRELATION OF HEAVY METALS AND THEIRS IMPACT TO EPIDEMIOLOGICAL SURVEY IN THE MINERS BLOOD DONORS AND OTHER HUMAN POPULATION

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Introduction: Miners who are blood donors, and work in mines for lead-zinc ores are constantly exposed to heavy metals (lead, zinc and cadmium) and this aspect is expected to increase or decrease many hematological parameters.
Aim of the Study: The concentration of lead, zinc and cadmium was studied in exposed blood donors and non-exposed blood donors (control group). Knowing the structure of various heavy metals, all of the analysis was carried out to examine the impact of these heavy metals on the occurrence and severity of certain epidemiological diseases and hematological parameters on the miners who are blood donors.
Material and Methods: In this research 120 miners were included who were blood donors (mining for lead and zinc) from the Republic of Macedonia and a control group of 30 participants that included blood donors not directly exposed to heavy metals, while living in the immediate vicinity of the lead and zinc mine. In this research biochemical analysis (inductively coupled plasma spectrometry (ICP) one of the most sensitive analytical techniques for the determination of elements in biological materials was applied and the basic haematological parameters were determined.
Results: The observation of increased blood lead level on blood donors in the exposed group (mean = 0.089 mg/l) and 20% on blood donors in the control group (mean = 0066), increased blood zinc level in the exposed (mean = 1391) and in the control group (mean = 1074), increased blood cadmium level in 62% of exposed (mean = 0007) and in 50% of the control group (mean = 0006); If the normal BLL (blood lead level) is 0.04–0.07 mg/l, we concluded that all male blood donors in the exposed group had above normal BLL. In the control group 20% of male blood donors had above normal BLL; if the normal BZL (blood zinc level) is 0.1 mg/l, we concluded that all male blood donors exposed in the control group had above normal BZL. If the normal BCL is 0.005 mg/l, we concluded that 62% of the male blood donors in the exposed group had above normal BCL. In the control group 50% of male blood donors had above normal BCL; The blood lead, zinc and cadmium level will rise during exposure at work. forty-eight percent of miners (exposed group) had an exposure period of 20 years, 29% between 10 and 20 years and the remaining 23% an exposure period under 10 years. Results showed negative correlation between the number of red blood cells and hemoglobin and blood levels of heavy metals; positive correlation between the number of leukocytes and blood heavy metals levels. Epidemiological survey showed that nearly all workers complained of headache. While 25 of 70 miners who were blood donors (with long exposure) were found to be suffering from various diseases such as asthma, respiratory tract, irritation and watering of eyes.
Conclusion: The research confirms that the increased content of heavy metals in blood donors affects the concept of professional risk that involves probability that as a result of exposure of workers to certain harmful agents in the work environment negative effects are manifested on their health. The change of some haematological parameters in the blood donors, results in the emergence of certain diseases with complex etiologiesand risks to their health.

P-319
SETTING STANDARDS TO CLINICAL TRANSFUSION PRACTICE; SUCCESSFUL IMPLEMENTATION OF A TRANSFUSION MONITORING PROGRAM IN A LOW RESOURCE SETTING

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Introduction: Despite being an essential component of the modern medical practice blood transfusion carries an inherent risk of serious adverse reactions. Therefore proper monitoring of patients during transfusions to identify transfusion reactions at an early stage plays a pivotal role in clinical transfusion. Several incidents of transfusion reactions due to patient misidentification during year 2010 in Wathupitiwala Base Hospital, Sri Lanka has raised concern over the necessity of an effective patient monitoring system during transfusions.

Aims: To evaluate and identify shortcomings in current practice of patient monitoring and record keeping during blood transfusions, plan and implement necessary interventions to rectify these shortcomings and assess the effectiveness of interventions by pre and post implementation audits in Wathupitiwala base hospital, Sri Lanka.
Methodology: A pre implementation audit was carried out using randomly selected blood units and several aspects of transfusion monitoring were recorded by direct observation in wards.
Based on these findings a printed monitoring chart was designed with structured format including check boxes and columns to be filled with patient information, blood component type and serial numbers, time of starting and ending the transfusion, pre transfusion, during transfusion and post transfusion vital signs clearly defining the intervals to be monitored. A system was implemented where this chart was sent to wards along with every blood unit issued from the blood bank. Implementation of the new monitoring system was followed by a post implementation audit.
Results: Fifty-seven transfusion events were audited during the pre implementation audit and it revealed that only 47.3% (n = 27) of the total transfusions were monitored. Monitoring was >90% in Intensive Care Units and theatres but <30% in general medical, surgical and other wards. Even in monitored patients Recording of Vital signs prior to starting the transfusion were done only in 22.8% (n = 13) and post transfusion values were recorded in none of the events. Even where monitoring was done temperature as a vital sign was monitored only in 5.2% (n = 3) of the events.
Most of the transfusions were not regularly monitored and there were lot of variation in the frequency of monitoring (from every 5–30 min). The starting time of the blood units were mentioned in 43.8% (n = 25) of the cases but the finishing time of the infusion of each unit was mentioned only in 15.7% (n = 9) of the cases. Separate monitoring charts rather than recording in the bed head tickets were used in only 29.8% (n = 17) of the events.
A total of 59 transfusion events were audited during the post implementation audit and 96.6% (n = 57) events were monitored after implementation of the new monitoring chart. In monitored transfusions recording of patient information, blood unit information, pre transfusion vital sign monitoring including temperature and regular monitoring of vital signs at least during two occasions has reached 100%. Post transfusion vital sign recording has increased to 69.4% (n = 41)
Conclusion: Successful and objective patient monitoring during transfusions can be implemented by simple interventions and education programmes and will play an important role in enhancing patient safety.

P-320
HAEMOVIGILANCE IN TAIWAN

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Background: Haemovigilance, in a broad scope, comprises the surveillance procedures for adverse reactions in donors or recipients, the adverse events during the collecting, testing, processing, storage and distribution of blood and blood components, and the epidemiological follow up of donors for potentially infectious donations. During 2010, Taiwan Blood Services Foundation (TBSF) distributes 4.8 million units (250 ml equivalent) of blood and blood components and 0.18 million units of single-donor platelets for the blood demand in Taiwan. On a voluntary basis, information related to the blood recipients that develop adverse transfusion reactions (ATRs) are reported to TBSF.
Aims: The aims of study are to determine the coverage and occurrence of ATRs, the key component of haemovigilance, in Taiwan.
Methods: The information of ATRs collected during 2008–2010 were analyzed. We determined 'the coverage' as the number of blood and blood components issued to the hospitals that reported ATRs divided by total number of those issued by TBSF, and 'the occurrence' as the number of blood recipients that developed ATRs per 100,000 units of blood and blood components issued to the hospitals contributing ATRs reporting. Since we collect both 250 and 500 ml whole blood, each 500 ml whole blood or the blood component derived from 500 ml whole blood was considered as two units in the analysis. The ATRs analyzed in this study were those considered as serious, including acute hemolytic transfusion reaction, delayed hemolytic transfusion reaction, anaphylactic shock, dyspnea, transfusion-associated graft-versus-host disease (TA-GVHD), transfusion-related acute lung injury (TRALI), bacterial and viral infection associated with transfusion.
Results: Of the total bloods and blood components issued, 43% were issued to the hospitals that contributed ATRs reporting. Between the geographic areas, the coverage of blood and blood components being reported were 44% in Taipei, 12% in Hsinchu, 70% in Taichung, 53% in Tainan, 19% in Kaohsiung, and 60% in Hualien. The overall occurrence of ATRs was 12 per 100,000 units of blood and blood components, attributed in the order to dyspnea (7.7/100,000), acute hemolytic reaction (3.4/100,000), anaphylactic shock (0.39/100,000), viral infection (0.22/100,000), TA-GVHG (0.16/100,000), delayed hemolytic transfusion reaction (0.10/100,000), TRALI (0.09/100,000) and bacterial infection (0.04/100,000). Further breakdown information by components show the occurrence of ATRs per 100,000 units of blood and blood components were 25 by whole blood, 20 by red cells, 32 by single-donor platelets, 3.9 by whole-blood platelets, and 6.1 by plasma components.

Table 1: Serious adverse events and blood products

	number	Unit Issued	1/100000
Whole Blood	19	76,376	25
RBCs	522	2,631,088	20
Leukocyte-reduced RBCs	12	62,377	
Washed RBCs	5	22,330	
Platelet concentrate	57	1,456,010	3.9
Apheresis platelets	49	157,338	32
Leukocyte-reduced Apheresis platelets	12	34,102	
FFP	122	1,736,200	6.1
FP	19	331,849	
Cryoprecipitate	1	242,027	
WBC Concentrate	0	1,138	0
Total	818	6,750,835	12

Conclusions: In this paper the information of ATRs collected are analyzed and reported. To achieve the goal for preventing recurrence of adverse events and reactions, a comprehensible guide for the incidents to be surveyed and reported should be established and national health authorities should contribute a role to the surveillance procedures.

P-321
FROZEN SYSTEM FOR ARCHIVE SPECIMENS FROM BLOOD DONORS IN TAIWAN

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Background: The retrospective investigation plays an important role in transfusion medicine. Basing on this point of view, Taiwan Blood Services Foundation built an automatic frozen system for archiving all specimens from blood donors in Taiwan. These are 1.8 million specimens yearly. The system had been enabled in September 2009.
Methods: Two compressors were installed for stabilizing the frozen temperature and as backups reciprocally. Shelves with 5616 spaces could accommodate thirteen million and four hundred seventy thousand sample tubes. Three automatic storage/retrieval machines are dominated by computer. For minimizing temperature difference, there is a room of 1–5°C between working area and storage and conveyor is installed to link. This great storage has 25 sensors to measure its temperatures which were consecutively recorded by central monitor. Moreover, an alert system is set for warning us when temperatures are about out of limitation and saving time for us to take action. Electricity has power generator to be the backup. Functions of the system were validated in the stability of storage temperature, the accuracy of temperature measured, temperature monitoring and alert system, the uniformity between outputs from the Blood Donation/Distribution System (BD/DS) and inputs to Automatic Storage/Retrieval System (AS/RS). It took 3 months to complete the validations.
Results: The storage is under −30°C and the capacity was designated to store donor specimens of 10 years. The specimens were arranged in order that one hundred specimens were lined up in a case and six cases in a basket. The electronic data of positions of every specimen were built by the BD/DS. The positions and the link of specimen number, case number and basket number were filed and sent to the AS/RS through internet. Only those basket numbers which had input to AS/RS were allowed to be archived. Specimens were frozen before shipping and were delivered under −5°C to archive storage periodically from all blood centers.
The specimen retrieving process is as follow: the application should be approved by medical direction first. Next is to obtain permission from Taiwan Blood Services Foundation. The specimen is retrieved only when the administration of archive storage received both of the application form and the permission.
Till May 2011, total of 3,187,200 tubes of specimens were archived in the storage and they occupied 23.65% of spaces.

Conclusions: This is the primary great automatic biological specimen storage in Taiwan. The manufacturer we entrusted had no such experience in building this kind of system. It happened that conveyor didn't work or storage/retrieval machine had trouble in orientation. The system was getting stable after 1 year of working. The automatic storage system has high efficiency in space and manpower utilization. The cost of land and human affairs were cut down. Automation lessens the time spent on storing and retrieving specimens and the opportunities of working under very low temperature environment. Even though the maintenance fee is as high as NT$ 3.5 million per year, automatic storage is sufficient and valuable.

P-322
DONOR DISCLOSURE – AN INDIAN EXPERIENCE

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Background: Rotary Bangalore TTK Blood Bank, collects 25,000–30,000 blood units per annum from voluntary donors. Each unit is screened for HBs Antigen, anti HIV and anti HCV using the fourth generation ELISA, and for malarial parasite & syphilis.
In India, disclosure of viral transfusion transmitted infections (TTI) to blood donor was not permitted until December 2004. Prior to 2004, government policy stated that blood banks discard HIV-seropositive blood without informing donors about their status in order to maintain donor confidentiality and avoid stigmatizing those with HIV/AIDS. The National Blood Transfusion Council now advocates the disclosure of TTI results to blood donors based on ELISA results. NAT and other confirmatory test are not mandatory in India.
India does not have a centralized Blood Transfusion Service. Most Blood Banks in India are either hospital based or operate as 'Stand Alone Blood Banks'. There is absence of well-defined notification processes and a lack of trained counselors. This could explain the intricacies and difficulties in maintaining uniform donor disclosure practises in India. Hence, there is a lacuna of information regarding counseling and referral follow-up in India.
This study describes our pioneering experiences in donor disclosure and counselling during the period 2007–2008 and challenges associated with them in a resource-limited environment.
Aim: To assess the success rate of donor disclosure.
Methods: All our donors are voluntary; 40% of them are repeat donors. Blood units are screened as per our testing algorithm. The units that test repeat reactive for viral TTI are discarded and donor is informed of his/her reactive status. These donors are requested to attend confidential counselling session(s) at our blood bank.
In the counselling session, donor is informed about their TTI test result and its significance. Information about the virus, risk factors and methods of transmission are explained. The counselor also tries to elicit information on the donor's sexual risk behaviour. The donor is advised on lifestyle changes and impact on immediate family. In case of HBV reactivity, HBV vaccine is recommended for immediate family. Finally, the donor is encouraged to get confirmatory tests and recommended to consult a physician or gastroenterologist.
Results: The number of units collected and tested at our blood bank in 2007 and 2008 were 21,700 and 22,479 respectively. Viral TTI reactivity in 2007 was 0.78%, 0.12%, and 0.18% for HBs Ag, anti HIV, and anti HCV. In 2008, it was 0.73%, 0.16%, and 0.18% for HBs Ag, anti HIV, and anti HCV.
Donors who attended counseling in 2007 were 41.18%, 11.11%, & 14.63% for HBs Antigen, anti HIV and anti HCV. In 2008, 48.17%, 16.22% and 14.63% attended counselling for HBs Antigen, anti HIV and anti HCV.
Conclusion: Our experiences were rewarding in that it directly benefited the donor and their families. Hurdles faced thus far in this programme were related to logistics of donor follow-up. We endeavour to achieve a 100% donor counseling rate. This will drastically reduce the incidence of viral TTI in the community.

P-323
TRANSFUSION TRANSMITTED INFECTIONS FOR THE PAST 2 YEARS (2009–2010) IN JAPAN

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Background: There are approximately 5 million blood donations and approximately 1 million blood recipients every year in Japan. Japanese Red Cross Blood Service Headquarters has been collecting information about suspected cases of transfusion transmitted infections (TTIs) and adverse reactions from medical institutions via medical representatives in regional blood centers since 1993. Analysis of the recipient's pre- and post-transfusion samples and repository samples of the implicated donation is performed to evaluate causal relationship between the event and transfusion. Repository samples of all the donations are stored for 11 years for the purpose of look-back study and analysis of the suspected TTI cases.
Methods and Results: The suspected TTI cases were evaluated using individual NAT of repository samples from the implicated donors or using blood culture of implicated components. If the repository sample was positive for the related virus, the viral genome sequence was compared to that from the recipient's sample. A total of 98 suspected cases of TTI were reported in 2009 and in 2010 respectively. A detailed breakdown of the reported cases of TTI by pathogen was 41.3% of HBV, 27.0% of HCV, 26.0% of bacteria and others (CMV, HEV, HAV, human parvovirus B19, HIV, herpes simplex virus). In these cases, 18 cases of HBV, two cases of HCV, one case of HAV and HEV respectively were evaluated to have a highly causal relationship with transfusion. Also, two cases of bacterial infections were highly likely associated with the contaminated blood components proven by blood culture. The number of implicated donor in 18 cases of HBV was 16 persons, so it means these two cases were come from the same donation or donor of another two cases. The two cases of HCV were caused by one donation as well. Of 16 implicated donations supposed to have relation with HBV, seven cases were donated in the window period, especially of which three cases were donated in the window period of individual NAT. The remained nine donations including two cases of individual NAT negative seem to have been associated with the donors with low viral load in chronic phase of infection. A detailed breakdown of implicated donations by genotype in the 16 HBV cases was three of genotype A, three of genotype B, and 10 of genotype C. The HAV infected case was revealed by post-donation information, and the HEV infected case was based on HEV positive result of quality control of the source plasma separated from the same donated blood for the manufacturing of plasma derivatives. Two bacterial infection cases were revealed to have a relationship with platelet concentrates contaminated with *Serratia marcescens* and *Streptococcus agalactiae* respectively although both recipients have been recovered with appropriate treatment.
Conclusions: Transfusion transmitted infections have been gradually reduced as a result of improvement of sensitivity on NAT or serological test. In order to improve transfusion safety further, it is highly necessary to monitor the safety of transfusion continuously by implementing haemovigilance system, carrying out reporting systems of adverse reactions and infectious disease and look-back study appropriately.

P-324
THE SURVEY OF 1 YEAR PERIOD OF IRRADIATED BLOOD COMPONENT ACTIVITY IN TEHRAN BLOOD TRANSFUSION CENTRE (TBTC)

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Objectives and Background: Pre transfusion inactivation of donor T cell by irradiation is the method of choice for prevention of TA-GVHD complication susceptible patients. In the present study, Tehran blood transfusion irradiation department activities were evaluated for a year since its establishment date.
Materials and Methods: The retrospective study was run by considering 1333 requests for irradiated blood products for 1 year period. Data including quantity and type of irradiated products, patients' age, irradiation indications, blood group of irradiated products and requesting hospitals were gathered. Descriptive statistical analysis performed by SPSS 18 software.
Result: Requested irradiated products in the aforementioned period of time include; platelets (71.3%), packed RBC (28%), FFP (0.4%), paediatric bags (0.2%) and whole blood (0.1%). Age groups of patients received irradiated products were adults (58.4%), children (26.5%), infants (6.7%) and neonates (3.4%). In 5% of cases the age were missed. Quantity and proportion of blood group in irradiated products comply with general population. Indications of prescription consist of leukaemia/lymphoma and Hodgkin (49.6%), allogeneic and autologous bone marrow transplantation (17.7%), immunosuppressive therapy (9.6%), congenital immunodeficiency (8.6%), premature birth (7.6%), aplastic anaemia (6.5%), relatives designated donation (0.3%) and HLA matched plateletphaeresis (0.1%). During the period of this study increasing trend in the quantity of requests of irradiated products and requesting hospitals were seen.
Conclusion: Considering the important prophylactic role of blood product irradiation on TA-GVHD, it is recommended to inform more hospitals about irradiation activities in TBTC and more education programmes for hospitals' staff about the proper prescription of irradiated products.

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This abstract has been withdrawn.

6.7 Alternatives to Blood Transfusion

P-326

PLATELET COUNT INCREMENT AFTER TRANSFUSION OF SINGLE DONOR APHERESIS PLATELETS IN THE HASAN SADIKIN HOSPITAL

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Backround and Aim: Patients in need for Platelets,can be transfused with platelet blood components derived from 6 to 8 whole blood donation(PRPs) or single donor platelets product derived by apheresis (AP). The choice for the component can be made because of economics (lower costs PRPs), and/or availability of sufficient donors. The aim of our ongoing study was to analyze the platelets count increment in patients after transfusion of single donor apheresis derived platelets.
Method: Apheresis platelet component were derived from 27 voluntary donors. In the patient hematology parameters (hemoglobin, white blood cells and platelets count) before and 1 h after the platelet were examened. Data before and after platelet transfusion were compared using Student *t*-test. Sixteen patients received apheresis derived platelet components between July 2009 and July 2010. In total 27 components were transfused.
Results: Sixteen patients (five males, 11 females) with various underlying disease were in corporate in our study. The reason for plateletpheresis transfusion was therapeutic in 15 (93%) subjects and prophylatic in 1 (7%) subject. The mean platelets increment after plateletpheresis transfusion in our patients was 48,111 + 5812/mm^3. Nine transfusion (33.3%) had a normal platelet increment (30,000–50,000/mm^3). Seven transfusion (22.3%) had below normal response (<30,000/mm^3). In 11 transfusions (40.7%) the platelet increment were above 50,000/mm^3. Four (14.8%) transfusions had platelet refractioness (platelet increment < 11,000/mm^3).
Conclusion: Mean platelets increment after 27 plateletpheresis transfusions was 48,111+5812/mm^3. Good response (platelet increment > 30,000/mm^3) was found in 74% (20/27) transfusions. Platelet refractioness (increment < 11,000/mm^3) was found in 14.8% (4/27). Most of of transfusions (21/27, 77.8%) are without adverse reaction. Based on this study we conclude that in our hands apheresis derived platelets can be safety transfused to our patients.

7. Cellular Therapies
7.1 Stem Cell and Tissue Banking, including Cord Blood

P-327

AN OPTIMIZATION STUDY ON THE SEPARATION AND CRYOPRESERVATION OF CORD BLOOD MONONUCLEAR CELLS

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Background: Umbilical cord blood has been widely used as a source of hematopoietic stem cells to treat a variety of diserse. The cell dose is critical and the available number of total nucleated cells (TNCs) plays a key role.
Aims: To optimize the procedure for separation and cryopreservation of TNCs from umbilical cord blood.
Methods: Cord blood units were collected and processed using either a double stem cell extraction method or a traditional method. The recovery rate of TNCs and other relevant factors affecting cryopreservation quality were analyzed.
Results: The average number of TNCs yielded by the double extraction method and the traditional method were (1.52 ± 0.89) × 10^9 and (1.11 ± 1.20) × 10^9 respectively. The recovery rate of TNCs using the double extraction method was 92.12 ± 5.78% and using the traditional method it was (86.94 ± 7.34)% (P < 0.05). The viability of TNCs, which were cooled at either 1°C/min or 5°C/min in a controlled-rate freezer from 4 to −40° was (92.4 ± 2.3)% and (84.7 ± 2.4)% (P < 0.05). The recovery rates of TNCs that were cooled at 1° or 5°C/min in a controlled rate freezer from −40 to −90°C were (91.1 ± 1.9)% and (90.3 ± 2.8)% (P > 0.05). The cells were cooled to either −90 or −130°C using a controlled-rate freezer, then moved to liquid nitrogen, and the recovery rates of TNCs were (91.1 ± 2.1)% and (92.3 ± 1.9%)%, respectively (P > 0.05).
Conclusions: The double extraction technique provides a high and consistent recovery of TNCs. The optimal method to cryopreserve cord blood mononuclear cells is to cool the cells at 1°C/min to −40°C and then at 5°C/min to −90°C using a controlled-rate freezer.

P-328

DIFFERENTIATION OF RAT BONE MARROW MESENCHYMAL STEM CELLS INTO HEPATOCYTES IN VITRO AND VIVO

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Background: Bone marrow mesenchymal stem cells (BMSCs) are highly plastic and differentiate into various cell types, including hepatocytes. To explore the mechanisms underlying these processes in vitro and in vivo, we focused on the initial responses of bone marrow to hepatic injury, using a rat model.
Aims: To investigate the mechanism and regulation of differentiation from (BMSCs) into hepatocytes and to find a new source for therapies of hepatic diseases.
Methods: We isolated BMSCs form whole bone marrow cells for subsequent differentiation in the presence of HGF or beta-NGF. Cells were subjected to the examination of hepatocyte characteristics by morphological and functional changes. Cell morphology was observed and cell surface phenotypings were detected by flow cytometry. α1-antitrypsin (AAT) expression of the hepatocyte was confirmed by immunocytochemistry, and the expression of albumin was validated by real time PCR and western blotting. The expression of high-affinity nerve growth factor receptor (TrkA) and the activation of Erk pathway were detected by western blotting. Hepatocyte functional activity was confirmed by uptake of indocyanine green (ICG) assay. Cultured BMSCs were labeled with 5-bromo-2-deoxyuridine (BrdU) 24 h before injecting 2 × 10^6 cells into the caudal vein of a rat model of prolonged toxic hepatic injury. Liver damage was induced by injection of carbon tetrachloride (CCl4) labeled engraft were researched by immunohistochemistry after transplantation 0, 1, 2, 3, 4, 5, 6 weeks. ALT, AST, AKP were detected by ELISA.
Results: Some small round cells appeared in the presence of HGF on day 10 (beta-NGF on day 12) and the cells increased in the course of differentiation for 21 days. Differentiated cells expressed albumin and had the functional characteristic of liver cells, such as uptake of ICG. BMSCs were positive for TrkA. HGF and beta-NGF significantly upregulated the protein levels of phospho-Erk. After 1 week of transplantation labeled engraft were detected in liver of rat model and positive labeled engraft were detected untill 4 weeks after transplantation. ALT, AST, AKP were different with the negtive control groups before 4 weeks after transplantation.
Conclusion: BMSCs could differentiate into hepatocytes in the differentiation media including HGF or beta-NGF. Combination of HGF and beta-NGF significantly increased the efficiency of hepatic differentiation. These hepatocytes were identified at the morphology, gene and protein levels. BMSCs may be differentiated into hepatocytes by HGF and beta-NGF. In vivo BMSCs had a notablely protective effects on the hepatocyte injured with CCl4. It makes a potential resource for the treatment of hepatic diseases.

P-329

GENERATION OF INDUCED PLURIPOTENT STEM (IPS) CELLS WITH ADENOVECTORS CARRYING EMBRYONICALLY EXPRESSED HUMAN GENES

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In recent years Scientists have reprogrammed the somatic cells to embryonic like cells named Induced Pluripotent Stem (iPS) cells. Regarding to genomic insertion of lenti- or retro-viral based vectors and subsequent adverse effects of these viruses, the current research focused on using safer gene transfer tools. Adenoviruses are the safe tools for gene delivery and expression in eukaryotic cells. In this study we construct adenovectors carrying embryonically active human genes and generate these recombinant adenoviruses aiming to produce safer transfer vectors as a nonintegrating viruses for reprogramming. cDNAs of embryonically active human cMyc, Klf4, Oct4, and Sox2 were amplified and then cloned individually into CMV-IRES-GFP transfer vector. Pme1 linearized transfer vector was co-transformed with pAdenoVator E1/E3 vector into rec+ BJ5183 cells to generate recombinant adenovectors. Production of adenoviruses was carried out by transfection of Pac1 linearized adenovectors into 293A packaging cell line. Cloning of desired genes was confirmed by PCR with specific primers for each genes, and sequencing after co-transfection of recombinant transfer vector and backbone adevirus plasmid, generation of recombinant adenovectors were confirmed by duplex PCR with specific primers for kanamycin resistance gene and adenovector plasmid. Transfection efficiency was determined by observation of GFP expression under fluorescence microscopy. As have suggested in several studies, adenovectors do not show insertional mutagenesis and they can produce high titer of

virus in host cells and broad types of target cells as well as dividing and non-dividing cells. Regarding to some limitations in using the retro- and lenti-viruses, we believe that adenovectors carrying human genes are the best and safe choice for gene delivery and reprogramming of somatic cells and generation of human pluripotent stem cells.

P-330
DETECTION OF ADDITIONAL INFECTIOUS UNITS AMONG SERONEGATIVE CORD BLOODS BY NUCLEIC ACID AMPLIFICATION TEST

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Background: Cord blood contains a sufficient number of hematopoietic progenitor cells allowing bone marrow restoration. To prevent the risk of viral infection from allogeneic donors, the cord bloods and/or their maternal bloods are tested for blood transmissible infection including HBV, HCV and HIV. Nucleic acid amplification test (NAT) has been implemented for blood donors either on mini-pooled or individual samples for detection of donors during window periods of serologic assays and those with chronic occult HBV infection (OBI), i.e. the donors containing a low level of viral DNA without detectable hepatitis B surface antigen in serum during later chronic stage.
Aims: The aim of the study is to detect infectious cord blood among those determined as non-infectious by serologic assays.
Methods: A number of 2156 units of cord blood, that were determined as non-infectious through detecting both cord bloods and maternal bloods for hepatitis B surface antigen and antibodies to HCV and HIV, are stored as the buffy-coat portion in liquid nitrogen and their plasma samples at −30°C. To carry out the study, individual plasma samples of these cord bloods were tested by Procleix Ultrio Assay (Novartis Diagnostics), the screening NAT that detect HIV-1, HCV and HBV simultaneously. Samples that were positive by screening NAT were further tested by Procleix Discriminatory Assays, the assay that detect these viruses each individually.
Results: Of the cord bloods analyzed, 6 units were found positive by screening NAT. Further discriminatory assays showed all the 6 units were negative for HBV, HCV and HIV-1. In view of the possibility that a low level of HBV DNA approximates the detection limit of NAT, we retested in triplicate the six samples for HBV DNA. All were found negative by retest.
Summary/Conclusions: Of the seronegative cord bloods, 0.3% (6/2,156) were determined as positive by screening NAT, however further tests were unable to identify the virus leading to the positive finding. Since that discriminatory assay is less sensitive than screening assay in detecting HBV at the lower levels of 30 and 100 copies/ml (as shown in assay manual), the reason for the non-discriminated results in this study could be the presence of HBV at a lower concentration approximating these levels. In view of immunosuppressive procedure to cord blood recipients, NAT should be implemented as a supplementary to serologic assays for detection of infectious cord blood.

P-331
STORAGE CONDITIONS FOR TRANSPORTING UMBILICAL CORD BLOOD TO CORD BLOOD CENTER

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Background: Conventionally, human hematopoietic stem cells (HSCs) have been purified on the basis of the expression of cell surface molecules such as CD34 and CD133. Recent data has shown that ALDH is highly expressed in primitive HSCs and its specific role in stem cell function. To assess the activity of ALDH in isolated HSCs from cord blood we study the expression level of this enzyme in various time during cord blood HSCs storing.
Methods: We compared the numbers of HSCs enumerated in samples processed immediately after acquisition (n = 20) with HSCs enumerated in specimens stored for 24, 48 and 72 h in 25°C, 4°C and after cryopreservation of CD133+/34+ HSCs isolated from cord blood with aldehyde dehydrogenase activity (ALDH br cells).
Results: ALDH activity was decreased during time in stored HSCs. We found significant difference in enzyme activity between HSCs stored at RT in compare with HSCs stored at 4°C. ALDH activity did not decrease in HSCs frozen in −20.
Conclusion: We suggest to transport and store cord blood HSCs under controlled cooling conditions.

P-332
DECOMPRESSION OF LUMBA-SACRAL NERVE ROOT AND STEM CELL TRANSPLANTATION FOR SPINAL CORD INJURY A CASE REPORT

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Background: Autologous Stem Cells from bone marrow have been shown to display some potential for tissue reconstruction in various neurodegenerative and muscle degenerative diseases. These cells are easily accessible from patients and can be expanded on a therapeutic scale. Although the mechanisms are not yet fully understood, some small open clinical trials with chronic Spinal Cord Injury (SCI) patients have demonstrated a positive effect of Autologous Stem Cells on their use and proved to be safe.
The combination of effective mobilization protocols (Granulocyte -Colony Stimulation Factor) and efficient use of apheresis methods have caused successful collection of peripheral blood progenitor cells (PBPC) for transplantation. Flow cytometric technology also can be used to assess target goal of CD34+ cell dose during harvesting of progenitor cells. The researchers think that following the surgery, a patient is grafted subarachnoidally, the grafted cells release a variety of axonal growth-stimulating, neurotrophic factors, and also participate immediately in restoring affected nervous communications.
Case Report: A 32-year-old young male who had a history of paraplegia with severe back pain following a gun shot injury between lumbar -2 and lumbar 3 levels 2. Mean time he had undergone two surgeries (Laminectomy with spinal fixation and removal calcified adhesions) for on and off severe pain. As he still did not respond, he was asked to admit for decompression and autologous stem cell transplantation by consultant neurosurgeon at National Hospital Sri Lanka. After referring to the Departments of Heamatology and Transfusion Medicine, he started 1 units of G-CSF(Granulocyte -Colony Stimulation Factor) daily for 4 days and target granulocyte counts were monitored by doing daily full blood counts and on the fifth day he underwent leukopheresis for collection of Stem cells. CD34+ count in the final product was assessed by Flow cytometry and then underwent decompression and infiltration of collected autologous stem cells into the affected site of the spinal cord. Results:
Since third day after operation, he complained that he felt sensation and less pain and 15 days after surgery he was referred to the rehabilitation unit as he complained low grade pain with more sensation compared with initial records.
Discussion: As this is the first case of autologous stem cell transplant for spinal cord damage in Sri Lanka, we don't have records to compare patient's outcome and long term follow up of this patient. The success rate cannot be compared with other patients in Sri Lanka as this was the first patient however when it is compared with international studies, it belongs to the good success. It is now known that bone marrow cells release a variety of factors that stimulate neuronal growth from progenitor cells. Additionally, bone marrow has been demonstrated to have the ability to directly differentiate into neurons. Thus, the stem cell-based technology opens new feasible opportunities for effective treatment of Spinal Cord injured patients.

7.2 Collection, Processing, Storage and Release

P-333
QUESTIONNAIRE SURVEY ON IN-HOUSE COLLECTION, PROCESSING AND STORAGE OF BLOOD CELLS FOR CELLULAR THERAPY IN JAPAN

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Background: More than 4000 allogeneic and autologous hematopoietic stem cell transplantations are annually carried out in Japan. The Japan Marrow Donor Program had confined volunteers to marrow donation since its establishment in 1991 but added peripheral blood as an option in October 2010. Also in 2010, the Japanese Society of Transfusion Medicine and Cell Therapy implemented Qualified Apheresis Nurse system, and disclosed a guideline for 'In-Hospital Blood Cell Processing' jointly with the Japan Society for Hematopoietic Cell Transplantation.
Aims: In order to figure out the situation and define the problems in in-house collection, processing and storage of blood cells in medical facilities in Japan.
Methods: The Japanese Society of Transfusion Medicine and Cell Therapy annually conducts Comprehensive Questionnaire Survey on Transfusion Medicine which

consists of general and supplementary questions. The former questions cover management system, laboratory tests and use of blood products, to which all hospitals are expected to reply, and the latter questions include those to which only applicable hospitals are expected to reply that include questions concerning cellular therapy.
Results: The society requested 11,449 hospitals for replying the questionnaire and 4352 provided their information. Autologous blood stem cells, related blood stem cells, unrelated blood stem cells, autologous bone marrow, related bone marrow, unrelated bone marrow, and unrelated cord blood were harvested at 138, 76, 3, 9, 39, 33 and four hospitals, respectively. Autologous blood stem cells from 1395 patients were processed, frozen and stored at 114, 122 and 125 hospitals. As to autologous peripheral blood cells, 56 hospitals conducted harvests at transfusion departments, 99 had standard operating procedures, 82 recorded working processes, 96 labeled containers for defined items, 82 identified and verified cell products according to procedures designed for blood products, 82 carried out flow-cytometric analyses at their own facilities and 93 used dedicated clean benches for open-system processing. Pre-storage and post-storage sterility tests were conducted at only 9 and 0 facilities, respectively. In aphereses, venipuncture was performed by patient-caring physicians in 111 hospitals, and cell-separators were operated by medical technologists or engineers in 40 or 65 hospitals, respectively. Processing, storage management and issuance of the cells were assumed by patient-caring physicians or medical technologists in 45 or 63, 17 or 93, and 26 or 89 hospitals.
Summary/Conclusions: This survey for 2010 revealed that process and quality control in cell processing in hospitals require major improvement, that patient-caring physicians still played significant roles, but at the same time, medical technologists and engineers were taking over the physicians roles. We plan to continue this survey to monitor the changes induced by the guideline, the Qualified Apheresis Nurse system and introduction of unrelated peripheral blood stem cell handling.

P-334
CRYOPRESERVATION OF PERIPHERAL BLOOD HEMATOPOIETIC STEM CELLS WITH LOWER DOSE OF DMSO BY ADDING NONTOXIC NATURAL CRYOPROTECTANTS

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Background: Cryopreservation of hematopoietic stem cells has become an important process due to the therapeutic protocol which includes peripheral blood stem cell transplantation after chemotherapy in many hematological malignancies. The conventional medium contains 10% dimethylsulfoxide (DMSO) as cryoprotectant, but it is reported to be related with many complications.
Aims: The usefulness of trehalose, catalase and zVAD-fmk for cryopreservation were analyzed with reduced concentration of DMSO to 5%.
Method: Peripheral blood stem cells were frozen in 10% DMSO as control and also in 5% DMSO with trehalose and catalase. After 3 weeks, the viability of each DMSO concentration was measured using Trypan blue staining and 7-AAD analysis. The colony forming potential was assessed using methylcellulose culture 14 days after thawing.
Result: Cryopreserved cells in 5% DMSO with trehalose and catalase showed similar survival with control. The viability of cells which were added with zVAD-fmk showed better result than without it after 24 h incubation. Colony forming assay showed similar colony formation in 5% DMSO with natural cyroprotectants.
Conclusion: Achieving better cell viability and reducing complications of high dose DMSO cryopreserved cells are important factors in successful stem cell transplantation. According to the results, lowering DMSO concentration to 5% in combination with nontoxic natural cryoprotectants and using zVAD-fmk is considerable to help cell survival. Thus we can expect better survival of hematopoietic stem cells and prevent many potential side effects of high dose DMSO when adding natural cryprotectants in the cryopreservation medium or adding caspase-inhibitor right after thawing.

P-335
THE SURVEY OF PLATELET PHERESIS YIELD AND FACTORS AFFECTING IT AMONG PLATELET DONORS IN SHIRAZ BLOOD TRANSFUSION SERVICE IN 2009–2010

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Millions of lives are saved each year through blood transfusions. With the improvement of medical science and increase the life potency, the demand of blood products increased. Due to the indication of platelet, the platelet products play vital role in patient's health. The single donor platelet is more effective and safer than random platelet products. Determination of platelet apheresis yields and the factors that affecting them help us to plan for more effective products. In this retrospective cross sectional survey, we studied 92 platelet donors referred to Shiraz blood transfusion service during 2009–2010 by random sampling. For all platelet donors after physical examination and performing HIV-Ag/Ab, HBs-Ag, HCV-Ab and RPR, we done Complete Blood count(CBC). The minimal acceptable of platelet count was 150*103. The apheresis was done by HAEMONETICS MCS+. The background variables and other data was recorded in their special form. The data was analyzed by using SPSS 16 (Chi-square, Independent *t*-test, One way ANOVA). We found that the mean platelet apheresis yields were 4.58 in female donors and 4.17 in male. We found significant difference between the yield (platelet counts of platelet apheresis unites) and the pre platelet apherisis platelet counts and blood donors hematocrite (P-value = 0 and 0.018 respectively). The difference was not significant about the weight, sex, age blood group and hemoglobin (P-value > 0.05). According to our findings the yield of single donor platelet apheresis is equal 8 units of random platelet. That yield is more than some studies that mention yield equal six times of random donor platelet. Female platelet donors and high platelet count is good donors for apheresis. Other studies showed that just precount platelet affect the yield, but in our study HCT affecting the yield. Because these products decrease the donor exposure and due to high quality of these products it's recommended for all patients require it.

P-336
VARIOUS ALDEHYDE DEHYDROGENASE ACTIVITY IN HSCS ISOLATED FROM UMBILICAL CORD BLOOD BEFORE & AFTER FREEZING

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Introductions: Aldehyde dehydrogenase (ALDH) is highly enriched in hematopoietic stem cells (HSCs) and is considered a selectable marker of human HSCs, although its contribution to stem cell fate remains unknown. ALDHbr Lin population contained committed progenitors as well as primitive repopulating cells. Recent data suggest that the practice of using frozen allogeneic grafts is becoming increasingly common among transplant centers. In this study, we assessed the disadvantage of HSCs freezing on ALDH activity for evaluation freezing effect in Blood Bank.
Methods: We compared the numbers of HSCs enumerated in samples processed immediately after acquisition (n = 10) and HSCs enumerated in specimens cultured for 7 days on MSCs feeder and cytokine cocktails. Cell harvested from these conditions and stored in cryopreservation. After that using three identification strategies: cell surface marker expression (CD34+/CD38−), aldehyde dehydrogenase activity (ALDH br cells) and colony assay.
Results: HSCs assessed in these four situations correlated with HSCs enumerated before and after CD34+/CD38− cryopreservation in both expansion on MSCs and without expansion condition. The percentage of ALDH br cells 26.8 ± 3.2 in the CD34+/CD38− cells before freezing compared to 23.9 ± 3.0 after thawing. However after expansion these cells on MSCs feeder the rate of ALDH br cells was decreased to 6.8 ± 1.5 for non -storage cells and 5.3 ± 1.2 for storage cells.
Conclusion: We revealed no significant difference between level of ALDH before and after freezing in CD34+/38− HSCs isolated from cord blood but in vitro expansion of HSCs result in decreasing ADLH activity. Since freeze immediately after harvested cord blood lead to remain of ALDH express level highly recommended. In feature study to expansion of HSCs we should make a condition to prevent diminishing ALDH activity.

P-337
ANALYSIS OF ANTI-L- NGFR, CD133 AND CD34 EXPRESSIONS IN BONE MARROW MONONUCLEAR CELLS BY FLOW CYTOMETRY WITH THREE COLOR FLUORESCENCE LABELLING

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Background and Objective: Stem cells hold great promise in tissue engineering for repairing tissues damaged by disease or wound. Mesenchymal stem cells (MSCs) are multipotent cells able to proliferate and differentiate into multiple mesodermal tissues

such as bone, cartilage, muscle, tendon, and fat. These MSCs can be derived from human bone marrow (BM) and identified by their ability to form fibroblast-like colony forming units that develop into stromal like cells when expanded in culture. These cells are characterized by their spindle-shaped morphology, their characteristic Immunophenotype [CD73(+), CD105(+), CD45(−), and CD34(−)]. However, the identification and purification of MSCs is hampered by the lack of suitable monoclonal antibodies (mAb). This study was purposed to detect the expressions of low-affinity nerve growth factor receptor (L-NGFR or CD271), CD133 and CD34, and to analyze the correlation of CD271 with CD133, and CD133 with CD34 expressions. To define the population more precisely, diverse surface markers have been used. We propose here to use CD271 as the sole marker for MSCs in bone marrow.

Materials and Methods: The human bone marrow cells (BMCs) and mononucleated cells (MNCs) were detected by flow cytomotry with CD45-PerCP, CD271-FITC, CD133-PE and CD34-FITC labelling according to different combinations of design, cells were located and selected repeatly by FSC, SSC and CD45 after acquirement, then the expressions of CD271, CD133 and CD34 were detected by flow cytometry.

Results: The results showed that the expressions of CD271, CD133 and CD34 in BMCs were 0.159%, 0.19% and 0.41% respectively, while their expressions were 0.69%, 0.44% and 1.05% respectively after isolation of MNCs. The co-expressions of CD271(+) CD133(+) before and after isolation of MNCs were (0.021 ± 0.01)% and (0.036 ± 0.02)% respectively. The co-expression of CD133(+) and CD34(+) before and after isolation of MNCs were (0.19 ± 0.11)% and (0.41 ± 0.21)% respectively ($P < 0.01$); mean while about 90% of cells with CD133(+) expressed CD34 and 40% of cells with CD34(+)expressed CD133.

Conclusions: It is concluded that the established method of detection using flow cytemetry with three color fluorescence labelling can be used to detect expression of CD271, CD133 and CD34 in BMCs. The cells with CD271 are different from cells with CD133 and CD34, which suggests that the CD271 may be of important role in evaluating and guiding the clinical application of BM MSCs. CD271(+) MSCs may be considered alternative candidates for tissue engineering and regenerative medicine applications.

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STUDY OF THE BONE MARROW MONONUCLEAR CELL APHERESIS PROCEDURE AND CLINICAL APPLICATIONS

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Background and Objective: To study the apheresis procedure of co llecting mononuclear cells from bone marrow for clinical treatment.

Materials and Methods: Bone marrow mononuclear cells were separated from bone marrow using COBE Spectra apheresis system. Cell morphology, cell counts, positive expression of CD34(+), CD133(+), and CD271(+) were detected, and the recovery rate of nucleated cell and mononuclear cell were also calculated. And the main parameters of the procedure with upper efficiency were set via data analysis.

Results: The efficiency of collection line with colouri-metric card of 3% was higher than that of 5% and of 7%. The recovery rate of mononuclear cell is 75% when the cycle times ranged from 7 to 9. The positive expression of CD34(+), CD133(+), and CD271(+) were 1.27%, 0.49%, and 0.57%, respectively.

Conclusion: This mononuclear apheresis procedure is safety, pollution free and efficient. The remaining part of the bone marrow could be retransfused to the donor.

7.3 Clinical Applications

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IMMUNOSUPPRESSIVE EFFECT OF TORISEL ON HUMAN DENDRITIC CELL FUNCTION: KEEP AN EYE ON DENDRITIC CELLS WHEN TREAT CARCINOMA

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Background: Dendritic cells (DC) are 'professional' antigen presenting cells (APC) of interest both as therapeutic targets and potential cellular vaccines due to their ability to regulate innate and adaptive immunity. However, the functional properties of human DCs differ, depending on microenvironmental factors as well as on their stage of maturation, especially in the patients who are treated with various drugs. Torisel is a novel mTOR inhibitor intravenous drug for the treatment of renal cell carcinoma (RCC) and approved by the US Food and Drug Administration (FDA) in late May 2007 and European Medicines Agency in November 2007. Although Torisel was reported would affect immune system and might bring patients a risk of getting an infection, it is still rarely known that which groups in immune system are targeted by it.

Aims: On these bases, we use human DC explored the hypothesis that Torisel bing down the immune response to infection through suppress DC, the key APC in immune system and subsequently inhibit downstream lymphocytes.

Methods: In our work, the human monocyte-derived DC (moDC), isolated and derived from buffy coat, were pretreated with Torisel for 1 day, with or without LPS. Lethal concentration 50 (LC50) was analyzed through Annexin V and PI staining assay. The treated cells were then analyzed surface mature marker CD83 and costimulatory molecule CD80, CD86 expression by flow cytometry. Endocytic activity was analysed through Dextron staining with FITC. T cell proliferation stimulating activity of DC was analyzed by Cell Trace assay. The supernatants of DC culture medium were analyzed cytokine secretion ability through cytokine array.

Results: In mixed cell culture with non-touched autologous T cells, torisel-treated DC showed almost loss the propensity to stimulate T cell proliferation even though pre-treated with an extra-low concentration. Later, we observed that CD83, CD80 and CD86 expression in LPS-stimulated mature DC were down-regulated by Torisel in a dose-dependent manner. Nevertheless, Torisel also reduced Endocytic activity of immature DC in a dose-dependent manner. Interestingly, 15 in 22 cytokines analyzed in our study were reduced by torisel and 1 was increased both in a dose-dependent manner when LPS present, but less was changed when LPS absent.

Summary: Accordingly, Torisel will systematically reduce the function of dendritic cells and shows collaborate its suppress effect with the toll-like receptor pathway. Our results give some information on the mechanisms of Torisel immune suppression and suggest that the patients treated with Torisol should be concerned whether need supply associated cell therapy in case.

P-340
IN VITRO EFFECT OF DIFFERENT CRYSTALLOID FLUIDS ON ERYTHROCYTE DEFORMABILITY AND AGGREGATION

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Background: Fluid therapy is associated with altered blood rheology. Aims This study aimed at investigating the in vitro effect of different crystalloids including normal saline (NS), lactated Ringer's solution (LR) and acetated Ringer's solution (also called plasmalyte A, AR) on RBC deformability and aggregation.

Methods: Whole blood was obtained from the jugular veins of the rabbits. The samples were centrifuged and the hematocrit was adjusted to 40%. The samples were diluted with NS, LR and AR at the volume ratio of blood to crystalloid of 1:2, 1:1 and 2:1, respectively. The blood without dilution was set as control. All samples were incubated for 1 h at 37°C. The hematocrit of all samples was again adjusted to 40% before the measurements of erythrocyte deformability and aggregation.

Results: Erythrocyte elongation index (EI) and aggregation index (AI) in all groups were not significantly different. When the volume ratio of blood to crystalloids was 1:2, Top time increased significantly in the NS group compared with the LR and control groups. The Amplitude of RBC aggregation was significantly lower in the NS, LR, AR groups compared with control group. When the volume ratio was 1:1, Top time in all groups was not significantly different. The Amplitude of RBC aggregation was significantly lower in the NS and AR groups compared with the control group. When the volume ratio was 2:1, Top time in all groups was not significantly different. The Amplitude of RBC aggregation was significantly lower in the NS group compared with the control group.

Conclusions: Crystalloids have no effect on RBC deformability, but will affect RBC aggregation by changing Top time and Amplitude. It is inferred that NS has the strongest ability of decreasing RBC aggregation, followed by AR and then LR.

8. Clinical Immunogenetics

8.1 HLA in Transfusion Medicine

P-341
GENETIC POLYMORPHISM OF THE TAROKO TRIBE, AUSTRONESIAN SPEAKERS ON THE NORTHEAST COAST OF TAIWAN

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Background: The Taroko people, recently officially recognized as a tribe on the basis of culture and language, live on the northeast coast of Taiwan. They are Austronesian

speakers, and their language belongs to a distinct branch of Atayalic which is spoken in the northern central mountain ranges by the Atayal tribe.
To this day, few genetic studies about the Aborigines of Formosa provide a clear understanding of the origin of each tribe. Also, the relationship between the tribes and to other neighboring populations still remains unclear.
Aims: Previous studies on the Taroko have been restricted to their language, cultural and social activities. Here we explored a new viewpoint using genetic profiles obtained from uni- and bi-parental descent.
Methods: The genetic affinity between the Taroko tribe and other Taiwan aboriginal and non aboriginal groups (15 groups in all) was analyzed using mitochondrial DNA (mtDNA), non recombining Y chromosome DNA (NRY) sequencing techniques, and human leukocyte antigens (HLA) reverse SSO techniques.
Results: Hundred and fifty-eight mtDNA haplogroups were determined in our pooled dataset. Six mtDNA haplogroups were seen in the Taroko (F4b, M7b3a, M7b3a1, B4a2a, E1a and N9a10new). These six haplogroups were also seen in the Atayal, who, in addition to language, also share with the Taroko, culture and legends. On the other hand the Atayal tribe displayed a much greater number of haplogroups (n = 16). This clearly indicates that the two tribes share a closed kinship and that the Taroko may be either a subset of the Atayal tribe or a different twig of the same branch.
Our Y- chromosomal DNA analysis on 765 individuals showed 27 Y-SNP haplogroups in the 14 populations studied. Interestingly all haplogroup except one Y haplogroup in Toroko were O1a1* (the other one was O3a3*). Both haplogroups were also seen in the Atayal tribe who similarly to mtDNA had more Y diversity (four different haplogroups). Allele frequencies at the HLA-A, -B, -DR loci among 18 Taiwan groups were used to construct a phylogenetic tree. Interestingly, we saw that the Taroko, Atayal, Bunun and Saisiat tribes belong to a unique cluster (the northern cluster) where, again, Taroko and Atayal showed the closest genetic relationship.
Summary: The Phylogenetic analysis showed that Taiwan groups belong to three main separate groups: the non Aboriginal group or Han Taiwanese, the north Aboriginal mountain tribes and the south aboriginal mountain tribes. In the northern group, Taroko and Atayal tribes show the closest kinship (followed by Bunun, Thao, Tsou and Saisiat).
The genetic affinity between Taroko and Atayal was the closest ever found between any two groups in Taiwan. These results support linguistic and anthropological findings and the Taroko appeared to be a subset of the Atayal tribes.

P-342
STRUCTURE OF Y-CHROMOSOME O1 HAPLOGROUP IN TAIWAN AND ISLAND SOUTH EAST ASIA

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Background: Human non recombining Y chromosome (NRY) is transmitted from father to son without recombination and the mutation rate of its Y-SNP is low. Analysis of NRY provides useful information for population genetics and human evolution studies. Here, we used Y-SNP (low mutation rate) and Y-STR (high mutation rate) to investigate the paternal genetic variation between populations of Taiwan (aboriginal and non-aboriginal), Southeast Asia (SEA) and Island South East Asia (ISEA).
Aims: As haplogroup O1 is the most commonly found in Taiwan aborigines, we investigated the distribution, genetic structure and variation of O1 among Taiwan and neighboring populations.
Methods: Blood or saliva samples were taken from 1593 unrelated healthy male's individuals from 22 populations comprised of 11 Taiwan aboriginal tribes, four Taiwan western plain tribes, other non Taiwan aboriginal groups (Minnan and Hakka) and neighboring groups from the Philippines, Indonesia, Thailand and Vietnam. According to the Y chromosome evolutionary tree published in 2008 (Karafet et al.), Y-SNPs were typed hierarchically by direct sequencing and Y-STR were defined using the AmpFlSTR (Yfiler kit) with the ABI 310 genetic analyzer. Allele scoring was obtained using the Genotyper software. Published data (Karafet et al. 2005, Delfin et al. 2010) were also used for comparative analysis.
Results: While most haplogroup found in Han belonged to haplogroup O3, over 80% of Taiwan aborigines belonged to O1. The frequency of this haplogroup decreased in the Philippines (42%), Indonesia (26%) and SEA (5%). More refined typing of the O1 haplogroup showed that sub-haplogroup O1a1* was the major Y-lineage in these populations (~70%). Interestingly, the highest diversity of O1a1* was found in south Taiwan and the Philippines. This may indicate southward gene flow between the regions. On the contrary, the Y-STR network of haplogroup O1 (not including O1a2) showed two major clusters: one star shaped cluster was centered on south Taiwan and Philippines populations with branches generally unique to Taiwan aborigines, plain tribes or the Philippines. The other cluster was multifocal with unshared foci composed of North Taiwan aborigines and shared foci composed of southern Taiwan aborigines, ISEA and Taiwanese Han.
On the other hand, haplogroup O1a2 showed high diversity and frequency among Taiwan aborigines. It decreased in ISEA (~5%), and was scarce in Han populations. STR network analysis showed that all populations shared four major haplotypes, except Bunun who were isolated from all others. This most likely indicated long isolation and gene flow restricted to Taiwan aboriginal tribes. Actually, AMOVA analysis also showed no distinct difference among aboriginal populations, strongly indicating a common ancestry.
Summary: O1 is one of the most prevalent haplogroups in South East Asia and ISEA. Extensive diversity was found in Taiwan and the Philippines. Here, using 7-STR network in combination with literature data, we could show that the South Taiwan genetic make- up plays a key role in the structure of other Taiwan regions and that gene flow between regions (from Taiwan to Philippines or from mainland to ISEA) requires more extended study to clear determine a major center of distribution.

P-343
HLA CROSSMATCHING BY IMMUNOCOMPLEX CAPTURE FLUORESCENCE ANALYSIS

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Background: Alloimmunization to donor class I HLA antigens is a major obstacle to successful platelet transfusion therapy. Therefore, we perform both HLA antibody detection and HLA crossmatching for platelet transfusion refractory (PTR) patients before HLA compatible platelet product shipment. Anti-human globulin lymphocytotoxicity test (AHG-LCT) is commonly used for HLA crossmatching in Japan, but it has drawbacks such as lower sensitivity and false positives caused by drugs, e.g. antithymocyte globulin (ATG). To overcome these problems, the Japanese Red Cross developed a new method in 2009; immunocomplex capture fluorescence analysis (ICFA). As ICFA is based on fluorescent microbead technology (Luminex), it allows high throughput and sensitive crossmatching. Moreover, because ICFA does not use complement, it is not affected by ATG. We therefore shifted from AHG-LCT to ICFA in December 2010.
Aims: To validate the reliability of ICFA as a routine crossmatch method, we analyzed retest and positivity rates. Moreover, we attempted to identify the causes of positive tests.
Methods: Crossmatching: The ICFA kit (Wakunaga Pharmaceutical Co., Ltd.) was used for crossmatching. White blood cells obtained by hemolysis of whole blood were sensitized with patient sera. Sensitized cells were then lysed. The immunocomplex in the lysate was captured by microbeads coated with HLA class I-specific monoclonal antibody. Finally, captured HLA antibody was labeled with phycoerythrin-conjugated secondary antibody and detected by Luminex.
Detailed Analysis: In the positive cases, donor HLA types were retyped. We usually detect antibody specificity in patients with a phenotype panel bead kit (Wakunaga Pharmaceutical Co., Ltd.). However, the single antigen panel bead kit (One Lambda, Inc.) was used for detailed analysis of HLA antibody in positive cases.
Results: The retest rate was 5.0% (102 cases) among 2059 cases from December 2010 to April 2011. All of these were positive on the first test. Duplicate retesting revealed that 48 cases were positive, and the others were negative, i.e. 54 cases gave false positives in the first test. Donor retyping revealed that all donor HLA types were correct. Donor-specific antibodies were newly detected by single antigen panel beads in four cases. One patient gave two positive results because of self-reactive HLA antibody. The remainder of positive cases have not yet been explained. The false positive rate was 8.2% in December 2010, but decreased gradually to 0.7% in April 2011. Thus, we believe handling was likely to be a major problem at the beginning.
Summary/Conclusion: We recently applied ICFA to routine HLA crossmatching. Although the retest rate was initially high, it is now <2%. Therefore, we conclude that ICFA is useful.

P-344
ANTI-PLATELET ANTIBODY DETECTION IN PATIENTS RECEIVING PLATELET TRANSFUSION

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Background: In Japan, universal prestorage leukocyte-reduction was implemented to all allogeneic blood products provided by the Japanese Red Cross Blood Center. Since 2004 the platelet concentrates transfused are supposed to contain $<1 \times 10^6$ leukocytes per bag. Patients with hematological disease usually require multiple platelet transfusions, and frequently develop anti-platelet antibodies, which are responsible for platelet transfusion refractoriness. In the present study, we aimed to investigate the performance of anti-platelet antibody testing in our hospital, and the frequency of antibody detection among the patients with hematological diseases receiving multiple,

defined as twice or more, platelet transfusions, in comparison with those receiving once or twice.
Methods: The 625 patients with hematological diseases requiring platelet transfusion were retrospectively analyzed. They were divided into cases receiving multiple, i.e. three or more platelet transfusions, and those receiving once or twice. The performance of anti-platelet antibody screening and the frequency of antibody detection in each group were analyzed.
Results: Among the 101 cases receiving 1–2 platelet transfusions, 25 (24.8%) were tested for anti-platelet antibodies, and antibodies were detected in only two. The antibody specificity was anti-GPIIb/IIIa in both. The remaining 76 cases (75.2%) were not tested. On the other hand, among the 524 cases receiving multiple platelet transfusions, 325 (62%) were tested, and antibody was detected in 60 (18.5%). The specificities were as follows: anti-HLA in 47, anti-HLA + GPIIb/IIIa in 3, anti-HLA + HPA-5b in 2, anti-GPIIb/IIIa in 5, anti-GPIIb/IIIa + HPA-4b in 1, anti-HPA-5b in 1, and in one case, the specificity was undetermined. The remaining 199 (38%) were not tested.
Conclusions: Although of the implementation of universal leukocyte reduction, the risk of platelet transfusion refractoriness (PTR) still exists, especially in those cases receiving multiple platelet transfusions. Whereas the risk of exposure to leukocyte antigens is theoretically significantly reduced, patients continue being exposed to platelet antigens. Therefore, we believe that frequent testing of anti-platelet antibodies is very important to diagnose and take the appropriate preventive measures to control PTR, especially in those cases receiving multiple platelet transfusions.

P-345
HLA ALLOIMMUNIZATION IN PATIENTS WITH END-STAGE RENAL DISEASE IN SOUTHERN TAIWAN

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Background: Alloimmunization to human leukocyte antigens (HLA) can occur with pregnancy and blood transfusion and have predictive value for kidney graft survival and the occurrence of rejection episode. The prevalence and risk factors for HLA alloimmunization in patients with end-stage renal disease (ESRD) in Taiwan are still unknown.
Methods: A cross-sectional study of HLA alloimmunization in ESRD patients in E-DA hospital, Kaohsiung, Taiwan. The volunteer blood donors were prospectively recruited by Kaohsing Blood Donation Centers. Donors provided a detailed history of pregnancy and transfusion Antibodies to class I and class II HLA were measured by an enzyme-linked immunosorbent assay (GTI Diagnostics).
Results: A cohort of 289 ESRD patients (138 females and 151 males) were recruited including 140 chronic hemodialysis patients, 98 chronic peritoneal dialysis patients and 51 pre-dialysis patients. Their mean age was 58.6 ± 12.3 years. The average dialysis duration was 3.0 ± 2.6 years. A total of 888 healthy volunteer blood donors (688 females and 200 males) were recruited as controls. The prevalence of any HLA antibody was significantly higher in ESRD patients (n = 67, 23.2%) and blood donors (n = 114, 12.8%), ($P < 0.001$). HLA antibodies were detected in 16.3% of all female healthy donors and in 22.4% of those with a history of previous pregnancy. The prevalence of HLA antibodies increased in women with greater numbers of pregnancy: 2.7% (zero), 14.0% (one), 23.3% (two) and 30.0% (three or more pregnancies; $P < 0.0001$). In ESRD patients, the presence of class I HLA antibody is highly correlated to the presence of class II HLA antibody ($P = 0.000$). While comparing the parameters between patients with positive and negative antibody (either to HLA class I or II), HLA antibody-positive patients had higher numbers of pregnancies ($P = 0.000$), higher numbers of blood transfusions ($P = 0.005$), and shorter time lag of the last transfusion ($P = 0.002$), than those of HLA antibody-negative patients. In a multivariate logistic analysis, both the pregnancy and the time lag of last transfusion had independent associations with positive HLA alloimmunization, especially, the time lag of last transfusion of <1 year had the highest odds ratio 10.06 (95% CI: 3.866–26.16, $P = 0.000$).
Conclusions: HLA Class I and Class II antibodies are detectable at low prevalence in male volunteer blood donors. In female, the prevalence of HLA antibodies increases significantly with number of pregnancy. The prevalence of HLA antibodies of ESRD patients was significantly high compared to healthy blood donors. Pregnancy and the time lag of last transfusion remained the strongest risk factors of HLA alloimmunization in ESRD patients.

P-346
ANALYSIS OF KILLER CELL IG-LIKE RECEPTOR (KIR) LIGAND MATCHING IN 21 CASES OF TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE (TA-GVHD) IN JAPAN

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Background: Transfusion-associated graft-versus-host disease (TA-GVHD) is one of the most severe and fatal complications in blood transfusion. TA-GVHD occurs when the human leukocyte antigen (HLA) types of the recipient heterozygous, but the donor is homozygous and one-way compatible to the recipient. As the donor T lymphocytes cannot be rejected by the recipient's immune system, they can then proliferate and react with the tissues of the recipient. However, the incident frequency of TA-GVHD was lower than expected, suggesting the existence of recipient protecting system. Alloreactivity of NK cells by killer Ig-like receptor (KIR) 'missing self' recognition of its HLA ligand is one of the candidate mechanisms but has not been clarified yet.
Aims: To analyze the state of NK cell alloreactivity in TA-GVHD cases, the patient and donor HLA KIR ligand were typed and evaluated KIR ligand matching.
Materials & Methods: Twenty-one cases of TA-GVHD in Japan between 1994 and 1999 that had been confirmed by microsatellite analysis were used for this study. TA-GVHD patient and donor genome DNAs were amplified using a whole genome amplification kit (illustra GenomiPhi HY kit, GE Healthcare). Genotyping of HLA-A, -B, -C, and DRB1 loci was then performed using the PCR-rSSO-Luminex method (WAK-Flow HLA genotyping kit, Wakunaga Pharmaceutical). KIR ligand type of KIR2DL (C1, C2) and KIR3DL (Bw4, A3 and A11) was determined from the HLA-A, -B, and -C genotypes.
Results: Confirmed or putative donor HLA types were all homozygous and one-way compatible to the recipient in 21 cases. Furthermore in 17 of these recipients, a change in HLA type after transfusion was detected, indicating proliferation of donor lymphocytes in the recipient's circulation. No KIR ligand mismatch of C1/C2, Bw4, and A3, A11 between donor and recipient was detected in any of the 21 cases.
Conclusions: The homozygocity and one-way compatibility of donor to recipient HLA types was confirmed as a risk factor for TA-GVHD. There was no mismatch of KIR ligand between recipients and donors, suggesting that NK cells could not function in the removal of donor lymphocytes in these recipients who developed TA-GVHD. Still, this is concordant with the notion that alloreactivity of NK cell is one of the mechanisms for donor T lymphocyte removal. For further understanding of the role of alloreactivity of NK cells in TA-GVHD cases, recipient KIR genotyping and haplotype analysis is being conducted.

8.2 Histocompatibility in Stem Cell Transplantation

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COMPARISON OF BIALLELIC POLYMORPHISMS AND SHORT TANDEM REPEATS FOR CHIMERISM MONITORING IN HEMATOPOIETIC STEM CELL TRANSPLANTATION RELAPSE PATIENTS ON DIFFERENT SAMPLES

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Background: Chimerism kinetics permits early detection of hematopoietic stem cell transplantation (HSCT) patients with high risks of graft-versus-host disease or those liable to relapse. Although short tandem repeats-PCR (STR-PCR) is the gold standard for quantitative chimerism analysis, STR-PCR has a relatively low sensitivity of 5%.
Aim: In this study, we developed a more sensitive real-time PCR method for chimerism analysis based on the informative biallelic polymorphisms (BP).
Methods: The informative biallelic polymorphic markers with high discrimination power in the Taiwanese population were identified. The TaqMan probe-based real-time BP-PCR for amplification of the informative loci was designed and the detection sensitivity was determined. Clinical application of real-time BP-PCR in chimerism monitoring was evaluated and was compared with the conventional STR-PCR from four allogeneic HSCT relapsed patients. Besides, we extend our study to analyse T-cell DNA and cell-free DNA samples collected on the new cases since 2007 and compared it between two kinds of these samples.

Results: Allele distribution analysis revealed that the loci of S01a, S03, S04a, S05b, S06, S07b, S08b, S09b, S10b and S11a had a relatively high discrimination power and were the informative BP for chimerism monitoring. Real-time BP-PCRs for these 10 BP loci were set up with the detection sensitivity equivalent to 0.003–0.006%. Real-time BP-PCR of the four HSCT patients revealed the presence of recipient-specific DNA at early time point than STR-PCR for three of the patients. However, the results from T-cell DNA and cell-free DNA samples did not show better results about early detection of relapse.
Conclusion: We conclude that real-time BP-PCR is a sensitive and reliable method for chimerism monitoring and is superior to the STR-PCR in identifying patients who are at high risk for relapse after transplantation. As to the efficacy, it is the same between whole blood, T-cell DNA and cell-free DNA.

9. Novel Development
9.1 Novel Development

P-348
CHANGE IN THE LEVEL OF SERUM CHOLESTEROL AND URIC ACID IN DONORS WITH ELEVATED VALUE FOLLOWING INTRODUCTION OF THE MEASUREMENTS

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Background: The supply of blood relies on non-remunerated voluntary blood donations. To reward blood donors, we implemented the measurements for cholesterol and uric acid to donors every 6 months. In addition, we provide donors with educational materials for the diet leading to elevated serum levels of cholesterol and uric acid.
Aim: The study is to assess the implementation of cholesterol and uric acid measurements in changing the serum levels of the analytes in the donors with elevated value.
Method: A retrospective analysis has been done using data from January 2009 to December 2010. Throughout the study, a level of cholesterol of ≥240 mg/dl and uric acid of ≥7.3 mg/dl were considered as elevated. Blood donors in the category of containing elevated level of the analyses and being provided with at least two subsequent measurements were further analyzed for the change in their serum level of the analytes. Data were analyzed using SigmaState version 1. For all analyses, statistical significance was set at $P < 0.05$.
Results: The data indicated the serum uric acid was significantly higher in male than in female donors (6.5 vs 4.9 mg/dl), but serum cholesterol was not (171 vs 170 mg/dl). The serum cholesterol significantly increased with age, but serum uric acid did not. The occurrence of hypercholesterolemia and hyperuricemia, male vs female donors, were 2.7% vs 2.8% and 27% vs 14%, respectively. Further analyses were done for the donor groups with an elevated level of either cholesterol or uric acid, following by two subsequent measurements. Of the donor group with an elevated cholesterol level, the mean cholesterol levels of the three measurements in order are 260, 240, and 239 mg/dl. Data breakdown by donor age and gender show a decreasing cholesterol level in the subsequent measurements for each breakdown donor group. The same results were found by analysis of donors with elevated uric acid. An overall decrease in uric acid level was shown in the elevated donor group (8.2, 7.5, and 7.6 mg/dl) and each of the breakdown donor group as well.
Conclusions: Provision of the measurements of cholesterol and uric acid together with educational materials lead to a decrease in serum level of the analytes in those donors with elevated level, probably due to changing their diet preference.

P-349
A VALIDATED OVINE MODEL FOR BLOOD COLLECTION, PROCESSING, COMPATIBILITY TESTING AND TRANSFUSION

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Background: In vivo animal models of blood transfusion are useful for investigating the mechanisms of both the positive as well as the negative clinical outcomes of transfusion. The majority of transfusion models have been small animal models, with their attendant and significant limitations for blood sampling volumes, the lack of suitable blood bags and difficulties with post-transfusion haemodynamic monitoring. To overcome these limitations, our aim was to develop a large animal ovine model of blood collection, processing, compatibility testing and transfusion which would be relevant to current clinical practice. The larger body mass and circulatory volume of sheep supports multiple blood sampling, the use of human blood bags and in-line haemodynamic monitoring using standard human hospital instrumentation. Compared to the small animals used previously, sheep also provide the added advantage of greater immunological and anatomical similarities with humans.
Methods & Results: Collection and processing: Whole blood (400 ml) was collected from healthy male sheep into Leukotrap WB bags (Pall Medical, England), leucodepleted, and then centrifuged. As ovine red blood cells (RBC) are smaller (MCV 28–40 fL) than human RBC, centrifugation force and time was modified (5000 g; 45 min) to ensure clear separation between plasma and packed cells. Plasma was then removed by manual expressor and frozen at −20°C. Additive solution (SAGM: saline-adenine-guanosine-mannitol) was added to the ovine packed red blood cell (PRBC) for storage at 4°C for up to 42 days.
Compatibility Testing and Transfusion: Using a serological ratio of one drop 10% donor sheep RBC: two drops undiluted recipient sheep serum, the compatibility test was performed under the following conditions, then examined and graded for visible macroscopic RBC agglutination and any lysis.
IgM Detection: immediate centrifuge, read, then 30 min at room temperature, read for agglutination and lysis IgG ('complete') antibody detection: 30 min at 37°C, read for agglutination and lysis IgG ('incomplete') antibody detection: 30 min at 37°C, centrifuge and remove supernatant, add 10% albumin, 30 min at 37°C, read for agglutination and lysis.
The PRBC was considered compatible when all three assays were negative.
Two units of PRBC were transfused by a standard giving set into a male sheep.
Results: A total of 24 units of whole blood were collected from healthy male sheep and processed into PRBC.
Seventy-seven compatibility suites were performed with sera from 24 potential male recipient sheep. Interestingly 14 of 77 (18.18%) were found to be not compatible, of which one incompatibilities were due to IgG, seven due to IgM and six due to both IgG and IgM antibodies.
With compatibility confirmed, 24 units of PRBC were transfused into 12 recipient sheep without incident.
Conclusions: Results from this ovine model of blood collection, processing, compatibility testing and transfusion substantiate its usefulness and relevance. As there is limited data on compatibility testing in animal models, the detection of incompatibilities in our cohort of young male sheep proves that our compatibility screening is able to detect the presence of any alloantibodies. These results validate the application of this ovine model for future blood transfusion modelling.

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CLINICAL OUTCOMES IN LEUKEMIC PATIENTS AFTER BLOOD COMPONENT TRANSFUSION DURING HEMATOPOIETIC STEM CELL TRANSPLANTATION THERAPY

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Objective: We aimed to evaluate the clinical outcomes and any influencing factors of the blood component transfusion in leukemic patients during hematopoietic stem cell transplantation.
Methods: A total of 75 leukemic patients who were undergoing hematopoietic stem cell transplantation were recruited in this study. Patients Platelet recovery rate (PPR) and corrected platelet count increment (CCI) were calculated 24 h after platelet transfusion. Hemoglobin (Hb) increment was also recorded 24 h after transfusion of red blood cell suspension. We evaluated the clinical outcomes based on the improvement in clinical symptoms in all the patients. Many factors, such as gender and blood type, were recorded and evaluated.
Results: Gender was not correlated with clinical outcomes of platelets and red blood cell transfusion ($P > 0.05$). The platelet transfusion outcomes were significantly better in leukemic patients after hematopoietic stem cell transplantation than that in the pre-transplant patients (61.91% vs 55.77%, $P < 0.05$), while the red blood cell transfusion outcomes were better in the pre-transplant patients than that in the post-transplant patients (71.43% vs 47.91%, $P < 0.01$). The blood transfusion outcomes of the patients, whose blood type matched the type of donors, were better than that of the patients, whose blood type didn't match that of donors (70.00% vs 55.77% for platelet transfusion, 58.00% vs 40.00% for red blood cell transfusion, both $P < 0.05$).
Conclusion: Timing of transfusion (pre-transplantation or post-transplantation) and blood type are two major influencing factors that my affect the clinical outcomes of blood component transfusion.