

2006 Immunohematology Reference Laboratory Conference

Summary of presentations

For more than 20 years, the AABB and the American Red Cross (ARC) have hosted the Immunohematology Reference Laboratory (IRL) Conference in mid-spring. Initially the conference was jointly hosted by the two organizations; it has since been hosted alternately, with each organization choosing the host city and planning the conference. The conference has been held in various cities throughout the United States, including Atlanta, Chicago, Las Vegas, New Orleans, Memphis, and this year, Orlando. The 2007 IRL Conference will be hosted by the AABB and held in Albuquerque, New Mexico, the weekend of March 27 to 29, 2007.

The conference begins on Friday afternoon with proctor-led case studies discussing advanced immunohematologic investigations, followed by a welcoming reception that promotes connecting and networking with fellow technologists and physicians from around the country and, typically, Canada. Saturday begins with breakfast followed by speaker presentations on various serologic, technical, clinical, administrative, quality, and regulatory issues that affect today's reference laboratories. These presentations extend until late afternoon with scheduled breaks and a provided lunch. The presentations continue on Sunday with the conference ending at noon. Attendees are encouraged to bring posters for viewing and those that do have the opportunity to present the information to all.

Following are summaries of the presentations given at the 2006 IRL conference that was hosted by the ARC from April 28 to 30 in Orlando, Florida.

Reference Laboratory's Joys and Woes— 30 Years (1975–2005)

I had been working for 8 years in blood banking at War Memorial Blood Bank (now known as Memorial Blood Center) in Minneapolis, Minnesota, when I came to Gamma Biologicals, Inc., in Houston, Texas, to work in the consultations service. I began as the supervisor of consultation on August 1, 1975, became the director of consultation and education in 1990, and was named the vice president of consultation and education services in 1996, a position I held until 1998 when I joined Immucor as the vice president of reference and education services.

There are several elements essential to having a successful immunohematology reference laboratory for RBC serology. The first element is to have staff that

is competent, dedicated, enthusiastic, and eager and willing to investigate all types of samples, whether they contain cold- or warm-reactive autoantibodies or a combination of the two, mixtures of alloantibodies, antibodies directed at low- or high-incidence antigens, or even the mundane Lewis, P1, or Bg antibodies. The second element is to have a facility where management is willing to support the many, and sometimes lengthy, investigations associated with resolving complicated serologic problems. Another element is to have the resources of unusual and rare RBCs and antibodies to perform special investigative studies. These are obtained by the collecting and sharing of samples by immunohematologists around the world. The SCARF program (organized by John Moulds) and other exchange programs have certainly helped to accomplish this. In addition, very importantly, a reference laboratory needs blood bankers in transfusion services, prenatal testing laboratories, donor centers, and even reagent manufacturing facilities who are willing to take the time to send samples for further studies. They are the ones who have the patients whose lives can be saved by providing the best possible blood for transfusion. They also have access to families and donors that could aid in furthering our knowledge of blood groups. And, last but not least, a key element to the success of an immunohematology reference laboratory is education. I was very fortunate to be able to attend meetings and seminars worldwide, as well as to be able to give talks on the special cases we investigated and the new methodologies and technologies introduced to blood banking that helped us to resolve the unusual cases.

There are many joys in working in an immunohematology reference laboratory. However, sometimes there are woes, such as regulations, assessments, validations, SOPs, corrective actions, etc. I have learned over the years to deal with these woes as best I can and look back on the good times and not the

bad. I am not always good at taking my own advice, but I do like to give it!!!

It has been a wonderful 30 years and I have many to thank for the support I have had in my career: from my mentors, the facilities that employed me, and the more than 20 staff members who worked with me at Gamma and Immucor and, before that, at War Memorial Blood Bank, Chadron Community Hospital in Chadron, Nebraska, and St. John's McNamara Hospital in Rapid City, South Dakota. Also, I extend a special thanks to the blood bank friends and customers who shared samples with our immunohematology reference laboratory. I wish all of you the best in your careers in the future and hope that there will always be those who have the desire and dedication to work on special serologic problems for the best patient care we can give.

Marilyn K. (Grandstaff) Moulds, MT(ASCP)SBB, Vice President Education, ImmucorGamma, Norcross, Georgia/Houston, Texas.

Serologic Results to Diagnostic Interpretation

Beyond the tests associated with antibody identification studies routinely performed in an immunohematology reference laboratory (IRL) are serologic tests that can lead to diagnosis. Serologic results alone, however, are not diagnostic. Their significance must be reviewed in conjunction with the patient's clinical condition. The following reviews serologic testing the author feels has a direct impact in diagnosis as well as comments on several requested tests that this author believes have limited value.

Detection of Mixed-Field Agglutination

Detecting mixed-field agglutination (2-cell populations) when performing ABO and D typings can be used to assess engraftment of marrow in a transplant recipient. Likewise, detecting mixed field in antigen typing can be used as an aid in assessing the survival of transfused RBCs.

Direct Antiglobulin Test

One of the most useful tests in the investigation of hemolysis is the DAT. Careful analysis of DAT results, in conjunction with the evaluation of serum reactivity, can lead to the diagnosis of warm autoimmune hemolytic anemia, cold agglutinin disease, and paroxysmal cold hemoglobinuria and can allow the physician to plan a course of treatment. Additional

testing can be performed to evaluate patients with so-called DAT-negative hemolytic anemia in an attempt to detect an antigen-antibody reaction. This type of information is valuable to the patient's physician because it can help confirm hemolysis is immune-mediated.

An area in which serologic testing can be misleading is the detection of newly forming antibodies in patients that have been recently transfused. A positive DAT posttransfusion with identification of a new antibody 7 to 14 days after transfusion alerts the laboratory to a possible delayed transfusion reaction. Clinical evidence of hemolysis is the key to differentiate a delayed hemolytic transfusion reaction from a delayed serologic transfusion reaction.

Elution

Elution procedures are performed to determine the specificity of the antibody coating the patient's RBCs. Eluates can confirm alloantibody specificity identified in the patient's serum. In rare cases, newly forming antibody can only be found coating the transfused RBCs in a recently transfused patient. A negative eluate in a patient with a strongly positive DAT (4+) may indicate that the patient has a drug-dependent antibody. Further review of the patient's clinical course and medication history may indicate the patient is experiencing drug-induced immune hemolytic anemia. Drug studies to look for the presence of drug-dependent antibodies will confirm this diagnosis.

Lectin Testing

Testing samples from infants and children with a panel of lectins may be valuable in detecting T-activated polyagglutinable RBCs, particularly in infants with a bacterial infection. However, routinely screening for polyagglutination in newborns with a diagnosis of necrotizing enterocolitis (NEC), for example, is generally not performed. RBCs from normal, healthy infants can show T activation and infants with NEC and possessing T-activated RBCs may show no hemolysis. Most experts believe that testing for polyagglutination should be selectively performed when the neonate has received RBCs or plasma products and has demonstrated hemolysis or an unexplained lack of rise in posttransfusion Hb.

There are rare examples of immune-mediated hemolysis in children because of T-activated RBCs and the child's own anti-T. The exact mechanism of hemolysis is not fully understood.

Ii Antigen Typing

Occasionally the IRL is asked to perform Ii antigen typing on a child. This is generally requested when a physician suspects stressed hematopoiesis. The i antigen is characteristic of fetal erythropoiesis and may be increased in cases of hereditary erythroblastic multinuclearity with a positive acidified serum (HEMPAS), aplastic anemia, and myeloblastic erythropoiesis, for example. There are several issues to consider before performing this typing. The first issue is that antisera are in limited supply. The second issue is that, since the level of I antigen expression increases and i antigen expression decreases with age, samples from children about the same age as the patient to be tested should be used as a control. Also, this testing can only be performed if the child has not been transfused within the past 3 to 4 months. Lastly, there are other tests that will provide more useful diagnostic results than determining the Ii antigen status.

ABO Titration in Transplant Patients

IRLs are being asked to assist transplant programs by performing anti-A and anti-B titration studies for ABO-incompatible kidney transplants and for ABO-incompatible heart transplants in infants.

In ABO-incompatible kidney transplants, the recipient is usually group O and the kidney donor is group A² or B. The procedure accepted by the United Network for Organ Sharing for ABO titration is peculiar to immunohematologists. Patient serum is treated with DTT to destroy IgM anti-A or anti-B and dilutions of patient serum are tested against pooled group A or B RBCs by incubating at room temperature for 10 minutes. If the titer is less than 8, the patient is considered able to receive a kidney from a group A² or B donor. ABO titers are performed periodically before transplant, immediately before transplant, and immediately posttransplant. If the titer increases following transplant, a plasma exchange may be performed to reduce the ABO-antibody titer.

The ABO titration performed to monitor infants less than 1 year old consists of diluting the patient's serum in saline, preparing doubling dilutions, adding known group A or B RBCs, and incubating the mixture at room temperature for 30 to 60 minutes. Interestingly, ABO antibodies often detected in infants are passively acquired from transfusion once maternal antibody clears.

Although more and more is being discovered about blood groups at the molecular and biochemical level, relatively simple serologic tests to detect antigen-

antibody reactions continue to play a major role in diagnosis and monitoring of a patient's disease. However, a serologic test result alone must always be correlated with the patient's clinical condition to provide the utmost value to the patient's physician.

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Special Notes When Using Anti-D Monoclonal Reagents

Commercial anti-D reagents using monoclonal antibodies began to be widely used in the early 1990s. Since then, differences among the reactivity of the various reagents with unusual or rare D+ RBCs have led to widespread discussion of the testing appropriate for blood donors, transfusion recipients, and obstetric patients. D+ RBCs giving varying reactions fall into the categories of weak D, partial D including the R₀^{Har} phenotype, and the Crawford (ceCF), ceRT, and the DEL (D_{cl}) phenotypes. Currently, there are several types of anti-D reagents available, including human blend, monoclonal/polyclonal blend, monoclonal/monoclonal blend, and monoclonal IgM used in the gel card. Another influence on the types of reactions observed is the technique used: tube, microplate, slide, gel cards, or automation, to name a few. Also, some reagents react best with these unusual RBCs at immediate spin (IS) and room temperature, some at IS and after incubation at 37°C, and some only in the IAT. In addition, the ethnic background of the person whose RBCs give these unusual varying reactions is significant.

Weak D

The incidence of weak D has previously been reported as 0.23 and 0.5 percent in Europe and 3 percent in the United States. These studies were performed before the introduction of monoclonal anti-D reagents. A study published in 2005¹ reported on the incidence of weak D blood donors typed as D+ by Olympus PK7200 as 0.4 percent (4 of 1005 donors).

Partial D

Judd et al. also reported in 2005² on the reactivity of FDA-approved anti-D reagents with partial D+ RBCs. They compared reactions in tube tests using anti-D reagents from Gamma, Immucor, and Ortho and with Ortho gel cards and found differences among Categories DVa, DBT, and R₀^{Har} RBCs in the various phases of testing.

Crawford (ceCF)

In 2003, Schlanser et al.³ reported on the Crawford phenotype that is present in African Americans and in two individuals from Colombia. RBCs with this unusual phenotype react with two monoclonal/monoclonal blend anti-D reagents but not with other anti-D reagents.

ceRT and Del

Even more recently, there have been several reports (a review, an editorial, and a forum)⁴⁻⁶ that focus on the issue of whether RBCs of blood donors with these phenotypes can elicit the production of anti-D in transfusion recipients and obstetric patients and whether we should be concerned with weak D. These publications can be consulted for the various opinions and other pertinent references.

References

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Cold Agglutinins

As serologists know, cold agglutinins found in normal individuals may interfere with pretransfusion testing. Cold agglutinins are also found in individuals with disorders including cold hemagglutinin disease (CHD) and paroxysmal cold hemoglobinuria (PCH). Reference laboratory serologists play a key role in helping to distinguish the former benign type of cold

agglutinins from the latter, more clinically significant type.

The focus of this discussion will not be on the serologic management of cold agglutinins. Rather, it will be on three clinically related topics: the association of cold agglutinins with patients undergoing cardiac surgery, with blood donors, and as critical values.

Should one be concerned with cold agglutinins in cardiac surgery patients? Over the last 10 to 20 years, the number of cardiac catheterization and percutaneous coronary intervention procedures has continued to increase, while the number of coronary artery bypass surgery procedures (CABG) peaked in 1997 and has since started to decline. 0.8 to 4 percent of cardiac surgery patients have been found to have some type of cold agglutinins. This compares with an incidence of 1 in 41,000 to 1 in 80,000 individuals with autoimmune hemolytic anemia (15.6% of these individuals have CHD).

The traditional CABG permits a cardiopulmonary bypass (CPB) machine to take over the functions of the cardiac and pulmonary systems (i.e., to pump blood through the body, while supplying oxygen and removing carbon dioxide). It also permits the surgeon to operate on a quiet, bloodless surgical field. Techniques initiated systemic hypothermia to 28 to 32°C, while cold (~5°C) potassium cardioplegic solutions were infused into the coronary arteries via the aortic root. By modifying the temperature or the manner in which cardioplegic solutions are infused, surgeons are now able to avoid complications that potentially may be induced by cold agglutinins.

Perhaps a more preferable way of referring to cold agglutinins is as cold-reactive proteins. There are three categories of cold-reactive proteins that may cause concern in cardiac surgery patients: cryoglobulins, cold agglutinins, and Donath-Landsteiner (DL) antibodies.

Cryoglobulins (Types I-III) are serum proteins that reversibly precipitate in the cold. Four cases of patients with cryoglobulinemia undergoing CPB surgery have been reported in the literature. The outcomes of these cases were all successful even though the techniques used varied. Some used plasma exchange to reduce the patients' cryocrit while others used temperature modifications in systemic or cardioplegic solutions.

Cold agglutinins are antibodies that typically bind to RBC antigens and cause agglutination and complement fixation over a particular temperature range. They are disturbing to the cardiac surgery

patient (and surgeon) for their potential to cause agglutination within the circuit or within coronary arteries that may result in hemolysis or organ damage secondary to agglutination or complement-mediated RBC destruction. Several case reports of hemolysis associated with CPB, as well as ways to avoid complications, have been published both in transfusion-medicine and cardiovascular-surgery literature. All serologists are encouraged to review these papers critically if requested to prescreen cardiac surgery patients for cold agglutinins.

The DL antibody is a biphasic IgG hemolysin that is classically associated with PCH. There is only one reported case in the literature of a patient with the DL antibody undergoing cardiac surgery.

Certain strategies may be considered when managing CPB patients with cold-reactive proteins. If cold-reactive proteins are detected before surgery, the surgical procedure can be modified to maintain normothermic systemic circulation and a crystalloid cardioplegic solution (rather than a blood-based cardioplegic solution) can be used. Plasma exchange may be helpful, for example, in cryoglobulinemia to reduce the cryocrit or in other cases of IgM-only antibodies. Patients with documented CHD may present special concerns. If cold-reactive proteins are not detected before surgery, the surgeon should observe for agglutination during cooling of the cardioplegic unit. If detected at that time, both systemic hypothermia and cold blood or cardioplegia should be avoided.

Individuals can be healthy enough to donate a unit of blood (allogeneic or autologous) and evidence of cold agglutinins may be found in these units, either grossly or microscopically. Units with gross evidence of cold agglutinins must be detected prior to issue. The use of digital imaging visual technology may prove helpful in the near future for communicating information between individuals in different facilities (e.g., does this unit have a clot or cold agglutinin?) and for education.

The College of American Pathologists transfusion medicine checklist now contains the following Phase II requirement: "Are critical values established for certain tests that are important for prompt patient management decisions?" There may be certain critical situations where discussion between the laboratory and the ordering physician should take place with regard to cold agglutinins; these may include evaluating a patient for autoimmune hemolytic anemia, resolving

an ABO discrepancy, and detecting cold alloantibodies that one considers clinically significant or that delay the procurement of blood.

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Polyagglutination

Polyagglutination is still present but rarely seen because of the use of monoclonal ABO typing reagents. Human-based ABO reagents contained polyagglutinins that interfered with the forward typing of RBC samples from patients with polyagglutinable RBCs. A basic review of polyagglutination will be presented.

Polyagglutinins are considered naturally occurring as they are most likely stimulated by normal flora found in the intestines. There are three types of polyagglutination: microbially induced, nonmicrobially induced, and inherited. The microbially-induced polyagglutination types are T, Th, Tk, Tx, VA, and acquired B. Microbial polyagglutination is transient and once the infection is eradicated, the polyagglutinable state goes away. One interesting note about T polyagglutination is that it may be a useful marker for hemolytic-uremic syndrome. Tk polyagglutination alters ABH, Ii, Lewis, and P1 antigens. Few cases of Th polyagglutination have been identified. Tx polyagglutination is also rare and is found in children with pneumococcal infections. Acquired B is usually found in patients with bowel disorders. The VA stands for Vienna and these polyagglutinable RBCs have reduced expression of the H antigen.

The nonmicrobial form of polyagglutination is Tn. Tn is permanent and irreversible. Tn RBCs acquire a weak A-like antigen. Tn has also been shown to be associated with leukemia.

The inherited forms of polyagglutination are CAD, HEMPAS, NOR, and Hemoglobin M-Hyde Park. HEMPAS stands for hereditary erythroblastic multinuclearity with a positive acidified serum test. HEMPAS RBCs have increased expression of i antigen and normal to increased I antigen expression. CAD 1, CAD 2, and CAD 3 phenotypes have been identified in which only CAD1 is considered polyagglutinable. RBCs with the autosomally inherited NOR form of polyagglutination were found not to react with cord sera. Polyagglutinable RBCs are usually identified by their reactivity with different lectins and with all adult ABO-

compatible serum or plasma, and by their nonreactivity with cord sera.

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Project Management

Immunoematology reference laboratories (IRL) provide a critical, value-added service to patients and to the healthcare community. The IRL commonly focuses on testing and operational issues; however, IRL staff and supervisors often serve as agents of change as well. Changes within the IRL, such as implementing new techniques or preparing for computer software upgrades, may benefit from application of project management techniques and tools.

Project management is anchored by the project management body of knowledge (PMBOK). The Project Management Institute maintains the guide to the PMBOK, which consists of 44 processes. These processes or activities fall into one of five process groups; initiation, planning, execution, closure, and monitoring or control. The processes or activities can also be grouped by knowledge area; integration, scope, time, cost, risk, human resources, quality, communication, and procurement. Full exploration of the PMBOK is beyond the ability of this review, and therefore, three selected process groups and 12 processes will be explored as an overview of project management.

Project Planning

Project planning includes defining the scope of the project. The scope statement should describe the work that will be done within the boundaries of the project, and where relevant, the work that is out of scope. Goals, objectives, and measurements are also defined during planning. Project goals are commonly established on the basis of one of four elements: schedule, cost, quality, or performance. Objectives will address decisions, inputs, or activities that are essential to meeting one of the project's goals. Measurements should be very specific and should detail how the objective will be measured and the success target value. Critical to measurement design is that the measurements take place during execution of the project; this ensures that corrective action can be taken during the course of the project in time to bring the project back on track. Tasks and activities for the project work are detailed with input from subject matter experts. Resources are assigned to each task or

activity on the basis of the required skills or abilities. Each task or activity is evaluated for an estimated duration and dependencies between tasks and activities are identified. A schedule is constructed by assigning estimated start and finish dates, starting with constrained dates, or with a chosen project start date. Risk planning is initiated by evaluating the scope and schedule as well as other sources, for "things that could go wrong." The identified risks are evaluated for probability of occurrence and impact on the project. Selected risks are assigned mitigation actions. A plan for monitoring the project is designed on the basis of the objectives and measurements, risk plan, and other component planning activities.

Project Execution

Project execution is the act of performing the tasks or work associated with the project. The project plan, containing elements described in the planning process, becomes the road map for the project. With a well-written plan, the project team should be able to work the plan and appropriately respond to events that signal an off-course project. During execution, periodic reports and communication should take place as well as celebration of project accomplishments.

Project Closure

During project closure, the team should review lessons learned, that is, what went wrong and what went well within the project. This information is captured for sharing with other current and future projects. Effectiveness data are collected and evaluated during closing as well as identification of future enhancements.

The appropriate application of project management skills, tools, and techniques increases the probability of project success. The extensive planning process should cumulate in a project plan that creates a common understanding of the project and its goals. The project plan becomes an active road map for the project.

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ISBT 128 for the IRL

ISBT 128 is an international information standard for blood, tissue, and cellular therapy products. It defines how information can be encoded for

transmission via different delivery mechanisms, including linear bar codes (Code 128 symbology), two-dimensional bar codes, radio frequency identification tags, or electronic messaging. It is currently in use in many countries with more countries implementing it each year. The AABB will require U.S. blood banks to implement the system for blood labeling by May 2008.

Data that can be encoded using the *ISBT 128* information standard include the unique donation identification number, ABO and Rh, product code, donation type, expiration date, HLA test results (genomic or serologic), RBC antigens, platelet antigens, CMV, IgA, HbS, patient identification number, patient date of birth, catalog and lot numbers of supplies, and staff identification codes. Specifics of how the information is encoded, as well as reference tables, can be found in the document available from ICCBBA titled *ISBT 128 Standard, Technical Specification*. Databases supporting the encoded information are maintained by ICCBBA, Inc., and are available on its Web site (www.iccbba.org). Specifics on labeling in the United States may be found in the *U.S. Consensus Standard*.

Of particular interest to immunohematology reference laboratories is the ability of *ISBT 128* to encode a RBC phenotype obtained by testing for antigens such as: A1, C, c, E, e, C^w, VS/V, K, k, Kp^a, Js^a, M, N, S, s, U, P1, Lu^a, Le^a, Le^b, Fy^a, Fy^b, Jk^a, Jk^b, Do^a, Do^b, Di^a, Mi^a as well as a long list of less commonly typed antigens. By encoding information for transfer via bar codes or electronic messaging, greater process control and accuracy can be achieved. In addition, automated transmission allows clear, unambiguous information to be transferred across language barriers, supporting the international sharing of rare blood, tissue, and cellular therapy products.

ISBT 128 is not merely a labeling system. By understanding and using its potential, secure information transfer for blood, tissue, and cellular therapy products is possible. This should lead to an increased level of safety for patients.

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Lean but Not Mean: Doing More with Less in the IRL

Continuous process improvement can be a valuable approach to achieve and sustain customer satisfaction as well as process excellence. There are many methodologies and tools for process

Table 1. Examples of waste in the IRL

Source of waste	IRL example
Overproduction	Antigen typing of donor units for stock i.e., without a customer order
Inventory	Storage of excessive amounts of test tubes or other supplies, such as blank forms
Defects	Incomplete documentation on testing worksheets, incorrect test result, delayed result
Over-processing	Academic "for fun" testing that is performed routinely, testing protocols beyond industry standard of practice, antibody reconfirmation, ABO and D testing performed after initial sample testing
Waiting	Time that elapses from sample notification to sample receipt, from sample receipt to initiation of testing
People underutilized	Highly trained staff performing filing or other clerical tasks, not including staff in problem solving or improvements efforts
Motion	Excessive up and down activity to obtain reagents and supplies during testing, cell washers located away from work stations, reagents stored in remote sites
Transportation	Movement of sample from patient to testing site, supplies from warehouse to laboratory, blood products from IRL to distribution department

improvement, one of which is Lean. Though many definitions exist for Lean, it can be described as a comprehensive evaluation of operations to identify and eliminate waste, decrease variation, and increase efficiency. Central to the Lean approach is to use the voice of the customer and evaluate processes on the basis of what the customer views as valuable. Table 1 lists sources of waste that are relevant to the immunohematology reference laboratory (IRL).

Lean has a "tool box" of tools that can be applied when evaluating and improving processes. Value stream mapping involves "walking" the process from beginning to end with intense observation and interviewing, as needed, to fully understand the process. Cycle times and measurement of wait are also included. The value stream map is evaluated for waste and a desired state or "to be" map is drawn. Gaps between the "as is" and "to be" are identified and prioritized for improvement efforts. The application of 5S can yield benefits: sort (clear out clutter), set in order (place remaining supplies or items in alignment with process flow), shine (clean the work area), standardize, and sustain. Other Lean tools include streamlined physical layout, standardized work, batch-size reduction, workplace teams, kanban, or pull system (perform work only when customer order is placed), and point-of-use storage.

Though Lean has many benefits and opportunities, it can fall prey to challenges that serve as obstacles to its successful use. These challenges include the human element; staff with a pervasive batch mentality, desire for autonomy, resistance to change, and perspective that “we are different; we save lives.” Other challenges include lack of staff training in the Lean tools and teamwork, not enough attention to voice-of-the-customer, and conflicting needs among different customer groups (FDA, AABB, patients, physicians, quality department, donors, etc). Last, but not least, of the obstacles is that Lean may not be a good fit for every organization. If appropriately chosen and applied, Lean can add value to the IRL through removal of waste, creating increased efficiency that allows for opportunities to enhance and expand service.

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D.R.U.G.S. in the Workplace

Drug-induced immune hemolytic anemia (DIIHA) is an uncommon finding. To confirm its diagnosis, drug testing is performed to identify drug-dependent antibody (DDA).

Drugs most often implicated in DIIHA today are second- and third-generation cephalosporins. Cefotetan leads the list in reports by Arndt and Garratty as well as in testing performed in this author's laboratory.¹ There are many other drugs known to cause DIIHA. Important in determining the testing needed to detect these drug-dependent antibodies is having knowledge of the drug's characteristics in laboratory testing. Some drugs, like the cephalosporins and penicillin group, bind tightly to the RBC membrane. Most others require that the drug be present in a soluble form. Many nonsteroidal anti-inflammatory drugs require drug metabolites for detection of DDA.

Initial serologic testing normally shows the DAT to be positive. Strength of reactivity is reported from strong (3–4+) positive because of IgG binding to weak positive because of complement binding only. Work performed in this author's laboratory has shown that the DAT is most often strongly positive (2–4+) regardless of the drug. Most often, the DAT is positive because of IgG and C3, less often because of IgG only, and least often because of only complement coating

the RBCs. The eluate is classically negative because drug is not present in the test mixture. However, there are several reports of eluates being disproportionately weaker ($\leq 2+$) as compared with the strength of the DAT (3–4+). Serum is also reported to be negative in routine antibody detection tests for the same reason. However, drug-independent “autoantibody” or drug present in the patient's circulation may cause a positive antibody detection test without drug.

Arndt and Garratty propose dividing DDAs into two categories, those that react with drugs bound firmly to RBCs, called the “drug adsorption mechanism” and those that react with drugs that do not bind firmly, known as the “immune complex mechanism.”¹

A new classification is proposed when referring to DDAs on the basis of testing methods: those that react with drug-treated RBCs or those reacting in presence of drug. This classification is suggested to eliminate confusion and controversy in using mechanisms of drug-dependent antibody binding to categorize these antibodies.

A careful drug history is important in the face of significant RBC hemolysis in a patient. DIIHA should be considered when there is serologic evidence of warm autoimmune hemolytic anemia with a strong positive DAT and positive IAT, or of cold agglutinin syndrome with a strong positive DAT because of C3 and serologic testing shows a positive antibody detection test at immediate spin. A thorough investigation for DDA requires knowledge of the characteristics of the putative drug to confirm the presence of DDA.

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Coagulation for Blood Bankers

Most blood bankers understand ABO blood groups and how to match a unit. Words like Kell, Kidd, RhIG, and transfusion reactions are commonplace. They are familiar with transfusion medicine and when to dispense platelets, FFP, and products that can help trauma victims or hemophiliacs. With all of that knowledge, why do they need to add another topic to

their plate? They are certainly busy enough! Well, when the blood bank is busy, so is the coagulation department! So what happens when the two departments meet and what do they have in common? There are many concepts in each department that can enhance the understanding of testing and benefit the most common denominator—patient care.

Hemostasis

Hemostasis is a system of checks and balances. It compromises the vascular system, platelets, and a series of enzymatic reactions that affect the coagulation factors. When the coagulation system is activated inappropriately, an individual will experience a bleed or a thrombotic event.

Primary hemostasis in coagulation deals with platelets. These small disc-shaped cells do not contain a nucleus; their activity is controlled by their granules. Platelets are the primary response to an injury. They undergo a shape change from a disc to a spiny sphere. They then adhere to the site of the vessel injury. Many factors are required in this process, including fibrinogen and von Willebrand factor (vW). This is considered primary aggregation and is reversible. The final phase is a release reaction whereby platelets release their contents of dense and alpha granules. This is called secondary aggregation and is irreversible.

Secondary hemostasis involves a series of enzymatic reactions that activate the coagulation factors, resulting in the formation of a fibrin clot. This complex reaction includes a system of inhibitors and activators and uses a complex mixture of relatively unstable proteins that are difficult to purify as well as phospholipids and calcium ions.

The Coagulation Laboratory

The coagulation laboratory evaluates secondary hemostasis by assessing the in vitro coagulation cascade by performing the screening tests: the prothrombin time (PT) and the activated partial thromboplastin time (APTT). This cascade does not reflect clotting physiologically; however it does play a role in the laboratory evaluation of a potential coagulation disorder.

The PT and the APTT provide a tremendous amount of information to the physician. They can be performed quickly and accurately. Abnormalities of the test results can assist the clinician in determining preoperative status and bleeding disorders, and in monitoring anticoagulation therapy.

Prothrombin Time

The PT test evaluates factors in the extrinsic pathway. It uses citrate anticoagulated plasma, and after the addition of an optimum concentration of calcium and an excess of thromboplastin, clot detection is measured by an automated device. The result is reported in seconds. The PT is exclusive for Factor VII, but assesses other deficiencies of factors II, V, and X, which are found in the common pathway. Therefore, if a patient presents with a prolonged PT and there is no other clinical abnormality or medication, the patient is most likely Factor VII deficient.

This test also looks at the monitoring of warfarin therapy; excessive dosage of this anticoagulant is the most likely reason for a prolonged result. Monitoring anticoagulation has a variable and unpredictable response. As a result, if the level is inadequate, the patient may experience thrombosis and if the level is excessive, the patient may bleed. So how does this affect the blood bank? Warfarin inhibits the carboxylation of the glutamate residues of the vitamin K-dependent factors (II, VII, IX, X, proteins C, and S), rendering them nonfunctional and impairing fibrin formation. Their loss of function is half-life-dependent. Factor VII has the shortest half-life, 4 hours, while Factor II has the longest, 2 days. For example, if a factor level is at 100 percent and it has a half-life of 4 hours, the activity of this factor will be at 50 percent after 4 hours. Understanding this becomes important when looking at replacement therapy as this will impair fibrin formation. Warfarin has a half-life of 35 hours. It can be administered for life and bleeding is a potential risk because of the influences of diet, vitamin K ingestion, body mass, and liver function. Eighty drugs interfere with coumadin and blood levels are only therapeutic 65 to 80 percent of the time. In addition, the results of the test are affected by the instrument and reagent system used in the laboratory. A system, the International Normalized Ratio (INR), has been developed to help standardize the monitoring so that treatment can be more precise. It uses a formula that combines the patient PT, the mean of the normal range, and the sensitivity of the reagent as determined by the manufacturer.

The therapeutic range of the INR is 2.0 to 3.0 for prophylaxis and treatment of thrombosis, a pulmonary embolism, or a myocardial infarction. A high dose range of 2.5 to 3.5 is used for treatment of a mechanical heart valve. (Table 1) So how does this information

Table 1. Guidelines for patient management: oral anticoagulation, target range INR is 2 to 3

INR value	Clinical risk	Patient management
3.1 to 3.9	No bleeding	Day 1: subtract 5-10% of total weekly dose (TWD)
4.0 to 5.0	No bleeding	Day 1: no warfarin, weekly reduce TWD by 10-20%
5.1 to 9.0	No bleeding but at risk for bleeding	Hold warfarin, monitor INR until it reaches upper limit of therapeutic range Weekly: reduce TWD by 20-50% Recheck INR in 72 hours
> 9.0	Significant risk for bleeding	Hold warfarin, give vitamin K, admit patient to hospital Monitor INR until it reaches upper therapeutic limit, reinstitute warfarin Recheck INR until stable
>3.0	With bleeding	Hold warfarin, give vitamin K by IV, give plasma Get hematology consult Test INR 6-12 hours

Guidelines from New York-Presbyterian pharmacy.

affect blood bankers? The AABB guidelines for using FFP are:

1. Bleeding or planned invasive or surgical procedure and 1 or more of the following:
 - a. PT greater than 1.5 times the mean of the normal range or greater than 17 seconds
 - b. APTT greater than 1.5 times the mean of the normal range or greater than 49 seconds
 - c. Deficiency of factor II, V, VII, X, or XI
 - d. Massive transfusion of more than 10 units
 - e. Disseminated intravascular coagulation
 - f. Thrombotic thrombocytopenic purpura or hemolytic-uremic syndrome

Activated Partial Thromboplastin Time

The APTT evaluates deficiencies of intrinsic factors VIII, IX, XI, and XII. The methodology involves the addition of a contact activator (e.g., celite, kaolin, microsilicate, or ellagic acid) and plasma. This mixture is incubated at 37°C, usually for 5 minutes. Thromboplastin preparation is added and mixed. CaCl₂ is added and the result is measured in seconds.

This test is also used to monitor heparin therapy. Heparin is an acidic mucopolysaccharide that inhibits all of the active serine proteases (IIa, Xa, IXa, XIa, and XIIa). It has a strong negative charge and a circulating half-life of only a few hours. It is stable for 24 hours in a 5% dextrose solution. Elimination is largely by the kidney so that heparin must be used cautiously in patients with impaired glomerular filtration. Heparin is best administered intravenously, intermittently, or,

better, as continuous infusion in a dosage of 400 to 500 units/kg body weight/day divided into every 6-hour dose so that 100 to 125 units/kg body weight are given each 6 hours. (APTT target range of 60 to 85 seconds or APTT ratio that is equivalent to a heparin level by antifactor Xa assay of 0.3 to 0.7 units/mL). Because of the biological variability that occurs with heparin, there has been no ability to standardize testing. Heparin is greatly affected by weight, is not well absorbed by the gastrointestinal tract, and is affected by liver function as well as by the concentration of antithrombin that is required for binding. There is no dose-response relationship, meaning that each patient will react differently to a dose.

The Common Pathway

The common pathway is the part of the cascade where the intrinsic and extrinsic pathways merge and factors I, II, V, and X are measured. However, it is important to know that the PT and the APTT will not detect qualitative or quantitative platelet disorders or a Factor XIII deficiency. Factor XIII is fibrin stabilizing factor; this is responsible for stabilizing a soluble fibrin monomer to form an insoluble fibrin clot. If a patient is Factor XIII deficient, the patient will form a clot but will not be able to stabilize the clot and bleeding will occur later.

Inhibitors

Inhibitors are soluble plasma proteins that act as natural anticoagulants; they prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control: the protease inhibitors and the protein C pathway. The protease inhibitors inhibit coagulation factors; they include antithrombin III, heparin cofactor, tissue factor pathway inhibitor, and alpha 2 protease inhibitor. The protein C pathway causes inactivation of activated cofactors; these include protein C, its cofactor of protein S, as well as activated protein C.

It is important for the coagulation laboratory to communicate to the blood bank the type of reagent that is used for coagulation studies and how it performs. When a lot of reagent is received from a manufacturer, it is assumed that a normal result obtained on running the PT or APTT will correspond to a normal amount of factor level reflecting about 50 percent of a factor level. Patients will do well with 30 to 40 percent of a factor level. Therefore, a normal PT and APTT should minimally be able to reflect a 30 to 40 percent factor level. These screening test results are

Table 2. Relationship of factor level to APTT results

Pooled Normal Plasma (%)	APTT (seconds)*
100	32.4
75	33.0
50	34.0
25	34.5 [†]
12	36.0

*normal range 24.3 to 35.4 sec.

[†]normal APTT; only 25% of factor; patient will bleed.

used by physicians to determine if a patient may bleed in surgery. However, reagents can vary in the amount and concentration of phospholipids. It is possible to obtain a lot of reagents from a manufacturer that is insensitive to certain factors. For example, a patient sample is tested preoperatively with an APTT result of 34.5 seconds, the upper limit of the normal range is 35.4 seconds; therefore, the patient result is within the normal range. The patient has a normal PT. The information from these tests tells us that all factors in the intrinsic, extrinsic, and common pathways appear to be normal. The patient is cleared for surgery. During the surgery the patient bleeds. There were no platelet problems. The problem is that the reagent used to test the APTT has a poor sensitivity to Factor IX, resulting in the inability of the APTT to detect an abnormal Factor IX level. It is important to understand how the reagent performs, as illustrated in Table 2.

Having a basic understanding of how coagulation testing works and its relation to blood bank outcomes can improve the understanding of patient results by both departments.

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Replacement Bodies

The vacancies of medical technologists (MT) and clinical laboratory scientists (CLS) in 2000 were frightening to everyone; many presentations and initiatives were implemented. The vacancy rate for MT and CLS staff members was 14 percent in 2000 but, since then, it has dropped to 4.3 percent according to

the 2003 wage and vacancy survey by the American Society for Clinical Pathologists. The need for replacement bodies in blood banking has been a concern as the number of specialist in blood banking (SBB) programs has diminished drastically since 1984. The average age of MT and CLS staff members has been estimated to be 51. The baby boomers are due to retire and they will take with them the knowledge and love of antibody identification. The question remains: What is the blood bank community doing to educate and encourage MT and CLS graduates to specialize in blood bank technology? The SBB programs have reviewed this question and some have decided to try a distance format for educating students. Currently there are six programs offering distance education in blood banking. The average number of people sitting for the SBB ASCP exam is 125 each year, with less than 40 percent passing. For graduates of a Commission on Accreditation of Allied Health Education Programs (CAAHEP), the pass rate increases to 75 percent for those passing the ASCP SBB exam. It was estimated that 9000 MT and CLS graduates will be needed in the year 2010. Currently, about 2000 people are taking the MT ASCP examination each year. There will be a shortage. For the first time ever, the workforce includes four generations: those over 60 are working alongside baby boomers as well as with Generation X and Generation Y individuals.

What can be done for a better future? At the national level, organizations need to support education and provide unity for the profession. At the hospital and program level, more attention needs to be paid to the needs of the Generation Y employees by looking at flexible scheduling, clearly defined employee roles, and rewarding achievements. As individuals, we need to encourage young people to join the field, to be mentors, and to be active in our organizations. Shortages in MT and CLS staff members seem to be looming on the horizon. New ways must be explored to educate and to keep employees interested in the field.

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