COMPARISON OF COMPOLAB TM AND HEMOCUE HB301 FOR HAEMOGLOBIN ESTIMATION OF WHOLE BLOOD DONORS

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine. It is also not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.

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LIST OF ABBREVIATIONS

CBC	Complete blood count
EDTA	Ethylenediaminetetraacetic acid
Hb	Haemoglobin
IPF	Immature platelet fraction
ICC	Intraclass correlation coefficient
IRF	Immature reticulocyte fraction
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
PLT	Platelet
POC	Point of care
QC	Quality control
RET	Reticulocyte
RET-He	Reticulocyte haemoglobin equivalent
RCPA	The Royal College of Pathologists of Australasia
WBC	White blood count
WHO	World Health Organisation

ABSTRAK

Latar Belakang. Bagi setiap pengumpulan darah dari mana-mana penderma darah yang berpotensi, pemeriksaan hemoglobin pra-pendermaan darah diperlukan. Penyaringan penderma berpotensi adalah penting bagi memastikan produk darah adalah selamat untuk penerima dan pada masa yang sama ia adalah selamat untuk penderma darah. Pemeriksaan hemoglobin dilakukan supaya penderma darah yang menpunyai tahap hemoglobin yang tidak normal tidak dibenarkan untuk menderma. Ini adalah untuk melindungi penderma darah dari kemerosotan kesihatan dan juga untuk melindungi kualiti komponen darah yang telah dikumpul. Tujuan kajian ini adalah untuk menilai *haemoglobinometer* yang lebih baru untuk pemeriksaan hemoglobin di kalangan penderma darah di Pusat Darah Negara, Malaysia.

Kaedah. 130 penderma darah yang layak di Pusat Darah Negara, Kuala Lumpur, Malaysia telah mengambil bahagian dalam kajian ini dari 1 Mac 2015 hingga 30 September. Hemoglobin setiap penderma darah telah ditentukan oleh Compolab TM dan HemoCue HB301 melalui jari-cucuk ujian darah kapilari dan seterusnya darah vena di hantar ke makmal untuk ujian darah dengan menggunakan Sysmex XN-1000 sebagai kaedah rujukan. Compolab TM dan HemoCue HB301 juga telah dibandingkan untuk kelajuan *haemoglobinmeter*. Kestabilan *cuvettes* yang telah dibuka pada 4 dan 7 bulan juga diuji.

Keputusan. Keputusan *haemoglobin* diambil dari 130 penderma darah yang layak. Purata hemoglobin bagi untuk Compolab TM, HemoCue HB301 dan Sysmex XN-1000 hemoglobin adalah masing-masing 14.8, 14.70, 15.1 g/dL. Analisis plot Bland Altman menunjukkan Compolab TM mempunyai bias yang lebih rendah (-0.37 g / dL) berbanding HemoCue (-0.44 g / dL) dan had sempit 95% *limits of agreement* yang lebih kecil. Kedua-dua kaedah mempunyai kepekaan yang tinggi iaitu 96% untuk Compolab TM dan 100% untuk HemoCue HB301. Ujian kebolehpercayaan dengan *Intraclass correlation* terhadap Sysmex XN-1000 adalah 0.993 untuk Compolab TM dan 0.996 untuk HemoCue, oleh itu, kedua-duanya adalah dianggap selamat untuk digunakan sebagai alat saringan hemoglobin. Kepekaan untuk Compolab TM adalah tinggi tetapi HemoCue HB301 tidak boleh dikira. Compolab TM mempunyai lima subjek yang dilabel secara salah tidak layak dan satu subjek dilabel secara salah layak untuk menderma darah. HemoCue HB301 mempunyai enam subjek yang dilabel secara salah tidak layak untuk pendermaan darah. Tiada perbezaan keputusan hemoglobin yang ketara apabila membandingkan cuvettes yang dibuka selama 4 bulan dan 7 bulan dengan cuvettes baru untuk Compolab TM dan HemoCue HB301. Compolab TM adalah lebih cepat daripada HemoCue HB301 pada min 9.67 saat ($P < 0.001$).

Kesimpulan. Kedua-dua Compolab TM and HemoCue HB301 boleh digunapakai di dalam ujian saringan Hb pra-pendermaan. Prestasi Compolab TM juga adalah setanding dengan kaedah semasa, iaitu HemoCue HB301.

ABSTRACT

Background. For every blood collection from any potential blood donor, pre-transfusion haemoglobin screening is required. It is important to screen potential donors so that the blood products are safe for recipient and at the same time safe for the blood donor. Haemoglobin screening is performed so that blood donors with abnormal levels of haemoglobin are not allowed to donate. This is to protect the donor from possible deterioration of health and also to protect the quality of the collected blood component. The aim of this study was to evaluate a newer haemoglobinometer for haemoglobin screening among blood donors in National Blood Centre, Malaysia.

Methods. 130 eligible blood donors in National Blood Centre, Kuala Lumpur, Malaysia participated in this study from 1st March 2015 to 30th September. Each blood donors' Hb was determined by Compolab TM and HemoCue HB301 through finger-prick capillary blood test and subsequently laboratory venous blood test using Sysmex XN-1000 as the reference method. Compolab TM and HemoCue HB301 was also compared for haemoglobin measuring speed and stability of opened cuvettes at 4th and 7th months .

Results. The Hb results were taken from 130 eligible blood donors. The mean Hb was 14.8, 14.70, 15.1 g/dL for Compolab TM, HemoCue HB301 and Sysmex XN-1000, respectively. Bland Altman plot analysis showed Compolab TM had lower bias (-0.37 g/dL) as compared to HemoCue (-0.44 g/dL) and narrower 95% limits of agreement. Both methods for Compolab TM had high sensitivity at 96%and HemoCue HB301 at 100%. Reliability with Intraclass correlation against Sysmex

XN-1000 was 0.993 for Compolab TM and 0.996 for HemoCue, therefore, both are considered safe to be use as screening tool. Specificity for Compolab TM was high but HemoCue HB301 was not calculable. Compolab TM had five subjects which were falsely ineligible and one subject to be falsely eligible for blood donation. HemoCue HB301 had five subjects who were falsely eligible for blood donation. There were no significant differences in Hb results when comparing cuvettes that were opened for 4 months and 7 months with new cuvettes for Compolab TM and HemoCue HB301. Compolab TM was faster than HemoCue HB301 in detecting Hb reading at mean of 9.67 seconds ($P<0.001$).

Conclusion. Both Compolab TM and HemoCue HB301 were reliable in measuring Hb level among blood donors. Performance of Compolab TM was comparable to current standard practice, which using HemoCue HB301.

Chapter 1

INTRODUCTION

Overview

For every blood collection from any potential blood donor, pre-transfusion haemoglobin (Hb) screening is required. It is important to screen potential donors so that the blood products are safe for recipient and at the same time safe for the blood donor. Hb screening is performed so that blood donors with abnormal levels of Hb are not allowed to donate. This is to protect the donor from possible deterioration of health and also to protect the quality of the collected blood component (Roback *et al.,* 2011). The acceptable value for pre-donation Hb is 12.5 g/dl – 18.0 g/dl in Malaysia (Ayob, 1998).

In most blood donation settings, point of care (POC) devices are used instead of the larger haematology analyser that are present in haematology laboratories. This is because the machines are too large for transport to mobile donations sites. Capillary blood samples are commonly used in POC devices at blood donation settings for Hb screening because it is quicker and less hassle than using venous blood samples. In a study, capillary blood Hb level was higher than the venous blood levels by +0.3 g/dl but concluded that for potential blood donors, capillary or venous blood is equivalent to using a haematology analyser (Schalk *et al*., 2007). In other studies, POC devices analysing finger prick capillary blood samples are susceptible to larger differences from standard laboratory measurements which using venous

blood sample, with differences up to 1.2 to 1.8 g/dl from reference standards (Gehring *et al.*, 2002; Gómez-Simón *et al.*, 2007; Patel *et al.*, 2007). Therefore, there is a need for newer POC devices that can decrease the differences when compared with reference standards.

In the National Blood Centre (PDN) in Kuala Lumpur, the common Hb screening for pre-blood donation is measured by using finger prick sampling of capillary blood either subjectively by Copper Sulphate method or quantitatively by HemoCue HB301. While the copper sulphate method may be quick and cost effective screening tool, it can produce the highest donor acceptance despite unacceptable Hb levels for donation (Gómez-Simón *et al*., 2007). The HemoCue Blood Hb haemoglobinometer is the second line of test if the copper sulphate method shows the donor's Hb level to be below the acceptable limit of 12.5 g/dl in PDN (Khor *et al*., 2010). HemoCue has been used commercially for some years and has been reported to be reasonably reliable (Bahadur *et al.*, 2010, Seguin *et al.*, 2011). Despite HemoCue popularity worldwide, there is still conflicting reports of its accuracy (Patel *et al.*, 2013, Bahadur *et al.*, 2010). The HemoCue Blood Hb haemoglobinometer display readings at only after approximately 10 seconds which may contribute to delays in the screening process especially during mobiles and the sample volume used in each test is 10 µl. The HemoCue haemoglobinometer's cuvette has expiration date for unopened cuvettes of 2 years but when opened it is only stable for 3 months.

The CompoLab TM system in this study can measure instantly at 1-2 seconds and uses only 10 µl of blood sample volume. The cuvettes used in this system have

an expiration date of opened cuvettes of 2.5 years. Similarly, the cuvettes do not contain any reagent for processing Hb levels. This study is carried out to assess the suitability of using this system for Hb screening at blood donation settings and to find a faster, cheaper and reliable instrument as an alternative to the current haemoglobinometer system used.

Research Justification

CompoLab TM haemoglobinometer will provide rapid result and will be easy to operate. It has a shorter timeframe (1-2secs) to process results compared to the current HemoCue haemoglobinometer (approximately 10 secs) used in National Blood Centre. In comparison, the HemoCue haemoglobinometer system's cuvettes have an expiry date of 3 months after opening its packaging compared to the CompoLab TM haemoglobinometer system's cuvettes which lasts for 2.5 year even after opening its packaging.

The National Blood Centre usually deal with high volume of blood donors. Thus, the centres requires reliable, accurate, rapid and cost effective portable haemoglobinometer for Hb level screening. This study is to justify the possible savings in costs and time of the CompoLab TM haemoglobinometer system compared to the HemoCue haemoglobinometer system.

Research Objectives

1.1.1 **General Objective**

To compare the CompoLab TM system to HemoCue haemoglobinometer system as a Hb screening tool in blood donation setting.

1.1.2 **Specific Objectives**

i. To determine the reliability of CompoLab TM haemoglobinometer system and HemoCue haemoglobinometer system in measuring Hb level among blood donors against the gold standard method (Sodium lauryl sulphate Method).

ii. To compare the measuring speed of CompoLab TM haemoglobinometer system in measuring Hb level among blood donor against HemoCue.

iii. To determine the cuvette time stability of CompoLab TM and HemoCue HB301 haemoglobinometer system in measuring Hb level among blood.

Hypothesis

i. CompoLab TM haemoglobinometer system and HemoCue are reliable in measuring Hb level among blood donors against the gold standard method (Sodium lauryl sulphate Method).

ii. CompoLab TM haemoglobinometer system is as reliable as HemoCue in measuring Hb level among blood donors.

iii. CompoLab TM haemoglobinometer system is faster than HemoCue in measuring Hb level among blood donors.

iv. CompoLab TM haemoglobinometer system has equally good time stable cuvettes than HemoCue in measuring Hb level among blood donors.

Study Endpoints

Primary Endpoint: To determine the reliability of the CompoLab TM haemoglobinometer system compared to the HemoCue haemoglobinometer system when testing donors blood haemoglobin level.

Chapter 2

LITERATURE REVIEW

Introduction

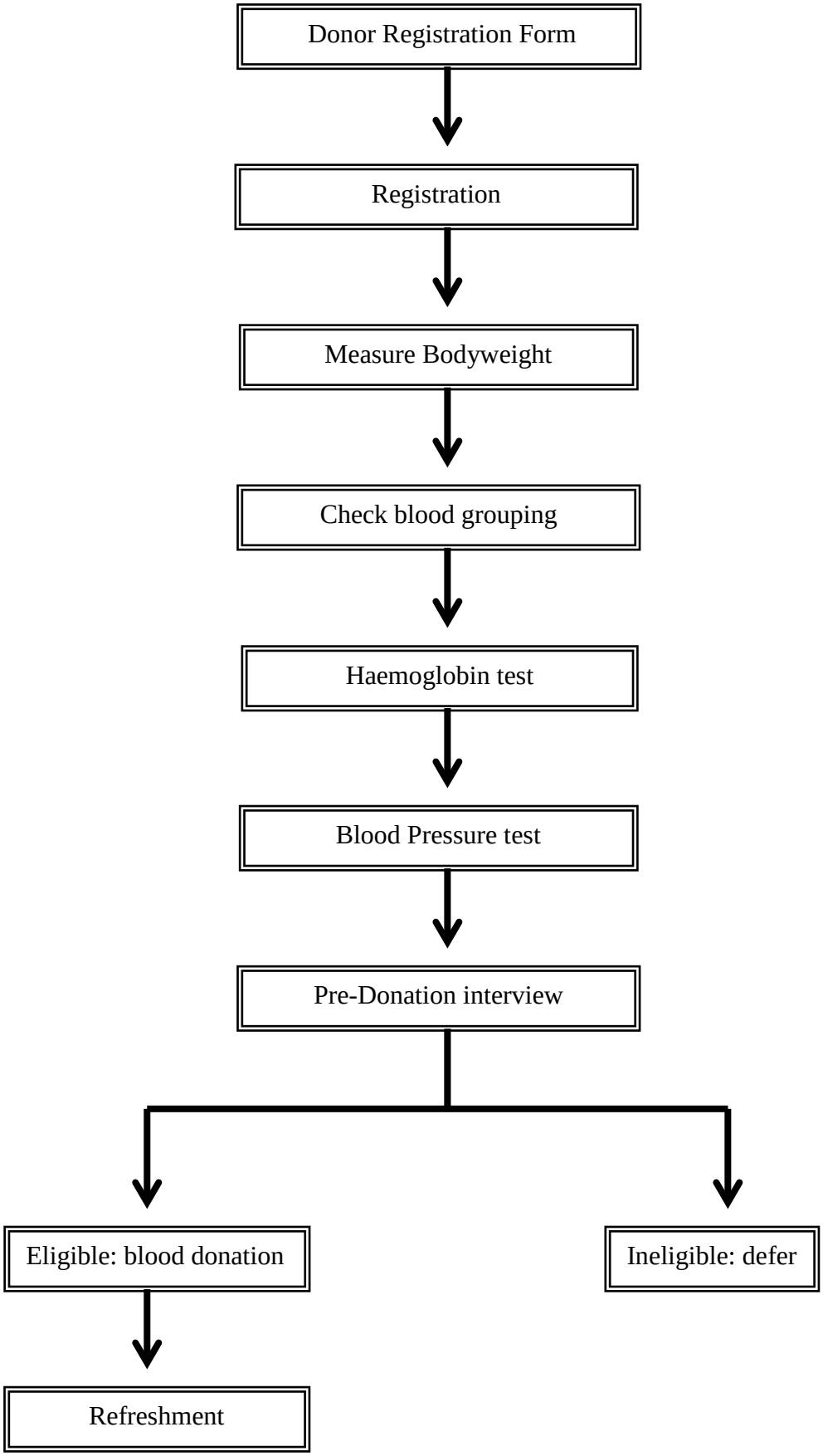
Blood transfusion has its place in modern medicine and over time it has become indispensible. Blood product usage has increase in modern medicine partly due to the increasing population and also the rise of accidents that require blood transfusion. Surgeries and diseases that require blood transfusion support pushes blood transfusion services to cope with the increasing demand of blood(Abolghasemi *et al.*, 2010). The blood transfusion service needs to retain as much as possible existing regular blood donors and recruit new blood donors. Blood transfusion services are also required to look after the health of their blood donors by monitoring their iron storage status especially for long term regular donors(Lynch *et al*, 2004). Blood transfusion service should have proper protocols adhered for vigilance towards their blood products to ensure safe and quality products are produced.

Blood Donation Process

2.1.1 **Donor Criteria**

Blood donors are required to fulfil blood donor criteria based on the blood donors' medical history, travel history, sexual history, family history, general examination, blood pressure and body weight. Another important aspect is that the blood donors must not be remunerated for their blood donation as stated by the World Health Organisation (WHO). This is to ensure blood donors are safe donors(Ayob,1998).

2.1.2 **Work flow for whole blood donation**



Deferral of blood donors

Based on the donor criteria, a blood donor can be deferred temporarily or pemanently. Examples of the cause of temporary deferral are like low haemoglobin level , high or low blood pressure, recent infection or lack of sleep. When the blood donor is temporarily deferred, the deferment period can be from one day to a few years depending on the reason of the deferral. Low haemoglobin value is the most common cause of blood donor deferral worldwide, particularly among women (Goncalez *et al.*, 2013; Ngoma *et al.*, 2013; Popovsky, 2012; Prados Madrona *et al.*, 2014; Smith *et al.*, 2013). Low haemoglobin is also the main cause of temporary blood donor deferral in National Blood Centre Kuala Lumpur followed by high blood pressure (BP), blood donors on medication, and low blood pressure. Each year around ten thousand of blood donor, estimated five to six percent of total blood donors were deferred due to low Hb.(Donor Procurement unit, 2014a)

When a blood donor is permanently deferred it means the deferred donor can no longer participate in blood donation for life. Examples of permanent deferral are blood donors who are at high risk of contracting sexually transmitted diseases(STI), intravenous drug users(IVDU), family history of hepatitis B infection or history of epilepsy(Ayob,1998).

In Malaysia, blood donors should have the minimum requirement for Hb level of 12.5 g/dL for male and female(Ayob,1998). Some countries might have different minimum requirements, for example 12.5 g/dL for female and 13.5 g/dL in Europe, while in Japan 12.0 g/dL for 200 mL blood bag and 12.5 g/dL for 400 mL blood bag but in the USA the minimum Hb level is similar to Malaysia (Gómez-Simón *et al.*, 2007).

Return to Donate

Blood donor deferral may discourage donors to return for blood donation. Deferral due to low Hb can cause the loss of potential blood donors and also lead to shortage of blood supply (Hillgrove *et al.*, 2011). This would most likely occur to first time donors as compared to the regular and repeat donors. There was a 5 year follow-up study that showed that 25 percent of first time blood donors who were temporarily deferred returned to donate and 47 percent first time eligible donors returned. The study also showed that repeat donors who were previously temporarily deferred had 81 percent return to donate whereas those who previously eligible donors 86 percent return to donate (Custer *et al.*, 2007). The study showed the importance of repeat donors who were more likely to donate and other factors like age, sex, race, and education level were linked with the return of donors (Custer *et al.*, 2007; Custer *et al.*, 2011; Zou *et al.*, 2008). A local study done at National Blood Centre showed that only 26.6% of the blood donors returned to donate within 7 months after being deferred (Siti Suzaina, 2013). The study also showed that repeat donors were more likely to return to donate with a return percentage of 90%. The reasons were some of the blood donors were afraid of being re-deferred (17.6%), too busy to donate (30.1%), pregnancy and having new medical illness.

Adverse Donor Reaction

The blood donation process is usually uneventful and rarely blood donors will suffer any adverse reaction due to blood donation but occasionally there would be exceptions. Data from donor vigilance National Blood Centre showed that vasovagal reaction is the most common adverse donor reaction. 171 donors suffered from vasovagal reaction in the first 6 month of 2013 and rose to 383 for the same period in 2014. The affected blood donors in 2013 who returned for blood donation was only 20.5% until end of October 2014 (Donor Procurement Unit, 2014b). Fear may be one of the commonest reasons that leads to adverse donor reaction (Oswalt, 1977). With the sight of blood, fear of needles or the fear of pain are few causes that may cause vasovagal reaction. The reaction may occur before venepuncture and others may have delayed presentation. Vasovagal reaction due to hypotension post blood donation is also one of the commonest reason. The adverse reactions can range from mild vasovagal reaction like light headedness, pallor and sweating to severe form of reaction like convulsive syncope and loss of consciousness. These can lead to serious accidental injuries like head injuries from fall or motor vehicle accidents if the blood donor was operating a vehicle when vasovagal attack occurs. Younger age, female gender, and first time donation are known risk factor of adverse donor reaction (Goldman *et al.*, 2013). Optimizing water intake has been suggested in a study to mitigate vasovagal reaction in which the blood donor was requested to ingest 500 ml of water prior to blood donation (Wieling *et al.*, 2011). It was noted that after drinking water, the healthy young subjects had improved orthostatic tolerance. The study also suggested 250 ml of sports drink or salt supplementation in oral fluids prior to blood donation will also mitigate adverse vasovagal reactions.

Haemoglobin

Haemoglobin is the most important and also the most abundant molecule in the red blood cells, approximately about 270,000,000 Hb molecules per red blood cells. It consists of two pairs of polypeptide chains (globins) and four-heme molecules with a ferrous iron inside each. It main function is as a carrier for oxygen (O_2) and carbon dioxide (CO_2). Each gram of Hb can hold up to maximum of 1.34 mL of O_2 (McPherson and Pincus, 2011). Since the O_2 is the vital component of human life, the measurement of O_2 and its carrier, which is Hb are the most routine laboratory investigations in healthcare systems. The Hb is measured in gram per deciliter (g/dL). Table 2.1 showed the variation of Hb according to gender in a multiethnic population.

Parameter	Mean	Median	Reference interval (mean±1.96SD)
Haemoglobin (g/dL)			
Males <60	15.45	15.5	13.5–17.4
Males >60	14.36	14.3	11.8–16.9
Females	13.33	13.3	11.6–15.1
RBC Count (10^{12}/L)			
Males <60	5.24	5.23	4.53–5.95
Males >60	4.74	4.75	3.86–5.62
Females	4.54	4.55	3.87–5.21
Haematocrit (%)			
Males <60	45.3	45.5	40.1–50.6
Males >60	42.3	42.2	35.7–48.9
Females	40	40	35.1–44.9

Table 2.1 Haematological parameters of a multiethnic population.(Ambayya *et al.*, 2014)

Principle of Haemoglobin Measurement

International Council for Standardisation in Haematology (ICSH) has determined a few methods of measuring haemoglobin concentrations. The haemiglobincyanide (HiCN) method was approved in 1995 as the reference method for haemoglobinometry in human blood. The HiCN method is currently the gold standard reference for Hb value measurement. Any new methods will need to compare with the HiCN method as the reference Hb value. (Zwart *et al.*, 1996)

HiCN (cyanmethhaemoglobin) is converted from haemoglobin lysed through a chemical process using reagents; potassium ferricyanide and potassium cyanide (Drabkin's reagent). Haemoglobin is converted into methaemoglobin by the oxidizing process of potassium ferricyanide. HiCN is formed after the Hi binds to cyanide ions from potassium cyanide. HiCN is a form of Hb pigment that can be measured by using a spectrophotometer. Examples of Hb pigments that can be measured with a spectrophotometer are shown in Figure 2.1 and Table 2.2. HiCN has a broad absorption maximum wavelength at about 540 nm which minimizes bias error. The HiCN method has a stable standard solution which is stable up to 15 years and approved by ICSH as a standard international reference for Hb concentration level. The HiCN method is able to measure other forms of Hb pigments like Oxyhaemoglobin (HbO_2), Methaemoglobin (Hi), and Carboxyhaemoglobin (HbCO) but not Sulfhaemoglobin (SHb) (Lewis *et al.*, 1991; McPherson and Pincus, 2011).

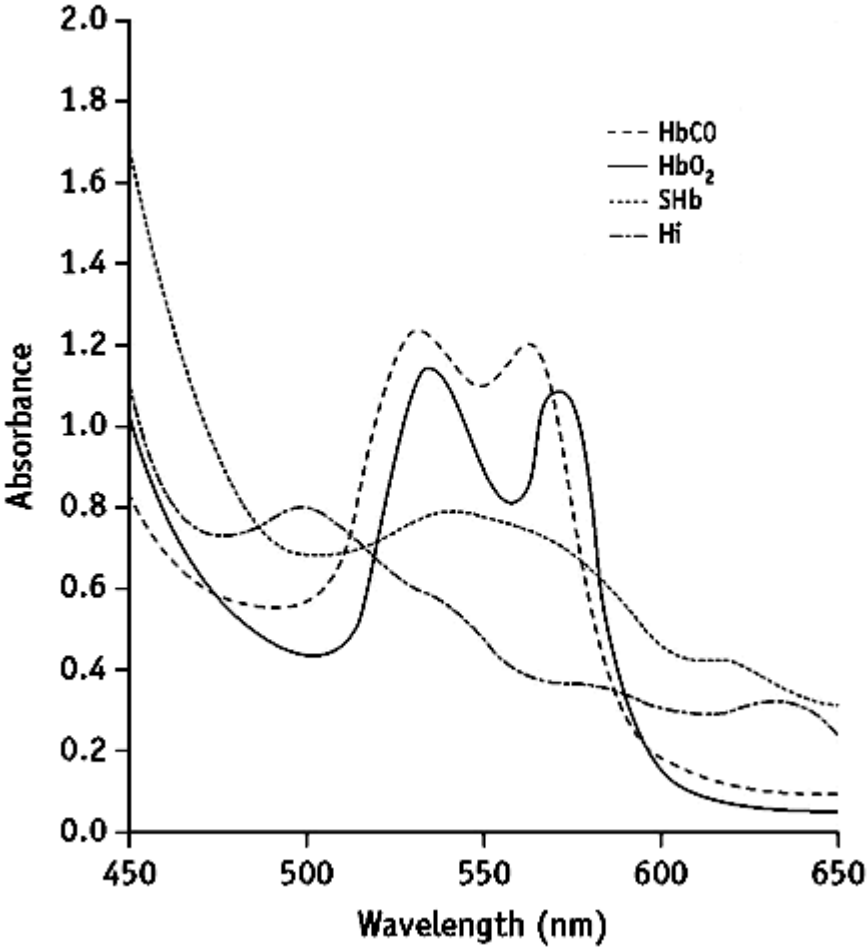


Figure 2.1 Absorption spectra of different form of Haemoglobin pigments. HbCO, carboxyhaemoglobin; HbO_2, oxyhaemoglobin; SHb, sulphaemoglobin; Hi, methaemoglobin (Dacie and Lewis, 2012)

Positions in spectrum for optimal absorbance of haemoglobin derivatives in absorption spectrometry (in nm)

Oxyhaemoglobin	542, 577
Deoxygenated haemoglobin	431, 556
Carboxyhaemoglobin	538, 568
Methaemoglobin	500, 630
Sulphaemoglobin	620
Methaemalbumin	624
Haemochromogen (Schumm's test)	558

Some approximations where slightly different figures have been reported in different studies.

Table 2.2 Absorption spectra of different form of Haemoglobin pigments. (Dacie and Lewis, 2012)

Formation of oxyhaemoglobin (HbO_2) occurs when each heme group is bound with one molecule of O_2. Methaemoglobin (Hi) is formed when ferrous iron in the heme group is in the ferric iron state, restricting methaemoglobin to bind with O_2. Formation of Carboxyhaemoglobin (HbCO) occurs when Hb binds with carbon monoxide (CO) and this form of haemoglobin is unable to bind with O_2 due to CO affinity is 200 times greater than O_2 toward Hb, which impairs the oxygen carrying capacity of Hb. Sulphaemoglobin (SHb) is formed during oxidative hemolysis and contained a mixture of partially denatured Hb (McPherson and Pincus, 2011).

Unfortunately, the reagents that the HiCN methods use contains potassium cyanide and it is toxic to the environment and laboratory personnel especially at high concentration (Shah *et al.*, 2011). The safe disposal of this reagent is troublesome especially when large volumes of laboratory waste contains cyanide. There are alternative non-hazardous reagents available like sodium azide, sodium lauryl

sulphate, imidazole, sodium dodecyl sulphate and dimethyl lauryl amine oxide which are safer and do not contain hazardous waste. These reagents have been validated and are used in laboratories (Lewis *et al.*, 1991). The reagents work almost similarly to the HiCN method; for example sodium lauryl sulphate Method (SLS) will convert the haemoglobin to haemiglobinsulphate almost immediately and the absorbance is read by using spectrophotometer at 534nm maximum absorbance wavelength. It is stable for several days without significant effect of storage. Its reliability is equal to that of haemiglobincyanide method with routine blood specimens, slightly more reliable when there is interference by leucocytosis or lipaemia. There is no significant difference in measurements on samples containing Hb F. It measures methaemoglobin but fails to measure sulphaemoglobin. It has a major advantage in that the reagent is a non-hazardous compound(Lewis *et al.*, 1991). The oxyhaemoglobin (HbO_2) method is the fastest method among all the other methods. The dilution reagent contains ammonia and the absorbance of the final solution is read by spectrophotometer at 540 nm wavelength (Barbara *et al.*, 2011). The disadvantage of current alternatives to HiCN method is the unavailability of stable standards; therefore, the calibration is done by using HiCN reference solutions(Zwart *et al.*, 1996).

Most of the automated haematology analysers are using the above-mentioned method. It produces accurate and reliable Hb value. Sysmex XN-1000 that was used in this research measure the haemoglobin value by the Sodium lauryl sulphate (SLS) method.

It is common that point of care (POC) haemoglobinometers uses spectrophotometry in haemoglobin concentration measurements. The haemoglobinometer uses a factory pre-calibrated spectrophotometer that is based on ICSH HiCN standard. The HemoCue 201 (HemoCue AB, Angelsholm, Sweden) for example uses the direct spectrophotometry method and uses cuvette that has been pre-filled with reagent (sodium nitrite and sodium azide). It converts haemoglobin into azidemethaemoglobin, and the spectrophotometer measures the absorbance wavelength of the azidemethaemoglobin at 565 and 880 nm. The disadvantage of HemoCue 201 is the disposable reagent pre-filled cuvettes are expensive and the reagent in the cuvettes deteriorates under suboptimal environmental conditions(Morris *et al.*, 2007).

In this study, HemoCue HB301 uses the Hb/HbO_2 isobestic point absorbance method that utilizes reagent-free microcuvettes. This microcuvettes is quite robust as it can be stored unopened at 10 – 40 °C until expiration date; short-term storage (six weeks) at -18 – 50 °C; and 3 months open vial stability based on a previous study(Morris *et al.*, 2007) but latest specification from HemoCue America shows stability up to 12 months(HemoCue America, 2014). The analyzer uses a double wavelength measuring method at 506 nm and 880 nm(Morris *et al.*, 2007). As for Compolab TM, it is calibrated according to ICSH Photometric method with broad spectrum wavelengths and also uses the similar Hb/HbO_2 isobestic point absorbance method. It measures Hb level in less than 2 seconds and has a measuring rage of 0.3-25.5 g/dL. Its cuvettes are reagent-free and can be stored at 0 - 50 °C up to 2.5 years or -30 - 70°C up to 24 hours even after the cuvette bag has been opened(Fresinius,2013).

In poor countries there is a much cheaper methods of measuring haemoglobin. The Haemoglobin Colour Scale (HCS) which is licensed by the World Health Organization (WHO) was released to the public in 2001 and each test only cost less than USD0.05. The Hb value is derived by comparison of the colour of a drop of blood with the set of printed colour shades representing Hb values between 4 to 14 g/dL with 2 g/dL increments (World Health Organization, 2004). The only disadvantage is that is when reading the haemoglobin result, errors can occur due to poor lighting and then the test requires time for the blood to dry out on the test paper matrix before it can be read. There was a study which used HCS as one of its haemoglobin screening methods and it showed 25.2% incorrect haemoglobin estimation (Tondon *et al.*, 2009).

Copper Sulphate solution ($CuSO_4$) is another low cost method to measure Hb concentration by the gravimetry principle and then determines the haemoglobin level semi-quantitatively (Philipps *et al.*, 1950). With the known specific gravity for the solution, the $CuSO_4$ solution can be prepared in house at the blood transfusion service centre. If the $CuSO_4$ is prepared with a specific gravity of 1.053, it is able to measure haemoglobin level which is equivalent to the value of 12.5 g/dL. A drop of whole blood is dropped into the solution and then the $CuSO_4$ will form a layer of copper proteinate around the drop of blood. This will maintain the drop of blood's specific gravity for 15-20 seconds. The haemoglobin value is known by observing whether the drop of blood floats or sinks in the solution. If the drop of blood sinks, the haemoglobin level is more than 12.5 g/dL . If it stays afloat then the haemoglobin level is either at 12.5 g/dL or less. This method is commonly used in blood donation mobiles or centres because it is cheap, relatively easy to perform and

provide rapid results. Unfortunately, this method requires proper technical knowledge to prepare the solution. There is a very narrow margin of error when preparing the solution, and any error can give a wrong results. Blood donation service centres must be careful when using this method at their centres (Deb *et al.*, 2002). If a blood donor is tested with the $CuSO_4$ method and fails, they will usually undergo a second test with a POC haemoglobinometer like the HemoCue (James *et al.*, 2003).

There are other newer alternative technology which may help to reduce cost of pre-donation haemoglobin screening which is by non-invasive haemoglobin screening method. This method is similar to the principle of pulse oximeter that measures the Oxygen saturation in the blood (SpO_2). These non-invasive haemoglobinometers do not require cuvettes or the need for finger pricking equipment. However, these haemoglobinometer requires a large initial cost outlay and may be prohibitive to poorer blood transfusion centres. The operating costs for predonation screening can be reduced if the centres are able to bear the initial costs. There are three non-invasive haemoglobinometer devices that are currently available in the market. The three devices are Haemospect (MBR Optical Systems GmbH & Co. KG, Germany), NBM-200 (OrSense, Israel) and products from Masimo, USA; Radical-7, Rad-87, Rad-57, Pronto-7 and Pronto. The non-invasive haemoglobinometers are based on transcutaneous reflection spectroscopy method. It projects a white light into the skin and underlying tissues, then the reflected transmission data will be captured and analysed. Some use an occlusion spectrophotometer-based haemoglobinometer monitor which create blood dynamics in which will generate an optical signal to analyse haemoglobin value.

Blood Sampling for Haemoglobin Measurement Test

Capillary blood sample for haemoglobin (Hb) determination is preferred than venous blood sample in blood donation setting due to ease of sample collection. A study regarding the use of capillary blood count parameters in adults showed capillary Hb was higher than the venous values (+0.30 g/dL), however they concluded that for potential blood donors, Hb screening is equivalent with either capillary or venous blood using a haematology analyser (Schalk *et al.*, 2007). During the predonation Hb screening, if there is a need for a repeat test from the same fingerpick due to borderline prerequisite blood donation Hb level; a faster point of care (POC) haemoglobinometer would be better to prevent the process of haemostasis from affecting the Hb level result.

Haemoglobin Measurement Devices

In blood donation setting, Point of Care (POC) devices is used instead of automated haematology analyser due to mobility factor as the latter is too big and heavy whereas the former is small and light handheld device. Apart from that, the cost and maintenance of POC devices is much cheaper compare to the automated haematology analyser. POC devices usually have reduced accuracy when compared with haematology analyzer and some haemoglobinometers require quality control reagent however, with newer technologies coming into the market, this has changed. Several studies show that results from POC devices which analysed finger prick capillary blood when compared to results from standard laboratory measurements