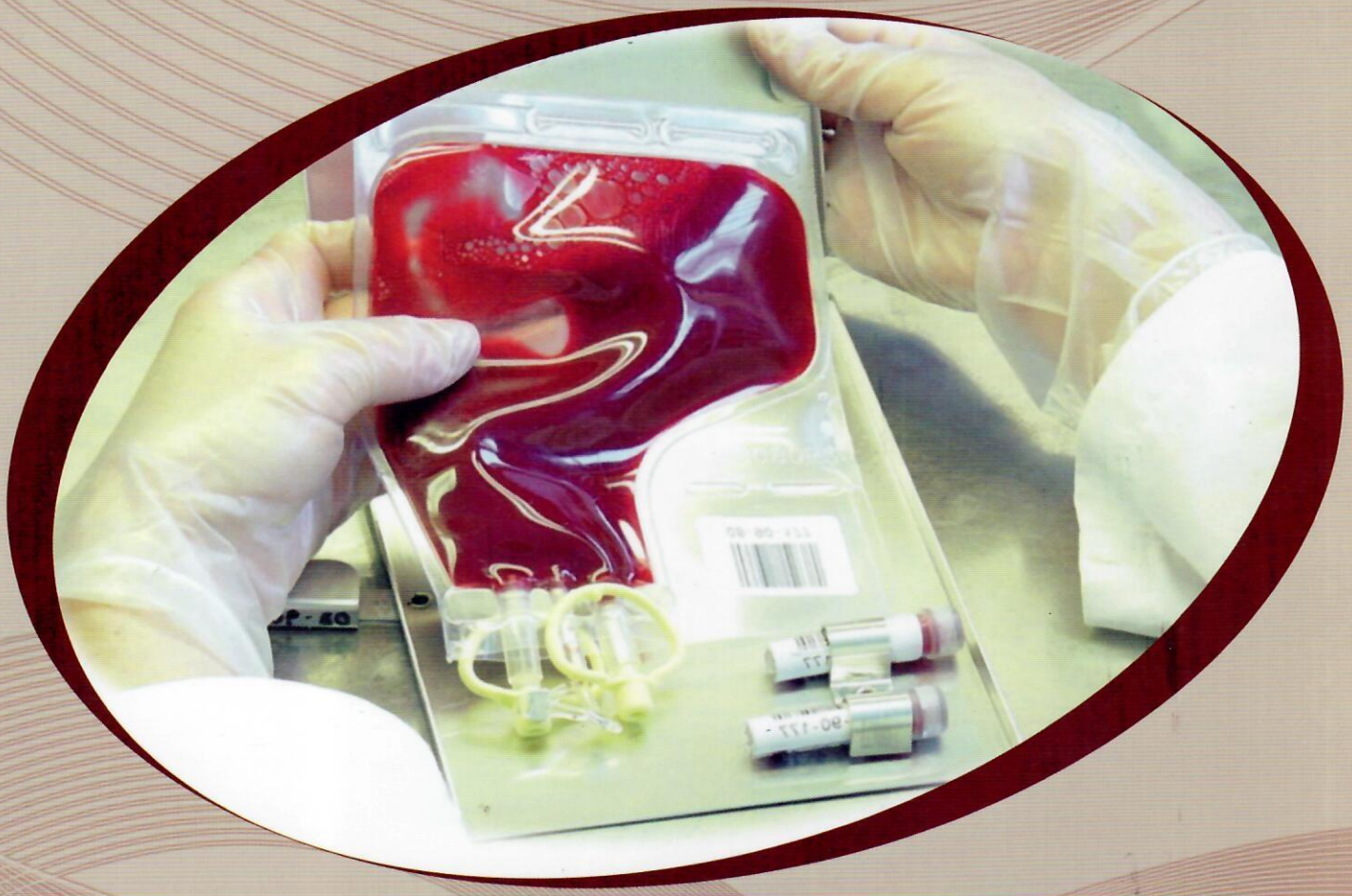
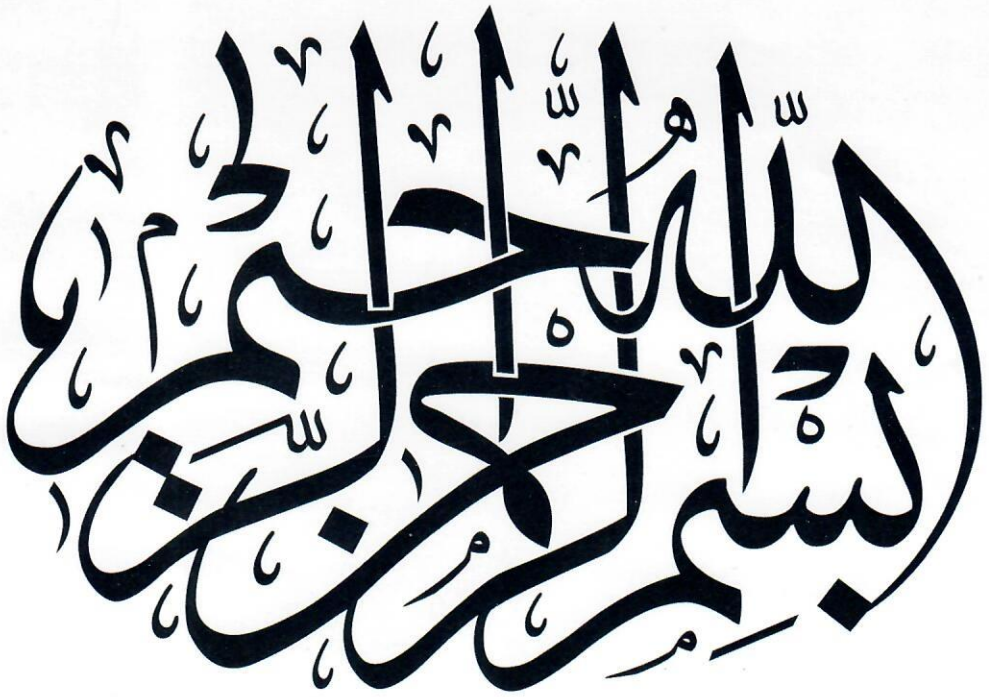
وزارة الصحة
Ministry of Health

الهيئة العربية لخدمات نقل الدم

The Unified Practical Procedure Manual For Blood Banks in The Arab Countries



Kingdom of Saudi Arabia
Riyadh
1434 - 2013


بسم الله الرحمن الرحيم

Kingdom of Saudi Arabia
Ministry of Health
General Administration of Laboratories and Blood Banks

The Unified Practical Procedure Manual

For Blood Banks in The Arab Countries

Kingdom of Saudi Arabia
1434 هـ - 2013م

In the name of God the Merciful

Introduction:

Praise be to Allah and peace and blessings be upon His Prophets and Messengers, our master Muhammad and his family and companions. .

The rapid development of the world in all fields of life and specially the most important The health requirements as an important role in human life. The blood transfusion services in the Arab world has been included by necessaity to this development to provide safe and sufficient blood and blood products to all the patients.This can be accomplished by the promotion of voluntary non rumenerated blood donors , proper blood testing for Transfusion Transmitted Diseases, adopting the latest methods for separating blood components and the filteration of the cellular blood components from the white blood cells that cause many complications as a result of blood transfusions, as well as rational use of blood and its components for patients in the hospitals and the application of the quality control methods on all the blood banks procedures. Therefore, it was necessary to have a scientific references to be a guide for those working in blood banks when they need to apply all of the above and other procedures in blood transfusion field .Therefor The Arab Association for Blood Transfusion Services sought to issue a Guide for Procedures Manual for performing The blood bank procedures and applying the quality control for all blood bank issues in all the Arab countries.

I hope to achieve the purpose for which this guide has been prepared for it, with sincere thanks and appreciation to all who contributed and cooperated in the preparation of this guide, and for all I wish the progress and success.

GOD Bless all of you,,,,

Your Brother

Dr. / Ali Bin Saad Alshamari

Director General of laboratories and blood banks,

Ministry of Health

Kingdom of Saudi Arabia

Riyadh, 1434 H – 2013

Contents

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Main Sources and References of The Unified Arab B. B. Practical Procedure Manual :-

1- Practical Procedure Manual of Blood Banks, MOH, KSA, 2005 and The Unified Arab B.B. Practical Procedure Manual, 2010

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PART ONE : Blood Donation and Collection

CHAPTER ONE: BLOOD DONATION PROCESS

1-Blood Donor Educational Materials:

a-MAKING YOUR BLOOD DONATION SAFE

Thank you for coming in today! This information sheet explains how you can help us make the donation process safe for yourself and patients who might receive your blood.

PLEASE READ THIS INFORMATION BEFORE YOU DONATE! If you have any questions now or anytime during the screening process, please ask blood center staff.

ACCURACY AND HONESTY ARE ESSENTIAL!

Your complete honesty in answering all questions is very important for the safety of patients who receive your blood. All information you provide is confidential.

DONATION PROCESS: To determine if you are eligible to donate we will:

-Ask questions about health, travel, and medicines

-Ask questions to see if you might be at risk for hepatitis, HIV, or AIDs

-Take your blood pressure, temperature and pulse

- Take a small blood sample to make sure you are not anemic

If you are able to donate we will:

- Cleanse your arm with an antiseptic. (If you are allergic to Iodine, please tell us!)

-Use a new, sterile, disposable needle to collect your blood

DONOR ELIGIBILITY – SPECIFIC INFORMATION Why we ask questions about sexual contact:

Sexual contact may cause contagious diseases like HIV to get into the bloodstream and be spread through transfusions to someone else.

Definition of "sexual contact":

The words "have sexual contact with" and "sex" are used in some of the questions we will ask you, and apply to any of the activities below, whether or not a condom or other protection was used:

1. Vaginal sex (contact between penis and vagina)

2. Oral sex (mouth or tongue on someone's vagina, penis, or anus)

3. Anal sex (contact between penis and anus)

HIV/AIDS RISK BEHAVIORS AND SYMPTOMS

AIDS is caused by HIV. HIV is spread mainly through sexual contact with an infected person OR by sharing needles or syringes used for injecting drugs.

DO NOT DONATE IF YOU:

-Have AIDS or have ever had a positive HIV test

-Have ever used needles to take drugs, steroids, or anything not prescribed by your doctor

Are a male who has had sexual contact with another male, even once, since 1977

Have ever taken money, drugs or other payment for sex since 1977

Have had sexual contact in the past 12 months with anyone described above

-Have had syphilis or gonorrhea in the past 12 months

-In the last 12 months have been in juvenile detention, lockup, jail or prison for more than 72 hours

-Have any of the following conditions that can be signs or symptoms of HIV/AIDS:

- **Unexplained weight loss or night sweats**
- **Blue or purple spots in your mouth or skin**
- **Swollen lymph nodes for more than one month**
- **White spots or unusual sores in your mouth**
- **Cough that won't go away or shortness of breath**
- **Diarrhea that won't go away**

b-MEDICATION DEFERRAL LIST

Please tell us if you are now taking or if you have EVER taken any of these medications:

-Proscar© (finasteride) – usually given for prostate gland enlargement
-Avodart© (dutasteride) – usually given for prostate enlargement
-Propecia© (finasteride) – usually given for baldness
-Accutane© (Amnesteem, Claravis, Sotret, isotretinoin) – usually given for severe acne
Soriatane© (acitretin) – usually given for severe psoriasis
-Tegison© (etretinate) – usually given for severe psoriasis
-Growth Hormone from Human Pituitary Glands – used usually for children with delayed or impaired growth
-Insulin from Cows (Bovine, or Beef, Insulin) – used to treat diabetes
-Hepatitis B Immune Globulin – given following an exposure to hepatitis B.

NOTE: This is different from the hepatitis B vaccine which is a series of 3 injections given over a 6 month period to prevent future infection from exposures to hepatitis B.

-Unlicensed Vaccine – usually associated with a research protocol

IF YOU WOULD LIKE TO KNOW WHY THESE MEDICINES AFFECT YOU AS A BLOOD DONOR, PLEASE KEEP READING:

- **If you have taken or are taking Proscar, Avodart, Propecia, Accutane, Soriatane, or Tegison, these medications can cause birth defects. Your donated blood could contain high enough levels to damage the unborn baby if transfused to a pregnant woman. Once the medication has been cleared from your blood, you may donate again. Following the last dose, the deferral period is one month Proscar, Propecia and Accutane, six months for Avodart and three years for Soriatane. Tegison is a permanent deferral.**
- **Growth hormone from human pituitary glands was prescribed for children with delayed or impaired growth. The hormone was obtained from human pituitary glands, which are found in the brain. Some people who took this hormone developed a rare nervous system condition called Creutzfeldt-Jakob Disease (CJD, for short). The deferral is permanent.**
- **Insulin from cows (bovine, or beef, insulin)** is an injected material used to treat diabetes. If **this insulin was imported into the US from countries in which "Mad Cow Disease" has been found, it could contain material from infected cattle. There is concern that "Mad Cow Disease" is transmitted by transfusion. The deferral is indefinite.**
- **Hepatitis B Immune Globulin (HBIG) is an injected material used to prevent infection following an exposure to hepatitis B. HBIG does not prevent hepatitis B infection in every case, therefore persons who have received HBIG must wait 12 months to donate blood to be sure they were not infected since hepatitis B can be transmitted through transfusion to a patient.**
- **Unlicensed Vaccine is usually associated with a research protocol and the effect on blood transmission is unknown. Deferral is one year unless otherwise indicated by Medical Director.**

2- BLOOD DONOR HISTORY QUESTIONNAIRE FORM
(Confidential)

NAME:.....

NATIONALITY:..... **AGE:**.....

I.D. NUMBER:..... **DATE OF ISSUE:** / /

PLACE OF ISSUE:.....

ADDRESS:..... **TEL. NO.** .....

MOBILE NO:.....

DONATION TYPE:

☐ **VOLUNTARY** ☐ **REPLACEMENT TO:**.....

☐**OTHERS**.....

Weight	Hgb	Temp	Pulse	BP	Detail Code	General appearance OK? Yes ☐ NO ☐ Arm inspection Right ☐ Left ☐	Interviewer ID

Phlebotomist ID	DHQ clerical Check OK? Yes ☐ NO ☐	Time start	Bag weight	Comments: ☐ Slow bleed ☐ Aspirin ☐ Relative ☐ Other.....	Reviewed by
Donor Photo ID Checked ☐	Visual inspection of bag OK? Yes ☐ NO ☐	Time end	Sealed by: ID:		Date

ATTENTION:	We are required by regulations, and for the safety of our patients to ask the following questions. They are not meant to be personal or offensive. If you do not feel that you are willing to answer them, please hand this paper to the Donor Center staff . All answers are confidential.

Please mark with (✓) where applies	Yes	NO
Are you:		
1-Feeling healthy and well today? And have you had a good sleep?		
2-Currently taking an antibiotic?		
3- Currently taking any other medication for an infection?		
4- Are you now taking or have you ever taken any of the following medications:Proscar , Avodart , Propeia , Accutane , Soriatane , Tegison , Growth Hormone , Hepatitis B Immune Globulin?		
5- Have you read the educational materials and have your questions been answered?		
In the past 48 hours:		
6- Have you taken aspirin or any medication that has aspirin in it?		
7- Female donors: Have you been pregnant or are you pregnant now? (Check if you are male") ☐ I am male		
In the past 8 weeks have you:		
8- Donated blood , platelets or plasma?	Yes	NO

9- Had any vaccinations or other shots?		
10- Had contact with someone who had a smallpox vaccination?		
In the past 16 weeks:		
11- Have you donated a double unit of red cells using an apheresis machine?		
In the past 12 months have you:		
12- Had a transplant such as organ, tissue, or bone marrow?		
13- Had a graft such as bone or skin?		
14- Come into contact with someone else`s blood?		
15- Had an accidental needle-stick?		
16- Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?		
17- Had sexual contact with a prostitute or anyone who takes money or drugs or other payment for sex?		
18- Had sexual contact with anyone who has ever used needles to take drugs or steroids , or anything not prescribed by their doctor?		
19- Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?		
20- Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Check if you are male") ☐ I am male		
21- Had sexual contact with a person who has hepatitis?		
22- Lived with a person who has hepatitis?		
23- Had a tattoo, ear, or body piercing?		
24- Had or been treated for syphilis or gonorrhea?		
25- Been in juvenile detention, lockup, jail, or prison for more than 72 hours?		
26- Performed Hijama (Blood Letting)?		
In the past 3 years have you:		
27- Been outside Saudi Arabia?		
28- Had malaria or visited a country with endemic malaria?		
From 1980 through 1996:		
29- Did you spend time that adds up to three (3) months or more in the United Kingdom? (Review list of countries in the United Kingdom)		
From 1980 to the present , did you		
30- Spend time that adds up to five (5) years or more in Europe? (Review list of countries in Europe)		
31- Receive a blood transfusion in the United Kingdom or France? (Review list of countries in the United Kingdom)		
From 1977 to the present , have you:		
32- Received money, drugs, or other payment for sex?		
33- Male donors: had sexual contact with another male, even once? (Check if you are female") ☐ I am female		
Have you EVER:		
34- Had a blood transfusion?		
35- Had a positive test for the HIV/AIDS virus?		
36- Used needles to take drugs, steroids, or anything not prescribed by your doctor?		
37- Used clotting factor concentrates or Insulin?		

38- Had hepatitis?		
39- Had Chagas , babesiosis or Brucellosis?		
40- Received a dura mater (or brain covering) graft?		
41- Had any type of cancer , including leukemia?		
42- Had any problems with your heart or lungs?		
43- Had a bleeding condition or a blood disease?		
44- Had sexual contact with anyone who was born in or lived in Africa?		
45- Been in Africa?		
46- Have any of your relatives had Creutzfeldt-Jakob disease?		
47- Are you donating blood for a family member who may receive blood products from?		
48- Have you ever given blood under a different name or I.D. number?		
49- Have you ever been refused as a blood donor or told not to donate blood?		
50- Have you ever requested that your donated blood would not be given to patients?		
51- Are you giving blood to reduce your Hemoglobin level?		
52- Are you giving blood to be tested for AIDS or any other blood tests?		

Donor Consent

I have read and understand the information provided to me regarding the spread of the AIDS virus (HIV) by blood and plasma. If I am potentially at risk of spreading the virus known to cause AIDS , I agree NOT to donate blood or plasma for transfusion to another person or for further manufacture. I understand that my blood will be tested for HIV and other disease markers. If this testing that I should no longer donate blood or plasma because of the risk of transmitting the AIDS virus , my name will be entered on a list of permanently deferred donors. I understand that I will be notified of positive laboratory test result (s). If instead , the results of the testing are not clearly negative or positive , my blood will not be used and my name may be placed on a deferral list without me being informed until the results are further clarified. I have been informed of the possibilities for withdrawal from the blood donation process AT ANY TIME before , during , or after the donation process if I believe that my blood is not suitable for transfusion. I have been informed that I may complete the donation process , but not have my blood transfused to a patient by selecting the "Use my blood for research only" box if I believe that my blood is not suitable for transfusion. I have been informed that there are circumstances in which infectious diseases tests may not be performed. I have been informed that in some circumstances in which infectious diseases tests may not be performed. I have bee informed that in some circumstances the blood may be transfused before all tests for infectious diseases are completed , and there may be circumstances in which in infectious disease tests are not performed at all before transfusing. I hereby grant permission to the Blood Bank to draw approximately 450 mL of whole blood , or perform Apheesis procedure , and the blood product may be used in such manner as the Blood Bank may deem desirable. The medical history I have furnished to the interviewer is true and accurate to the best of my knowledge

Use my Blood for Research ONLY, and do not transfuse to Patients ☐

Name:..... Signature:.....

Date:.....

إستبيان التبرع بالدم

العمر:..... الجنسية:..... الاسم:.....

تاريخها: / / هـ مصدرها:..... رقم بطاقة الأحوال:.....

أخرى:..... □ لمريض: اسم المريض/.....□ لوجه الله تعالى□الغرض من التبرع

Weight	Hgb	Temp	Pulse	BP	Detail Code	General appearance OK? Yes □ NO □ Arm inspection Right □ Left □	Interviewer ID

Phlebotomist ID	DHQ clerical Check OK? Yes □ NO □ Visual inspection of bag OK? Yes □ NO □	Time start	Bag weight	Comments: □ Slow bleed □ Aspirin □ Relative □ Other.....	Reviewed by
Donor Photo ID Checked □		Time end	Sealed by ID		Date

ملاحظة	نحن مطالبون بموجب التعليمات وحرصاً على سلامة المرضى بطرح الأسئلة التالية والتي لايقصد بها التدخل في أمور المتبرع الشخصية أو إلحاق أي إساءة به وإذا شعرت بعدم الرغبة في الإجابة عليها الرجاء إعادة الورقة إلى الموظف مع العلم بأن كافة الإجابات ستعامل بسرية تامة

(الرجاء وضع علامة✓في خانة الإجابة)	نعم	لا
هـــل؟		
1. تتمتع بالصحة والعافية اليوم؟وهل حصلت على قسط كاف من النوم؟		
2. تتناول أي مضاد حيوي؟		
3. تتناول حالياً أي أدوية أخرى لعلاج الالتهاب؟		
4. هل تتناول حالياً أو سبق لك أن تناولت أي من الأدوية التالية :بروسكار-افودارت-بروبيشيا-اكيوتان-سورياتان-تيجيسون-هرمون النمو اميونوجلوبلين الكبد الوبائي (ب)؟		
5. هل اطلعت على المواد الثقافية وحصلت على إجابات لتساؤلاتك؟		
خلال الـــ48ساعة الماضية هـــل:		
6. هل تناولت الأسبرين أو أي دواء يحتوي على الأسبرين؟		
7. للإناث فقط : هل سبق لك الحمل أو أنك حامل الآن؟ □ إنا ذكر		
خلال الـــ 8 أسابيع الماضية هـــل؟		
8. تبرعت بالدم أو بصفائح الدم أو ببلازما الدم؟		
9. تلقيت أي لقاح أو أي ابرة؟		
10- كان لك إتصال مع شخص تلقى لقاح الجدري؟		
خلال الـــ 16 أسبوع الماضية هـــل؟		
11- تبرعت بوحدتي خلايا دم بإستخدام جهاز سحب وفرز مشتقات الدم؟		
خلال الـــ 12 شهرالماضية هـــل؟		
12- خضعت لعملية زرع عضو أو نسيج أو نخاع عظم؟		
13- خضعت لعملية ترقيع عظمي أو جلدي؟		
14- لامست دم شخص آخر؟		
15- تعرضت لحادثة وخز عرضي بإبرة؟		
16- كان لك إتصال جنسي مع أي شخص مصاب بفيروس نقص المناعة المكتسب "الايدز" أو كانت نتيجة اختبار فيروس نقص المناعة المكتسب "الايدز" لديه إيجابية؟		
17- كان لك اتصال جنسي مع مومس أو أي شخص يتقاضى المال أو المخدرات أوغيرها مقابل الجنس؟		
18- كان لك اتصال جنسي مع شخص اعتاد على استخدام الابر لتعاطي المخدرات أو الستيرويدات أو أي مستحضر لم يصفه الطبيب؟		
19- كان لك إتصال جنسي مع أي شخص مصاب بالنزاف "الناعور" أو يستخدم محاليل عوامل التجلط المركزة؟		
20- للإناث فقط: هل كان لك اتصال جنسي مع رجل له اتصال جنسي مع رجل آخر؟ أنا ذكر □		

21- كان لك إتصال جنسي مع أي شخص مصاب بإلتهاب كبدي؟		
22- أقمت مع شخص مصاب بالتهاب كبدي؟		
23- خضعت لوشم أو ثقب للأذن أو أي منطقة من الجسم؟		
24- خضعت أو تخضع حالياً للعلاج من مرض الزهري أو السيلان؟		
25- تم إيقافك في سجن الأحداث أو سجن عادي لمدة تزيد عن 72 ساعة؟		
26- أجريت عملية حجامة؟		
خلال فترة الـــ3 سنوات الماضية هـــل:		
27- سافرت خارج المملكة العربية السعودية؟		
28- أصبت بالملاريا أو زرت بلداً تتفشى فيه الملاريا؟		
منذ عام 1980 إلى 1996م هـــل:		
29- هل أمضيت (3) أشهر أو أكثر في المملكة المتحدة (فضلاً راجع قائمة المناطق في المملكة المتحدة)		
منذ عام 1980 م وحتى اليوم هـــل:		
30- قضيت (5) سنوات أو أكثر في اوروبا؟ (فضلاً راجع قائمة الدول الأوروبية)		
31- نقل لك وحدات دم في المملكة المتحدة أو فرنسا؟ (فضلاً راجع قائمة المناطق في المملكة المتحدة)		
منذ عام 1977 م وحتى اليوم هـــل:		
32- تلقيت المال أو المخدرات أو غيرها مقابل الجنس؟		
33- للذكور فقط : هل كان لك اتصال جنسي برجل آخر أو لمرة واحدة فقط؟ أنا أنثى ☐		
هل سبق لك أن :		
34- تم نقل وحدات دم إليك؟		
35- كانت نتيجة فحص فيروس نقص المناعة المكتسب "الايدز" موجبة؟		
36- استخدمت الابر لأخذ حقن أو ستيرويد أو أي مستحضر دوائي لم يصفه لك الطبيب؟		
37- اتخدمت محاليل عوامل التجلط المركزة أو الانسولين؟		
38- أصبت بالإلتهاب الكبدي؟		
39- تعرضت للأمراض التالية : (شاغاس – بابيزا – الحمى المالطية)؟		
40- أجريت ترقيع الأم الجافية (غطاء الدماغ)؟		
41- تعرضت لأي نوع من السرطان بما في ذلك سرطان الدم "اللوكيميا"؟		
42- تعرضت لأي مشكلة في القلب أو الرئتين؟		
43- تعرضت لحالة نزيف دموي أو مرض دموي؟		
44- كان لك إتصال جنسي مع أي شخص ولد أو عاش في أفريقيا؟		
45- قمت بزيارة أفرقيا؟		
46- كان أحد أقاربك يعاني من مرض (كروتزفيلد – جاكوب – جنون البقر)؟		
47- هل تتبرع بدمك لأحد أفراد عائلتك (أبناء – أخوة - والدين) من الذين سيحصلون على الدم؟		
48- تبرعت بالدم تحت اسم مختلف أو رقم بطاقة مختلف؟		
49- تقدمت للتبرع بالدم ورفضت أو طلب منك عدم التبرع؟		
50- تبرعت بالدم وطلبت عدم إعطاء الدم للمريض؟		
51- هل تريد التبرع بالدم لإنقاص نسبة هيموجلوبين الدم (نسبة الحديد بالدم)؟		
52- هل تريد التبرع بالدم من أجل إجراء فحص الايدز أو أي فحوصات أخرى فقط؟		

إقرار المتبرع

قرأت وتفهمت المعلومات المقدمة لي حول انتشار فيروس الايدز من خلال الدم والبلازما ، وإن كنت أشكل خطراً لانتشار الفيروس المسبب لمرض الايدز فإنني أوافق على الامتناع عن التبرع بالدم أو البلازما بغرض نقلهما إلى شخص آخر أو بغرض تصنيعها ، أدرك بأنه سيتم إخضاع عينة من دمي لاختبار الايدز وغيره من الأمراض وإذا تبين بنتيجة الاختبار بأنه ينبغي علي الامتناع عن التبرع بالدم أو البلازما بسبب خطر نقل فيروس الايدز فإن اسمي سوف يدرج على لائحة المتبرعين الممنوعين من التبرع بالدم بشكل دائم ، أدرك أيضاً بأنه سيتم إبلاغي في حال كانت نتيجة الاختبار إيجابية وإذا لم تظهر نتيجة إيجابية أو سلبية واضحة للاختبار فإن دمي لن يتم استخدامه واسمي قد يدرج على قائمة المؤجلين دون إبلاغي بذلك إلى حين توضيح النتائج بشكل مستفيض ، أعلم أيضاً بإمكانية انسحابي من عملية التبرع في أي وقت (قبل - أثناء أو بعد التبرع بالدم) إذا شعرت بأن دمي غير صالح للنقل إلى المرضى ، أعلم أيضاً بأنه يمكنني إتمام عملية التبرع مع عدم إعطاء الدم المتبرع به إلى المرضى وذلك بوضع علامة على مربع (أرجو استخدام دمي بغرض الأبحاث فقط) أسفل الصفحة ، أعلم أيضاً أنه في بعض الحالات الطارئة يتم صرف الدم أو البلازما الذي تبرعت به من دون إجراء بعض أو حتى كل الفحوصات للأمراض المعدية ، أفوض مركز التبرع بالدم بسحب 450ملليتر (تقريباً) من دمي أو لإجراء عملية سحب مشتقات الدم الآلية وأمكنهم من التصرف بدمي بالطريقة المناسبة ، لقد قمت بالإجابة على جميع الأسئلة بكل مصداقية والمعلومات التي أدليت بها صحيحة وعلى مسؤوليتي.

☐ أرجو استخدام دمي بغرض الأبحاث فقط وعدم إعطاءه للمريض

الاسم..... التوقيع..... التاريخ / / 14هـ

3-BLOOD DONATION PROCEDURES

I. REGISTRATION:

1) The interview should be inaudible to the rest of the staff and the other donors.
2) All potential donors must be provided with information about HIV, HBV, HCV and other TTD so that those at risk will refrain from donation.
3) The information obtained from the donor during registration must fully identify the donor.
4) Current information must be obtained and recorded for each donation, single use donation form (see before) is used for recording the information.

II. DONOR MEDICAL HISOTRY QUESTIONNAIRE EXPLANATION

1. In the past 8 weeks have you donated blood or its components?

a. Frequency of whole blood donation is every 8 weeks for one unit and every 16 weeks for two red cell units by an automated Apheresis method, but not more than five whole blood or RBC's units in a year.

b. Frequency of apheresis donation can be as frequent as every 48 hours, but not more than twice in a week or more than 24 times in a year.

2. Have you ever been rejected as a blood donor? Why?

a. If he answered yes, we should know the reason and carefully revaluate the whole issue of donation.

3. Donors should be deferred permanently if they suffer from or if they have, had.

1. Bleeding abnormalities/Blood clots.
2. Cancer
3. Chaga's disease
4. Diabetes/Insulin
5. Epilepsy
6. Heart disease/chest pain
7. Hepatitis
8. Human growth hormone or beef insulin
9. Kidney disease
10. Leishmaniasis
11. Lung disease
12. SARS
13. Positive HIV, serology (Aids Patients)
14. LV. drug users or used intranasal cocaine.
15. Family member with Creutzfeldt-Jacob's disease
16. Dura matter transplant or reside in UK for 6 months.
17. Tegison medication for psoriasis
18. T.B 19. Stroke 20. Symptoms of AIDS.
    - Prolonged fever or diarrhea.
    - Enlarged lymph nodes
    - Unexplained weight loss (more than 5kgm)
    - Night sweats
    - Persistent cough
    - White spots in mouth.

4. Donors should be deferred for 3 years if they have:

1. Been from countries with endemic malaria
2. Had been diagnosed and treated from Malaria.
3. Soriatane medication, because of its long acting teratogenic effect.
4. Has been diagnosed and treated from Brucellosis.

5. Donors should be deferred for 1 year (12 months) if they suffer from or they have, had:

1. Himself or his spouse received blood or organ transparent.
2. Rabies shots
3. Been a nurse for kidney dialysis unit.
4. Been a rape victim.
5. Been incarcerated in a prison more than 72 hours .
6. Been a patient in a mental hospital
7. Tattoo
8. Acupuncture
9. Ear or Nose piercing
10. Needle stick
11. Stab wound
12. A contact with AIDS patient
13. Body fluid splash to mucous membrane.
14. Gonorrhea, after treatment
15. Syphilis, after treatment
16. A contact with hepatitis patient, or receive Anti HB immune globulins.
17. Been treated with anti-malarial treatment as prophylaxis.
18. Been travelled to Malaria endemic area without symptoms.
19. Animal bite.
20. Been outside the kingdom for leisure trip (Not married, or without his family)
21. If they have had any surgery or severe illness.
22. Have sex with hemophilia A or B or taking money or drug for sex.

6. Female donors should be deferred for 6 weeks if they have been pregnant or delivered a baby.

7. Donors should be deferred for 4 weeks if they have had:

1. Low hemoglobin (less than 12.5g/dl)
2. High pulse rate (more than 100 beat/minute)
3. Low pulse rate (less than 50 beat / minute)
4. High blood pressure (more than 180mm Hg for systole and or more than 100mm Hg for diastole)
5. Vaccination, travel to endemic area or in contact with SARS patient
6. Acutane Medication for Acne.
7. Proscar mediation for prostate.
8. Propecia or Prozac medications.

8. Donors should be deferred for one week if they have had.

1. Mild Fever
2. Flu or common cold
3. Sore throat
4. Dental extraction
5. Antibiotics

9. Donors should be deferred for 72 hours if they have had:

1. Asprin or feldene or any Asprin containing medication,if we intend to separate platelet concentrate.

10. The confidential unit exclusions (CUE):

The confidential unit exclusions can be accomplished by giving the donors a piece of paper with their blood unit numbers and options of giving permissions to use or not to use their blood in transfusion under confidential measures.

Note: Donor deferral rates should be monitored by the blood bank physician to ensure they are within a reasonable range. Donors who are deferred should be given a full explanation of the reason and be informed whether or when they can return.

11- Accepted criteria for donor physical examination:

1. Age:

a) Between 18 and 65 years

b) Below age of 18 and above age of 65 can donate after been evaluated by medical director.

c) Below age of 18 should submit a written agreement for donation from his father or his guardian.

2. Weight:

a) Should be more than 5Okg.

b) If less than 50 kg adjust anticoagulant level, if blood volume is less than 300m1, e.g. if donor weight is 30kg

$$\text{blood volume} = \frac{30\text{kg}}{50\text{kg}} \times 450 = 270\text{m1}.$$

$$\text{required anticoagulant} = \frac{270 \times 14}{37.8\text{m1}} = 100$$

c) Donors shall donate not more than 10.5m1 per kilogram of body weight.

3. Temperature:

a) Oral temperature shall not exceed 37.5°C (99.5°F)

4. Pulse:

Should be regular between 50-100 beat/minute.

5. Blood pressure:

a) Systolic B.P. between 100-180 mmHg

b) Diastolic B.P. between 60-100mm Hg.

6. Arm Inspection.

a) Both arms should be free of lesions, needle puncture marks, sclerotic veins, boils, purulent wounds and severe skin infections

b) Mild skin disorders as acne and psoriasis, not in the antecubital area are accepted for donation.

7. General appearance:

Donor should look healthy, not under influence of drugs or alcohol and not excessively nervous.

8. Hemoglobin Evaluation:

1. **Perform hemoglobin determination:** by copper sulfate method (specific gravity), or any available method for Hb. estimation.

Accepted hemoglobin level: Routine donor: 12.5-18gm/d1= 1.053 sp.gr.

Autologous donor: 11 gm/d1= 1.049 sp.gr.

A. Copper sulphate ($CuSo_4$) method:

a) *Principle:*

- This is not a quantitative test
- It estimate Hb. content of blood from its specific gravity
- Specific gravity of 1.053 corresponds to Hb. of 12.5g/dl.
- If drop of blood in contact with CuSo4 solution of 1.053 sp.gr, sink within 15 Seconds, it means that blood Hb. is higher than 12.5g/dl.

b) Reagents and materials:

- Copper sulphate CuSo4 solution at specific gravity of 1.053.
- Sterile 2x2 gauge.
- Alcohol swab
- Sharps disposal container
- Capillary tubes coated with anticoagulant and dropper bulb or plastic transfer pipettes.
- Sterile lancet.
- Protective medical gloves.

c) Procedure:

1. Copper sulfate solution preparation
   - Dissolve 86gm of CuSo4 in 1000cc of distilled water.
   - Adjust specific gravity to 1.05
   - Store CuSo4 solution in tightly capped bottles away from direct Sunlight or extreme temperature.
   - Dispense well mixed SOmI of solution in dry vial.
2. Finger puncture
   - Use the 4th Finger (ring finger)
   - Clean with sterile alcohol swab
   - Puncture the tip of the finger by sterile lance
   - With free blood flow, discard the first drop of blood and use second drop.
3. Collect the blood in anticoagulated capillary tube, or by plastic transfer pipettes.
4. Release one drop of blood to the center of CuSo4 vial, from a height of 1 cm above surface of solution.
5. Observe the blood drop for 15 seconds.
6. Change CuSo4 solution after 25 tests or 2 hours.

d) *Interpretation:*

1. If blood drops sinks within 15 seconds; Hb. for donation O.K.
2. If blood drop does not sink or sinks after 15 seconds; Hb. not OX, for Donation.

e) *Safety:*

1. Wear gloves
2. Dispose lancets, capillary tubes, alcohol swabs, gauze and used CuSo4 solution in biohazard container and sharps container.
3. No blood contamination to work surfaces, donors clothing or equipme

f) Quality control:

1. **Principle:** Observe behaviour of blood drops of known hemoglobin concentration.
2. **Procedure:**

a)Obtain 6 blood samples with known hemoglobin levels in range around 12.Sgm/dl.

b) Gently place a drop of each blood sample into a vial of CuSo4 sol. Of stated sp.gr. of 1.053.

c) Drops with Hb. At or above 12.5g/dl must sink and drops with Hb below 12.5g/dl must float.

d) Record :

- Date of testing
- Lot number
- Expiration dates
- Results
- Person performing the test

e) Document corrective actions if desired results are not achieved.

B. Blood Hemoglobin Photometer

- The system consists of disposable microcuvettes with reagent in dry form and a single purpose designed photometer.
- The microcuvitte is used for measuring the sample, as reaction vessel and as measuring cuvette. No dilution is required.
- Reading of hemoglobin takes place in the photometer, which follows the reaction and presents the result only when the reaction has stopped.
- The photometer is calibrated at the factory against the hemoglobin-cyanide-(HiCN) method, which is the international reference method for the determination of the total hemoglobin concentration in blood.
-Photometer, transformer (battery eliminator) and control cuvette are delivered with the machine.
- The photometer may be powered by batteries.

Control Cuvette :

- The control cuvette, which is an optical interference filter, is used to verify that the calibration is stable, e.g. not changing from day to day.
- When checking the value received, it should not deviate from the assigned value on the control cuvette card more than ±0.3 g/dl (± 3g/l,0.2mmol/l).

Sampling Collection:

Make sure that the patient sits comfortably. The hand should be warm and relaxed. The patient's fingers should be straight but not tense, to avoid stasis.

Use the middle finger or the ring finger for sampling. Avoid fingers with rings for sampling. Clean the puncture site with disinfectant and allow to dry.

Using your thumb, lightly press the finger from the top of knuckle to the tip. This stimulates the blood flow towards the sampling point.

The thumb has moved up to tip of the finger and use very gentle pressure. Prick at the side of the fingertip. Not only the blood flow is best at this point, it also causes the least pain.

Wipe away the first two or three drops of blood. This stimulates the blood flow. If necessary applies light pressure again, until another drop of blood appears. Avoid milking.

Make sure that the drop of blood is big enough to fill the cuvette completely. Introduce the cuvette tip into the middle of the drop.

Fill the cuvette in one continuous process.

Measuring procedure:

Wipe off the excess blood on the outside of the cuvette tip.

The filled cuvette should be visually inspected for air bubbles. Small air bubbles around the edge do not influence the result.

Place the filled cuvette into the cuvette holder immediately and push it into measuring position.

The filled cuvette should be analyzed immediately and at the latest 10 minutes after it has been filled.

After 15-45 seconds the result is displayed.

9. ABO/Rh Typing (Slide or tile method)

A) *Principle:*

1. The four main blood groups A, B, O and AB are determined by red cell typing with known antisera (Anti-A, Anti-B), depending on presence or absence of two antigens A and B on the red blood cell.

2. Rh. Typing .

B) Policy:

determine the presence or absence of the D antigen on the donor's red blood cells by using Anti-D antisera.

This is a preliminary ABO blood grouping and Rh typing, to be confirmed by tube method and reverse serum typing.

C) Sample:

by finger puncture(use the same puncture site done for Hb evaluation.

1. With free blood flow discard the first drop of blood and use second drop

2. Collect the blood in anticoagulant capillary tube or plastic transfer pipettes.

D) *Reagents and materials:*

1. Anti A grouping serum (Monoclonal) .
2. Anti B grouping serum (Monoclonal) .
3. Anti D grouping serum (Polyclonal) .
4. Rh Control .
5. Glass slides or ceramic tiles.
6. Wooden applicator sticks .
7. Sterile 2x2 gauze.
8. Alcohol swab .
9. Protective medical gloves.
10. Capillary tubes coated with anticoagulant and dropper bulb.
11. Sharps disposal container and biohazard container.
12. Rh View Box.
13. Stop Watch .
14. Plastic transfer pipettes .
15. Donor medical history form .
16. Rejected donors log sheet.

E) Procedure:

ABO Typing (slide Method)

1. At room temperature, place one drop of Anti A and Anti B reagent on the opposite ends of a clean properly labelled slide (by marker pin).

2. Using collected blood from the donor by finger puncture by anticoagulated capillary tube or plastic transfer pipette, place one drop of whole blood to each drop of Anti-A and Anti-B reagent on the slide.

3. Mix using separate applicator sticks over a circular area 20mm in diameter, within 2 minutes (2 minutes, gives false positive results)

4. Read macroscopically for agglutination and record the test results on the donor medical history form.

Rh(D) Typing (slide Method):

1. Check Rh view box temperature (45°C-47°C).

2. Place on drop of Anti D and Rh-Control reagent on the opposite ends of a clean. properly labelled slide on top of lighted Rh. view box.

3. Add one drop of whole blood to each drop of Anti D and Rh-control reagents.

4. Mix using separate applicator sticks, within 2 minutes. (>2 minutes gives false positive results).
5. Read macroscopically for agglutination and record test results on the donor medical history form.

F) Interpretation of results:

1. Agglutination is positive reaction.
2. No agglutination is negative reaction.
3. If Rh- control show agglutination, do not interpret blood group results.
Donor cells with:

Donor cells with				*Blood Group and Rh Type*
Anti. A	*Anti B*	*Anti D*	*Rh Control*	
+	-	+	-	**A Positive**
-	+	-	-	**B negative**
+	+	+	-	**AB positive**
_	-	+	-	**O positive**
+	-	+	+	**Un determined**
	±	-	+	**Un determined**

G) Limitations of procedure:

False test results may occur due to:
1. Improper incubation time or temperature.
2. A cell suspension is too light or too heavy.

H) Safety:

1. Wear protective gloves
2. Dispose all materials used in biohazard and sharp containers.
3. Avoid any blood contamination
4. No attempt to pick up broken glass with fingers.
5. All blood and blood products must be treated as potentially infectious as per laboratory safety manual.

I) Quality Control:

1. Refer to daily Quality Control of Antisera.
2. Daily check of Rh view box temperature.
3. Confirm the results by tube method and reverse grouping.

III. PHLEBOTOMY AND COLLECTION OF BLOOD

1- Principle:

Donor blood will be collected using a sterile, pyrogen-free, properly identified FDA OR CE approved container. The venipuncture site must be aseptic with blood and anticoagulant mixed continuously throughout the procedure using a properly calibrated blood mixer. After the donation is completed, the donors recovery will be observed and monitored, refreshments will be given and the donor will receive the post phlebotomy care instructions prior to being allowed to leave the donation room.

2- Reagents, Supplies and Equipment: (Materials.)

1- Iodophor- PVP 2% scrub solution swab stick.
2- Iodophor- PVP 10% prep solution swab tick.
3- 4x4 Sterile Gauze Square.
4- 1" Surgical tape / plastic bandage.
5- Sterile blood collection sets.
6- Unit number sheet.
7- Blood mixer scale.
8- Dielectric heat sealer, or metal clips and hand sealers.
9- Daily calibration of blood mixer scale sheet.
10- Whole blood collection bag lot number log sheet.
11- Clean scissors, hemostats and forceps.
12- 7 ML red top (plain) vacutaniner tubes and holder .
13- Donor registration, identification, medical history questionnaire, physical examination and donor reaction record form.
14- Post phlebotomy care form.
15- Tourniquet.
16- Public services emergency numbers.
17- Medical emergency supplies box.

3- Safety:

All blood and it's products must be treated as potentially infectious as per laboratory safety manual.

4- Quality Control:

1- Complete whole blood collection bag lot number log sheet daily.
2- Prepare unit numbers log sheet daily.
3- Complete donor reaction records daily.
4- Complete and check all the information in the donor medical history and physical examination Form.
5- Inspect bags for defects and discoloration for each donor.
6- Check blood mixer scales for accuracy by trying different known weights every day and endorse the results for the shaker calibration in Q.C log sheet.

5- Procedure:

1- Prior to phlebotomy write unit number and donor name on the main collection bag and all transfer bags, donor medical history form, and three red top tubes.
2- Place the blood collection set on the blood mixer scale, and thread donor tubing on the blood mixer scale.
3- Inspect arm for suitable vein (usually in the antecubital fossa.)
4- Apply tourniquet, identify suitable vein, and release it.
5- Prepare donor arm:
   a- Scrub 4 cm area in all directions from intended site with 2% PVP iodine solution for 30 seconds (use exidine scrub or green soap for donors sensitive to iodine, and if not available use alcohol swab.)
   b- Apply 10% PVP iodine swab stick, start at the center with concentric spiral outward for 30 seconds.
   c- Cover area with sterile 4x4 gauze, and do not touch the skin.
6- Apply tourniquet or blood pressure cuff approximately 2 inches above the ante cubital area

in case of blood pressure cuff , the tubing should be directed away from the ante cubital area and the cuff should be inflated to 40-60 mm Hg.

7- Before venipuncture, the phlebotomist must check the blood bag and the tubing for evidence of leaks, discoloration, particulate contamination, kinks or other defects.

8- The phlebotomist will clamp the tubing near the needle before the needle cover is removed in order to prevent air from entering the system. The system must be contaminated if this is not done.

9- Remove the needle cover. (16-gauge needle), and perform phlebotomy, by inserting the needle with it's bevel upward in straight steady motion in the vein.

10- Remove the clamp from the tubing and observe the blood flow through the tubing (fairly rapid and steady).

11- If more than one skin puncture is needed another blood bag must be used.

12- Tape needle in place on arm with adhesive strips.

13- Switch on blood mixer, mixing blood with anticoagulant may be done manually by gentel lift and tilting the bag every 100 ml, the collection bag may also be hung portside down, since blood mixes with the anticoagulant as it enters the bag, less agitation is necessary.

14- Have donor open and close fist (squeezing foam ball every 10-12 seconds.) & do not leave the donor unattended at any time during the phlebotomy procedure.

15- When 585 gm (469 ml) of blood has been collected, the device will automatically stop the blood flow, and alarm will sound (complete draw within 10-15 minutes; to separate all blood components collection time must not exceed 10 minutes, if it reach 15 minutes we can separate only PRBCs and discard platelet rich plasma, and if it exceed 15 minutes we stop donation discard blood due to slow bleed).

16 - Apply hemostat to tubing near venipuncture and make a tight knot from previously prepared loose knot just distal to inline needle and hemostat.

17- Cut tubing by scissor between the tight knot and hemostat and separate the blood collection set.

18- Obtain blood samples by unclamp the hemostat, fill the tubes with blood and reclamp again.

19- Release the tourniquet, withdraw the needle, and apply pressure with a gauze pad, and have donor raise arm.

20- Discard needle assembly into sharp container.

21- Record time started and finished and unit weight.

22- Seal tubing next to the knot, strip donor tubing three times into the bag to prevent the blood from clotting in the tube, invert the bag several times, then allow tubing to be refilled with anticoagulated blood and heat seal into 5 segments at least, making sure that the lot number is present on each segment. The seal between each segment should be clean and allow for easy separation.

23- Remove the first segment and label it with unit number and place it into daily segment tray.

24- Put all blood collection set with rubber band and send to component preparation.

25- Place red top tubes in rack for sending to donor processing and infectious disease screening.

26- Complete the donor medical history form.

27- Assess donation site. If satisfactory, apply band-Aid.

28- Allow donor to sit up and stay with him

29- Provide donor with juice and cookies and observe him.

30- Allow donor to leave after 10 minutes rest, and in absence of any adverse reaction.

31- Give the post donation instructions to the donors:-

a) Increase fluid intake for the next 24 hours.
b) Do not smoke for the next hour.

c) Leave the bandage on the venipuncture site for a few hours.
d) Do not lift or carry heavy objects with the donating arm for the next few hours.
e) If bleeding occurs from the phlebotomy site, raise your arm straight up and reapply direct pressure on the bandage until bleeding stops.
f) If you feel dizzy or faint, sit down with your head lowered between your knees or lie down with your feet elevated.
g) Donors who work in certain occupation like construction workers or at heights should be cautioned that dizziness may occur if they return back to work .
h) If you become ill in the next 3-4 days contact our blood bank staff with information on your illness.

32- Thank the donor for an important contribution and encourage him to donate again.

6- Reporting. Results:

1- Record Adverse reactions.
2- Record any exception.
a- Double phlebotomy. b- Incomplete bleed. c- Slow bleed (more than 15 minutes).
d- Arterial puncture. e- Contamination. f- Overweight unit (>522 g) +wt of set.
g- Low volume unit (<316g)

7-Procedure Notes:

1- Units take more than 15 minutes not suitable for preparation of components.(only WB or PRBC'S)
2- In case of cardiac arrest call emergency room team in the hospital.
3- In mobile site cardiac arrest cases call ambulance (dial 997), or Police (dial 997) or Fire (dial 998)
4- Contamination of venipuncture site, repeat entire procedure.
5- Double phlebotomy, use different site and set.
6- Units from donors ingested aspirin within last 3 days, not suitable for platelet preparation.

IV. DONOR ADVERSE REACTION PREVENTION AND TREATMENT

1-Principle:

Trained Phlebotomists must be alert in observing and recognizing signs of impending reactions and provide immediate proper treatment.

2-Policy:

The staff on duty in donor areas must be trained to observe for signs of impending reactions and how to prevent and treat them and how to do CPR.

3-Materials:

1. Cold Compresses.
2.Emesis basin or equivalent.
3.Emergency Supplies Box, which contain:
a- Normal saline 500 ml, Dextrose 5% 500 ml and Transfusion sets.
b-Ca-Gluconate 10 ml amp, hydrocortisone amp and 10 ml and 5 ml disposable syringes.
c- Paracetamol tab.
4- Aromatic spirit of ammonia.
5- Towels, Tourniquet, alcohol swabs, cotton, gauze, band Aid.
6- Oropharyngeal airway, plastic or hard rubber.
7- Oxygen and mask.
8- Donor medical history and donor reaction form.
9- Public Services Emergency numbers (Ambulance, Police. and Fire.)
10- Hospital Incident Report.

4-Safety:

All blood and it's products must be treated as potentially infectious safety manual.

5- Procedure:

1. ***In all cases of donor reactions inform blood bank physician, to examine him as soon as possible.***
2. **Mild Reactions:**
   - **a- Symptoms: increased Nervousness, increased respiration (yawing and sighing) Thready pulse, decreased blood pressure, pallor and mild sweating.**
   - **b- Treatment:**
     1. **Stop phlebotomy.**
     2. **Maintain adequate airway (Loosen clothing.)**
     3. **Raise donors feet.**
     4. **Talk to the donor.**
     5. **Apply cold compress to his forehead.**
     6. **Monitor vital signs.**
3. **Moderate Reactions:**
   - **a- *Symptoms:* Nausea and vomiting, dizziness, Rapid shallow breathing, slow pulse and hypotension.**
   - ***b- Treatment:***
     1. **Apply cold compresses to forehead.**
     2. **Instruct donor to breath slowly and deep.**
     3. **Turn donor head to either side.**
     4. **In case of vomiting offer him a basin & tissues.**
     5. **Do Not Give Oxygen.**
4. **Severe Reactions:**
   - **a- Symptoms: loss of consciousness, involuntary movements, generalized tetany, Rhythmic muscular contractions, grasping breathing and cessation of muscle activity.**
   - **b- Treatment:**
     1. **Check vital signs until return to normal**
     2. **Blood bank physician will evaluate the symptoms and may apply aromatic spirit of ammonia, or any other medication accordingly.**
     3. **If no response call hospital emergency.**
5. **Hematoma:**
   - **a-Prevention:**
     1. **Select large firm vein.**
     2. **No good vein no venipuncture.**
     3. **Prevent needle movement by tapping it to donor arm.**
   - **b- Treatment:**
     1. **Loosen tourniquet and remove needle.**
     2. **Apply sterile gauze with pressure for several minutes.**
     3. **Donor hold arm above heart level.**
     4. **Apply ice to the area for 5 minutes.**
     5. **Inform donor that bruising and slight discomfort may occur.**
   - **c- Arterial Puncture:**
     1. **Remove needle and apply firm pressure for 10 minutes.**
     2. **Check radial pulse and if absent inform blood bank physician.**
6. **Convulsions:**
   - **a- With help from staff prevent donor from injuring him self.**
   - **b- Allow adequate airway and position donor on side.**

c- Elevate donor feet above head level.
d- Inform blood bank physician.

7. **Cardiac or Respiratory difficulties:**
   a- Very rare incident.
   b- D.D. between vasovagal reaction and cardiac shock.
   c- For vasovagal attack, raise donor feet,
   d- For cardiac shock, rapid pulse and cyanosis is apparent.
   e- In cardiac arrest:
      1- Start CPR.
      2- Call hospital emergency.
      3- Inform blood bank physician who may give oxygen, or LV Fluids or hydrocortisone injection accordingly until emergency team arrive.
   f- Record all the events in donor adverse reaction report.

8. **Donors who experience moderate, severe reactions, convulsions or cardiac shock may be excluded from future donation.**

6- Procedure Notes:

1- Adverse reactions may occur at any time during the process of medical history, examination, phlebotomy and after donation.
2- Syncope (fainting) is the most common reaction and can be treated by Aromatic sprit of ammonia.
3- Frequency increased for the first time donors, donors who have not eaten for three hours before donation and those who are not aware about the procedure of donation.

Donor Adverse Reaction Prevention and Treatment

NAME:	ID#:	UNIT#:

LOCATIONS

- □ History Area
- □ Donor Area
- □ Refreshmet Arrea
- □ Other……

MILD REACTION

- □ Pale
- □ Perspiration
- □ Fast Breathing
- □ Nausea
- □ Vomiting
- □ cold
- □ Dizziness
- □ Light Headedness
- □ Other……

MODERATE REACTION

- □ Slow Heart Rate (Pulse)
- □ Low Blood Pressure (less than 60 systolic)
- □ Dizziness
- □ Time for Recovery …….. Minutes

SERIOUS REACTION

- □ Stiff arms and legs
- □ Shaking of arms and legs
- □ Unconscious How Long? …….. Seconds
- □ Color change pale to dark blue around lips
- □ Incontinence • Urine • Stool
- □ Convulsions:
  - • Mild: Short time unconscious, shaking of arms and legs.
  - • *Severe:* Body stiff, Noisy breathing, arms and legs moving

TREATMENT

- □ Reassure Donor
- □ Uncross Legs
- □ Protect from injury
- □ Donation Discontinued
- □ Cold Towel to Forehead
- □ Maintain Clear Airway
- □ Give Fluids after Recovery: Water/juice
- □ Calcium Gluconate
- □ Elevate Feet
- □ Loosen Light Clothing

INJURIES

- □ None
- □ Hit Head
- □Other
- □ Arm Bent with Needle
- □Bruising ……..
- □ Fall
- □ Lacerations ……..

FOLLOW UP

- □ Start Time: …….. End Time: …….. Recovery Time: …….. Min
- □ Evaluated before Leaving 11 Sent Emergency Room
- □ Follow up: Call Tel #: ……..

CONTRIBUTING FACTORS

- □ First Time Donation.
- □ Weight <120 lb.(54.5 kg)
- □ Long Waiting Lines (>30 min.)
- □ >5 Years Since Last Donation
- □ History of previous Reaction
- □ Noisy, Crowded Collection Site
- □ Donor Area: • Hot • Cold • Humid
- □None of the above
- □ Last Meal > 3 hours
- □ Anxiety
- □ Needle Manipulation / Double Stick.
- □ <20 Year Old
- □ Emotional Stress
- □ Other ……

- SENIOR RN / SIGNATURE:……
- BLOOD BANK PHYSICIAN SIGNATURE:……
- DATE:……

V. MOBILE BLOOD BANK

Principle:

The mobile blood bank is an important aspect of blood banks activities . It encourages voluntary donation and by which we can have safe, voluntary donors.

Policy:

1-The In charge of the mobile blood banks arranges campaigns with different governmental and private establishments all over the country.

2- An annual program for the activities of the mobile blood bank is arranged and submitted to the director of blood transfusion services.

3- Periodical maintenance for the mobile blood bank car is carried out by Company service and related reports are kept in special file.

4-Equipment list for the contents of the mobile blood bank Vehicle is available in the related file and should be updated periodically.

5- A check list for consumable and in use items should be checked and filled by the in charge of the mobile blood bank before every campaign to ensure the availability of the required items in the car.

6-Quality control tests be performed prior to every campaign to ensure the suitability of the available equipments to work properly.

How to run a successful blood drive?

A) Dates:-

1- To select a date and time for the drive, consider factors like competing events and try to hold the drive when the most people can attend.

2- Remember that the blood supply is lowest during holidays and summer months so blood drives during these times are particularly important.

B) Sites:-

1- Be sure the site is inviting so donors will feel comfortable and will want to attend future drives.

2-When choosing a site, make sure the space is large enough, clean, easily accessible and with proper lighting.

C) Organization:-

1- One to two days before the drive, make sure you call people to confirm the dates and site arrangements.

2- Posters and other materials will help give the drive immediate exposure.

3- Meet the group leadership and give him all information he needs.

4- Obtain a list of the people, who have previously donated, these people will most likely donate again.

5- Don't ignore people, who have not donated in the past, they may have had a change of circumstances that prevented them from donation.

6- Make arrangements to have an article publicizing the blood drive in the place's newsletter.

7- Decorate the donation site to make it attractive, pleasant and fun.

8- Encourage senior managers to walk through the day of the drive.

9- Asking face to face provides a chance to deal with specific questions and inform the potential donors that by giving blood they save lives.

10- Some people are not permitted to give blood; let them know they can still help by encourage other people.

D) Donor Encouragement :-

1- Remember to thank all donors for their lifesaving contributions.

2- Give him a suitable small gift to remind him about his generous blood donation.

3- Invite the regular donors and key-persons to social events.

E) Thank you note:-

1- Thanks should also go out to the members of your team who worked so hard to make the drive a success.

2- Letters of appreciation and certificates from you to the key-persons and the manager of the site of donation will make everybody feel great.

F) Assessment:-

1- As soon as possible; hold a team meeting for feedback and review the entire process:

Did you make your goal?

What went right?

What went wrong?

What would you change?

2- This meeting will improve future blood drives.

CHAPTER TWO: AUTOLOGOUS / DIRECTED DONATION

I. PREOPERATIVE AUTOLOGOUS DONATION (PAD) AND DIRECTED DONATION

1. PRINCIPLE:

A- Autologous: Preoperative Autologous Donation: (PAD)

1- Definition: PAD units maybe drawn from a prospective surgery patient, and stored in anticipation of his need.

2- Advantages:

a) Prevent transfusion Transmitted diseases (TTD).
b) Prevent alloimmunization.
c) Prevent allergic, febrile and hemolytic transfusion reactions
d) Supplement blood supply
e) Reassure patients concerned about blood transfusion risks.
f) Provide compatible blood for patients with alloantibodies.

3- Indications:

a) Orthopedic surgery
b) Vascular surgery
c) Cardiac or thoracic surgery
d) Radical prostatectomy
e)Pregnant women with alloantibodies to multiple or high incidence antigens f) Pregnant women with placenta previa.

4- Disadvantages:

a) Bacterial contamination risk as allogeneic donation
b) Risk of ABO incompatibility errors.
c) More costly if frozen.
d) Blood wastage (50% not transfused.)
e) Increased prevalence of donor adverse reaction.
f) Preoperative Anemia.
g) Volume over load.

5- Contraindications :

a) Evidence of bacteremia.
b) Active seizures.
c) Uncontrolled hypertension.
d) Unstable angina and myocardial infarction.
e) Cyanotic and coronary heart disease.
f) Aortic stenosis correction surgery.
g) Cardiac or pulmonary disease.
h) Procedures that seldom require transfusion as cholecystectomy, herniorrhaphy, vaginal hysterectomy and uncomplicated obstetric delivery.

B- Directed Donation:

1. Definition: donations are recipient-specific designated donations.

2. Indications :

a) Patients anticipating transplantation (improved graft survival.)
b) Patients with high incidence antigen or combination of antibodies

2. POLICY:

A. Autologous and directed donation must be requested by patient's physician in consultation with blood bank medical director, and should contain the following information:

1) Patient name, and (Donor name if directed donation)
2)Patient ID number or medical record number.

3) Number of units.
4)Whole blood or component.
5)Date of surgery.
6)Surgical procedure.
7)Physician name and signature.

B. Donor selection and physical examination and preparation.

1) Directed donation as routine donation.
2) Autologons donation: as routine donation except.
a) Medical interview concentrate on medications, medical illness, cardiovascular fitness and presence of bacteremia.
b) Not necessary to ask about TTD.
c) Hemoglobin should not be less than 11 g / dl.
d)Schedule: every week, no sooner than 3 days before surgery.
e) Rescheduling surgery: options. -discard autologous unit or - reinfuse units to the patients or - freeze the units.
f) Erythropoiesis stimulation : if Hb<11 g / dl or there are urgency for collection by giving patient (Donor)
- Recombinant Erythropoietin (EPO), Subcutaneous twice weekly, at a dose of 300 mg/kg /dose.
-Oral iron: 125-300 mg / day.

3. REAGENTS:

Supplies and equipments:

As in phlebotomy and blood collection procedure + labels for autologous donation, for autologous use only, Biohazard and Directed donation.

4. PROCEDURES:

A. Autologous donation:

1-Written request from patient's physician approved by blood bank physician.
2- Registration and identification as routine donation.
3- Donor selection, medical history, physical examination and preparation as in policy for autologous donation.
4- Label all bags with green Autologous Donor label, and autologous use only, and if found to be positive label or mark all bags with biohazard label, and label must include
a) Patient name
b) Patient medical record number or LD. No.
c) Expiration date
d) Collection date
e) Facility name
f) Results of laboratory testing.
5- Phlebotomy and blood collection as in procedure for phlebotomy and blood collection.
6- Laboratory Testing :
a) Determine ABO and Rh. Type of both the patient and the unit before transfusion.
b) Antibody screen and cross matching are optional.
c) All infections diseases HIV I / II, HBs Ag, HIV Ag P24 Anti HCV, Anti HBC., but not HTLVI / II.
d) Patient's physician must be notified of any positive results.
7-Storage and Shipping:
a) Store PRBC's units for 6 weeks and whole blood for 5 weeks in 2-6C° refrigerators.
b) For shipping a written request from patient physician to use positive units.
8- Transfusion:

Autologous units transfused before allogeneic.

B. Directed donation:

1. Written request from patient's physician approved by blood bank physician.
2. High light directed donation on donor questionnaire form.
3. Registration, identification, criteria of donor selection, history, physical examination and infectious disease testing as routine donation and processing.
4. Perform phlebotomy, labeling and blood collection as per phlebotomy and blood collection procedure.
5. Affix a directed donation tag to the unit completed with
   a) Full name of intended patient recipient.
   b) Patient medical record number.

5. SAFETY:

All blood and blood products must be treated as potentially infections as per laboratory safety manual.

6. CONTINOUS QUALITY IMPROVEMENT:

1) Reduction of allogeneic transfusions is an indicator for autologous blood practice.
2) Monitor wastage rate of autologous units.
3) Criteria of efficient autologous blood program:
   a) Standardized indications.
   b) Utilize whole blood only.
   c) Limit the use of freezing.
   d) Application of the same transfusion guidelines as allogeneic blood.
   e) Do not test for infectious disease markers, units collected within 30 days from testing the first autologous unit.

II. AUTOLOGOUS/DIRECTED DONATION REQUEST FORM

1. This form is to be completed for each donation.
2. Once completed and copied, please forward to the blood bank medical director for review. Maintain a copy for donor distribution, if approved.
3. Once approved, provide each potential donor with a copy of the completed form. <u>One form is required for each donation.</u>
4. Instruct the potential donors to bring a completed form with them each time they come to

---

DATE: - ----------
REQUESTING PHYSICAIN: ----------
DONOR NAME: ----------
DONOR ID#:---------- UNIT#:----------
PATIENT NAME: ----------WEIGHT: ----------
PANTIENT MRN#:----------
PATIENT ABO/Rh: ----------

REASON FOR TRANSFUSION	DOSE	DATE NEEDED

PRODUCTS REQUIRED: Packed Red Cell (PRC) □ Fresh Frozen Plasma □
Platelet □ Granulocytes □
Platelet (pheresis) □ other (specify) ----------
REQUESTING PHYSICAIN: _____ ID # ----------
(Signature / Date) Pager # ----------

III. DONOR / PATIENT REGISTRATION FORM (For Autologous Donation)

Patient/Donor Name: ---------- MRN#:----------
Sex: ---------- A_{ge}: _____ Bodyweight: ---------- Diagnosis: ----------
Scheduled Surgery: ----------Scheduled Date of Surgery: ----------
ESTIMATED BLOOD USAGE (specify different blood products and quantity)
□ PRC □ PLT □ FFP □ CRYO □ OTHER: ----------
-

Do patient / donor have previous record of blood donations with the blood bank □ yes □ No

Medications	Yes	No
Anti-hypertensives		
Beta blockers		
Calcium Blockers		
Anti-convulsives		
Antibiotics		
Iron Supplement and date		
Replacement Fluid* Type:		

Special Risk Factors	Present	Not Present
Coronary Artery Disease		
Cardiac Failure		
Ventricular Arrhythmia		
Atrio-Ventricular Block		
Aortic Stenosis		
Recent Myocardial Infarct		
Unstable Angina		
Idiopathic Hyperthopic Subaortic Stenosis		

Predonation disease marker testing (HBsAg, HCV, HIV I/II, HTLV I/II) □ Done
PATIENT ELIGIBLE FOR AUTOLOGOUS DONATION: □ Yes □ NO
Submitting Physician:---------- pager #:----------
(Please print name)
Sign / Date: ----------
The completed form is forwarded to the blood bank director for review

---

Patient data reviewed and patient found: eligible for autologous blood donation program:_
□ No□ Yes□
Blood Bank Director:----------Signature:..... Date ----------

CHAPTER THREE:APHERESIS.

Definition:-

Apheresis is defined as the separation of whole blood into its components. During this procedure, a selected component is removed and the remaining elements are recombined and returned to the donor or patient.

Purpose:-

1)To collect a blood product generally intended for transfusion when performed on a donor.

2)To provide a therapeutic effect when performed on a patient.

Separation Techniques:-

A)Centrifugal technology:-

Currently there are a variety of apheresis machines available to perform apheresis procedures:-

1)Continuous flow centrifugal devices:- Whole blood is drawn from the donor / patient at one access site, is processed continuously and is returned through a second access or return site.

2) discontinuous flow centrifugal devices:- Whole blood is drawn from one site, processed as a discrete volume and then returned to the donor / patient often through the same site.

Notes:- Both of these devices use centrifugal force to separate whole blood into plasma and formed elements. Each blood component has specific density, specific gravity and will separate accordingly.

B)Membrane technology:-

Separates plasma and the cellular components by sieving the cells from plasma. Membrane technology is limited to plasmapheresis.

C) Adsorption technology:

Both membrane and centrifugal devices can be adapted to protocols that selectively remove specific soluble plasma constituents by exploiting the principles of affinity chromatography.

[I] COMPONENT COLLECTION

(A) DONOR SELECTION

PRINCIPLE:

Potential routine aphaeresis donors are screened to ensure that they meet all established requirements. In addition to general criteria applicable to whole blood and aphaeresis donation, donors are selected depending on venous sites and complete blood count result.

POLICY:

Donor selection for plasma apheresis and cytapheresis procedure shall be in compliance with selection criteria for whole blood donors per AABB standards. In addition, apheresis donors are required to donate at least one unit of whole blood within the last two years and at least 56 days prior to first aphaeresis.

REAGENTS, SUPPLIES, EQEUIPMENT: -

- Specimen tubes -Vacutainer adapter needle - Betadine / alcohol swab stick
- 4x4 gauze - Tourniquet - Band-aid - Donor identification card

PROCEDURE:

1- Check to ensure normal donor status.
2- Check donor arms for two acceptable venous access site in each arm.
3- Draw specimen, record ID number, date and send to hematology.
4- Obtain CBC result.
5- Accept or defer the donor based on test results.

REPORTING RESULTS:

1-Write donor pre-donation CBC results on donor card acceptable values are
- HGB: 12.5g/L to no grater than 18g/L
- HCT: 38% to no grater than 50%
- PLT count: 200x 10^9/L- double product 150x10^9/L- single product - WBC <12-9/L

2.Testing of the aphaeresis donor's blood must be identical to that performed for whole blood if the component to be prepared is intended for transfusion the following tests must be negative to prevent transmission of disease: HBs Ag, anti HTLV I/ II, HIV P24 Ag, anti HIV I/ II, anti HCV and serological test for syphilis, and malaria.

3- Donation Interval

a- The interval between procedure should be at least 2 days

b- A donor should not undergo the procedure more than two times in a week or twenty four times per year except in unusual circumstances as determined by blood bank physician.

c- The donor must have a quantitative immunoglobulin and total protein drawn and results charted if

1-The donor is cytapheresed more than two times per week.

2-The donor is plasma pheresed more than once per eight weeks.

3- Minimum acceptable values are: - IgG 6.8g/L - IgA O.5g/L - IgM 0.3 g/L - TP 60g/L

d- The donor's CBC result must be monitored to detect a developing cytopenia; abnormal result shall be reviewed by a blood bank physician to determine suitability for continued donation.

Procedure notes:

1.Donor vital signs are monitored and recorded approximately every 20 minutes during procedure. 2.Donors intra vascular volume deficit may not exceed 10.5 ml /kg of the donor's weight at any time during the procedure.

3.Potential aphaeresis donors who have not donated product for a year or longer must be tested for disease markers prior to their admission to the program. Any travel may have predisposed them to contacting transmissible disease they must be retested and accepted or deferred.

(B) DONOR PREPARATION AND CARE DURING APHERESIS

PRINCIPLE:

Careful preparation of Apheresis pre-donation is required.

POLICY:

In accordance with AABB standards the donor /patient will be prepared before the procedure.

PROCEDURE:

1- Donor should have an adequate meal before donation.

2- He/ She should empty his/ her urinary bladder just prior to donation.

3- Obtain written consent from the donor for aphaeresis procedures.

4- Make sure that the donor is feeling comfortable on the couch.

5- Prominent vein for vein puncture.

6- Serve the donor with fruit juice, talk with him and supply him with reading material or ask him to watch TV.

NOTE: Be sure that the donor is not on any medication, especially aspirin or antibiotics for the last 72hr.

(C) PLASMAPHERESIS

PRINCIPLE:

Plasmapheresis involve the removal of whole blood from donor veins, separation by centrifugation, collection of the plasma, and return of red blood cells, leukocytes and platelets to the donor.

POLICY:

Plasmapheresis will be performed in adherence to AABB standards.

1. For donors in an occasional plasmapheresis program donor selection and monitoring are the same as for whole blood donation.

2. For serial Plasmapheresis, more frequent than every 4 weeks the following principles apply.

- Donor must provide informed consent
- Red cell losses related to the procedure, including samples collected for testing must not exceed

25 ml / week
• If the donor RBCs can not returned during an aphaeresis procedure, hemapheresis or whole blood donation should be deferred for 8 weeks
• Volume limits for automated collection of source plasma maximum annual plasmapheresis collection "excluding anticoagulant" will be 12 L for donors under 175 lb. and 14.4 L for donors over 175 lb.
• At least 48 hr interval between successive procedures and no more than two procedures within a 7 day
• Serum or plasma must be tested for total protein and quantitative Ig, result must be within normal.
• A qualified, licensed physician, knowledgeable in all aspects of hemapheresis.

SPECIMEN:

Donor pre donation

• 5ml purple top for hematology for CBC.
• 5ml purple top for donor processing.
• 7m1 red top for donor processing.

REAGENT, SUPPLIES, EQUIPMENT

1- The machine.
2- The Disposable kit.
3.Venipuncture materials
4. Hemostats.
5. ACD-A

SAFETY PRECAUTIONS:

All blood and blood pro duct must be treated as potentially infectious as per laboratory safety manual.

PROCEDURE:

1- Register donor
2- Complete donor medical history
3- Complete consent
4- Verify the protocol selected on the Machine.
5- Prepare the donor and perform vein puncture
6- Order, label and collect sample
7- Connect, the donor to the machine
8- Follow the instruction of the operating manual.
9-At the end seal and remove product weigh and record on product label
10-Disconnect donor, discard contaminated needles
11- Send product to component preparation

QUALITY CONTROL:

• Before each procedure the machine goes through a series of diagnostic test to ensure that the machine is operational and functioning properly.
• The operator shall carefully observe the plasma collection lines for pink discoloration suggestive of hemolysis.

(D) RED BLOOD CELL APHERESIS

PRINCIPLE:

RBCS apheresis involves removal of RBC from donor's veins, separation by centrifugation.

POLICY

RBC pheresis will be performed in compliance with AABB standards.
• Removal of 2 allogeneic or autologous RBC's units every 16 weeks.
• Saline infusion is used to minimize volume depletion

• The procedure is limited to persons who are larger and have higher hematocrits than current minimum standards for whole blood donors.
For male wt 130 Ib, ht 5.1", hematocrit 40%
For female wt 150 Ib, ht 5.5", hematocrit 40%

SPECIMEN:

• 5 ml purple top tube for hematology
• 5 ml purple top tube for donor processing
• 7 m1 red top tube for donor processing

REAGENT, SUPPLIES, EQUIPMENT

1) The machine.
2) The Disposable kit.
3) Venipuncture materials.
4) Hemostats.
5) SAGM additive solutions.
6) 0.9% Normal saline.
7) Anti coagulant (ACD-A).

SAFETY PRECAUTIONS

All blood and blood product must be treated as potentially infectious.

PROCEDURE:

1) Register donor.
2) Complete donor medical history.
3) Complete consent.
4) Verify the protocol selected on the Machine.
5) Prepare the donor and perform veinipuncture.
6) Order, label and collect sample.
7) Connect the donor to the machine.
8) Start the procedure by following the instruction of the operating manual.
9) At the end seal and remove product weigh and record on product label
10) Disconnect donor, discard contaminated needles
11) Send product to component preparation

QUALITY CONTROL:

Before each procedure the machine goes through a series of diagnostic test to ensure that the machine is operational and functioning properly.

(E) PLATELET PHERESIS

PRINCIPLE:

Thrombocytapheresis involves the removal of whole blood from donor veins, separation by centrifugation collection of platelet layer and return of red blood cells leukocytes and plasma to the-donor.

POLICY:

Platelet pheresis procedures will be performed in accordance with AABB standards.

SPECIMEN:

a- Donor pre donation

• 5 ml purple top for hematology
• 7 m1 red top for viral marker
• 5 ml for donor processing b- Product
• 5 ml purple top for hematology

REAGENT, SUPPLIES AND EQUIPMENT:

1) the disposable kit.
2) Veinipuncture materials.
3) Two hemostats.

4) ACDA anticoagulant.
5) Aphaeresis donor summary sheet.
6) Aphaeresis consent.
7) Donor medical history.

PROCEDURE:

1) Register donor.
2) Complete donor medical history.
3) Complete consent.
4) Place tourniquets on donor arm, perform veinipuncture.
5) Order, label, collect and send specimens.
6) Verify the protocol selected on the machine.
7) follow the instruction in the operating manual.
8)Monitor the procedure
9)Terminate the procedure at the end by clamping the anticoagulant and blood lines
10)Remove veinipuncture needle and apply pressure
11) Seal and remove product, weigh and rest product for 10 minutes.
12) Calculate yield and record on donor chart

SAFETY PRECAUTION:

All blood and blood products must be treated as potentially infectious.

QUALITY CONTROL:

Before each procedure, the machine goes through a series of diagnostic test to ensure that the alarm systems are operational and functioning properly.

1. The operator shall carefully observed plasma collection lines for pink discoloration suggestive of hemolysis.
2. Component preparation is responsible for product quality control "platelet count, Ph, WBC count"

(F) STEM CELL APHERESIS

PRINCIPLE: '

Peripheral blood stem cell harvesting by continuous flow separation is used to collect hematopoietic progenitor cells for autologous transfusion after chemotherapy or bone marrow transplantation.
Hematopoietic progenitor cells enhance marrow production of all cell lines.
Stem cell harvest procedure is also performed to collect T-Cells as a directed donation for bone marrow transplant recipients and oncology patients when indicated.

POLICY:

Stem cell harvest will be performed in accordance with applicable section of AABB standards for Hematopoietic progenitor's cells. This procedure may be performed on donors who do not meet the usual requirements for blood donation only when the harvest cells are excepted to be cases the physician must document in the donors chart that their health permits the procedure.
Donors or patient who have reaction to ACDA during prolonged apheresis procedure, which can not be alleviated with oral titralac, may be given calcium glucoante intravenously if a second procedure is to be scheduled i.e. PBSCH donors, patients. The primary physician medical director will grant approval prior to administration.

***Possible indications for hematopoietic* transplantation.**

Congenital immune deficiencies:
Severe combined immunodeficiency disease.
Wiskott- Aldrich syndrome.
Marrow failure syndromes:
Severe aplastic anemia

Fanconi's anemia
Diamond-Blackfan anemia (congenital hypoplastic anemia)
Inborn disorders:
Mucopolysaccharidoses
Adrenoleukodystrophy
Osteopetrosis
Hemogloinopathies:
Thalassemia
Sickle cell disease
Malignant or premalignant disease of marrow:
Acute leukemia
Chronic myelogenous leukemia
Hodgkin's and non-Hodgkin's lymphoma
Myelodysplastic / myeloproliferative disorders
Multiple myeloma
Solid tumors:
Neuroblastoma
Wilm's tumour
Breast cancer
Ovarian cancer
Testicular cancer.
Other:
Paraxysmal nocturnal hemoglobinuria
Acquired aplastic anemia
Autimmune disorders

SPECIMEN:

Samples are collected by the BMT/oncology clinic prior to procedure. Post samples are collected and sent by immunopathology lab.

SAFETY PRECAUTIONS:

All blood and blood products must be treated as potentially infectious. Patient receiving calcium gluconate intraveneously should be monitored closely. Parenteral calcium should be avoided in a patients receiving digitalis glycosides.
If calcium must be given, administer slowly in small amount to avoid high serum concentration.

QUALITY CONTROL:

1. Before each procedure, the machine goes through a series of diagnostic alarm test to ensure that the alarm system is operational and functioning properly.
2. The operator shall carefully observe the plasma collection lines for pink discolouration suggestive of hemolysis and the medical director will be consulted if hemolysis is excessive.

MATERIALS AND PROCEDURE:

Follow the instruction of the operating manual.

PROCEDURE NOTES:

1. This procedure is for the collection of stem cells.
2. A reaction to ACDA will reveal perioral and extremity numbness not relieved by titralac (maximum dosage up to 24 tablets), tetany spasm of hands which is not relieved by titralac or breathing into a paper bag, or nausea/vomiting which is not associated with the patients illness.
3. Calcium gluconate will be ordered with apheresis orders and sent with patient from the floor.
4. Do not irradiate this product.
5. Immunology lab will collect samples form product and send to hematology and flowcytometry lab.

6. Collect product according to oncology protocols.
7. Product not to exceed 150m1 unless ordered specially by primary physician.

(G) THERAPEUTIC/DONOR LEUKAPHERESIS

PRINCIPLE:

This procedure removes whole blood from the donor separates granulocytes (polymorphonuclear cells) by centrifugation and returns red cells, plasma and platelets to the donor.

POLICY:

Leukaphereiss will be performed in accordance with AABB standard section H2 000. This procedure may be performed on donors who do not meet the usual requirement for blood donation only when the product is expected to be of particular value to an intended recipient. In these cases the physician must document in the donors chart that their health permits the procedure and be approved by the blood bank medical director.

Indications of granulocytes transfusion:

1. Adult with neutropenia and documented gram negative infection that did not respond to adequate antibiotic treatment.
2. Septic infant

Drugs administered for leukapheresis:

1. Corticosteroids:

Oral corticosteriods preparation (hydrocortisone, prednisone methyl predinisolone or dexamethason) may be given before cytapheresis to increase the donors circulating granulocytes count. A protocol using 20mg of oral prednisone at 17, 12 and 2hrs before donation gives superior granulocytes harvest with minimal systemic steroid activity.

2. Growth factors:

G-CSF or GM-CSF can result in collection of up to l Ox 10^{10} granulocytes per apheresis procedure. 3. Hydroxyethyl starch: (HES)

Granulocytes harvest can be improved by more complete separation between granulocytes and the red cells. (HES) is a synthetic polymer of amylopectin that when present in the donor circulation greatly increase separation between red cells and granulocytes.

SPECIMEN:

Donor pre-donation:

5 ml purple top for CBC
5 ml purple top for viral markers
7m1 red top for viral markers

Donor product:

5 ml purple top for CBC D with comment "Leukocytes collection, product samples".

Safety precautions:

All blood and blood products must be treated as potentially infectious. Follow safety precautious.

Reagents, supplies equipments and procedure:

Follow the instruction of the operating manual.

Calculation:

1. WBC yield in unit.

$$\frac{(\text{WBC/L} \times 10^9/\text{L}) \times (\text{Volume of unit ml})}{1000\ \text{mL}} = \text{Total WBC} \times 10^9/\text{L}$$

2. Granulocytes yield in unit:

$$\frac{\%\ \text{granuloc ty(from differential} \times \text{WBC yield of unit}}{100} = \text{Total granulocytes} \times 10^9/\text{L}$$

3. Lymphocytes yield in unit

$$\frac{\%\ \text{jzranulocytes (from differential)} \times \text{WBC yield of unit}}{100} = \text{Total granulocytes} \times 10^9/\text{L}$$

Reporting results:

Product yield is calculated and charted by medical director. Acceptable product should have $1x10^{1^{\circ}}$ granulocytes/unit. Results are to be charted on the donor summary sheet.

QUALITY CONTROL:

1. Before each procedure, the machine goes through a series of diagnostic alarm test to ensure that the alarm system is operational and functioning properly.

2. The operator shall carefully observe the plasma collection lines for pink discolouration suggestive of hemolysis. The medical director will be consulted if hemolysis is excessive.

3. Quality control of products collected for transfusion is assured by cellular counts performed on the product. These counts are recorded by the designated labs and the medical director of the specific lab notified of the results.

Hospital:... :	**Health Care No.:**.....
Name:.....	**Nationality:** .....
Age: ...../.... Yrs./Months Sex: 0 M [I F	**Consultant In-Charge:**.....
Department:	**Unit:**.....

THERAPEUTIC APHERESIS REQUEST FORM

Diagnosis:. ... .:. ....

Type of Apheresis procedure required: ... .

Frequency of treatment: ....

Replacement fluids and additives: . ...

Total volume to be exchanged:.....:..... Number of Exchange sessions: .....

Date AND time exchange needed: .....

Laboratory results:... ....

.....

.....

Blood group:..... Weight: ..... kg. Height: ..... cm

Physician:..... Bleep No:..... Sig..... Date ..... Time:.....

II. THERAPEUTIC APHERESIS

Principle: .

The procedure involves the removal and replacement of plasma or cells. Replacement fluids include colloids, such as 5% albumin, thawed FFP or plasma, Cryoprecipitate reduced, or crystalloids, such as 0.9% normal saline or red cells.

The volume of plasma (cells) removed and the volume / type of replacement fluids is specific to each patient's clinical requirement and determined by the blood bank physicians. Therapeutic exchange must be ordered by the primary physician through a blood bank physician.

POLICY:

Therapeutic aphaeresis shall be done only at the written request of the patient's physician. The blood bank physician must decide the volume of plasma / cells to be removed and type and volume of replacement fluids all consents and requests must be in writing and signed and dated.

All therapeutic procedures will be performed in accordance with AABB the blood bank physician will perform an assessment of the patient, including the explanation of the procedure.

Indication for therapeutic aphaeresis:

a. Replacement with normal plasma to supply an eventual substance

b. To reduce the patient's load of a pathologic substance to levels that will allow improvement.

I- Hematologic Conditions

1. Serum hyperviscosity syndrome resulting from multiple myeloma or waldenstrom's macro globulinemia.
2. Hyper leukocytosis as in acute leukemia with total circulating WBC above 100,00 / ml and circulating blast above 50.000 / ml
3. Thrombocythemia:- Platelet counts above 1.000.000 / ml in patient with evidence of thrombosis or bleeding secondary to thrombocythemia.
4. Thrombotic thrombocytopenic purpura / Hemolytic uremia syndrome TPE with FFP or plasma, cryoprecipitate become the treatment of choice for TTP / HUS.
5. Complication of sickle cell disease

- Priapism
- Stroke
- Acute chest syndrome
- Multi organ failures

II- Cryoglobulinema

III- *Neurologic Conditions*

1- Myasthenia Gravis

TPE is used as

- Adjunctive treatment for patients experiencing exacerbation not controlled by medications
- For a patient being prepared for thymectomy.

2- Acute Guillain-Barie Syndrome

TPE if initiated early can decrease the period of minimal sensorimotor function.

3- Chronic inflammatory demyelinating polyneuropathy seen in HIV patient, TPE and IV lg have equivalent efficacy in-patient unresponsive to corticosteroids

4- Polyneuropathy associated with monoclonal gammopathy of undetermined significance.

IV-Renal Diseases

1. Rapidly progressive glomerulornepheritis, usually responds to TPE as an adjunct to immunosuppressium drugs.
2. Good pasture disease.

V- Other Conditions

1. Homozygous type II familial hypercholesterolemia prolonged reduction in circulating lipids can be achieved with repeated TPE.
2. Refsum's disease, rare inborn error of metabolism on having toxic level of phytanic acid causing neurologic, cardiac, skeletal and skin abnormalities TPE is useful in conjunctions with phytanic acid deficient diet.
3. Separation of lymphocytes by apheresis, subject them to irradiation and then transfuse it to the patient as a treatment for cutaneous T. cells lymphoma. (Photopheresis).

Therapeutic Plasmapheresis

Calculations:

Volume of exchange fluid for one-volume exchange TPE = Patient's weight (Kg) x 60ml / Kg

Example Patient weight = 50Kg

Volume of exchange = 50Kg x 60ml / Kg = 3000ml=3liters fluid

Calculated and ordered by blood bank physician.

SPECIMEN:

- 5ml purple top tube for CBC
- 3m1 blue top tube for " PT,PTT,Fib".

• 7ml red top tube for chemistry " serum protein, lipids

MATERIALS:

• The machine
• Plasma exchange disposables
• Veinipuncture materials
• Hemostats
• Anti coagulant ACD-A, 4% tri sodium citrate
• Replacement fluids prescribed by physician
• Consent form

SAFETY PRECAUTIONS:

All blood and blood products must be treated as potentially infectious.

QUALITY CONTROL:

• Before each procedure, check the machine to ensure that they are operational and functioning properly
• The operator shall carefully observe the plasma collection lines for pink discoloration suggestive of hemolysis, the medical director will be consulted of hemolysis is excessive.

PROCEDURE:

1. Assess patient
• Review chart for vital signs and patient consent
• Vein access
• Medications
• Discontinue ACE inhibitors at least 24hr prior to the procedure, as they may cause flushing, sever hypo tension, dyspnea, bradycardia and cardiac arrest.
• If the patient is on anti cholinesterase medication, the dose preceding the procedure should be decreased to half to avoid complication of bradycardia hypotension, pallor and respiratory arrest
• Record the patient wt, height
• Patient should have a light meal 1-2hr prior to procedures.
2. Arrange for time of the procedure
3. Order appropriate exchange solution as determined by patient physician.
4. Obtain the volume of exchange fluid for one-volume exchange TPE, calculated and ordered by blood bank physician. Volume of exchange=patient wt (Kg)x60m1 / kg
5. Verify the protocol selected "TPE protocol" on the machine.
6. Prepare the donor and perform veinipuncture.
7. Open the blood line
8. Start the procedure by pressing draw
9. Follow the instruction in the operating and maintenance manual.
10. When the procedure is completed:
• Clamp the anticoagulant and blood line
• Remove the veinipuncture needle, apply pressure to occlude blood flow
• Seal and remove the plasma bags from the disposable set.

GENERAL NOTES:

a. Take temperature, pulse and blood pressure every 10 min Notify primary physician of positive or negative results.

b. Comparison of replacement fluids

Replacement solution	*Advantage*	*Disadvantage*
Crystalloid	Low cost	2-3 volumes required
	Hypoallergenic	Hypo-oncotic
	No hepatitis risk	No coagulation factors
		No immunoglobulin
Albumin	Iso-oncotic	High cost
	No contaminating "Inflammatory	No coagulation factors
	Mediators"	No immunoglobulin
Fresh frozen plasma:	Maintain normal levels of IgG.	- Hepatitis & HIV Risk
	- Complement	- Citrate load
	- Antithrombin	- ABO incompatibility risk
	- Other proteins	- Allergic reaction
		- Sensitization

III. APHERESIS DONOR ADVERSE REACTIONS

PRINCIPLE: -

To recognize donor / patient reaction and to provide initial treatment.

POLICY:

In accordance with AABB standards, the donor / patient will be observed closely during the procedure and any adverse reaction will be treated and documented.

REAGENTS, SUPPLIES AND EQUIPMENT:

- **Cloths**
- **Emesis basin**
- **Ice packs**
- **Paper bags**
- **-Titralac tablets**
- **Donor reaction list**
- **MCS3P procedure sheet**
- **Oral fluid**
- **Normal saline .9%**
- **Spirits of ammonia**
- **Normal saline for injection**
- **Sterile syringe / needles**
- **Alcohol pads**
- **Ca-Gluconate lOml. LV. amp.**
- **Dilantin, phenobarbital or valium IM or LV.**

QUALITY CONTROL:

Donor charts are reviewed prior to commencing procedure.

PROCEDURE:

1-Fainting or syncope

•Symptoms may include a feeling of weakness and dizziness, loss of consciousness and Generalized convulsion, the most useful signs to watch for are facial pallor, perspiration, slow pulse, and restlessness and low blood pressure.

Treatment:

• Stop procedure and keep veins open with normal saline
• Place donor in trendelenberg position
• Loosen tight clothing
• Ensure patent airway
• Apply cold compresses to forehead and / or back of neck, both are usually effective.
• Administer aromatic spirits of ammonia, donor should respond to the ammonia by coughing which elevates the blood pressure".
• Monitor vital signs every 5-6 min until stable. Offer and insist that the donor drink at least one glass of fluids and encourage more if the symptoms are progressive discontinue the procedure.
• Keep the donor in this position for a minimum of ten minutes and raise head gradually and only as the donor tolerate it.
• Have donor sit upright for ten minutes before permitting them to stand.
• If symptoms are persistent or if there is a persistent fall in blood pressure notify the blood bank physician.

NOTE:

If the donor is not responding to these measures with the blood bank physician order, you may initiate a saline bolus.

2- Nausea and vomiting:

Treatment:

• Stop procedure and keep a vein open with normal saline.
• Make the donor as comfortable as possible.
• Instruct the donor to breath slowly and deeply.
•Apply cold compresses to the fore head and /or the back of neck.
•Turn donors head to one side to prevent aspiration.
• Have an emesis basin ready and wet towel to wipe the donor `s mouth.
• After vomiting, offer the donor water to rinse his / her mouth.
• Check the donor's blood pressure. If elevated, notify the blood bank physician.

3- Twitching or muscular spasms

• Nervous donors may hyperventilate causing faint muscle twitching or titanic spasm on their hands or face.
• Donors should be watched closely during and immediately after donation. This may also be the result of ca binding by anticoagulant citrate "see paresthesia below"

Treatment:

• Engage the donor in conversation.
• Give the donor cold liquid to drink. If these all fail, and donor is getting light headed, keep the donor's attention on you by speaking slowly to them while telling them to take a deep breath and hold it until the count of five.

NOTE: Do not give oxygen

4-Paresthesia / muscle cramping

• Symptoms may include abnormal sensation as tingling, prickling, burning, tightness, a feeling of band or girdle around the limb or trunk. Most common sensation, tingling around the lips and finger tips, is usually the result of ca deficiency because of ca binding by citrate.

Treatment:

• Treat by slowing the reinfusion and centrifuge and do not begin another cycle until symptoms disappear.

• Give four titralac tablet to be chewed, may be repeated every 15-20 minutes.

• If symptoms still do not disappear, notify the blood bank physician. LV. Injection of ca gluconate may be indicated.

5- Hematoma, during or post donation

Treatment:

• Remove needle and place three or four sterile gauze over the veinipuncture site and apply firm digital pressure for 7-10 minutes, with the donor's arm held above heart level,

• Apply cold compresses to the area for about 5 minutes

• If arterial puncture is suspected, apply firm pressure for ten min and notify blood bank physician. Check and apply pressure dressing after there is evidence that no hematoma is occurring. Check for the presence of radial pulse. If pulse is not palpable or weak, notify the blood bank physician

6- Convulsion

Treatment:

Stop procedure and remove needles

• Call for help immediately prevent the donor from injuring him or herself. During sever seizures, some people exhibit great muscular power and one difficult to restrain

• Remove IV needles

• Ensure the donor has a patent airway

• Notify the blood bank physician

• IM / IV dilantin or Phenobarbital or valium may be indicated

7- Cardiac arrest or symptoms

Treatment:

• Stop procedure.

• Immediately call for emergency cardiac care. Notify blood bank physician, initiate CPR

8- Chills

Treatment:

Treat symptomatically with blankets. If chills persist and become progressively worse, pause procedure, keep the vein open with normal saline and notify the blood bank physician.

9- Headache:

Treatment

• Slow the re-infusion. Check the donor's blood pressure. If elevated, notify the blood bank physician

10- Irregular pulse

Treatment:

Discontinue the procedure and notify the blood bank physician.

11- Suspicion of air embolism

• Lower head of bed / chair, elevate legs, turn donor on left side and call blood bank physician immediately.

PROCEDURE NOTES:

1- Close observation of donor and promote treatment of mild reaction will prevent reaction from progressing to moderate or sever.

2- If a donor has not eaten for more than 3hr prior to donation, he should be offered some nourishment (cookies and juice)

3- Donor who experience moderate to sever reaction must be seen by blood bank physician immediately and should be deferred from future donation.

4- Classification of reaction:

Mild reactions

- Chills
- Hematoma
- Hyperventilation
- Nausea
- Perioral paresthesia
- Vasovagal syncope

Moderate reactions

- Allergic reaction
- Hypertension / Hypotension
- Urticaria
- Vomiting

Sever reactions

- Suspect air embolism
- Dyspnea
- Anaphylaxis
- Cardiac arrhythmia's
- Convulsions
- Chest pain
- Respiratory Distress.
- Tetany

IV. CARE OF DONOR POST PROCEDURE PRINCIPLE:

Careful observation of aphaeresis donors post-procedure is required.

POLICY:

After the donation is completed the donors will be cared for and informed of post donation care.

1- Check the phlebotomy sites and apply bandage or pressure dressing once the bleeding stops. 2- Have donor remain in donor chair a few minutes under close observation by the staff. 3- Allow donor to sit up when his/ her condition appears satisfactory. Staff member should stay with donor as he/ she assumes upright position.

4- Send to donor room and provide donor liquid refreshment.

5- Ask donor to remain in the area for 10 minutes.

6- Note on the procedure sheet any adverse reaction and treatments that occulted and if the donor leaves the area before recommended time.

V. PRE DONATION ADMINISTRATION OF GCSF "GRANULOCYTES COLONY STIMULATING FACTOR" TO HEALTHY DONORS:

Principle:

Granulocyte stimulation with GCSF is done to boost cell count for apheresis collection 2-24hr later.

Policy:

The clinical service is responsible for providing candidates for granulocytes donation with GCSF. All prospective donors must be examined and found suitable to donate according to AABB standards. GCSF will be administered at a specified interval prior to granulocytes collection.

Reagents, supplies and equipment:

1. Consent for the collection of blood specimens from donors/family donors.
2. Alcohol swabs.
3. Guaze swabs.
4. GCSF "Filgrastim-Neupogen" pre filled syringes in 30 MU doses administration ready stored in refrigerator at 2-8°C until expiration date shown on package.
5. Band-aid.
6. Possible reaction to GCSF and suggested treatment sheet.

Safety precautions:

All blood and blood products must be treated as potentially infectious.

Procedure:

1. All prospective donors must present to apheresis area two days prior to collection. Assess venous access and draw blood specimens for CBC and viral marker testing.
2. The blood bank physician/medical director must examine all donors ensure that there is documentation of the examination by the physician, finding the donor to be physically capable of GCSF treatment.
3.Open a donor file for each donor found to be suitable.
4. Obtain the donor to get the injection from his physician o the blood bank physician.
5. Determine the timing of the dosage according to the granulocytes collection schedule.
6.Inform the donor of the details of the procedure and side effects of the GCSF.
7. Have the donor read an sign the consent form, which will include consent for administration of GCSF. Ensure that he/she understands.
8. Determine the dosage according to donor weight
9. Prepare the site "usually upper arm" and inject the determined dose subcutaneously.
10. Observe the donor for 30min post injection for possible reactions
a. Muscle pain, bone pain
b. Diarrhea
c. Headache d. Dizziness e. Nausea/vomiting.
11. Instruct the donor when return for the apheresis procedure.

Calculation:

Total dose GCSF(ug) = 5ug GCSF/kg body weight (l0ug = 1MU) Example Dose for a 50kg patient = 5ug /kg x 50kg = 250 u = 25MU GCSF.

NB donors who weight more than 60kg will need to use more than one syringe of neupogen. Due to the injection mode this will need to be given in 2 separate sites.

Reporting result

1. Chart the details of the injection including GCSF lot number and expiry dates in the donors prepared apheresis chart and on the GCSF supply. Sign the entry and write employee ID number.
2. Have the blood bank physician/medical director sign the chart entry.

Limitation of procedure:

l. Drugs to facilitate leukopheresis shall not be used for donors whose medical history suggests that such drugs may exacerbate previous or intercurrent disease.
2. Contraindications
a) Donors with heart or lung disease
b) Donors with hypersensitivity to medication
c) Donors with WBC count > 12 x 10^9/L.

Treatment of possible reactions:

1. Headache-Acetaminophen.
2. Bone pain- Acetaminophen
3. Muscle pain- Acetaminophen
4. Nausea/vomiting-self limiting/ Fluid replacement, if persist cessation of drug administration
5. Dark urine-cessation of drug administration

VI. BLOOD BANK / APHERESIS PROCEDURES INFORMATION FOR PHYSICIANS AND NURSES

Apheresis procedures are arranged by the patient's physician through the blood bank physicians. These procedures include:-

1. Therapeutic Plasma Exchange.
2. Red Blood Cell Exchange.
3. Therapeutic Leukopheresis.
4. Therapeutic Plateletpheresis.
5. Stem Cell Harvest.
6. Lymphocyte Procedures.

The attending physician and / or Nurse looking after the patient must arrange the following:

1. Signing and dating of informed consent by patient or next of kin.
2. Completing and signing therapeutic apheresis request form and returning it to the aphaeresis department (see attached).
3. Aphaeresis nurse will arrange the time for he procedure.
4. Aphaeresis nurse will book location for procedure and will notify the unit of the location. The unit nurse is responsible to arrange transportation to and from the procedure site.
5. The primary and / or the unit nurse is responsible for checking blood products with the aphaeresis nurse upon arrival of the patient to the procedure site.
6. Insert a stiff central line catheter (double lumen Quinton recommended) if apheresis staffs have determined the patient's peripheral veins are unsuitable.
7. All specimens must be ordered stat. Routinely, CBC, PT, PTT, Fib, Albumin, CA and TP may be ordered. The Apheresis nurse will notify the unit.
   Red Blood Cell Exchanges require the patient to have a crossmatch for PRBC's:
   Adult patients: Four (4) units Pediatric patients: Two (2) units
   Results should be available at least two hours before the aphaeresis procedure is scheduled. When PT,PTT and Fibrinogen are ordered, please comment: for TPE in the order or it will not be resulted. 8. Record patient's weight and height in chart and send patient chart with the patient. Daily weights are required to be taken and recorded on the patient chart, when possible. 9. Discontinue ACE inhibitors at least 24 hours prior to the intended apheresis procedure, as they may cause flushing, severe hypotension, dyspnea, bradycardia, and cardiac arrest. If any of these medications are given 24 hours prior to procedure, please notify the apheresis nurse unit.

ACE INHIBORS INCLUDE:	
Short Acting	Long Acting
Captopril (Capoten)	Benazepril (Lotensin)
Capo side (Captopril and HTZ)	Enalpril (Vasotec)
Fosinopril (Monopril)	Lisiopril (Prinivil or Zesril)
Quinapril (Accupril)	Prinzide or Zestoretic (Lisinopriland HTZ)
	Ramipril (Altace)
	Vaseretic (Enalpril and HTZ)

10- If the patient is on anticholinesterase medications, the dose preceding the procedure should be decreased to half to avoid complications of bradycardia, hypotension, pallor, and respiratory arrest. The remainder of the dose, if so ordered by the primary physician, may be given post procedure.

Anticholinesterase Drugs Include			
Mestinon	**Provocholine**	**Prostigmine**	**Urecholine**
Antilirium	**Pilocarpine hydrochloride**		

11- Ensure the patient has a meal or drinks a glass of milk, if possible, 1-1/2 to 2 hours before procedure to minimize possible side effects of hypocalcaemia induced by the anticoagulant, ACD:A.

Please encourage high calcium content foods-cheese, laban, yogurt, milkshakes, milk.

12- When the procedure is finished, the apheresis nurse will contact the primary nurse and give a herbal report of patient's condition. The unit nurse must arrange for transport of the patient back to the nursing unit.

13- Some medications may not be given during the procedure because they will be removed by the procedure.

Refer to primary physician or to blood bank physician if in doubt.

NB: It is recommended that the dose of antcholinesterase meds be split to give the patient one half the dose at the usual time before the procedure and the remainder after the procedure. Some of the medication will be removed during the procedure. It is the primary physician's responsibility to order: a) The dose be split. b) The dose not be split.

Either order should be documented on the patient's chart. It is the apheresis nurse's responsibility to ensure the primary physician is aware of he medication recommendation.

CHAPTER FOUR : DONOR BLOOD TESTING

1 - ABO BLOOD GROUPING FOR ALL BLOOD DONORS.

2 - Rh. TYPING FOR ALL BLOOD DONORS.

3 - DETECTION OF UNEXPECTED ANTIBODIES TO RED CELL ANTIGENS FOR ALLOGENIC BLOOD DONORS (Antibody Screening Test) : Qurantine all positive antibody screening test donor blood.

4 - TRANSFUSION TRANSMITTED DISEASE TESTING :

The infectious disease screening and confirmatory tests done in the blood banks of Ministry of Health can be changed annually according to the circumstances of medical supply tenders of M. O. H. So, you have to follow at all times the instructions give by each and every particular manufacturer as guideline for the performance of each test.

General Instructions and Policies

1. All blood units must be screened to the (TTDs) following Transfuction Transmitted Disease markers according to AABB policy Number : 5.8.4, by Certified FDA or CE ELISA tests:

1) HBsAg
2) Anti - HBC
3) Anti - HBs for all Anti - HBc Positive samples.
4) Anti - HCV
5) HIV I / II
6) HIV RNA
7) HBV DNA
8) HCV RNA
9) Anti-HLTV I/II
10) Serological test for syphilis (RPR)
11) Malaria by thick film or any other MOH approved tests.

2. All blood component units must be stored in separate secured proper storage places until all TTD test results released.
3. All blood component units with positive screen TTD test results must be quarantined from the first screen test results.
4. All blood component units with positive screen TTD test results must be repeated in duplicate one sample from blood unit and another sample from the test tube, before the release of the whole Batch of donation, to solve any problems about labelling.
5. All repeat TTD positive screen test results must be confirmed by neutralization or western Blot tests approved from FDA or CE with blood sample from the donor.
6. Notify the Health authorities with all the confirmed TTD positive test results.
7. Notify the donors will all the confirmed TTD positive test results.
8. Follow up donors through special infectious Disease Management clinics and Social consultation and assistance offices.
9. Defer permanently all blood donors with positive screening or confirmatory results for HIV I/II, HTLV I/II, HBV and HCV.
10. Defer for three years all blood donors with positive Malaria test from the date of treatment and cessation of symptoms.
11. Defer for one year all blood donors with positive syphilis test or history of Gonorrhea from the date of treatment and cessation of symptoms.
12. Anti-HBs test should be performed to all Anti-HBc. Positive samples, and discard blood if Anti-HBs result is negative and use blood if Anti-HBs result is positive due to presence of Natural Immunity in this donor for HBV.

(A) SCREENING TESTS

1. EIA FOR QUALITATIVE DETERMINATION OF HEPATITIS B SURFACE Ag "HBsAg" (EIA, Qualitative):

Principle:
All donations are screened for hepatitis B surface antigen "HBsAg' immunoassay as a means to

eliminate potentially infectious donor and provide a safer donor pool. The method is a direct, non competitive sandwich assay based on the ELISA technique.

The presence of HBsAg allows the enzyme tracer to bind to the solid phase. The enzyme activity is therefore proportional to the HBsAg concentration present in samples or control.
The measurement of bound enzyme activity is performed by adding a chromogen /substrate solution. The enzyme action on chromogen / substrate produces a color which can be detected with a photometer.

2. THE QUALITATIVE DETERMINATION OF TOTAL AB TO HEPATITIS B CORE Ag "ANTI-HBc" (EIA, Qualitative):

Principle:

All donations are screened for anti-HBc to eliminate potentially infectious donor and provide a safer donor pool.

The method for qualitative anti-HBC determination is simultaneous competitive assay based on the ELISA technique as given below.

1. Well coated with recombinant HbcAg.
2. Anti-HBc from sample or control.

3. Enzyme traces: anti-HBc antibodies (human) conjugated to horseradish peroxide. Anti-HBc present in the sample and labeled anti-HBc antibodies complete for fixed quantity of enzyme tracer bound to the solid phase and consequently the enzyme activity are inversely proportional to the anti-1-113c concentration present in samples or controls. Measurement of enzyme activity is performed by adding chromogen substrate solution. The enzyme action on substrate produces a color which is measured with photometer.

3. QULITATIVE DETERMINATION OF ANITBODIES TO HEPATITIS B SURFACE Ag "Anti-HBs" (EIA, Qualitative):

Principle

All donations are screened for Anti-HBs as a mean to eliminate potentially infectious donors and provide a safer donor pool.
The method for qualitative Anti-1-113s determination is a direct non competitive sandwich assay based on the Elisa technique as given below.
The presence of Anti-1-113s allows the enzyme after tracer to bind to the solid phase. The enzyme activity is therefore proportional to the concentration of Anti-1-113s present in the sample of calibrator. Measurement of enzyme activity is performed by adding chromogen substrate solution. The enzyme action on substrate produces a color which is measured with photometer.

4. DETECTION OF ANTIBODIES TO HUMAN HEPATITIS C VIRUS, Anti-HCV:

Principle

The HCV antibody EIA assay is used for the detection of antibodies to proteins expressed by putative structural and non structural regions of the HCV genome. The presence of these antibodies in the serum or plasma of donor blood indicates that the individual has been infected with HCV, may harbor infectious HCV, and may be capable of transmitting non-A, non-B hepatitis and these donations should be eliminated from the blood supply.
The wells of the plates are coated with a mixture of HCV antigens: cores, NS3, NS4, NSS. The test samples are incubated in such well. Virus specific antibodies to HCV, if present in the sample will bind to the solid phase antigens subsequently rabbit anti human IgG labeled with enzyme horseradish peroxidane is added. Upon a positive reaction this labeled AB becomes bound to any solid phase Ag, AB complex previously formed. Incubation with enzyme substrate produces a blue color in the test well which turns yellow when the reaction is stopped with sulphuric acid. If the

sample contains no HCVAB, then the labeled AB cannot bind specifically and only a low background color developed.

5. HIV I & II EIA AG/AB (EIA)

Principle

HIV Ag/Ab combination is based on microwells coated with a synthetic peptide representing an immunodominant region of HIV-1 [0], recombinant protein derived from the envelope proteins of HIV-1 and HIV-2 and an HIV Pol protein and monoclonal AB reactive with p24 and p26. The conjugate is a mixture of the same antigen epitopes and different monoclonal AB to p24 and p26, all labeled with horseradish peroxidase. Test specimens and control sera are incubated in the wells and p24 or p26 and/or/AB to HIV in the sample or control sera bind to the AB and/or Ag on the microwell, in subsequent step, conjugate is added which in turn binds to any p24, p26 and/or specific antibody already bound to the reagent on the well sample not containing p24, p26 or specific AB will not cause the conjugate to bind to the well. Unbound conjugate is washed and solution of TMB and hydrogen peroxide is added to the well, wells with bound conjugate developed a blue green colour which is converted to an orange colour which may be read at 450nm after the reaction has been stopped with sulphuric acid.

6. QUALITATIVE DETECTION OF ANTIBODIES AGAINST HUMAN HTLV I/II

Principle

The HTLV I/II EIA is an enzyme immuno-assay for the qualitative detection of Ab to human T-lymphotropic virus type I and type II "HTLV I/II". It is intended as a screen for donated blood to prevent transmission of HTLV I/II to recipients of cellular blood component and as an aid in clinical diagnosis of HTLV I/II. The test specimens and control sera are incubated in the wells "which are coated with synthetic peptides representing immunodominant regions from HTLV I and HTLV II envelope proteins and a recombinanat transmembrane protein from HTLV II". The Ab to HTLV I or II in the sample or control serum bind to the Ag on the micro well. Sample and any excess Ab is then washed away. Conjugate mixture of the same peptide Ags and recombinant transmembrane protein from HTLV I, each of which has been labeled with horseradish peroxidase" is added, which in turn binds to any specific Ab already bound to the Ag on the well. Samples not containing specific Ab will not cause the conjugate bind to the well, unbound conjugate is washed away and a solution containing (TMB) and hydrogen peroxide is added to the wells. Wells with bound conjugate develop a purple colour, which is converted to an orange colour when the reaction is stopped with sulphuric acid. The concentration of Ab to HTLV in the sample can be read spectrophotometrically at 450nm.

7. SYPHILIS "RPR"

Principle:

RPR test is a macroscopic non-treponemal flocculation test for the detection and titration of reagins "Circulating Ab produced as a result of the tissue damaged caused by the disease" in serum or plasma. The antigen employed in the kit is a modification of the VDRL antigen, in the presence of reagins agglutination occurs which is visibly enhanced by the presence of the micro particulate carbon.

8. MALARIA PARASITES

Policy:

In Compliance to the ministry of health regulations a sample of blood from each donation will be tested for malaria parasite. Whole blood and blood components will not be used for transfusion unless the result is negative.

Principle:

Thick films should be prepared for all donors and stained unfixed after drying at 37'C FOR 15

min using Giemsa stain, then film examined for the presence of malaria parasite by microscope.

Specimen:

Sample of venous blood from the donor.

Reagent and supply:

l. Giemsa powder 3.8gm
2. Methyl alcohol 250 ml
3. Glycerol 250 ml
4. Slide for film preparation
5. Light microscope

Safety and precautions:

Treat blood and its products and its potentially infectious.

Quality control:

1. All new batches of Geimsa stain should be tested with a known p.vivax or p.ovale infection to ensure that schuffner's dots are stained and that parasitized cells are decolorized. Blood film for this purpose can be wrapped in Para film and frozen. Frozen films must be brought to room temperature before unwrapping.
2. Films on all positive cases should be examined by two observers.

Procedure:

A- Stain Preparation:

Add stain and glass beads to bottle. Add glycerol and alcohol. Shake vigorously and place at 37C' for 24h. with further frequent shaking remove from the incubator and shake again over 24h. the Stain is then ready for use filter small amounts when required.

B- Thick film preparation:

1. A drop of blood, 3-Smm in diameter is put into the center of a 76x26mm slide and spread with the corner of another slide or swab stick to cover an oval area of approximately 1015mm in diameter. 2. Thoroughly dry the smear, horizontally, in an incubator at 37'c for one hour.

C- Staining methods:

1. Do not fix the dry film, but place it gently in coplin jar containing buffered water and allow to lyse until no more haemoglobin can be seen falling away from the smear.
2. Remove from water, place face - down on staining dish, and stain with Giemsa diluted 1 in 10 with water.
3. Stain for 30 min then rinse briefly with tap water and drain dry.
4. Examine the film by microscope under 100 high power (x 100) fields for the presence of malaria parasite.

(B)CONFIRMATORY TESTS

1. HEPATITIS B SURFACE ANTIGEN CONFIRMATORY ASSAY

Principle:

The hepatitis B surface antigen "HBsAg" confirmatory assay is used for testing a repeat reactive hepatitis B surface antigen enzyme immunoassay samples in which the hepatitis B surface Ag is neutralized. The assay uses the principle of specific AB neutralization to confirm the presence of HBsAg.

A) In the first stage: Sample allowed to react with specific AB bound to a solid phase, whereupon either the complex solid phase AB/Ag or the complex solid-phase AB/interfering substance is formed.

B) In the second stage: A specific unlabeled anti HBs AB is allowed to react with one of th two duplicate series, while a serum non-reactive for anti HBs AB is allowed to react with the second duplicate series.

C) In the third stage: The labeled anti HBs AB is added to both duplicate. If the positive reaction in the screening test was caused by an interfering substance, the unlabeled specific AB will not inhibit the reaction of the labeled AB with the solid phase complex. Conversely, if the positive reaction in the screening test was caused by the presence of HBsAg , the unlabelled specific AB will inhibit the binding between the labeled AB and the solid phase complex. A reduction of the value of the neutralized sample with respect to the value of the non neutralized sample will confirm the presence of HBsAg.

2. HCV Ab. Confirmatory Test

Principle

Is based on the principle of an enzyme immunoassay. A diluted test sample is incubated in trough together with the LIA test strip. If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip. Subsequently, an affinity purified alkaline phosphatase labeled goat anti human IgG (H+L) conjugate is added and reacts with specific HCV Ag/Ab complexes if previously formed, incubation with the enzyme substrate produces a chestnut like color, the intensity of which is proportionate to the amount of HCV. Specific antibody captured from the sample on any given line color development is stopped with sulfuric acid.

3. Anti-HIV I/II Confirmatory Test

For the confirmation of antibodies to HIV-I, including group O, and HIV-2, as well as for the differentiation between HIV-1 and HIV-2 Ab.

Principle

Recombinant proteins and synthetic peptides from HIV-1 and HIV-2 and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing.
Five HIV-1 antigens are applied: sgp 120 and gP41, which detect a specific Ab to HIV-l, and p31, p24 and p l7 which may also cross-react with Ab to HIV-2. Group O peptides are present in the HIV-1 sgp 120 band. The antigens gP36 and sgp 105 are applied to detect specific Ab to HIV-2.

In addition to these HIVAg's four control lines are coated on each strip:2 cut off lines (all human Ig Gs) 1 strong positive control line which is also the sample addition control line "anti human Ig" and one streptavidin control line.
The test sample is incubated in a test trough together with the multiple antigen coated test strip. Specific HIV Ab if present in the sample, will bind to the individual HIV Ag lines on the strip afterwards, a goat anti human IgG labeled with alkaline phosphate is added which will bind to any HIV Ag/Ab complex previously formed.
Incubation with enzyme substrate and the chromogen BCIP produces a dark brown colour in proportion to the amount of specific Ab present in the sample. Colour development is stopped with sulfuric acid. If the sample contains no HIV-specific Ab, the labeled anti human Ab will not be bound to Ag/Ab complex so that only a low standard background color develops.

4. Anti-HTLV I/II Confirmatory Test

Principle

Based on the enzyme immunoassay principle, a diluted test sample is incubated in a test trough together with the multiple antigen-coated strips. Specific HTLV Ab, if present in the sample, will bind to the HTLV antigen lines on strips, subsequently, a goat anti-human IgG labeled with alkaline phosphatase is added and will bind to any HTLV Ag/Ab complex previously formed. Incubation with a chromogenic substrate produces a dark brown colour in proportion to the amount of specific Ab present in the sample. The colour development is stopped with sulfuric acid. If the sample contains No HTLV specific Ab, only a low background colour develops.

5- SYPHILIS SEROLOGY TEST (TPHA)

Principle:

When diluted positive samples are mixed with sensitized erythrocytes, Ab to sensitizing antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of micro titration plate well. In the absence of Ab they form a compact button in the well.

C. UNIT QUARANTINE

PRINCIPLE:

Blood units or any prepared components are quarantined under the following circumstances:

1. **Positive serological test result for any one of the transfusion transmitted disease, on the first screening test, even if become negative by repeat screening or confirmation tests.**
2. **Confidential unit exclusion request by Donor. (CUE).**
3. **A discrepancy exists between a units ABO/Rh type and the corresponding donors historical or tube results.**
4. **Permanently deferred donors units will be quarantined upon donation.**
5. **Unsuitable units for transfusion:**
   - **a. Quantity not sufficient (QNS).**
   - **b. Heavy units, weight>525gm (>495m1)**
   - **c. Closed system compromised**
   - **d. Hearsay information**
6. **Polycythemia patients units for therapeutic donation.**

POLICY:

The donor room senior, senior nurse apheresis, senior technologist infectious disease testing, and blood bank supervisor will manually quantitative donated units in response to any of the above reasons, or to variances in procedure, resulting in units being assessed unsuitable for transfusion.

MATERIALS:

1. **Quarantine label.**
2. **Biohazard label**
3. **Variance documentation 4. Quarantine report.**

SAFETY:

All blood and blood components must be treated as potentially infectious.

QUALITY CONTROL:

Verify quarantine report and final disposition of quarantinable units and components everyday before released units for transfusion.

PROCEDURE:

1. **Identify units and components need to be quarantined, from a given donation number.**
2. **Attach to the unit a quarantine and biohazard labels.**
3. **Isolate quarantinable units in a separate secured storage place.**
4. **Complete variance documentation and quarantine report, stating reason for quarantine and final disposition (autoclaving or Incineration) and date of Quarantine and the person received the Quarantinable units and his signature, after consultation with medical Director or Blood Bank**

(C) Nucleic acid Amplification Technology (NAT) Tests

The NAT tests are a qualitative in vitro tests for the direct detection of Hepatitis B Virus (HBV) DNA, Human Immunodeficiency Virus Type 1 (HIV-1)RNA and Hepatitis C Virus(HCV)RNA in human plasma from donations of whole blood and components for transfusion.

The test is intended for use in screening individual donor samples of human plasma or pools of human plasma composed of equal aliquots of not more than 12 individual donations. These tests are intended to be used for detection in conjunction with licensed tests for detecting antibodies or antigens. The ultimate objective of implementation of NAT is to assure a safer blood supply.

NAT technology generates multiple copies of specific nucleotide sequence from a target organism. It also provides a mechanism to detect extremely low levels of viral material present before the body begins producing antibodies and reduce the window period .NAT undergoes into three main steps process referred to as a cycle, that is repeated a specified number of times. One PCR cycle consist of Denaturation, Annealing and Extension. The process takes place in a thermal cycler and also called Amplification. This process of amplification allows the scientists to detect the presence or absence of a specific virus or bacterium and in some cases the quantity of pathogens. The implementation of antigens or antibodies test in patients blood infected with HIV, HCV and HBV has reduced but not completely eliminated the risk of transmitting of viral infections by transfusion of blood products.

Principles of the Procedure

The HBV Test is based on four major processes while HIV and HCV tests are based on five steps.

1. Sample processing

Two specimen processing are used as follows:

a. Multiprep specimen processing procedure for the testing of mini-pool specimens, using pool of 12.

b. Standard specimen processing procedure for preparation of individual donor samples.

In the standard specimen processing HBV DNA, HCV RNA or HIV RNA is isolated directly from plasma by lysis of the virus particles with Multiperp Lysis Reagent followed by precipitation of the DNA (HBV) or RNA(HIV & HCV) with alcohol. In the Multiprep specimen processing procedure, the viral particles are first depelleted from the plasma sample by high speed centrifugation (at 23,000 x g for 60 + 4 minutes at 4 ^{0}C)

Followed by lysis of the depelleted virus with Multiprep Lysis Reagent and precipitation of the DNA or RNA with alcohol.The Multiprep internal control (MP IC) is introduced into each sample with the Multiprep Lysis Reagent and serves as an extraction and amplification control for each processed specimen and control. The internal control is a DNA plasmid (HBV) and RNA plasmid (HIV & HCV) with primer binding regions identical to those of the target sequence, a randomized internal sequence of similar length and base composition as the target sequence and a probe binding region that differentiates the internal control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the internal control and the target DNA or target RNA.

2. Reverse Transcription

Reverse transcription of target RNA to generate complementary DNA (cDNA) in HIV and HCV tests are performed with the thermostable recombinant enzyme Thermus thermophilus DNA polymerase (r Tth pol). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions. rTth pol has both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture. Reverse transcription using rTth pol produces a cDNA copy of the HIV or HCV target and the HIV or HCV internal control RNA. This stage isn't applied to HBV DNA.

3. PCR Amplification

The amplification reactions are performed with the thermostable recombinant enzyme Thermos aquaticus DNA polymerase (Taq pol) the reaction mixture is heated to separate double-stranded DNA. As the mixture cools, primers anneal to the target DNA and in the Presence of Mg^{2+} (HBV test) and Mn^{2+} (HIV &HCV tests) and excess deoxynucleotide triphosphates (dNTPs), the Taq pol extends the annealed primers along the target templates to produce a double stranded DNA

molecule termed an amplicon. The Analyzer automatically repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA. The required number of cycles is preprogrammed in the Analyzer.

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, the AmpErase enzyme is added to the test, the AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine but not DNA containing deoxythymidine. AmpErase enzyme is inactive at temperature above 55^0C i.e.,througout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation solution, thereby preventing the degradation of any target amplicon.

4. Hybridization Reaction

Following amplification, the analyzer adds Denaturation solution to the A-tubes to denature the amplicon and the multiprep internal control amplicon to form single stranded DNA. Aliquots of denatured amplicon are then transferred to two detection cups (D-cups).A suspension of magnetic particles coated with an oligonucleotide probe specific for HBV, HCV or HIV is added to the individual D-cups.

5. Detection Reaction

Following the hybridization reaction,the analyzer washes the magnetic particles in the D-cups and then adds avidin-horseradish peroxidase conjugate which conjucate binds to the hybridized biotin labeled amplicon.

The Analyzer removes unbound conjucate by washing the magnetic particles and then adds substrate solution to each D-cup.In the presence of hydrogen peroxide the particle bound horseradish peroxidase catalyzes the oxidation of 3,3,5,5-tetra methyl benzidine (TMB) to form a colored complex.The absorbance is measured by the analyzer at a wave length of 660 nm.

Materials and Reagents

i) 2ml vacutainer sterile tube
ii) Powder Free Gloves
iii) 1.5 ml eppendorf screw–cap tubes
iv) 200 and 1000µl eppendorf pipettes and tips
v) Refrigerated Centrifuge
vi) Micro Centrifuge
vii) Ethanol 90%
viii) Isopropyl alcohol
ix) Distilled Water
x) Vortex Mixer
xi) A-Rings and D-Cups
xii) Plastic Pasteur Sterile Pipettes

The reagent Kits are divided into four main separate boxes:

i) Multiprep Speciman Preparation and Control Kit.
ii) Amplification Reagents.
iii) Detection Reagents.
iv) CA Wash Buffer.

Reagent Preparation

All reagents except for MMX must be at room temperature before use.

1. Normal Human Plasma (NHP), Mutiprep Internal Control (MP IC), Mutiprep Positive Control (MP (+) C), Multiprep Negative Control (MP (-) C) and Mutiprep Diluent (MP DIL) Warm all mentioned above to room temperature (25°C-30°C) before use.

2. Working Lysis Buffer Keep the MP LYS for 30 minutes at room temperature to dissolve the precipitate prior to use. Vortex the MP IC up and down before using and tap vial to collect the solution in the base.

3. Working Amplification Master Mix (Perform in Pre-Amplification Area)

Pipette 100 ul of Mg^{2+}or Mn^{2+} into one bottle of MMX. Mix well by inverting 15-20 times. Store at 2-8°C and use within 4 hours of preparation.

4. Working Probe Suspension Detection Reagents

Prepare working probe suspension as follow:

For HBV

Mix BH PS1 well by vortexing briefly to suspend the microparticles and pour whole bottle BH PS1 into one BH4 cassette, and mix BI PS1 well and pour it into BI4 cassette.

For HCV

Mix CH PS1 well by vortexing and pour it into CH2 cassette and mix CI PS1 well and pour it into CI2 cassette.

For HIV

Mix IH PS1 well by vortexing and pour it into IH2 cassette and mix II PS1 well and adding it to II2 cassette.

5. DNA-Denaturation Reagent and CN4-Conjugate Reagent

Once DN4 and CN4 cassette are opened, they are stable for 30 days at 2-8°C or until the expiration date.

6. Working Substrate Reagent

Working substrate must be freshly prepared daily. Pipette 5ml of SB into SB3 cassette Mix it gently and check the cap of the cassette, if it is wet dry it with tissue paper.

***7.* Wash Buffer Reagent**

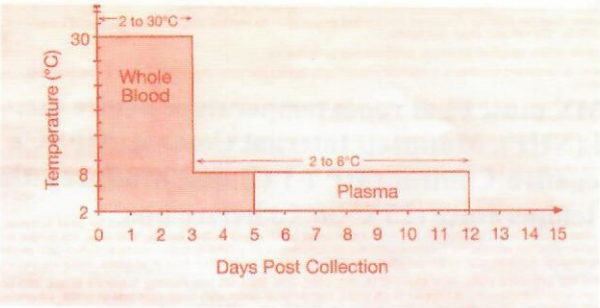
Check the WB Before Dilution, if any precipitate appears warm at 30-37°C. Add 1 volume of WB to 9 volumes of distilled water and mix well. Wash buffer is stable for 2 weeks from the date of preparation.

***8.* 70% Ethanol**

Prepare daily70% Ethanol fresh, 1ml of 70% ethanol is needed for each specimen. Mix 11.7ml 90% ethanol and 3.3ml of distilled water in a conical polypropylene screw-cap flask.

Specimen Collection,Storage and Pooling

The blood sample may be collected in EDTA,CPD, CPDA-1 and ACD-A or 4% Sodium Citerate. Heparin has been shown to inhibit PCR. Blood collected in EDTA may be stored for up to 72 hours from time of draw at 2-30°C followed by two days at 2-8°C. For storage longer than five days, remove the plasma from the red blood cells by centrifugation at 4000 rpm for 20 minutes. Following removal, plasma may be stored at ≤-18°C up to one month. Before using warm pooled or individual donor specimens to room temperature. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.



Specimen Pooling

The pooling system for use with the AmpliScreen test performs pooling operations that combine aliquots from 12 individual samples into a single primary pool. The pooling requires preparation of secondary pools as well as individual specimens for follow-up testing in the event a primary pool test is positive. If less than 12 specimens are available, testing is performed using the individual specimens.

Daily 120 plasma specimens required to perform 12 pools test containing the negative and positive controls. The main step should be perform in the early time is pre-cool the centrifuge to 4°C and switch on the UV box. During that take out the plasma samples from the cold room and warm it to room temperature before using. Then start labeling each rack with the pool number.

Pooling Procedure

Thoroughly clean and disinfect all surfaces. Confirm that all pipettes are correctly set to the specified volume and avoid contaminating gloves when manipulating specimens.

Pipette175µl of each individual donor specimen into 2ml vacutainer sterile tube (2100µl for each pool). Take these pools into the UV box chamber, and change the gloves to avoid contamination. Vortex well the vacutainer tubes to insure proper mixing .Label the sterile screw-cap tubes and make an orientation mark on each tube with permanent maker. Usually two more sterile screw-cap tubes are taken one for negative control and other for the positive control with orientation mark.

Multiprep Specimen Processing Procedure

Pipette 1000µl of each pool into a labeled screw-cap tube .Use a permanent marker to make an orientation mark on each tube.Vortex *NHP* upward and downward. Pipette 1000µl NHP into an appropriate screw-cap tube for each negative and positive control and cap the tubes.

Place specimens and control tubes into the pre-cooled centrifuge all tubes orientation mark facing outward at 4°C for 23,000-24,000xg for 1 hour. The pellet will form on the outer wall as indicated by orientation mark.

Remove the tubes from the centrifuge slowly and carefully aspirate 900µl of the supernatant from each tube leaving approximately 100µl of supernatant. Avoid contact with the pellet. Discard the supernatant and pipette tip appropriately. Use a fresh pipette tip for each tube.Prepare a working *Lysis* buffer by pipetting 100µl of *MP IC* (vortex briefly) into one bottle MP LYS and vortex.

Pipette 600µl working lysis buffer into each specimen and control tube, vortex briefly.

Prepare the controls as follow:

Negative Control

Vortex MP (-) control briefly. Tap vial to collect the solution in the base. Pipette 20µl of MP (-) control to the labeled negative tube containing lysis buffer and NHP vortex briefly.

Positive Control

Vortex MP (+) control briefly. Tap vial to collect the solution in the base. Pipette 20µl of MP(+) control to the labeled positive tubes containing lysis buffer and NHP. Vortex briefly.

Incubate all tubes for 10-15 minutes at room temperature. After the incubation period vortex all tubes briefly.

Pipette 700µl of isopropanol into each tube and vortex briefly.

Place the tubes into the microcentrifuge at 14,000 rpm for 15-20 minutes at room temperature with orientation mark facing outward to align with pellet that will form.

Slowly aspirate the supernatant from each tube. Remove as much liquid as possible without disturbing the pellet.

Slowly aspirate the supernatant from each tube without disturbing the pellet.

Pipette 1.0ml of 70% ethanol into each tube and vortex briefly.Place the tubes into a microcentrifuge with the orientation marks facing outward to align with the pellets that will form and centrifuge at 14,000xg for 5-10 minutes at room temperature.
Short spin to remove any remaining supernatant residual, ethanol can inhibit amplification.
Pipette 200µl *MP* DIL into each tube. Use a pipette to break apart the pellet. Vortex briefly, the processed specimens can be stored up to one month at 70°C.
Perform Preparation of Working Master Mix in Pre-Amplification, dead air box.
Create the appropriate number of *A-ring* needed for the tests.
Place the *A-ring* on the A-ring holder.
For each *A-ring* prepare one Working Master Mix.

Pipette 50µl Working Master Mix into each A-tube and donot cap the A-tube.
Place the A-ring containing the Working Master Mix in a sealable bag and seal the plastic bag.
Label the plastic bag with the test name HBV, HCV, or HIV and the date.
Store the A-ring containing Working Master Mix at 2-8°C until the specimens and controls preparation is completed. The A-ring containing Working Master Mix must be used within 4 hourse.

Loading the A-ring

Create an A-ring work list record for each A-ring to identify the A-tube with the appropriate specimen and controls to be pipette.
If processed specimen and control were stored frozen thaw at room temperature before proceeding.Vortex briefly.Pipette 50µ1of each processed specimen and control into the appropriate A-tube containing specific Working Master Mix for a specific test. Immediately cap the A-tube and repeat this step for all 12 A-tubes to complete the A-ring loading.
Transfer the A-ring with sealed tubes containing the processed specimens and controls in Working Master Mix to the Amplification/Detection area.

Amplification and Detection

Daily maintenance procedure should be performed before the start up of the system, which includes:
Cleaning the instrument.
Removing the internal parts of the system such as the reagent racks, D-cups racks, and other accessible internal parts should also be wiped clean with a damp cloth.
Check waste container and empty if necessary.
Check wash buffer reservoir and add prepared wash buffer if necessary.
Prime the AMPLICOR ANALYZER as mentioned in software operation.

Instrument And System Operating

Place the generic reagents (DN4, CN4 and SB3) cassettes in the labeled rack for generic reagent platform. Record the date when each cassette was opened.

Place the specific reagents in the specific rack and place it on the specific reagent platform.
If a new cassette replaced in the rack identify it by saving each cassette number in the AMPLILINK software or by barcode scanner.
Place the D-cup rack on the D-cup platform. Tow D-cups are required for each A-tube and two D-cups are required for each working substrate cassette to allow for blanking by ANALYZER.
Create a B-ring order using the software, as described under the software operation.
Place the A-ring into the thermal cycler segment of the AMPLICOR ANALYZER and close the cover on the thermal cycler segment.
Create a B-ring order using the software as described under the software operation.
Place the B-ring into the thermal cycler segment of AMPLICOR ANALYZER and close the cover on the thermal cycler segment.

Start the AMPLICOR ANALYZER as described under the software operation.
AMPLICOR ANALYZER automatically performs amplification and detection. Results are expressed as absorbancy values at 660nm as positive or negative.

Software Operation

- Switch on the electricity from right (main switch) to left (AMPLICOR ANALYZER) direction and switch off the system in the opposite direction.
- Wait for the AMPLICOR ANALYZER to complete the self-priming cycle,which takes approximately one minute and it will stop automatically. During the prime,inspect syringes and valve block for air bubbles,leakage or discoloration.Check transfer tip and resuspension probe tips for proper flow of working wash buffer.
- Click service icon, when the cycle is completed choose Tools then prime extended which takes approximately six minutes.
- Click order icon enter the A-ring ID# then double clicks on the test and enters test profile.
- Click save.
- Click status and choose Analyzer.
- Repeat same procedure for the A-ring for another test.
- Click again status and choose cassette icon and check that the generic and specific reagents are enough for test run and located in the proper position.
- Back again to analyzer and click start.
- Wait for AMPLICOR ANALYZER to give a massage that load check pass.
- Click start and choose Parallel Mode for Amplification only.
- Later on, choose Basic Mode after removing the two A-rings from TCA and TCB to TCD1 and TCD2 detection location and place the third A-ring for Amplification and Detection in TCA position.

Interpretation of Results

1.Quality control procedure

At least one Multiprep (-) control and one Multiprep (+) control must be processed with each A-ring. Both controls must be processed in the same manner as a sample.

Negative control MP (-) C

The absorbance for the MP (-) C should be less than 0.2 at 660nm and its associated MP IC should be greater than or equal to 0.2 at 660nm for the negative control to be valid. If the absorbance value for the MP (-) C is greater than or equal to 0.2 its associated MP IC is less than 0.2, the entire A-ring is invalid, and the entire test procedure for that A-ring (specimen and control preparation, amplification and detection) must be repeated.

Positive Control MP (+) C

The absorbance for the MP (+) C should be greater than or equal to 1.0 at 660 nm and its associated MP IC should be greater than or equal to 0.2 at 660nm for the positive control to be valid. If the absorbance value for the MP (+)C is less than 1.0 and /or its associated MPIC is less than 0.2, the entire test procedure for the A-ring (specimen and control preparation, amplification and detection) must be repeated.

2. External Control

An External Control is required in the laboratory, and it must be processed in the manner as the sample. The external control should contain a defined number of target sequence copies for HBV, HCV, or HIV and the level of this control should be a multiple of the cut-off value of the test system. The HBV, HCV, or HIV absorbance and the MP IC absorbance for the external run control must both be valid for the external control to be valid. The HBV, HCV, or HIV absorbance of the external run control should be greater than or equal to 0.2 and the MP IC

absorbance for the external run control should be greater than or equal to 0.2 at 660nm. If the absorbance of the external run control doesnot meet the above criteria the negative samples may be in question. Therefore, the laboratory should follow their established standard operating procedure for the appropriate action.

3. Specimen Results

Two absorbance values are obtained for each specimen, one for the HBV, HCV, or HIV target and one for the internal control MP IC. For a sample with an absorbance less than 0.2, the MP IC absorbance for that specimen must be greater than or equal to 0.2 at 660nm for a valid negative specimen test result. If the absorbance for the HBV, HCV, or HIV target is greater than or equal to 0.2, the MP IC result is disregarded and the test result is valid and positive.

Testing of pooled samples for the AmpliScreen HBV, HCV, or HIV requires a single level of testing for primary pools that are negative for the HBV DNA, HCV RNA, or HIV RNA. and report the results for all associated individual donor specimens in that primary pool as negative e.g. HBV DNA negative and three levels of testing (Primary pool, Secondary pool and Tertiary Resolution) for primary pools that are positive HBV DNA, HCV RNA, or HIV RNA. When the primary pool is positive prepare four secondary pools containing the associated donor specimens. If one or more of the secondary pools tests positive report the results for the donor specimens in the negative secondary pools as negative. For positive secondary pools follow the positive primary pool, positive secondary pools tertiary resolution testing. test each of the individual donor specimens in that secondary pool. If one or more of the individual donor specimens is positive the positive donor specimens are reported as positive and the remaining negative donor specimens associated with the positive secondary pool are reported as negative.

If all the individual donor specimens are negative the individual donor specimens in that primary pool may be reported as negative.

4. Quality Assurance Program

As part of an overall Quality Assurance program we conduct additional testing to determine the cause of the initial positively of the primary pools and secondary pools for which the associated individual samples were negative. Positive pools resolved as negative at the tertiary resolution testing level is usually a result of a contamination event to the secondary pool, but theoretically may be due to viral load below the limit of detection.

5. Sensitivity

Reliable results are dependent on adequate specimen collection, suitable anticoagulant and proper transport procedures. The AmpliScreen HBV, HCV or HIV test demonstrates that using the Multiprep sample processing procedure the limit of detection level is as low as 30 copies/ml on the NIBSC and 5 IU/ml on the WHO international standard. In the standard sample processing procedure can detect 100 copies/ml on the NIBS and 20 IU/ml on the WHO international standard.

2- Routine Quality Control Tests :

Routine QC. Tests are carried out as per required dates and are documented in special forms (see attached Forms).

CHAPTER FIVE: PREPARATION, STORAGE AND Q.C. OF BLOOD COMPONENTS

[I] BLOOD COMPONENT PREPARATION

1) Red Blood Cells

PRINCIPLE:

1. Donor whole blood (WB) is collected in doube, triple or Quadrable bags with integral tubing within 10 - 15 minutes.
2. RBCs Are prepared from freshly collected whole blood by removal of 200-250 ml of plasma at first 6-8 hours after collection .
3. They are stored at 1-6 C in a variety of different anticoagulant / preservative solutions. The resultant RBCs components have different haematocrits and shelf lives.
4. RBCs stored in additive solutions (AS) have haematocrit of 52 -60 % and a shelf life of 42 days ,wheres RBCs stored in CPDA -1 have haematocrit of 70 -80 % and can be stored for 35 days .RBCs stored in CPD have same haematocrit as those stored in CPDA-1 but with shelf life of 21 days .

POLICY:

1. The sterility of all components shall be maintained during processing. Components will be prepared in accordance with AABB standards 5.7.5.

MATERIALS:

1. Refrigerated Centrifuge.
2. Plasma Expresser.
3. Dielectric Tube Sealer.
4. Electronic Weight Scale.
5. Plastic Tubing Clips, and F.F.P. Boxes.
6. Rubber Centrifuge Balance Weights.
7. Blood Component Log Sheet.
8. Blood Bank Refrigerator.

SAFETY:

All blood and blood products must be treated as potentially infectious. Follow universal precautions detailed in the laboratory safety manual.

QUALITY CONTROL:

Refer to the Q.C. procedures for blood components.

PROCEDURE:

1. Four or six W.B. units received from donor room.
2. Balance units in pairs using electronic scale.
3. Place balanced units in plastic centrifuge cup liners and place the cups opposite one another in the refrigerated centrifuge.
4. centrifuge whole blood using a heavy spin with a temperature setting of 4C except in the case of preparing plt using a soft spin with a temperature setting of 18 – 22C.
5. After completion of centrifugation, remove the units gently from the centrifuge using the ports at the top of the bag.
6. Place the primary bag containing centrifuged on plasma expressor ,and release the spring , allowing the plate of the expressor to contact the bag .
7. Clamp the tubing between the primary and satellite bags with a hemostat or, if a mechanical sealer will not be used , make a loose overhand knot in the tubing.
8. If two or more satellite bags are attached , apply the hemostat to allow plasma to flow into only one of the satellite bags. Penetrate the closure of the primary bag . A scale may be used to measure the expressed plasma.
9. Reapply the hemostat when the desired amount of supernatant plasma has entered the satellite bag. Seal the tubing between the primary bag and the satellite bag in two places .

10.Check that the satellite bag has the same donor number as that on the primary bag and cut the tubing between the two seals .

11. if using a red cell additive solution, transfer the additive to the red cells

2)Fresh Frozen Plasma

Principle :

Plasma is separated from cellular blood elements and frozen within 6 – 8 hours of phlebotomy to preserve the activity of labile coagulation factors . FFP Unit Volume: 200 – 250 ml.

Procedure :

1. Centrifuge the blood soon after collection, using a " heavy spin ". Use a refrigerated centrifuge at 1-6 C unless also preparing platelets(see platelets preparation).
2. Place primary bag containing centrifuged blood on plasma extractor and place the attached satellite bag on a scale adjusted to zero .Express the plasma to the satellite bag and weight the plasma .
3. Seal the transfer tubing with a sealer. Place another seal nearer the transfer bag.
4. Label the transfer bag with the unit number before it is separated from the original container . Record the volume of the plasma on the label .
5. Cut the tubing between the two seals . Leave a segment for any test desired .
6. Place the plasma at - 18 C to ensure it is frozen solid within 8 hours of phlebotomy and stored for one year.

3)Platelets Concentrate

Principle

Platelets-rich plasma is separated from whole blood by " light –spin "centrifugation and the platelets are concentrated by " heavy –spin " centrifugation with subsequent removal of supernatant plasma. **Procedure**

1.Whole blood is collected (450 ml) in triple bag configuration (primary + 2 satellite bags connected with integral tubing).

2. Do not chill (4+/- 2 C) the blood at any time, before or after centrifugation. If cryofuge is at 4 C, set the temperature control at 22 C and perform a blank run in order to bring the cryofuge to 22 + /- 2 C (important) .

3. Centrifuge the whole blood at 22 +/- 2 C using a soft spin (2650 rpm ,for 5 minutes duration. Express the supernatant platelets –rich plasma (PRP. 200 ml) into the transfer bag intended for the platelets storage. Temporarily seal this bag from the other two, and add Adsol (100 ml) to the erythrocytes in the primary bag. Now heat – seal the tubing twice between the primary bag and Y – connector of the two satellite bags and cut between the two seals. This is important to maintain the sterility of the products. Promptly store the RBCs in the blood refrigerator at 4 +/- 2 C . Shelf life of erythrocytes concentrate is 42 days .

4-Centrifuge the RPR at 22+/- 2 C using a Heavy spin (3160 rpm for 6.45 minutes and this is the second spin) .

5-Express the platelets –poor plasma into the second transfer bag ,leaving behind 35 –50 ml of plasma and seal the tubing . Determine exactly the weight of concentrated platelets . Check for identification on the bags ,such as the unit No., expiratory date, the blood group and others ,then detach the bags .

6-Cell free plasma thus separated must be promptly shock frozen at –55 or - 80 C horizontally for at least 4-5 hours ,then stored vertically at – 37 C as Fresh Frozen Plasma (150 –200 ml).

7-In order to re suspend the pelted platelets in the plasma without undue irreversible aggregation , the plastic bag containing concentrated platelets are left stationary with label side down at room temperature (22+/- 2 C) for approximately one hour ,and then place in horizontal shaker with gentle agitation . Re suspended platelets are stored at 22 +/- 2 in the horizontal shaker until their expiry. Shelf life is 5 days ,and the volume is 35 –50 ml.

Whole Blood Processing By Second Spin Bypassing The First Spin:

1. In inadequate separation of plasma and red cells.
2. Unsuitable unit for platelet production due to: -Slow bleed
   - Aspirin ingestion by the donor
   - Low volume blood unit.
3. The unit for pediatric PRBC's production, using quadrable pediatric bags, 100m1 each.
4. CPD unit preparation for neonatal patients undergoing cardiac surgery.

Note: CPD shelf life is 21 days.

REPORTING RESULTS:

Complete the blood component log sheet and retain it for five years.

PROCEDURE NOTES:

1. Exception units are identified by donor room staff, and handled by component preparation staff as follows:
   a. Low volume (300 - 404m1) labeled. (Low volume AS - 5 Red Cells)
   b. "QNS" units weighing less than 320gm.
   c. Heavy units weighing more than 525 gm.
   d. "QNS" and "HEAVY" units disposed by component preparation staff next day.
2. All components will be stored in unprocessed storage equipments until labelling and release of infectious disease screening results, then released for cross match and transfusion.
3. Access to component storage area and authorization for removal of contents is restricted to assigned blood bank personnel.

BLOOD COMPONENT LOGSHEET

BATCH #:

Centrifuge	Blood unit#	Time blood collected	Time component separated	Type of Component						
				PRBC's	PC	FFP	CRYO	WBC's	Filt. PRBC'S	Filt. PC.
Batch prepared by:			Reviewed by:				Date:			

4) CRYO PRECIPITATES AND CRYOPRECIPITATE REDUCED PLASMA

PRINCIPLE:

1. Cryoprecipitate is the cold insoluble portion of plasma protein when FFP is thawed between 1-6°c.
2. It contains 50% of F. VIII (both VIII C-Procoagulant activity +VIII VWF.) 20 - 40% of fibrinogen and some of F XIII.

3. Cryoprecipitate reduced plasma is the plasma remaining after separation of (cryo) from FFP. Which can be used in patients of TTP undergoing therapeutic plasma exchange procedures.

POLICY:

Cryoprecipitate and cryoprecipitate - reduced plasma will be prepared in accordance with AABB standards 5.7.5.14 within 1-6°c and maintaining sterility and refreezing within one hour.

MATERIALS:

l. Refrigerator 1 -6°c.
2. Refrigerated centrifuge.
3. Electronic scales.
4. Dielectric heat sealer.
5. -80°c freezer and -18°c freezer.
6. Cryoprecipitate production thawing records sheet.
7. Fresh frozen plasma boxes.
8. Cryoprecipitate boxes.

SAFETY:

All blood and blood products must be treated as potentially infectious.

QUALITY CONTROL:

Refer to cryoprecipitate quality control procedure.

PROCEDURE:

1. Batch preparation:
a. Batch of cryoprecipitate consists of 12 units.
b. Process oldest units first.
c . Complete cryoprecipitate production batch thawing record sheet.
2. Thawing:
a. Remove FFP with attached satellite bag, from the freezer and place it in the blood bank refrigerator to be thawed.
b. Total thawing time will be 16 - 18 hours.
c. Allow centrifuge to come down to 4°c overnight.
3. Centrifugation:
i. When thawing is complete, remove units from refrigerator, balance units in pairs and place balanced units in the centrifuge cups opposite one another.
ii. Select the appropriate program:

a. Speed	4000 RPM
b. Time	20 minutes
c. Temperature	4°c.

4. Express supernatant plasma into the satellite bag and adjust cryoprecipitate weight in the parent unit to 25 ± 30gm.
5. Disconnect cryoprecipitate unit and the cryoprecipitate reduced plasma unit and label each one properly.
6. Print list of quarantined units and label them.
7. Place cryoprecipitate units within ONE hour of removal from refrigerated centrifuge in matching ABO / Rh type labeled cryoprecipitate boxes and store for one year, In 40-80°C freezer,
8. Record the date, the total time and the temperatures cryoprecipitate unit take from centrifuge to freezer in cryo. production batch thawing records sheet and sign it.

REPORTING RESULTS:

The senior technologist component preparation must review the completed cryoprecipitate production batch thawing record before release of the product, and this record should be

retained for 5 years.

PROCEDURE NOTES:

1. Maintenance of temperatures during storage, thawing and centrifugation is essential for recovery of labile procoagulants in cryoprecipitate.
2. Both cryoprecipitate and cryoprecipitate reduced plasma:

a. Have shelf life of 12 months from date of blood collection.

b. Storage temperature: -18°c or lower (-80°c).

REFERENCE:

1. AABB technical manual, 14th edition, Bethesda, MD, 2002.
2. AABB standards, 21 St edition, Bethesda, MD, 2002.

CRYOPRECIPITATE PRODUCTION BATCH THAWING RECORD

BATCH NUMBER	TIME UNITS REMOVED FROM CENTRIFUGE	TIME UNITS RETRUNED TO FREEZER	CENTRIFUGE TO FREEZER TIME (must not exceed one hour)	SEPARATED BY
	Date:	Date:		
	Time:	Time:		
	Temp:	Temp:		
	Date:	Date:		
	Time:	Time:		
	Temp:	Tem :		
	Date:	Date:		
	Time:	Time:		
	Temp:	Temp:		
	Date:	Date:		
	Time:	Time:		
	Temp:	Temp:		
BATCHES PREPARED BY:		REVIEWED BY:		DATE:

5) GRANULOCYTE PREPARATION

PRINCIPLE:

Granulocytes are usually collected by aphaeresis techniques; however, Buffy coats harvested from fresh whole blood can provide 1 x 10^9 of granulocytes in urgent neonatal situation.

POLICY:

l. Granulocytes must be blood grouped and screened for infectious diseases prior to preparation in order to be ABO/Rh compatible and negative for transfusion transmitted diseases.

2. Final product by apheresis must contain > 1.Ox $10^{1°}$ granulocytes, in 75% of units, and 1x10^9 granolyctes in buffy coat.

3. Storage in RT (22-25C°.), FOR 24 HOURS.

4. Indication for granulocytes transfusion:

a. In adults:
- Reversible neutropenia. (Count <500/mm)
- Gram-negative infections not responding to antibiotics.
- Myeloid hypoplasia
- Chronic granulomatous disease (granulocyte dysfunction)
b. In infants
- Septic infants.
5. Complication of Granulocytes transfusion.
a. In adults:
- HLA allo-immunization.
- CMV transmission.
- GVHD, if not irradiated
b. In infants:
- is less because of absence of HLA allo-immunization.

SPECIMEN:

Whole blood stored at RT. (22-25C°.), collected within 6 hours

MATERIALS:

1. AS-5 quad collection unit.
2. Plasma Expressor.
3. Electronic scale.
4. Plastic tubing clips.
5. Refrigerated centrifuge.
6. Dielectric sealer.

SAFETY:

All blood and blood products must be treated as potentially infectious.

PROCEDURE OF PREPARATION OF BUFFY COATS:

1. Select the donor from family member, ABO and Rh typed and screen for TTD, and make sure he will be ABO/Rh compatible and negative for TTD.
2. Collect AS-5 quad WB unit and separate within 6 hours from collection.
3. Centrifuge WB at 4000 RPM, for 10 minutes at 20°C,
4. Transfer plasma into platelet transfer bag, by plasma expressor, and close the line by the plastic tubing clip.
5. Transfer buffy coat into another transfer bag adjust volume to 50-100m1, heat seal and separate from parent unit.
6. Process PRBC's and FFP as usual.
7. Store in RT (22-25°C.) FOR 24 HOURS.
8. Inform the requesting department when the product is available.

6)LEUKOCYTE DEPLETED BLOOD COMPONENTS

A. Introduction:

Reduction of leukocytes in transfused blood components may reduce the risk of:
l. Febrile non haemolytic transfusion reaction.
2. CMV transmission.
3. HLA allo-immunization.
4. Platelet refractoriness.
5. Immune-modulation, cancer recurrence and bacterial infections in some surgical procedures.
6. Prion disease (CJD)
7. Yersinia enterocolitica contamination of RBC's. B. Acceptable leukoreduction level in blood components:

1. RBC's < $5X10^6$
2. Platelet pheresis < $5X10^6$
3. Random platelet <$8.3X10^5$
4. Platelet pooled <5X1 06

B. Methods for leukoreductions

1. Blood Washing:

a) Methods:

1. Automated: - Expensive - Time consuming
2. Manual.

b) Washed RBC's

1. One to two liters of sterile normal saline removes 99% of plasma proteins, electrolytes and antibodies.
2. Remove some leukocytes but does not prevent HLA alloimmunization.
3. Loss of 20% of RBC's
4. Must be transfused within 24 hours because:
   i. Open system
   ii. Anticoagulant preservative removal

c) Washed Platelets:

1. By normal saline.
2. Loss of 33% of platelet.
3. No changes in leukocyte content.
4. Must be transferred within 4 hours.

d) Advantages:

1. Remove 99% of plasma.
2. Decrease prophylaxis in IgA deficient patients.
3. Decrease FNHTR.

2. Post storage leukoreductin filtration:

a. By using bedside filters which remove >99.9% of leukocytes to reach a residual leukocytes of <5X106/unit.

b. Technology: Multiple layers of synthetic non-woven fibres which retain leukocytes by:

1. Cell size.
2. Surface tension.
3. Difference in surface charge.
4. Density of RBC's.
5. Cell to cell interaction.
6. Cell adhesion and activation properties.

c. Advantages:

1. Decrease the risk of HLA alloimmuniztion.
2. Decrease the risk of FNHTR
3. Decrease the risk of CMV transmission
4. Available when indicated within the reach of nurses. d. Precautions:
   1. Need strict priming.
   2. Do not use infusion pump or pressure.

e. Disadvantages:

1. Degranulation of leukocytes during storage release cytokines which may cause FNHTR.
2. Not effective in multiple platelet transfusion.

3. Hypotensive reaction may occur with negatively charged filters.
4. Difficult QC performance.

f. Q.C. by:
1. Nageotte Brite line chamber.
2. Flow cytometer.

3. Prestorage leukoreduction filtration:

a. In line filtration of whole blood:

1. As-5 whole blood is filtered
2. Then processed to PRBC's and LR plasma
3. Platelet retained by filters and cannot be prepared.

b.Prestorage leukocyte filters using sterile connecting device (SCD) to be connected to the desired RBC's unit for filtration.

c. inline filtration of Red cells:

1. Plasma removed from CPD whole blood unit.
2. AS-5 is added to red cells.
3. RBC's then filtered into secondary storage container.
4. Must be within 5 days of collection.

d. inline filtration of Platelets:

1. Prepared from platelet rich plasma.
2. Then using inline leukocyte reduction filters.

e.Platelet pheresis filtered by the machine while collected from the donor automatically.

f. Platelet pheresis not filtered during collection, then can be filtered using leukocyte filter and SCD.

g.Random pooled platelet can be filtered without using SC; must be used within 24 hs.

h. Advantages of prestorage leukocyte filtration:

1. More effective in preventing risk of FNHTR.
2. More effective in multiple platelet transfusion.
3. Easy QC performance in blood bank.
4. No hypotensive reactions noticed.

(1)FILTRATION OF RED CELLS (Pre-storage)

PRINCIPLE:

Red cells are filtered from white cells, to prevent HLA Alloimmunization, febrile non haemolytic transfusion reaction and Transfusion transmitted CMV infection, using commercial WBC's filers.

POLICY:

1- In accordance with AABB standard 5.7.5.6 RBC'S will be filtered:

a) Retain >85% of RBC'S
b) WBC'S <SX10^6/unit

2- Use sterile connecting Device, in accordance with AABB standard 5.7.2.

SPECIMEN:

Red blood cell unit.

MATERIALS:

1- Red cell leukocyte removal filters.
2- Sterile connecting Device (SCD).
3- SCD wafers.
4- Dielectric heat sealer.
5- Labels.

SAFETY:

All blood and it's products must be treated as potentially Infectious.

QUALITY CONTROL:

4 units to be tested monthly for residual WBC'S and RBC'S survival.

PROCEDURE:

1- Label filter bag with original unit number, collection and expiration dates, facility ID and Component type. (Leukocyte filtered RBC' S)

2- Mix the red cell unit by inversion.

3- Attach the cell unit to the labelled filter set and use the sterile connecting Device.

4- Place filter bag hotizotal, while red cell unit and the filer vertical, and allow blood flow by gravity until filtration complete.

5- Expel the air through the bypass loop.

6- Heat seal the tubing twice, match unit numbers with filter bag numbers, and separate the filtered RBC' S unit.

7- Strip the tubing and segment every 5 cm.

8- Place filtered unit in separate unprocessed refrigerator until TTD results, then label and release for cross matching.

PROCEDURE NOTES:

1- If filters become blocked, return blood to parent unit and label unfiltered blood.

2- Document weak or leaking welds by SCD.

3- RBC'S should be filtered within 8 hours from collection and before storage in 1-6°c.

4- Component processing can determine the number of units to be filter every morning with consultation of BB. Supervisor and medical director.

5- Expiry within 24hs to a11 units filtered without SCD.

(2) PLATELETS FILTRATION (Pre-storage)

PRINCIPLE:

Platelet Products are filtered from white cells to prevent HLA Alloimmunization, FNHTR and CMV,

Using commercial WBC' s filter.

POLICY:

1- In accordance with AABB standard 5.7.5.14 and 5.7.5.16, platelet will be filtered:

a) Residual leukocytes:<8.3 X 10^5 in random units and <SX 10^6 in apheresis platelet units.

b) Used for all patients < 4 month of age and those requiring CMV negative products.

2- In accordance with AABB standard 5.7.5.14, residual leukocytes in platelet pooled leukocyte Reduced must be<SX10^6.

SPECIMEN:

1- Platelet pheresis products. **2- Random platelets.**

MATERIALS:

1- Platelet leukocyte removal filter.

2- SCD and it's wafers.

3- Dielectric tube sealer.

4- Platelet agitator and horizontal rotator.

5- 5 ml red top tub for Q.C.

6- Label.

SAFETY:

All blood and it's products must be treated as potentially Infectious.

QUALITY CONTROL:

1- 4 platelet products will be tested monthly for Residual WBC'S and platelet recovery.

2- Random platelets who does not demonstrate swirl effect" must be discarded, because it will

clog the filter.
3- Discard units show leakage or discoloration.

PROCEDURE:

1- Label filter bag with original unit number, collection and expiration dates, facility ID and Component type (Leukocyte filtered pooled random platelet or LR apheresis platelet).
2- Select platelet units show "swirl effect" and pool them.
3- Weigh the unit and document the pre-filtration weight on Q.C. form and calculate the volume by dividing by specific gravity (1.03)
4- Strip the tubing and detach 10 cm long segment by heat sealer, dispense content in 3 ml red top properly labelled tube and send for prefiltration platelet count.
5- Attach pheresis platelet unit to the labelled filter bag set using SCD, while in case of pooled random platelet you can spike the platelet bag with the filter bag without using SCD.
6- Place filter bag horizontal, while platelet unit and the filter vertical and allow platelet to flow by gravity until filttation complete.
7- Expell the air through the bypass line.
8- Heat seal and separate filter bag and ensure that lab has correct information.
9- Strip the tubing and detach 10 cm long segment by heat sealer, dispense content in 3 ml red top properly labelled tube and send for post filtration platelet count.
10- Weigh the filtered unit and document the post filtration weight on Q. C. form and calculate the volume as before.
11-Upon Return of the Q. C. result, calculate the residual WBC'S and platelet recovery, and document on Q.C. form and file it.
12- Calculation:

a) $\underline{\text{Weight to volume:}}$

$$\frac{\text{Wt(gm)}}{\text{S.G. (1.03)}} = \text{volume in (ml)}$$

b) $$\frac{\text{Residual WBC's}}{\text{WBC's X106 /bag}} = \text{WBC's X } 10^6\text{/L X } \frac{\text{Vol. filtered unit (ml).}}{1000}$$

c) Platelet recovery:

$$\text{(P1) P1at.X}10^9\text{/bag (prefilt.)} = \text{prefilt. Pit. X } 10^9 \text{ X } \frac{\text{Vol. of prefilt. Unit}}{1000}$$

$$\text{(P2) P1at.X}10^9\text{/bag (prefilt.)} = \text{postfilt. Pit. X } 10^9 \text{ X } \frac{\text{Vol. of postfilt. Unit}}{1000}$$

$$\text{\% platelet recovery} = \frac{\text{P2}}{\text{P1}} \text{ X } 100\%$$

PROCEDURE NOTES:

1. Place filtered platelet on platelet agitator and make sure that temperature in vicinity is 2024°C.
2. Ensure unit is issued prior to expiry.
3. Obtain sample from tubing does not affect sterility as long as aseptic techniques are used.
4. Platelet pheresis ptoducts are filtered during collection however some are not filtered, and these will be filtered upon request.
5. Filteration platelet pheresis into platelet storage bag retain original expiry date, while filteration into non platelet storage bag using SCD, expired within 8 hours.
6. Platelet pools have 4 hour expiration only (Do not use SCD)

7) IRRADIATION OF BLOOD PRODUCTS

PRINCIPLE:

1. Irradiation of blood and cellular components is recommended for prevention of graft versus host disease caused by lymphocytes present in most blood products specially to the immuno compromised patients.

2. These patients include:

- **a. B.M. transplant recipients (blood products not B.M.)**
- **b. B.M. donors receiving blood transfusion prior to and during harvest of Marrow.**
- **c. Intrauterine transfusion.**
- **d. Post intrauterine transfusions during neonate period.**
- **e. Patient receiving directed donation from family members.**
- **f. Patients with severe combined immune deficiency syndrome.**
- **g. A plastic anemia, acute leukemias and Hodgkins disease.**

POLICY:

1.In accordance with AABB standards J8. 400 all patients at risk of developing graft versus host disease will receive irradiated cellular blood components.

2. The product will be permanently labelled as irradiated and the expiry of PRBC's will not exceed 28 days.

SPECIMEN:

PRBC's, platelet products and granulocytes.

MATERILAS:

l. a Gamma irradiation source.
2. Canister
3. 25 GY blood irradiation indicator label.
4. Irradiated label
5. Gamma Irradiation request form.

SAFETY:

l. Irradiator must be located under constant supervision 24 hours a day, seven days a week.

2. The irradiator is only operable with a key that is kept in secure area (senior technologist desk) and only blood bank personnel have access to it.

3. In the event of irradiator upset or irradiation leakage.

- **a. Evacuate the staff from irradiator area.**
- **b. Notify irradiation physics, safety and security teams.**
- **c. Do not enter the area until declared safe from irradiation leakage.**

4. The irradiated units should be placed in any ordinary nylon bag when placed in the stainless steel beaker,so to prevent the damage of the machine parts if leakage happen during irradiation.

5. Once you install the beaker and start irradiation, you should leave the area until you hear the alarm for the end of cycle.

6. The door of the room to be closed once the unit is not in use .

CALIBRATION:

Every six months, the biomedical physist predetermines the required number of seconds for the expected dose. (25 GY = 2500 rads).

QUALITY CONTROL:

The biomedical physist perform the following Q.C. checks:

1. Recalculate the decay of the source every six months according to the information given in the operation manual.

2. Monitor the irradiation leakage every six months.

3. Required dosage must reach all areas within the product compartment.

PROCEDURE:

1. Ensure that the electrical cord supply is plugged in.
2. Turn the power key switch to reset position and release.
3. Ensure the switch remains in the ON position.
4. Check that the time is set correctly to deliver 2500 rads (25 GY).
5. Attach irradiation indicator label, to the product unit.
6. Insert the product into the canister.
7. Place the canister onto the turntable and adjust its position according to the red lines and close the radiation chamber door.
8. Press the start switch.
9. The irradiation is terminated automatically.
10. Remove the blood bag from the canister and check irradiation indicator label to ensure irradiation.
11. Expiry of PRBC's will not exceed 28 days, or the original expiry date whichever is first.

PROCEDURAL NOTES:

1. Irradiated units are not radioactive and require no special handling. Irradiated components can be transfused to other patients if not transfused to intended recipient.
2. Red Cells Can be irradiated at any time up to 14 days after collection.
3. For neonates use, it is preferable to use red cell products within 24 hours of irradiation. There may be an increase risk of hyperkalaemia in neonates particularly when large volumes are required e.g. exchange transfusion.
4. Platelets may be irradiated at any time and will have a normal shelf life.
5. Frozen products (FF, Cryo) don't contain viable lymphocytes and do not require irradiation.
6. Granulocytes for all recipient should be irradiated as soon as possible after production and thereafter transfused with minimal delay .
7. If irradiation indicator label is not available, write or attach IRRADIATED label to the unit, when taking it out of the irradiator.
8. Severe combined immune deficiency syndrome (SCID) required irradiation to FFP.
9. Discard the irradiated unit if:

a. Irradiation exceed the required time.

b. Irradiation time is unknown.

TEMPERATURE QC FORM

Read and record temperature every four hours:

Acceptable range: Platelet incubator 20°C-24°C
Blood refrigerator 1°C-6°C
Plasma freezer -20°C

Equipment name: ..... **Equipment number:.....**

Reason for manual temperature: ... .

DATE	TIME	TEMPERATURE		TECH
		UPPER	LOWER	

Senior Review..... **Date: ..... Q.A.**
Review..... **Date:.....**

[II] BLOOD COMPONENTS QUALITY CONTROL

[1] QUALITY CONTROL OF RED BLOOD CELLS

PRINCIPLE:

4 CPD recently expired PRBC's units without additives checked for HCT every month, to ensure the presence of sufficient plasma and anticoagulant for RBC's survival.

POLICY:

1. In accordance with AABB standard 5.7.5.1, PRBC's must be prepared with final Hct <_ 80%. 2. If QC requirements are not met, immediate corrective action must be taken.

SPECIMEN:

CPD RBC's units.

MATERIALS:

1. l0 ml syringes with needles.
2. 5 ml Red top collection tube.
3. RBC's Q.C. form.

SAFETY:

All blood and blood products must be treated as potentially infectious.

PROCEDURE:

1. 4 CPD recently expired RBC's units.
2. Record unit's numbers in RBC's Q.C. form.
3. Mix units by horizontal platelet rotator for 30 - 60 minutes.
4. Label a 5 ml Red Top tube with unit number your ID number or initial, date and time.
5. Draw Sml of RBC's by syringe and empty content in the corresponding Red Top labeled tube. 6. Send the sample for HCT ratio check in hematology section.
7. Enter HCT result in RBC's Q.C. form.
8. Discard units in Red biohazard bags for incineration.

REPORTING RESULTS:

1. Acceptable values: 90% of units tested < 80% HCT.
2. If one unit >80% HCT, test 10 units and all must < 80% HCT, otherwise corrective action should be implemented by Q.C. coordinator and BB supervisor.

PROCEDURE NOTES:

Unit with additive solutions should not be used because they will have HCT < 80%

RED BLOOD CELLS-QUALITY CONTROL FORM

DATE:

PERFORMED BY:

Unit	HCT RATIO	COMMENTS
% Acceptable		

AABB STANDARDS: < 0.80 in all units tested

At least 4 units to be tested monthly

Senior Review:---------- **Date:----------**

QA Review:---------- **Date:----------**

[2] CALIBRATING CENTRIFUGES FOR PLATELET SEPARATION

PRINCIPLE:

Appropriate calibration of centrifuges gives adequate yield of platelets.

POLICY:

1. Centrifuges used for platelet preparation should be calibrated when:

- **a. Receipt of new centrifuge.**
- **b. Major repair of centrifuge was done.**
- **c. Unacceptable platelet Q.C.**

2. In compliance with AABB standard 5.7.5.13, preparation of platelet should yield > 5.5x10" platelet in 75% of tested units.

MATERIALS:

l. Refrigerated Centrifuge.
2. EDTA tubes and 4WB units.
3. Dielectric heat sealer.
4. Electronic Scale.
5. Plastic tubing clips.
6. 3 cc. Syringes with needles.
7. Electronic Calculator.
8. % Platelet yield - PRP worksheet.
9. % Platelet yield - PC. Worksheet.
10. 3 ml Red Top blood collecting tube.
11. 300 ml pipette and Tips.
12. 15x75 mm test tubs.

SAFETY:

All blood and blood products must be treated as potentially infectious.

PROCEDURE:

1. 4 WB units, with 4 EDTA tubes from donors.
2. Enter unit numbers in the 2 Q.C. worksheets (PRP and PC.)
3.Weigh each unit, calculate volume, enter in PRP Q.C. form.
4. Withdraw 1 ml from well mixed EDTA tube, dispense in 3 ml red Top tube properly labeled,

send for platelet count (platelet count must be > 133 x 10^9 / L.)

5. Select speed, time and temperature for slow spin, and prepare PRP, and enter these settings on PRP Q.C. form.
6. Weigh each PRP unit and calculate its volume and enter on PRP Q.C. form.
7. Draw 1 ml. of PRP, in properly labeled red top tube and send for platelet count, and enter on PRPQ.C. Form.
8. Prepare P.C at selected time, speed and temperature and enter in P.C QC form.
9. Weigh each P.C, calculate its volume and enter on QC form.
10. Allow P.C. unit to rest at R.T for 1 hour, then place on horizontal platelet agitator another 1h.
11. Draw 1 ml of P.C in 12x75 mm glass tube, withdraw 300 µl and dilute 1:3 by normal saline in properly labeled red top tube, send for platelet count and enter result on P.C .Q.C form.
12. Repeat procedures 3-4 times; select the shortest time, lowest speed which give highest yield of platelets in PRP and P.C., with minimal RBC's contamination.
13. Calculation:

a. Platelet counts:

$$\text{In WB} = \frac{\text{WB vol. x plt. count x}(10^9/\text{L})}{1000}$$

$$\text{In PRP} = \frac{\text{PRP vol. x plt. count x}(10^9/\text{L})}{1000}$$

$$\text{In P.C} = \frac{\text{P.C. vol. x plt. Count x}(10^9/\text{L})}{\text{factor } 1000} \quad \text{X dilution}$$

b. Platelet yield:

$$\text{of WB : PRP} = \frac{\text{Plt. Count in PRP x } 100}{\text{Plt. Count in WB}} = \%$$

$$\text{of PRP : P.C.} = \frac{\text{Plt. Count in P.C x } 100}{\text{Plt. Count in PRP}} = \%$$

14. Accepted Values:
    a. % yield of platelet in PRP > 75%
    b. % yield of platelet in P.C. > 90%

CENTRIFUGE CALIBRATION
FORM 1- % PLATELET YIELD-PLATELET RICH PLASMA

CENTRIFUGE NO:

	WHOLE BLOOD			PLATELET RICH PLASMA					
UNIT NO.	PLT CT	VOL (mL)	# of X 10^9	SPEED (RPM)	TIME (Min)	PLT CT (X 10^9)	VOL (mL)	# of X10^9	PLT YIELD

REVIEWED BY:---------- **DATE:** ----------

COMMENTS:----------

CENTRIFUGE CALIBRATION
FORM 2- % PLATELET YIELD-PLATELET CONCENTRATE

CENTRIFUGE NO:

PLATELET CONCENTRATE

UNIT NO.	# of Plts (X 10^9)	SPEED (RPM)	TIME (MIN)	PLT CT (X 10^9/L)	DILN (X3)	VOL (mL)	# of plt (x 10^9)	PLT YIELD

REVIEWED BY:---------- **DATE: ----------**

COMMENTS:----------

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[3] PLATELET PRODUCTS QUALITY CONTROL

PRINCIPLE:

4 expired (6^{th} day) units of platelet products (random, pheresis and leukoreduced pooled randoms), Checked for platelet count and PH, every month to ensure the therapeutic efficiency of the products.

POLICY:

1. **Q.C. will be performed on platelet products in accordance with AABB standards 5.7.5.**
2. **Unacceptable Q.C. results must be followed by immediate corrective action.**
3. **Platelet products that contain >2mls of RBC's will be cross matched with the recipient, in accordance with CAP transfusion medicine checklist # 40760.**

SPECIMEN:

1. **Platelet concentrates.**
2. **Platelet pheresis products.**
3. **Leuko reduced, pooled platelets.**

MATERIALS:

1. **l0 ml syringes with needles.**
2. **5, l0 ml Red Top Tubes.**
3. **300 µl pipette with tips.**
4. **Normal saline.**
5. **Electronic scale.**
6. **Platelet Q.C. worksheet.**
7. **">2mls of RBC's in product, cross match required" label.**

SAFETY:

All blood and blood products must be treated as potentially infectious.

PROCEDURE:

1. **Select 4 expired P.C. units, and 4 expired PPP units (6^{t}h day of life).**
2. **All units demonstrate "swirl" effect.**
3. **Each PPP from separate donor.**
4. **Enter unit numbers on Q.C. worksheet.**
5. **Place the chosen units on platelet rotator.**
6. **Label Red Top tubes with unit number, your ID number or Initial, date and time as follows:**
   a. **Label 5m1 tube with XCBC.**
   b. **Label l0 ml tube with plt. count and PH.**
7. **Weigh each platelet unit, calculate volume, enter in QC worksheet.**
8. **Resuspend platelets, draw 7m1, and dispense in l0 ml corresponding Red Top tube, draw300 µl, then dispense in 5 ml Red Top tube properly labeled and add 600 µl normal saline to dilute 1:3, cap and mix and send to Hematology section for platelet count.**
9. **Check pH for each unit.**
10. **Enter platelet count and pH for each unit in Q. C. worksheet.**
11. **Red cell contaminated platelet:**
    a. **Draw 5 ml and dispense in XCBC red top tube.**
    b. **Send to Hematology section for HCT.**
12. **Calculation of total platelet/ bag:**

$$\text{Volume of unit} = \frac{\text{wt (gm)}}{\text{S.G of plasma (1.03)}}$$

$$\text{Plt. count/bag} = \text{plt. count x 109/L X 3 (dilution) X } \frac{\text{VOL}}{1000}$$

13. Calculation of Absolute Red Cells volume (ml):

Volume of unit = $\frac{\text{wt (gm)}}{\text{S.G of plasma (1.03)}}$

Red cells volume in unit = HCT % X volume of unit.

14. Accepted value:

a. Random plt. c:

- 75% of units > $5.5x10^{10}$ plt / bag.
- All units ≥ 6.2 pH.

b. Platelet pheresis products (PPP):

- 75% of units > $3x10^{11}$ plt / bag.
- All units ≥ 6.2 pH.

c. Contaminated platelet products of more than PRBC's 2m1 need to be cross matched with the intended recipient.

LIMITATIONS OF PROCEDURE:

1. Platelet products should be well mixed before sampling.
2. Non - Swirling platelets will not meet QC requirements.

PLATELET QC RESULTS FORM

Date:---------- Performed by:---------- Product:----------

UNIT	WEIGHT gm	VOLUME mL	pH	Plt Count X 10^{10}/unit
% Acceptable				

ACCEPTABLE LIMITS:

Random Platelet Concentrates:

1) Platelet count = ≥ 5.5 X $10^{1°}$ Platelets in at least 75% units tested.
2) pH = ≥ 6.2 in all units tested

Apheresis Platelet Units:

1) PLT Count = ≥ 3.0 X 10^{11} Platelets in at least 75% units tested.
2) pH = ≥ 6.2 in all units tested.

Senior Review:---------- Date:----------

Quality Assurance Review: ---------- Date:----------

[4] CRYOPRECIPITATE QUALITY CONTROL

PRINCIPLE:

FACTOR VIII and fibrinogen assays for cryoprecipitate ensure it's proper preparation procedures.

POLICY:

1. 4 Units of Cryo. Will be assayed for factor VIII and fibrinogen every month and within 30 days of preparation.
2. In Accordance with AABB Standards, all units tested must contain > 150 mg of fibrinogen and > 80 IU of F VIII.
3. Unacceptable Q.C. results must be corrected and documented immediately.

SPECIMEN:

Cryo. Units within 30 days of preparation.

MATERIALS:

10 ml Syringes, and needles.
2. Water bath 37°C.
3. 12 mm x 75 mm plastic tubes with caps.
4. Electronic Scale.
5. Cryo. Q.C. Worksheet.

SAFETY:

All blood and blood products must be treated as potentially infectious.

PROCEDURE:

1. Select 4 Cryo. Units within 30 days of preparation.
2. Remove units from deep freezer 30 minutes earlier.
3. Units represent all ABO groups and from different dates of preparation.
4. Place each unit in a disposable plastic bag and thaw in 37°C water bath.
5. Enter unit numbers on Q.C. worksheet.
6.Weigh each unit, calculate it's volume by dividing wt. By plasma specific gravity (1.03), and enter volume in Cryo. Q.C. worksheet.
7. Mix well, draw 3 ml into properly labeled tube, and send for assay of F VIII and fibrinogen.
8. Calculation:
a. F VIII: FVIII IU /ml x volume (ml)= IU / bag.
b. Fibrinogen: Fibrinogen gm / L x volume (ml)= mg / bag.
c. Enter results in Cryo. Q.C. worksheet.
9. Accepted Values:
a. All units > 80 IU / bag for factor VIII.
b. All units > 150 mg / bag for fibrinogen.

PROCEDURE NOTES:

1. Unacceptable results must be followed by immediate corrective action, and repeating of Q.C. on another 4 Cryo. Units from same month.
2. Q.C coordinator, and Medical Director must review Q.C results every month.
3. Testing samples must be processed close together and quickly due to labile nature of FVIII
4. Group A plasma has higher F. VIII than group O.
5. Fibrinogen level may reach up to 800 mg in Cryo. Unit.

CRYOPRECIPITATE CONCNETRATE Q.C. FORM

DATE:---------- PERFORMED BY:----------

S.NO	UNIT	Prep	Cryo		Fibrinogen g/L	Fibrinogen mg/Bag	Factor VIII mg/BAG	Factor VIII IU/Bag	Group
			Wt	Vol					
1.									
2.									
3.									
4.									
5.									
6.									
7.									
8.									
9.									
10.									
11.									
12.									

AABB STANDARDS

1. ≥ 80 IU/BAG Factor VIII in each unit tested.
2. Fibrinogen ≥ 150 mg/unit in each unit tested.

% ACCEPTABLE:----------

Senior Review:---------- Date:----------

--Quality Assurance Review:---------- Date:----------

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[5] RESIDUAL WHITE CELLS COUNT (NAGEOTTE BRITE LINE CHAMBER)

PRINCIPLE:

Residual leukocytes in leukoreduced blood components can be calculated by first lyse the RBC's then count WBC's by Nageotte chamber after staining their nuclei with crystal violet.

MATERALS:

1. Nageotte Britte line chamber with SOmI counting volume.
2. Crystal violet stain: 0.01% w/v crystal violet in 1% v/v acetic acid (e.g.Turks solution).
3. Red cell lysing agent (e.g. Zapoglobin Coulter Electronics, Haileah, Fl.)
4. Pipettor (40 µl, 100 µl) with disposable tips.
5. Talc free gloves, clean plastic test tubes, plastic Petri dish, filter paper.
6. Light microscope with l0 X ocular lens and 20 X objective.
7. Residual WBC's count QC worksheet.

PROCEDURE:

1. For red cell containing components:
   a) Pipette 40µl of lysing agent into a clean test tube.
   b) Place the sample in a clean test tube (HCT < 60%).
   c) Pipette 100 µl of the sample into the lysing agent tube, and mix completely.
   d) Pipette 360 µ1 of crystal violet stain into the mixture and mix to reach final volume , of 500 µl.
2. For platelets:
   a) Place 100 µl of platelet sample in clean test tube.
   b) Pipette 400 µl of crystal violet stain into the 100 µl platelet test tube and mix to reach final volume of 500 µl.
3. Fit the hemocytometer with coverslip and load the counting area with the mixture.
4. Put hemocytometer inside Petri dish with the peace of damp filter paper, and let rest

for 10-I5 minutes (WBC's settle in counting area).

5. Remove the moist lid, place the hemocytometer on the Microcope, and by using 20 X objective count the WBC's in SO,uI volume of counting chamber.
6. Calculate and record results:

a) WBC's/ml = $\frac{\text{WBC's in 501!1 counting chamber}}{\text{10 (dilution factor).}}$

b) WBC's/unit = WBC's /µl X 10^3 (WBC's/ml) X unit vol. (ml)

7. *Record component identity, date and the ID of the person performing the testing.*

PROCEDURE NOTES:

l. Inaccurate results may occur due to WBC's deterioration by refrigerator storage. `
2. Do not use gloves with talc powder particles to avoid misread as WBC's.
3. Validate the accuracy of counting method by using reference sample with serial dilutions and compare with calculation method.
4. This counting technique is not accurate at < 1 WBC/µl.

[6] GRANULOCYTES QUALITY CONTROL

PRINICPLE:

QC for Granulocytes must be performed to ensure the proper therapeutic granulocytes level.

POLICTY:

l. QC on each granulocyte product.
2. Granulocyte product must contain > $1x10^{1°}$ granulocytes in at least 75% of tested units, in accordance with AABB standards 5.7.5.20.

SPECIMEN:

Granulocyte product.

MATERIALS:

1. Granulocyte pheresis QC form.
2. Autologous/directed donation procedure form.

PROCEDURE:

1. Determine granulocyte count for all granulocyte products.
2. Send aliquot of granulocyte product to Hematology section for CBC and differential leucocytic count.
3. Enter unit number in granulocyte QC form.
4. Weight the granulocyte pheresis unit and calculate the volume and enter on QC form.
5. Enter WBC x 10^9 /L, % of polymorphnuclear cells and enter on QC form.
6. Calculate the number of granulocytes X 10^9 / unit:

Granulocyte X 10^9 / unit = WBC (x 10^9 /L) X $\frac{\text{Poly}}{100}$ X $\frac{\text{Volume (ml)}}{1000}$

7. Review results every month by Apheresis senior Nurse and by Blood Bank QA.
8. Acceptable limits:

a) 75% of tested units contain> $1.0x10^{1°}$ granulocyte/unit.
b) Units must be labeled with granulocyte content.

9. Do corrective action for unacceptable QC results.

[III] TRANSPORT AND SHIPPING

1. Package:

a. Temperature, date, time and name, ID and signature of the person who perform the shipment.
b. Labelled (human blood, PRBCs, Platelet Concentration, FFP, or other).
c. Insulated and keep close all the time (adhesive tape).
d. Identity (human blood), sending facility.
e. Enclosed list of blood unit numbers, and statement that blood found to be non reactive by

screening tests for transfusion transmitted diseases, and approved by sending facility.
f. Blood units placed on the bottom, covered by cardboard, then securely bagged wet ice or coolant on the top and keep in up wright position.
2. Temperatures for PRBC's and whole blood should be maintained between 1-10°C while transport. 3 Thermometers should by enclosed inside transport containers.
4. Transport of platelet should maintain temperature between 20-24°C without ice, in transport containers.
5. Transport of FFP or cryoprecipitate should maintain temperature below -20°C with dry ice and special packaging and labeling requirements as hazardous shipment.
6 Inspection on receiving:
a. Temperature, date and time of shipment and receiving.
c. Variance over 10°C, or pertinent temperature according the type of the component.
d. Appearance of the unit.
e. Age of the unit.
f. Probability of storage before transfusion.
7. Records should be maintained about:

a. The mean of transport: - Transport containers. - Cooler boxes.
b. Name of the sender and receiver facility.
c. Name and title of the persons who issue the blood and who also receive it.
d. Date, time and temperatures on issue and on receive.
e. Blood unit numbers.

8. Notify sending facility of any unacceptable temperatures.

PART TWO: PRINICPLES OF SEROLOGY IN BLOOD TRANSFUSION MEDICINE

CHAPTER ONE: PRE TRANSFUSION TESTING

[I] TRANSFUSION REQUEST

Blood Transfusion Request Form

Hospital:	**Health Care No.:.....**
Name:.....	**Nationality:.....**
Age: ...../..... Yrs./Months Sex:□ M □ F	**Consultant In Charge:.....**
Department:.....	**Unit:.....**

This form must be completed and forwarded to laboratory at least 24 hrs. prior to transfusion (expect in emergencies)

Clinical Diagnosis and reason for transfusion:

.....

Hb:..... Date:.....

Blood group if

TYPE SCREEN AND TRANSFUSION

□ T/s Previous Tm: □ yes □ no
crossmatch: □ WB □ PRBCs
Pregnancy: □ yes □ no
□ FFP □ Cryp □ P1t.C Trn Rn : □yes □no
No. of units:.....

This blood will be held reserved for 8 hours only in a well controlled Ref(1-6°C) and can not be in RT more than 30 minute without transfusion and if not utilized will be returned to the blood bank.
- Transfusion should be finished in 4 hours.

Consultant:.....

Blood requested by:
Blood sample taken and labeled by:
Date and time blood needed:

Patients Blood Group	Anti-A	Anti-B	Anti-AB	A cells	B cells	Anti-D	Rh-control	DCT	Ab SCREENS	Ab-ID

Cross – Matched Units

	Unit No.	Grou	RT	37	AH	Signature	Received by	Time
1								
2								
3								
4								
5								

EMERGENCY TRNASFUSION REQUEST

Ll LTNCROSSMATCHED 0 URGENTLY CROSSMATCHED 0 ABO & Rh 0 GROUP O (Free of haemolysins)
I believe this patients life be in jeopardy without an emergency transfusion; I accept the responsibility the blood bank personnel of the responsibility for any adverse patient reaction resulting form this may have been prevented by pretransfusion routine compatibility testing.

PHYSICIAN: ..... Bleep No:.....

Sig:..... Date::..... Time:.....

REMARKS

Date & Time started: Date & Time completed:	Reaction: □ YES □ NO Signature of Technologist:	Date:

[II] BLOOD SAMPLE & SPECIMEN RECEIPT

Principle:

Proper collection and labeling of blood blank specimens are important to ensure patient safety. Policy:

All samples received in the blood blank will be labeled in accordance with AABB standards (5.11). Samples not meeting the labeling requirements must be recollected. In certain situations it may be necessary to process an inadequately labeled sample, but only with approval of the blood bank supervisor or medical director.

Although samples of insufficient quantity should generally not be received, it may be necessary to process these samples when difficulties in collection were encountered.

Specimen:

CROSSMATCH TEST	TYPE OF TUBE	ADULT VOL. (ml) (MINIMUM VOL)	PEDIATRIC VOL. (ml) MINIMUM VOL.
Type and screen*	Red top	10(7)	5(3)
Cross match*	Red top	10(7)	5(3)
Ab titre	Red top	10(7)	5 (3)
ABI Iso	Red top	10(7)	5(3)
DAT	Lavender top	5(3)	3 (1)
Red cell phenotype	Red top	10(7)	3(1)
Group confirmation	Red top	5(3)	5(3)
Transfusion reaction	Red top	10(7)	5(3) 3(1)
Cord blood	Lavender top		5(3)
Fetal blood	Lavender top		3(1)
Blood group only	Lavender top	3 (3)	3(3)
RhIG	Red Top Lavender Top	10(7),5	

- For type and screens or cross matches on newborns (collected by heels stick), a minimum of two full bullets is required.

DONOR PROCESSSING TEST	TYPE OF TUBE	ADULT VOL. (ml) (MINIMUM VOL)	PEDIATRIC VOL. (ml) MINIMUM VOL.
HIV I/11	**Red top**	**7(3)**	**7(3)**
HTLV 1/11	**Red top**	**7(3)**	**7(3)**
Hl3sAg	**Red top**	**7(3)**	**7(3)**
HCV & RIBA	**Red top**	**7**	**7**
Hepatitis	**Red top**	**7(3)**	**7(3)**
HIV antigen	**Red top**	**7(3)**	**7(3)**
Western blot (HIV 1 & HTLV I)	**Red top**	**7(3)**	**7(3)**
Viral marker (combination order)	**Red top**	**7(3)**	**7(3)**
PCR	**Green top**	**7**	

Safety precaution:

All blood and blood product must be treated as potentially infectious. Follow universal precaution procedure:

1. Check specimen to ensure that the following requirement have been met:

- **Tube is firmly stoppered.**
- **Label is firmly attached.**
- **Label information is complete.**
- **Label information matches the information on the order.**
- **Label information include:**
  1. **Patient full name.**
  2. **Patient medical record number.**
  3. **Date/time drawn.**
  4. **ID number of phlebotomist.**

2. Do not receive any sample which:

- **The tube is cracked or broken.**
- **The label is inadequately attached.**
- **The label information is incomplete.**
- **Has inadequate volume.**
- **The specimen date is different than date collected.**
- **The information on label does not match on the request.**

3. If the sample must be rejected for one of the above reasons or if hemolyzed

- **Phone the floor to notify them the sample must be recollected.**
- **Tell the floor to reorder the specimen.**
- **Cancel the order.**

4. If the sample does not meet criteria but must be accepted, get medical director or supervisor to approve acceptance.

Procedure notes:

1. The sample should not be drawn from the tubing used for infusion of IV fluid or from the contigous vein, but from a fresh venipuncture site.

2. Sample must be drawn from an arm without LV. If site not available, draw below LV.

3.Do not allow nursing staff or phlebotomy staff to re-label improperly labeled specimens.

4. Do not accept samples for cross matching and/or type and screen if the specimens date is not the same as the date collected.

Exception:

This does not apply to group confirm samples cold bloods, or samples ordered and drawn close to midnight. The collection date must be hand written if it does not match the specimen date.

5. If computer generated labels are not available, imprinted labels e.g. address graph labels may be used provided all required information is included. The following items must be hand written, as they are not included on the address graph label:

- **The specimen number.**
- **Specimen date.**
- **Collection time.**
- **ID # of the person drawing the specimen.**

6. Store cross match samples for (Donor & Recipient) and donor segments for 7 days at 1-6°C.

7. Store patient samples for viral marker testing at 1-6°C labeled with date of testing for at least 4 days after testing.

8. If the specimen is improperly labeled, phone floor to explain what is wrong.

[III] BLOOD GROUP AND Rh TYPING

PRINCIPLE:

a) The four main blood groups, A,B,O and AB are determined by:

1. Forward grouping to detect presence or absence of A and B antigens on red blood cell by testing red cells with anti A anti B antisera and Anti AB.

2. Reverse grouping to detect presence or absence of corresponding antibodies in the serum by testing serum with known A1 and B cells.

b) Rh positive or Rh negative red blood cells are classified by presence or absence of D antigen which can be detected by anti D (monoclonal-polyclonal blend). Weak D (Du) antigen can be detected by antiglobulin test.

POLICY:

ABO an Rh type will be determined in accordance with AABB standards 5.12.1 and 5.12.2

SPECIMEN

1) Clotted whole blood or an anti-coagulated specimen is acceptable.
2) Test the specimen as soon as possible; store at 2°- 8°c if delayed.
3) Test blood drawn into oxalate, EDTA or heparin within 2 days.
4) Test clotted specimens within 14 days.
5) Test donor blood within expiration date of donor unit.

REAGENTS, SUPPLIES, EQUIPMENT:

1) Anti-A antisera (monoclonal.)
2) Anti-B antisera (monoclonal.)
3) Anti AB antisera (monoclonal)
4) Anti-D (monoclonaUpolyclonal blend.)
5) A1 cells.
6) B cells.
7) Antihuman globulin serum.
8) Coombs control cells.
9) Isotonic saline. (0.9% Nacl.)
10) Rh control.
11) 12 mm x 75mm disposable glass test tubes.
12) Centrifuge.
13) Disposable pipettes.
14) Marking pen.
15) Cell washer.

SAFETY PRECAUTIONS:

1) Flush sodium azide with large volume of water if discarded into sinks, to prevent azide buildup, which react with lead and copper plumbing to form highly explosive metal azide.
2) Do not open centrifuge while rotor is running.
3) All blood and blood products should be treated as potentially infectious.
4) Dispose glass fragments in sharp disposal container and do not pick up broken glass with fingers.

QUALITY CONTROL:

1) ABO grouping must include both forward and reverse procedures.
2) Reverse grouping is not performed on cord blood or fetal samples.
3) Rh control is used as an autocontrol in Rh typing.
4) Daily quality control of antisera and cells.

PROCEDURE:

A- Forward grouping:

1) Prepare 3-5% red cells suspension in isotonic saline. Wash cord blood 4 times, capillary specimens 3 times.
2) Label five test tubes with patient ID number and with antisera to be added (anti A, anti B, anti AB, anti D, Rh control).
3) Add one drop each of antisera to the first four tubes and one drop of Rh control to the corresponding tube.
4) Add one drop of the prepared cell suspension to each test tube using a transfer pipette.
5) Mix well and centrifuge at 900-1000xg for 30 seconds.
6) Gently resuspend the cell button and examine for hemolysis and agglutination (see the table of grading of agglutination reaction.)
7) Read, interpret and record test result. and compare with results of reverse grouping.

Table of: Grading of agglutination reaction.	
Appearance	Grade.
1- Red supernatant, few or no intact red cells.	Hemolysis (H)
2- Single agglutinate (one button.), no free cells, clear background	4 + Agglutination.
3- Number of large agglutinates, clear Background	3 + Agglutination.
4- Large agglutinates with multiple smaller clumps, slightly cloudy background	2 + Agglutination.
5- Numerous small agglutinates, cloudy red back- ground	1 + Agglutination.
6- Easily dispersed very small agglutinates, cloudy red background	Weak agglutination.
7- Macroscopically appears negative, microscopically few agglutinates in most fields.	Microscopic agglutination

B-Reverse Grouping:

1) Label two test tubes with patient ID number and the cell type to be added (Al cells and B cells)
2) Add 2 drops of patient serum or plasma to each tube, using a transfer pipette.
3) Add 1 drop of appropriate well-mixed reagent cells to its corresponding test tube.
4) Mix well and centrifuge at 900-1000 xg for 30 seconds.
5) Gently resuspend the cell button and examine for hemolysis and agglutination (see the table of grading of agglutination reaction.)
6) Read, interpret and record test result and compare with results of forward grouping.
7) If grading of agglutination for reverse typing is <2+, you must check blood groups of all red cells crossmatched with that specimen.

C- Weak D (DU) procedure:

1) If no agglutination is observed with anti D after immediate spin, add one drop of anti-D, incubate test and Rh control tubes for 15 minutes at 37° C.
2) Mix, centrifuge both tubes at 900-1000 xg. For 30 seconds, resuspend the cells and examine for agglutination. If the test red cells are agglutinated in the anti D tube but not in the control tube, record the test sample as D-positive and does not proceed with antiglobulin phase test.
3) Wash cells four times with isotonic saline and decant supernatant completely and add 2 drops of anti human globulin, mix centrifuge at 900-1000 xg for 30 seconds.
4) Resuspend cells and examine for agglutination, record graded reaction.
5) Add coombs control cells to all negative AHG tests. Mix, centrifuge and check for presence of agglutination. If agglutination is not present, repeat weak D testing.

INTERPRETATION:

1- Interpret ABO results as follows:

Patient cells with			Patient serum with		Interpretation
Anti-A	Anti-B	Anti AB	A cells	B cells	
-	-	-	+	+	O
+	-	+	-	+	A
-	+	+	+	-	B
+	+	+	-	-	AB
+/-	+/-	+/-	+/-	+/-	Invalid.
	-	+	+	+	

+ = Agglutination. **- = No agglutination.**

a- resolve weak reactions or discrepancies before recording result and / or issuing components.

2-Interpret Rh results as follows:

Patient cells with:			Interpretation
Anti D	Du test (AHG)	Rh Control	
+	N/A	-	Rh- positive
-	+	-	Rh- positive
-	-	-	Rh- negative
-	+	+	Invalid test
+	N/A	+	Invalid test

a) Report any agglutination of tested cells with anti D even in Du test as Rh- positive (not Rh-negative Du positive.)

b) No agglutination of tested cells with anti D and AHG must be reported as Rh-negative.

c) If test and control are positive, perform DAT., if positive do not test for weak D (Du.) by indirect antiglobulin.

PROCEDURE NOTES:

1) If correct Rh (D) type of the patient is in question, consider the recipient as Rhnegative.

2) Perform forward and reverse typing at room temperature (20° - 30°c).

3) Bacterial contamination of specimen may cause false results.

4) When mother is Rh. Negative and cord blood for newborn give positive DAT., suspect false negative result with anti D because the Rh-positive newborn red blood cells are covered with maternal anti-D, leaving no D sites available.

[IV] CROSS MATCHING TESTS

Principle:

l. The crossmatch is an in vitro procedure to determine serologic compatibility between donors red cell suspension and recipients serum without agglutination and or hemolysis in the various phases.

2. Massive transfusion is an infusion of 10 units of blood [=or> recipients total blood volume] within 24 hours.

Policy:

l. Patient history of ABO/Rh., antibodies or severe reactions must be reviewed before issuing blood.

2. Immediate spin crossmatch is acceptable for patient with no clinically significant antibodies (AABB standard 5.13.1).

3. Anti P1;M;N;Lea;Leb, Sda, Bga, and Anti A1 are considered clinically insignificant because they react only at the immediate spin phase unless they are reactive at 37°C or AHG, then they will be considered clinically significant.

4. Patient with currently detectable clinically significant antibodies must receive full crossmatch compatible antigen negative units.
5. Massive transfused patients must receive ABO/Rh compatible blood which is antigen negative for any existing or pre existing antibodies (AABB standard 5.16.4).

Specimen:

1. Properly labeled clotted whole blood from patient within 3 days of scheduled transfusion.
2. Donor cells from segment originally attached to the unit being crossmatched.

Materials:

1. 12mm x 75mm glass test tubes, disposable Pasteur pipettes.
2. Saline (0.9%)
3. Anti human globulin (AHG)
4. IgG sensitized RBC's (Coombs control cells)
5. Serofuge and or automatic cell washer
6. Agglutination viewer, Microscope, water bath.
7. 22 % bovine albumin

Safety:

1. All blood and blood products must be treated as potentially infectious.
2. Use a dustpan or scooping implement to pick up broken glass and dispose it in sharps disposal container.

Quality Control:

1. See B.B. reagent Q.C.

Procedure:

(1) *Immediate spin crossmatch (LS.)*

1. Notes: a) For patients without clinically significant antibodies.
   b) LS is not for neonates (less than 4 months of age) or, for patients with clinically significant antibodies.
2. Place 2 drops of patient serum in a properly labeled test tube (one tube for each unit)
3. Add one drop of 3.5% saline suspension of donor red cells, and mix.
4. Centrifuge immediately, examine for hemolysis, resuspense, read macroscopically for agglutination and record the results.

(2) *Coombs (AHG) Crossmatch*

l. Prepare 3-4% twice washed donor red blood cell suspension from donor blood unit segment in 0.9% of normal saline.
2. Place 2 drops of patient serum in a properly labeled test tube (one tube for each unit to be cross matched).
3. Add one drop of 3-4% twice washed donor red cell suspension, and mix.
4. Spin and observe for agglutination or hemolysis, read and record results.
5. Add 2 drops of 22% bovine albumin.
6. Incubate at 37°C for 30 minutes.
7. Centrifuge, examine for hemolysis, resuspend and observe for agglutination, record results.
8. Wash 4 times with saline and decaunt after last wash.
9. Add 2 drops of AHG, and mix.
10. Centrifuge, resuspend and examine for agglutination and hemolysis, record the results.
11. To all negative tests, and one drop of CCC, centrifuge and examine for agglutination If no agglutination, repeat the test.

(3) *Massive Transfusion*

1. If patient's antibody screen is negative, group check on ABO compatible red cells for all subsequent crossmatches, with indate specimen.
2. After 24 hours from massive transfusion, a new type and screen must be requested.

3. If patient has clinically significant alloantibodies full cross match is required with ABO compatible and antigen negative units.

Reporting Results:

1. Both hemolysis and agglutination in any phase of the cross match indicate incompatibility.
2. Enter the units numbers, record reaction results and blood group in the crossmatching worksheet.
3. For each unit compatible for the patient:

a) Print an Issue Transfusion form, handwrite the unit number and date of cross match and patient name and ID number and attach it to the blood bag.

b) Place labeled unit in the refrigerator for crossmatched units.

Procedure Notes:

1. Give priority to STAT crossmatch orders, which should be available in 30 minutes.
2. Routine crossmath orders, should be available in one hour.
3. Do nor use an immediate spin crossmatch for
- Neonates
- Patients have positive immediate spin crossmatch due to cold agglutinins or clinically insignificant alloantibodies.
4. In immediate spin crossmatch, you should recheck the unit blood group.
5. Acceptable blood groups of red cells for transfusion.

Patient blood group	First choice	Acceptable Alternative	Acceptable with approval of medical director.
O +ve	O+ve	O-ve	-----
O -ve	O-ve	--------	O +ve
A+ve	A+ve	A-ve, O+ve, O-ve	-----
A-ve	A-ve	O-ve	A+ve, O+ve
B+ve	B+ve	B-ve, O+ve, O-ve	------
B-ve	B-ve	O-ve	B+ve, O+ve
AB+ve	AB+ve	AB-ve, A+ve, A-ve,B+ve,B-ve, O+ve, O-ve	-----
AB-ve	AB-ve	A-ve, B-ve, O-ve	AB+ve, A+ve, B+ve, O+ve.

6. Negative patients can receive Rh-positive red cells only by approval of blood medical director.
7. Rh-negative patients transfused by Rh-positive red cells will be serologically monitored by:

a) Performing DAT using polyspecific AHG on all patient red cell specimens until further notice.

b) Comments will be recorded into the patient's history about his response to Rh positive cells.

8. 10 units of packed red cells is considered a replacement of original blood volume in normal adult, while it is difficult to calculate it in poediatric cases.
9. If serum is not sufficient for crossmatch, do blood groups check on units until you get new sample.

CROSSMATCHING TESTING

		SI	SII	SIII	Auto-control
Pat. Serum	1 drop	1 drop	1 drop	1 drop	1 drop
Pat. Cell Susp	-------	-------	-------	-------	1 drop
Donor Cell Susp	1 drop	-------	-------	-------	-------
SI Cells	-------	1 drop	-------	-------	-------
SII Cells	-------	-------	1 drop	-------	-------
SIII Cells	-------	-------	-------	1 drop	-------

	Cross MATCH	SI	SII	SIII	AUTO-CO	INTERPRETATION
IS	+	-	-	-	-	Error, weak AB0-Ag, anti-A1, in A2, A2B individuals, anti-M, anti-I. Donor polyagglutination, rouleaux.
IS	-	+	-	+		Auto. Anti-H (-IH), anti-Le bH, reagent problem, unit single dose of A g.
37C	-	-	-	-	-	Compatible
AHG	+	-	-	-	-	Donor DAT +ve, Kidd, duffy, MN, P1, Rh dosage Ag, low incidence Ag, anti-A or anti-B in incompatible platelets
AHG	+	+	+	-	+	Alloantibodies, IVIG alloatibodies, cold or warm Ab, rouleaux formation
C.C.C				+		Must give +ve with all -ve AHG results.

Limitations of Procedure:

Compatible crossmatch will not:

a) Guarantee normal survival of transfused cells.

b) Prevent immunization of the recipient

c) Detect all unexpected antibodies in recipient serum protein interference.

[V) ANTIBODY SCREENING

Principle

1. Immunization to fore red cell antigens may occur through pregnancy, transfusion or injection of immunization materials.
2. Antibody screening is performed on:

a) Obstetric patients: to identify women with alloantibodies that might cause hemolytic disease of new born.

b) Potential candidates for blood transfusion: to detect alloantibodies that might cause a hemolytic transfusion reaction.

3. Antibody screening tests allow serum of the patient or the donor to react with selected red blood cells under conditions that demonstrate antibodies activity either in LS., or 37°C or antiglobulin phase.

Policy

1. Antibody screens will be performed on all samples received with a request for transfusion, and when specially requested.
2. In accordance with AABB standards 5.12.3, samples will be tested by a method that will demonstrate all clinically unexpected antibodies.
3. Pre-transfusion testing will be added to all AHG tests interpreted as negative.
4. IgG sensitized red cells will be added to all AHG tests interpreted as negative
5. The sample must be obtained from the patient within 3 days of the scheduled transfusion.

Specimen

Serum and cells from l0 ml (minimum 7 ml) clotted whole blood.

Materials

1. 12 x 75 mm glass test tubes
2. Saline (0.9%)
3. 22% bovine albumin.
4. AHG
5. Antibody screening cells.
6. CCC (IgG sensitized RBC's).
7. Serofuge and /or automatic cell washer.
8. Agglutination viewer.
9. Microscope, 37°C water bath.
10. Disposable pipettes

Safety Precautions

1. All blood and blood products must be treated as potentially infectious
2. Follow universal precautions detailed in the laboratory safety manual.

Quality control

See reagent quality control

Procedure

1. Place two drops of serum to be tested into three properly labeled test tubes (one tube for each screening cell I, II, III)
2. Add one drop of 2-5% RBC's suspension to the corresponding tube and mix, centrifuge, resuspend, read and record the result.
3. Add two drops of 22% bovine albumin, additive and mix
4. Incubate at 37°C for 30minutes in water bath, centrifuge, resuspend, read and record the results.
5. Wash 4times with saline, and add two drops of AHG, mix, centrifuge, read and record the results.
6. Add one drop C.C.C. to all negative tests, centrifuge, resuspend and examine for agglutination, if negative test must be repeated.

Interpretation of results

1. Negative test means that no antibodies present in the serum to react with screening cells.
2. Positive test should be followed by antibody identification testing.
3. In case of discrepancy between screening cell results and cross matching results, one of the following antibodies may be responsible.
   a) Anti -H in A1 and A_1B individuals may agglutinate "O" and AZ cells.
   b) Anti-L3 ^{bh} react only with O Leb+ cells but not with A_1 or A1B that are Leb+
   c) Anti A_1 in A2 individuals will not react with O cells (screening cells) but will react with Al cells during cross matching.
   d) Low incidence antigens.
   e) Antigens on homozygous cells only react with the corresponding antibody.
4. In false negative results you may use:
   a) Enzymes: as ficin panel.
   b) Adjustment of incubation time to not more than 30 minutes with 22% bovine albumin
   c) Equal amount of serum and cells with 22% bovine albumin

Reporting results

1. Record result immediately
2. Repeat any test that is negative with coomb's control cells
3. Do not increase serum to cell ratio with bovine albumin.

[VI] ANTIBODY TITRATION

PRINCIPLE:

1. It is a semi quantitative method to determine antibody concentration in a serum sample.
2. Applications:
   a) Estimation of antibody activity in alloimunized pregnant women for early detection of HDN.
   b) Elucidating autoantibody specificity.
   c) Titer and avidity of antibodies to certain antigens.
   d) Determine antibody class (IgG or IgM) by the effect of sulfhydryl reagents.

SPECIMEN:

Serum or plasma antibody to be titrated.

REAGENTS:

1. Red cells (2-5% suspension), that express the antigens corresponding to the antibody specificity.
2. Saline or albumin for dilution.

PROCEDURE:

1. Label 10 test tubes according to the serum dilution
   l, 1/2, 1/4 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512.
2. Deliver one volume of saline to all test tubes except the 1^{st} tube (1/1).
3. Add an equal volume of serum to 1^{st} and 2^{nd} tubes.
4. Using a clean pipette, mix the contents of 2^{nd} tube (1/2) and transfer one volume into the 3' tube (1/4).
5. Continue the same procedure from 3^{rd} tube (1/4) to tube number 10, then remove one volume from 10^{th} tube (1/512) and save it for further dilution.
6. Label 10 tubes for the appropriate dilutions.
7. Transfer 2 drops from each dilution to the appropriate tube and add 1 drop for RBC's suspension to all tubes.
8. Mix well and screen for antibodies by immediate spin, 37°C water bath and AHG steps.
9. Examine for agglutination macroscopically from higher to lower dilution to exclude prozone
   phenomena in lower dilution (higher concentration).

INTERPRETATION:

1. Observe the highest dilution that produces 1 + macroscopic agglutination.
2. Report the titer l, or 2, or 4 and so on.
3. If agglutination observed in all 10 tubes, prepare more dilutions and test it.
4. Observe strength and agglutination and give score
   (l+ =5, 2+=8, 3+=10, 4+=12)
5. From titer and strength determine antibody titer and avidity, (e.g. high titer, low avidity antibody has high titer and weak reaction).

NOTES:

1. Careful pipetting is essential.
2. Consistent time, temperature and centrifugation.
3. Age of patient, phenotype and concentration of cell suspension will influence results.
4. Same serum dilutions must be used for different RBC's samples
5. Adequate volumes of serums needed to attain accurate results.

[VII] RESOLVING ABO DISCREPANCIES

PRINCIPLE:

1. Discrepancies occur due to technical errors, absence of expected antigens on RBC's, Unexpected reactions with Anti-A and Anti-B, or due to unexpected serum reactions.
2. History of pregnancy or previous transfusion should be questioned.

POLICY:

In accordance with AABB standards 5.12.1, the blood shall not be released until any discrepancy in ABO grouping is resolved.

SPECIMEN:

1. Freshly drawn clotted whole blood.
2. Anticoagulated (EDTA) specimen (7m1).
3. Segment from discrepant blood unit.

MATERIALS:

1. Anti-A, Anti-B.
2. Al, A2 and B cells.
3. Anti-Human Globulin (AHG).
4. Screening cells.
5. ABO compatible cord serum or plasma.
6. Anti-Al (lectin).
7. Other Antisera as indicated.

QUALITY CONTROL:

Routine daily quality control of Antisera and cells to be tested against known cells and antisera positive and negative for the corresponding antigen and antibody.

CAUSES OF ABO DISCREPANCEIS:

A-CAUSES IN CELL TYPING:	RESOLUTION
1- Chimerism: more than one ABO group, due to blood transfusion, or BM transplant.	obtain the patient history of diagnosis, blood transfusion, bone marrow transplantation and Medication.
2- Weak expression of A and B antigens As in leukemia.	1- Incubate patient washed cells with Anti A, Anti B, and Anti- AB in room temperature for 30 minutes. 2- Treat patient RBCs with ficin, papin or bromolin.
3- Poly agglutinable state to inherited or acquired abnormalities of red cell membrane.	Use monoclonal Anti A or Anti B reagents.
4- Non specific aggregation of red blood cells, which simulates agglutination due to presence of concentrated serum proteins as in Wharton's Jell contaminated cord blood sample.	Wash patient cells. And make 5% suspension with isotonic saline.
5- Rauleaux formatting which simulate agglutination.	Wash cells and dilute serum with saline
6- False positive reaction with Anti A or Anti B reagents due to presence of high concentration of A and B blood group Substances in the serum Which will neutralize Anti A or Anti B before Getting to RBC's membrane.	wash patient cells and make 5% cell suspension with isotonic saline.
7- False positive reaction due to presence of anti-bodies in patient serum against coloring dyes used to color Anti A and Anti B reagents.	wash patient cells with saline
8- Spontaneous auto agglutination in presence of diluents, due to cold reactive autoagglutinins.	1-wash patient cells. 2- Incubate in 37 c. For 30 minutes.

9- False positive reaction due to presence of PH or diluents dependent auto antibody.	1- Wash patient cells.

B- CAUSES IN SERUM TYPING:	RESOLUTIONS
1- False positive results due to presence of small fibrin clots.	Obtain a new blood specimen on EDTA.
2- False positive results due to red cell aggregation in presence of high molecular Weight plasma expanders (colloids).	Dilute serum.
3- False positive results due to presence of Antibodies other than Anti A or Anti B. (e.g. Anti M., Anti N, Anti S).	Perform antibody screening.
4- False negative result due to absence of ABO antibodies in presence of immunodeficiency state.	Obtain the patient history.
5- False negative result due to absence of ABO antibodies. In infants under 4-6 months.	Obtain the patient history.
6- False negative result due to very high titer of complement in samples lacking EDTA.	Obtain a new blood specimen on EDTA.
7- False negative result due to absence of ABO antibodies in Bone marrow transplants. (Group A. patient receive group O BM, will have O cells but will produce only Anti B).	Obtain the patient history about B.M. transplant.
8- False positive result due to FFP transfusion which contain ABO antibodies.	Obtain the patient history about FFP transfusion.
9- False positive result due to antibodies against diluents preservative of reagent A, and B cells.	wash reagent cells with isotonic saline and make 5% cell suspension with Saline.

INVESTIGATIONS FOR ABO DISCREPANCY RESOLUTION:

A-In General:

1. Obtain a new blood specimen to avoid mislabeled and contaminated specimens.
2. Obtain the patient's medical history and diagnosis, previous blood transfusion, Bone marrow transplantation and medications.
3. Wash the test and reagent red cells with isotonic saline to avoid false positive reaction against serum antibodies or any chemicals.
4. Test red cells with Anti AB, AntiA, or Anti.H as required.
5. Test serum with A1 and A2 red cells, if anti A1 is suspected.
6. Perform Antibody screening to detect cold reactive allo-or auto antibodies.
7. Incubate tests and control at room temperature for 30 minutes to detect weak antigens or antibodies.

B- In Absence of expected antigens:

1. Incubate washed red cells with Anti-A and Anti-AB at room temperature for 30 minutes.
2. Treat the patient's red cells with ficin, papain or bromelain enzymes to enhance the reaction.

C- Unexpected reactions with Anti A and Anti B:

1-Acquired B phenotype:

a-Check patient diagnosis: as in colon infection.

b-Monoclonal anti B will not react with acquired B.antigen.

c-Acidified human Anti B serum to Ph 6.0 will not react with acquired B antigen.

2-Acquired A like antigens:

Use enzyme treated cells to abolish reactivity with anti A.

3-Mixed-field agglutination: as in.

a-Blood transfusion, for the life of transfused cells.

b-B.M transplantation, until patient own cell stop.

c-Chimerism: remain for life.

d-Group A3 red cells with anti-A.

4-Antibody-coated Red cells:

a-Heat or acid elution removes most of 1gG antibodies.

b-Incubation of red cells at 37c and washing them with warmed saline at 37c, remove most of cold reactive IgM, autoantibodies.

Unexpected reactions with anti-A and anti-B

RBC's + known antisera					Serum + known RBC's				Blood group
Anti A	Anti Al	Anti B	Anti AB	Anti H	A1	A2	B	O	
+	+	-	+	-	-	-	+	-	Al
+	-	-	+	+	+/-	-	+	-	A2
+ Mf	-	-	+ Mf	+	+/-	-	+	-	A3
-	-	+	+	-	+	+	-	-	B
+	+	+	+	+	-	-	-	-	AB
				+	+	+	+	-	O
-	-	-	-	-	+	+	+	+	Bomby
Mf: mixed field agglutination									

D-Unexpected Serum Reactions:

1. Check age and patient diagnosis to detect absence of antibodies in immnodeficient patients, newborns or elderly persons.
2. Dilute the serum in cases of high ABO antibody titer to avoid prozone and false negative reaction.
3. Individuals who have anti-A1, agglutinate A1, cells but not A2 or O red cells, and should be given A2 or O PRBCS only.
4. If you expect cold autoagglutinins as anti-I and anti-H, warm the serum and reagent red cells to 37c
5. If you expect alloantibodies as anti P1 or antiM, identify the antibody and incubate the serum and reagent cells in 30-37C°.
6. Dilute serum 1:3 in isotonic saline, if you expect rouleaux formation (stack of coins), in cases of high protein concentration in the serum.

[VIII] Introduction to Column Technology & the Gel Microtyping System

Principle:-

A specific red blood cell solution is added to the gel contained in a special micro tube. The gel acts as a trap, the free red blood cells pellet in the bottom of the tube while agglutinates are trapped (fixed) in the top of the gel for hours. A clotted or anticoagulated whole blood sample can be used.

Different Gel tests:-

There are 3 types of Gel:

1) Neutral Gels: No specific antiserum used.

Mode of Action: gel traps the agglutinates during centrifugation.

Types of tests: Reverse ABO grouping, antibody screening and Identification by saline and enzyme technique, antibody titration.

2) Specific Gels: Gel plus anti sera e.g. anti-A, anti-B, anti-AB, Anti-D, anti-C anti- K

Types of tests: For antigen determination e.g. ABO, Rh, Kell blood group

3) Antiglobulin Gels: Gel plus mono or polyspecific aniglobulin e.g. anti- IgG+C3d

Types of tests: Compatibility testing, Antibody screening, Antibody identification by Coombs technique.

Samples:-

Anticoagulated samples allow ideal access to plasma and cells and provide the best productivity and ease of use in the DiaMed-ID system. Plasma is not detrimental to the quality of antibody detection.

No special preparation of the patient is required prior to sample collection. Draw blood samples using acceptable phlebotomy techniques. Preferably blood samples should be taken into citrate, EDTA, Heparin or CPD-A. For reliable results use freshly collected blood samples.

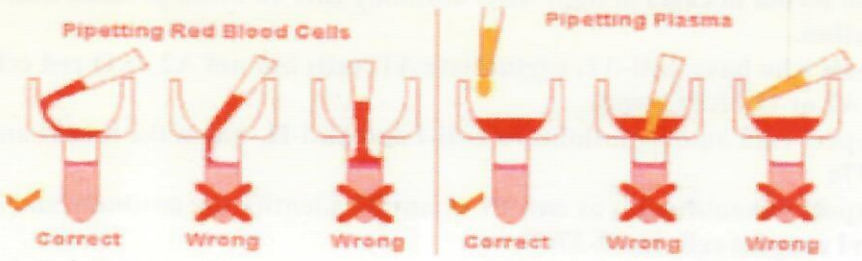
When the use of serum instead of plasma is required, the serum must be well clarified by centrifugation at 1500g for 10 minutes before use so as to avoid fibrin residues which may interfere with the reaction pattern.

Cell Suspensions:-

It is best to prepare cell suspensions from cell concentrates (packed cells) to maintain greater consistency. The use of whole blood to prepare cell suspensions will be more variable as the PCV differs between blood samples.

Pipetting:-

Pipetting technique is important to get optimal results from the DiaMed-ID system. Cells should be added first, using the FP2 or EP3 pipette held at an acute angle. Allow the tip to rest on the edge of the well and dispense the solution into one side of the cup. The fluid should run down into the cup and form a meniscus over the micro-tube to create a 'bubble' between the cells and the test gel. To prevent cross contamination between microwells avoid touching the tip on the side of the cupule during pipetting.



Secondly pipette the test sample, plasma or serum using the pipette held upright, directly above the cells and slightly to one side of the cupule. Under no circumstances pipette directly onto the gel surface or into the cell suspension, this may lead to false results.

Limitations

- Certain drugs are known to cause positive Coombs tests.
- Some pathological conditions are also reported to cause positive Coombs tests
- Bacterial and other contaminants of materials used can cause false positive and negative reactions. Do not use reaqgents which have become turbid or show precipitates.
- Fibrin residues in the red cell suspension may trap non-agglutinated cells causing a fine pink line "red-line" on the top of the gel while most are seen at the bottom of the microtube after centrifugation.

· Strict adherence to procedures and recommended equipment is essential. The equipment should be regularly checked according to GLP procedures.
· Use of other test cells reagents or suspension solutions for red cells other than ID-diluents may modify the reactions patterns.
· Too heavy red cell suspensions can cause false positive or false negative results.
· Use of other test sera than the described ID test sera may modify reactions. All other test sera must be fully validated for use in the gel test before actual test results are accepted.
· The ADB confirmation tests do not replace a complete ABO/Rh type determination. It should only be used as a confirmatory test of previously determined ABO/Rh type. ABO/Rh and reverse typing is validated by a negative 'ctl' test.
· Poly agglutinable cells may react with all human antisera - further investigation is required.

Storage of the ID Cards:

The cards should be stored the correct way up and may be kept at room temperature. The reagent gel is stable for 1 year. Centrifuge the cards before using to pack unsettled contents.

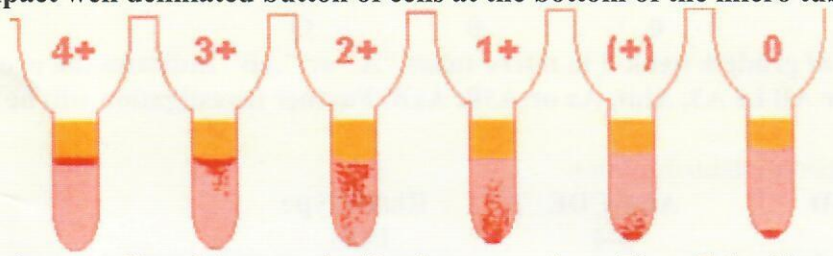
Cold Antibodies:-

Cold antibodies may be enhanced by incubating the ID card at or below room temperature and the detection of many, such as Lewis, HI may also be optimised by using enzyme techniques. (MNS and Duffy antigens are damaged by enzymes)

For ID tests at 4°C for cold agglutinins special care is needed: before use place the ID cards at 4°C for 2 hours and use similarly cooled reagents and samples.

Positive: agglutinated cells form a red line at the top of the gel or agglutinates dispersed within the gel matrix.

Negative: A compact well deliniated button of cells at the bottom of the micro tube.



- The cards must be centrifuged for exactly 10 minutes at about 70g. False Negative results are obtained if centrifugation is too long or fast. False positive results are obtained if centrifugation is too slow or short.

DETERMINATION OF ABO AND RHESUS (D) BLOOD GROUPS

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Working Table
-ID Dispenser
-ID Pipetor EP-3/FP-2/FP-3
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 1 (Bromelin Solution)

MICROTYPING CARDS

ABO/Rh (Profile:- A-B-AB-D-CDE-ctl) Human
or
ABO Confirmation (Profile:- A-B-D/A-B-D) Human
or
ABO/Rh DD (Profile:- A-B-AB-D-D-Ctl) Human & Diaclon

SAMPLE:

Red cell concentrate/Whole blood

TEST PROCEDURE:

1. Allow all reagents to reach room temperature before use.

2. Identify the ID-microtyping card with the patient name and number. Remove the aluminium foil.

3. Prepare a 5% suspension of test red cells in ID-Diluent 1 (Bromelin)

25µl red cell concentrate + 0.5ml ID-Diluent 1

or

50µl whole blood + 0.5ml ID-Diluent 1

4. Mix well, incubate at room temperature for 10 minutes.

5. Add 10µl of test red cell suspension to each microtube in the ID-microtyping card.

6. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.

7. Interpret the results.

INTERPRETATION

Results can only be accurately interpreted if the control ("Ctl") micro tube gives a negative reaction. A positive reaction may indicate the presence of an autoantibody. The test should be repeated after elution of the autoantibody.

ABO blood group determination

	Anti-A	Anti-B	Anti-AB	Group
Tube	4	0	4	A
Reactions	0	4	4	B
0-4	4	4	4	AB
	0	0	0	O

Positive reactions of grade ± weak-3 in micro tubes "A" or "AB" indicates the presence of a subgroup of A or AB i.e A3, Aint, Ax or A3B, AxB. Further investigation will be required to confirm this.

Rhesus (D) blood group determination

	Anti-D	Anti-CDE	Rh(D)Type
Reaction	4	3-4	POS
Grade	0	0	NEG
0-4	0	3-4	NEG

Positive reactions of grade ± 3 in micro tube "D", indicates the presence of weak D varient expression. Further investigation may be required to confirm this.

A negative reaction in micro tube "D", with a corresponding positive reaction in micro tube "CDE", indicates the presence of the Rhesus antigens C and/or E.

For the ABO/Rh D-D ID card with anti-D of both Human and monoclonal origin, a discrepancy in the Rh(D) type may indicate a weak D varient, for example category DVI.

DETERMINATION OF ABO BLOOD GROUP WITH REVERSE GROUPING

EQUIPMENT/MATERIALS

-ID Centrifuge

-ID Working Table

-ID Pipetor EP-3/FP-2/FP-3

-ID Dispensor

-ID Suspension Tubes

-ID Disposable Tips

REAGENTS

-ID Diluent 1 (Bromelin Solution)
-ID DiaCell A1
-ID DiaCell B

MICROTYPING CARDS

ABD + reverse group (Profile:- A-B-D-ctl-A1-B) Human

SAMPLE

Red cell concentrate/Whole blood - ABO group
Serum or Plasma - Reverse Group

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-microtyping card with the patient name and number. Remove the aluminium foil.
3. Prepare a 5% suspension of test red cells in ID-Diluent 1:-
   25µl red cell concentrate + 0.5ml ID-Diluent 1
   or
   50µl whole blood + 0.5ml ID-Diluent 1
4. Mix well, incubate at room temperature for 10 minutes. 5. Add 10µl of the test red cell suspension to microtubes "A", "B", "D" and "ctl".
   Add 50µl of ID-DiaCell A1 to microtube "A1".
   Add 50µl of ID-DiaCell B to microtube "B".
6. Add 50µl of test serum or plasma to microtubes "A1" and "B".
7. Incubate the card at room temperature for 10 minutes.
8. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.
9. Interpret the results.

INTERPRETATION

Results can only be accurately interpreted if the control ("Ctl") microtube gives a negative reaction. A positive reaction may indicate the presence of an autoantibody. The test should be repeated after elution of the autoantibody.

ABO blood group determination

Anti-A	Anti-B	A Cell	B Cell	ABO
4	0	0	3-4	A
0	4	3-4	0	B
4	4	0	0	AB
0	0	3-4	3-4	O

Positive reactions of grade ±1-3 in microtubes "A" or "B" indicates the presence of a subgroup of A or AB i.e A3, Aint, Ax or A3B, AxB, or of B3, Bx. Further investigation will be required to confirm this. A positive reaction of grade 4 in tube 3 "D" indicates Rh(D) Positive. Grades of 1-3 in tube 3 indicate Rh(D) Du or weak D varient. A negative reaction is Rh(D) Negative.

ANTIBODY SCREENING
(37°C ENZYME + INDIRECT ANTIGLOBULIN TEST)

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Cell I-II-III (Antibody Screen Cells for Indirect Antiglobulin test)
-ID Cell I-II-IIIP (Papainised Antibody Screen Cells for Enzyme test)

MICROTYPING CARDS

Coombs/Enzyme Test Combined (Profile:- 3 enzyme tests + 3 coombs tests) Each card contains 3 "Neutral" gels for Enzyme tests and 3 "Antiglobulin" gels for Indirect Coombs test.

SAMPLE

Serum or Plasma

TEST PROCEDURE

1. **Allow all reagents to reach room temperature before use.**
2. **Identify the ID-microtyping card with the patient name and number. Remove the aluminium foil.**
3. **Add 50µl of ID-Cell I, II and IIIP Papainised Antibody Screen Cells to micro tubes "1", "2" and "3" respectively.**
4. **Add 50µl of ID-Cell I, II and III Coombs Antibody Screen Cells to micro tubes "4", "5" and"6" respectively.**
5. **Add 25µl of test serum or plasma to each micro tube.**
6. **Incubate the micro typing card for 15 minutes at 37°C in the ID-Incubator.**
7. **Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.**
8. **Interpret the results.**

INTERPRETATION

Positive reactions, grade 1-4, in the micro tube indicates the presence of an antibody. Further investigation using an Antibody Identification Panel by the appropriate technique, will be required to confirm this.

Negative reactions in the micro tubes indicates the absence of an antibody.

ANTIBODY IDENTIFICATION
37°C INDIRECT ANTIGLOBULIN TEST and 37°C ENZYME TEST.

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 1 (Bromelin Solution)
-ID Diluent 2 (Modified LISS)
-ID DiaPanel (Antibody Identification Cells (1-11) for Indirect Antiglobulin test)
-ID DiaPanelP (Papainised Antibody Identification Cells (1-11) for Enzyme test)

MICROTYPING CARDS

LISS/Coombs (Profile:- 6 IgG/C3d Polyspecific Coombs tests)
or
LISS/Coombs IgG (Profile:- 6 IgG monospecific Coombs Tests)
NaCl/Enzyme Test (Profile:- 6 enzyme tests)

Each LISS Coombs card contains 6 "Antiglobulin" gels for Indirect Coombs tests. Each NaCl/Enzyme card contains 6 "Neutral" gels for enzyme tests. 2 of each card are required per test sample. Microtubes "1-11" are used for Antibody Identification Cells 1-11, microtube "12" is used for the Autocontrol.

SAMPLE

Red Cell Concentrate/Whole Blood
Serum or Plasma

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Label the microtyping card with sample identification.
3. Prepare Autocontrol red cell suspension:-
10µl red cell concentrate + 1.0ml ID-Diluent 2
or
20µl whole blood + 1.0ml ID-Diluent 2
Mix well.
4. Add 50µl of ID-Panel Antibody Identification Cells 1-11 to micro tubes "1-11" of the LISS/Coombs cards.
5. Add 50µl of ID-PanelP Papainised Antibody Identification Cells 1-11 to micro tubes "1-11" of the NaCl/Enzyme cards.
6. Add 50µl of Autocontrol red cell suspension to micro tube "12" of both cards.
7. Add 25µl of test serum or plasma to each micro tube.
8. Add 25ul of ID-Diluent 1 (Bromelin) to micro tube "12" of the Na Cl/Enzyme cards. (Enzyme Autocontrol)
9. Incubate the micro typing card for 15 minutes at 37°C in the ID-Incubator.
10. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.
11. Interpret the results.

INTERPRETATION

Results can only be accurately interpreted if the Autocontrol ("Auto") micro tubes give a negative reaction. A positive reaction may indicate the presence of an autoantibody. The test should be repeated after elution of the autoantibody.
Positive reactions, grade 1-4, in the micro tube indicates the presence of an antibody. The specificity of the antibody can be determined by comparing the reaction pattern obtained with the antigen profile of the Antibody Identification Cells. (See Antibody Identification)
Negative reactions in the micro tubes indicates the absence of an antibody.

COMPLETE COMPATIBILITY TEST

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 2 (Modified LISS)
-ID Diluent 1 (Bromelin)

MICROTYPING CARDS

Complete crossmatch (Profile:- A-B-D-Enz-AHG-AHG) Clon Monoclonal.
Confirmation of donor and recipient ABO/Rh blood groups, confirmation of compatibility between donor red cells and recipient serum using enzyme and indirect antiglobulin test (IAT) test with recipient autocontrol is performed on a single microtyping card. A single microtyping card is required for each donor unit.

SAMPLE

Red Cell Concentrate/Whole blood - Donor and Recipient

Serum or Plasma – Recipient

TEST PROCEDURE (Both Donor and Recipient) Schematic

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient and donor unit name and number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of donor unit and recipient red cells:-
20µl whole blood + 1.0ml ID-Diluent 2
or
10µl red cell concentrate + 1.0ml ID-Diluent 2
Mix well.
4. Add 50µl of donor unit red cell suspension to microtubes "A", "B", "D", "Enz/NaCl" and "Coombs" (microtubes 1, 2, 3, 4 and 5.)
5. Add 50µl of recipient red cell suspension to microtubes "A", "B", "D" and "Auto" (microtubes 1, 2, 3, and 6.)
6. Add 25µl of the recipient serum or plasma to microtubes "Enz/NaCl", "Coombs" and "Auto". (microtubes 4, 5 and 6.)
7a. For a Enzyme phase Crossmatch, add 25µl of ID-Diluent 1 (Bromelin) to microtube "Enz/NaCl" (microtube 4).
or
7b. For a 37°C Saline phase Crossmatch continue to step 8.
8. Incubate the microtyping card for 15 minutes at 37°C in the ID-Incubator.
9. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.
10.Interpret the results.

TEST PROCEDURE (ABO/Rh(D) of Donor Only) Schematic

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient and donor unit name and number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of donor unit and recipient red cells:-
20µl whole blood + 1.0ml ID-Diluent 2
or
10µl red cell concentrate + 1.0ml ID-Diluent 2
Mix well.
4. Add 50µl of donor red cell suspension to micro tubes "A", "B", "D", "Enz/NaCl" and "Coombs" (micro tubes 1, 2, 3, 4 and 5.)
5. Add 50µl of recipient red cell suspension to micro tube "Auto" (micro tube 6)
6. Add 25µl of the recipient serum or plasma to micro tubes "Enz /Na Cl", "Coombs" and "Auto". (micro tubes 4, 5 and 6.)
7a. For a Enzyme phase Crossmatch, add 25µl of ID-Diluent 1 (Bromelin) to micro tube "Enz /Na Cl" (micro tube 4).
or
7b. For a 37°C Saline phase Crossmatch continue to step 8.
8. Incubate the microtyping card for 15 minutes at 37°C in the ID-Incubator.
9. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.
10.Interpret the results.

INTERPRETATION

Compatible Incompatible
ABO/Rh blood group compatibility
ABO/Rh blood group compatibility results can only be accurately interpreted if the "Auto" control gives a negative reaction.

Dual Cell procedure: A single positive reaction, grade 4, or negative reaction in each micro tube "A", "B" and "D" indicates compatibility between the donor and recipient ABO/Rh(D) blood groups.
Different positive reactions, grade 4 and negative reactions occurring together in the same micro tube "A", "B" or "D" indicates the presence of a dual population of red cells and possible incompatibility between the donor and recipient ABO/Rh(D) blood groups.
Double populations of red cells are clearly visible in micro tubes "A" and "B". In micro tube "D", single red cells may be partly caught within a strong agglutination and in the case of a double population, the negative reaction may be weaker than normal. A double population (negative reaction in the presence of a positive reaction) indicates incompatability of the Rhesus D group of donor and recipient.
Donor Only Procedure: The AB/D group check must be confirmed as compatible against the donor pack label and recipients blood group.
Donor red cell/Recipient serum compatibility
Negative reactions in micro tubes "Enz / Na Cl" and "Coombs" indicates that the donor red cells and recipient serum are compatible.
Positive reactions, grade 1-4, in micro tubes "Enz" and/or "Coombs" (micro tubes 4 & 5) indicates an incompatibility between donor red cells and recipient serum. Further investigation using an Antibody Identification Panel by the appropriate technique, will be required to confirm the presence of an antibody.
Positive reactions in the "Auto" control may indicate a auto reacting antibody and must be further investigated.

DIRECT COOMBS (ANTIGLOBULIN) TEST (DAT)

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS-ID Diluent 2 (Modified LISS)

MICROTYPING CARDS

LISS/Coombs (Profile:- 6 IgG/C3d Polyspecific Coombs tests)
or
LISS/Coombs IgG (Profile:- 6 IgG monospecific Coombs Tests)
Each card contains 6 "Antiglobulin" gels for Direct Coombs tests.

SAMPLE

EDTA Red Cell Concentrate or Whole Blood

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient and donor unit name and number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of test red cells:-
10µl red cell concentrate + 1.0ml ID-Diluent 2
or
20µl whole blood + 1.0ml ID-Diluent 2
Mix well.
4. Add 50µl of test red cell suspension to a micro tube.
5. Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.

6. Interpret the results.

INTERPRETATION

Positive reactions, grade ± to 4, in the micro tube indicates a positive DAT. Classification of a positive DAT can be performed using mono specific Coombs anti sera.

Negative reactions in the microtube indicates a negative DAT.

CROSSMATCH (37°C INDIRECT ANTIGLOBULIN TEST)

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 2 (Modified LISS)

MICROTYPING CARDS

LISS/Coombs (Profile:- 6 Coombs tests)

Each card contains 6 "Antiglobulin" gels for Indirect Coombs tests.

SAMPLE

Red Cell Concentrate/Whole Blood - from donor unit

Serum or Plasma - from recipient

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient name and number and each micro tube with the donor unit number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of donor unit red cells:-
   10µl red cell concentrate + 1.0ml ID-Diluent 2
   or
   20µl whole blood + 1.0ml ID-Diluent 2
   Mix well.
4. Add 50µl of each donor unit red cell suspension to the appropriate micro tube.
5. Add 25µl of recipient serum or plasma to the appropriate micro tube.
6. Incubate the micro typing card for 15 minutes at 37°C in the ID-Incubator.
7. Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.
8. Interpret the results.

INTERPRETATION

Positive reactions, grade 1-4, in the micro tube indicates an incompatibility between donor and recipient. Further investigation using an Antibody Identification Panel by the appropriate technique, will be required to confirm the presence of an antibody.

Negative reactions in the micro tube indicates that the donor and recipient are compatible.

Compatibility NaCl/Enzyme

CROSSMATCH (37°C ENZYME or Na Cl TEST)

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor

-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 1 (Bromelin Solution) for Enzyme test
-ID Diluent 2 (Modified LISS) for Na Cl test

MICROTYPING CARDS

NaCl/Enzyme Test (Profile:- 6 enzyme tests)
Each card contains 6 "Neutral" gels for enzyme or Na Cl tests.

SAMPLE

Red Cell Concentrate/Whole Blood - from donor unit Serum or Plasma - from recipient

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient name and number and each micro tube with the donor unit number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of donor unit red cells:-
   10µl red cell concentrate + 1.0ml ID-Diluent 2 (Modified LISS)
   or
   20µl whole blood + 1.0ml ID-Diluent 2 (Modified LISS)
   Mix well.
4. Add 50µl of each donor unit red cell suspension to the appropriate micro tube.
5. Add 25µl of recipient serum or plasma to the appropriate micro tube.

6a. For a Saline crossmatch continue to step 7.
6b. For a One Stage Enzyme Crossmatch add 25µl of ID-Diluent 1 (Bromelin) to each micro tube.

7. Incubate the micro typing card for 15 minutes at 37°C in the ID-Incubator.
8. Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.
9. Interpret the results.

INTERPRETATION

Positive reactions, grade 1-4, in the micro tube indicates an incompatibility between donor and recipient. Further investigation using an Antibody Identification Panel by the appropriate technique, will be required to confirm the presence of an antibody.
Negative reactions in the micro tube indicates that the donor red cells are compatible with the recipients plasma.

DETERMINATION OF BLOOD GROUP ANTIGENS:-
D, Lea, Leb, P1, K, k, Jka, Jkb Kpa, Kpb, Lua and Lub.

EQUIPMENT/MATERIALS

-ID Centrifuge
-ID Incubator 37°C
-ID Working Table
-ID Pipetor EP-3/FP-2/FP-3
-ID Dispensor
-ID Suspension Tubes
-ID Disposable Tips

REAGENTS

-ID Diluent 1 (Bromelin Solution)

ID-MICROTYPING CARDS

Anti-D, Anti-Lea, Anti-Leb, Anti-P1, Anti-K, Anti-k, Anti-Jka, Anti-Jkb, Anti-Kpa, Anti-Kpb, Anti-Lua or Anti-Lub.

Select the appropriate micro typing card for the antigen to be determined.
Each card allows up to 6 single tests for determination of the antigen.

SAMPLE

Red cell concentrate/Whole blood

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient name and number. Remove the aluminum foil.
3. Prepare a 5% suspension of test red cells in ID-Diluent 1 as follows
   25µl red cell concentrate + 0.5ml ID-Diluent 1
   or
   50µl whole blood + 0.5ml ID-Diluent 1
4. Mix well, incubate at room temperature for 10 minutes.
5. Add 10µl of test red cell suspension to the appropriate micro tube.
6. Centrifuge the microtyping card for 10 minutes in the ID-Centrifuge.
7. Interpret the results.

INTERPRETATION

Positive reactions in the micro tube indicates the presence of the corresponding antigen.
Positive reactions, of grade 1-3 in micro tube "D", indicates the presence of the Du or weak D antigen. Further investigation will be required to confirm this.
Negative reactions in the micro tube indicates the absence of the corresponding antigen.

DETERMINATION OF BLOOD GROUP ANTIGENS:- M or N

REAGENTS

-ID Diluent 2 (Modified LISS)
MICROTYPING CARDS
Anti-M or Anti-N
Select the appropriate micro typing card for the antigen to be determined.
Each card allows up to 6 single tests for determination of the antigen.

SAMPLE

Red cell concentrate/Whole blood

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient name and number. Remove the aluminum foil.
3. Prepare a 5% suspension of test red cells in ID-Diluent 2:-
   25µl red cell concentrate + 0.5ml ID-Diluent 2
   or
   50µl whole blood + 0.5ml ID-Diluent 2
   Mix well.
4. Add 10µl of test red cell suspension to the appropriate micro tube.
5. Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.
6. Interpret the results.

INTERPRETATION

Positive reactions in the micro tube indicates the presence of the corresponding antigen.
Negative reactions in the microtube indicates the absence of the corresponding antigen.

DETERMINATION OF BLOOD GROUP ANTIGENS:- S, s, Fya or Fyb

REAGENTS

-ID Diluent 2 (Modified LISS)
-ID Anti-S, Anti-s, Anti-Fya or Anti-Fyb
MICROTYPING CARDS

Antigen Test cards for S, s, Fya or Fyb
Select the appropriate micro typing card for the antigen to be determined.
Each card allows up to 6 single tests for determination of the antigen.

SAMPLE

Red cell concentrate/Whole blood

TEST PROCEDURE

1. Allow all reagents to reach room temperature before use.
2. Identify the ID-micro typing card with the patient name and number. Remove the aluminum foil.
3. Prepare a 0.8% suspension of test red cells in ID-Diluent 2:-
10µl red cell concentrate + 1.0ml ID-Diluent 2
or
20µl whole blood + 1.0ml ID-Diluent 2
Mix well.
4. Add 50µl of test red cell suspension to the appropriate micro tube.
5. Add 50µl of the appropriate ID anti sera to each micro tube.
6. Incubate the micro typing card for 15 minutes at 37°C in the ID-Incubator.
7. Centrifuge the micro typing card for 10 minutes in the ID-Centrifuge.
8. Interpret the results.

INTERPRETATION

Positive reactions in the micro tube indicates the presence of the corresponding antigen.
Negative reactions in the micro tube indicates the absence of the corresponding antigen.
Automated blood Grouping by Micro-typing System
Sample collection:
1.Collect 4.5 ml of venous blood in a VACUTAINER EDTA tube with bar- code label.
2.Centrifuge the sample at 3500 RPM for 10 min

Operation of instrument:

1.Keep the required micro-plates, DILUENT bottles, cell suspension A & B from the refrigerator for 30 min before starting the test
2.Switch on the machine & the computer
3.Click on ID SAMPLER II F Password- sharjah- operator ID

Take out the carousel:

1. **Select Accessories menu, then the washing command to open the corresponding window**
   - **Click on fast wash N a C l**
   - **Click on normal wash N a C l**
   - **Click on fast wash H2o**
   - **Click on normal wash**
2. **Priming procedure**
3. **Connect the DILUENT bottle .**
4. **Perform priming with DILUENT 1.by bringing the needle down. Press the diluent#1 to reject first 3-4 ml from the DILUENT**
5. **Click the needle up**
6. **Arrange sample tubes so that bar code facing to the operator**
7. **Place the cell suspension tubes in corresponding positions.**
8. **Place the cell suspension tray according to no. of sample**
9. **Place the carousel in position**
10. **Close the carousel lid.**
11. **Open the table protection lid**

12. Place the required micro plates
13. Close the protection lid
14. Switch the ID sampler 11 F ON
15. In the dispense program selection window, select the following _options:
- Single - patients:1
16. Click on GO AHEAD button
17. Select the test A-B-AB-D(V1-)-D(V1+)-CTL/A1B-1
18. Click on GO AHEAD BUTTON
19. Click YES to continue
20. In dispense management window appears with a message prompting to install suspension trays
21. Click on start button
22. Click run dispense button
23. After end of dispense click continue button
24. Wait for 10-15 minutes
25. Take out the plate and centrifuge it

Reading the micro plate.

1.click I HMP readerVER3.51 button
2.Click IIF
3.Insert the micro plate-click OK
4.In micro plate agitation ? Menu click YES.
5.After agitation click print
6.Take the print out

Daily cleaning:

1.Click daily cleaning menu
2.Remove the transparent tray
3.Clean the needle by isopropyl alcohol
4.Shut down the computer and switch off the machine.

CHAPTER TWO: OTHER SEROLOGICAL TESTS

[I] ACID ELUTION TEST

Principle

Elution using the commercial Elu-kit is a procedure for recovery of antibody from coated RBC's.

1. RBC's coated with antibody are first washed in a buffered wash solution to remove all unbound protein.

2.The washed RBC's with attached antibodies are suspended in a low pH glycine buffer.

3. After centrifugation, the supernatant containing any dissociated antibody is separated from red cells. By adding tris buffer we achieve normal ionic strength and pH.

Policy

1.An acid elutin will be performed on patient RBC's samples with positive DAT due to IgG.

a. If 6 months have passed since last elution.

b. In patients with auto immune disease, in first presentation and every 6 months.

c. If DAT strength increase

d. If patient transfused in the past three months.

Specimen

5 ml (minimum 3m1) whole blood in a lavender top tube

Materials

1. **Centrifuge, timer.**
2. **Normal saline, distilled water.**
3. **Disposable Pasteur pipettes and 15 x 75 mm glass test tube.**
4. **Elu -kit:**

a)Concentrated wash solution preparation: one volume of concentrated wash solution + nine volumes of distilled water. Mix and store at 2-8°C in stoppered glass or plastic container good until turbid.

b) Eluting solution: Low pH glycine buffer.

c) Buffering solution: Tris-aminomethane solution to neutralize acidity of eluting solution.

Safety

1. **All blood and its products must be treated as potentially infectious.**
2. **Do not pick up broken glass with fingers.**

Quality control

1. **Test last wash against reagent screening cells to exclude any unbound antibody remaining as a result of inadequate washing.**
2. **If antibody detected in the last wash, repeat the elution procedure after more through washing of the cells.**

Procedure

1. Preparing the eluate:

a) Separate excess plasma or serum by centrifugation.

b) Wash the RBC's four times with saline, and decant the supernatant each time.

c) Reserve a small aliquot of last wash solution for Q.C.

d) Place one ml (20 drops) of washed RBC's in a clean 12 x 75mm test tube. Add one ml (20 drops) of eluting solution and mix by inversion four times, then centrifuge for one minute.

e)Transfer supernatant eluate to a clean test tube. Add one ml of buffering solution to eluate and mix and add additional buffer one drop at a time with mixing until it turn from light blue to blue.

f) Centrifuge to separate any cellular debris and transfer eluate to a clean properly labeled test tube.

g) The eluate is now ready for testing to detect or identify antibodies.

Policy

1. A full reagent red cell pane! Is performed for all patients who have a positive antibody screen.
2. For previously identified antibodies serum is tested against selected antigen negative cells to these antibodies to exclude any additional clinically significant antibodies.
3. An autocontrol is run with all serum panels.

Specimen

1. Serum and cells from l0 ml (min 7m1) clotted whole blood.
2. Eluate

Materials

1. 12mm x 75mm test tubes, Pasteur pipettes.
2. 0.9% saline, LISS.
3. AHG, IgG sensitized red cells.
4. Commercially prepared red cell panel and corresponding panel sheet.

Safety

1. All blood and its products must be treated as potentially infectious
2. Do not pick up broken glass with fingers.

Quality control

1. Reagent Q.C
2. Procedure

I. Select an indate panel and its sheet with the same lot number

II. Fill in all information on the panel sheet.

b) Date of testing
c) Tech ID #
d) Patient name and medical record number
e) Phases of testing, additive and conditions:
IS = immediate spin phase
37 = 37°C incubation phase
AHG = Antihuman globulin phase
CCC = coombs control cells or (check cells).

III. New Antibody

1. Select set of cell panel of minimum 11 cell panel.
2. Label reagent red cell tubes with patient ID, and the last tube "AC" (Auto control)
3. Place two drops of patient's serum in each of the tubes.
4. Mix cell panel, add one drop of reagent red cells to the labeled tube, and mix well with serum.
5. Add one drop of patient 2.5% RBC's cell suspension to "AC" tube, and mix well with serum.
6. LS. and examine for hemolysis, resuspend and examine for agglutination and record the result in LS. column.
7. Add two drops of LISS to each tube and mix (but not with eluate or enzyme panel)
8. Incubate the tubes of 30minuts in 37°C water-bath, then centrifuge, examine for hemolysis and resuspend and examine for agglutination and record results in 37°C column on panel sheet.
9. Wash all tubes 4 times with saline, after last wash decant completely
10. Add 2 drops of AHG to all tubes, and mix, then centrifuge, resuspend, examine for agglutination and record the results in AHG column or panel sheet.

11. To all negative tests add one drop of C.C.C centrifuge, examine for agglutination and record results in C.C. column on panel sheet. If no agglutination repeat the test.

Interpretation

1. Positive result is agglutination or hemolysis in LS., or 37°C phases or agglutination in AHG phase which indicate the presence of antibodies.
2. Negative result is absence of agglutination or hemolysis which indicates absence of antibodies.
3. If a pattern of positive and negative reaction is observed, antibodies can be eliminated to the antigens present on the non reactive cells.
4. If only one antigen is remaining:
   a) If the match is observed and there is minimum of 3 positive and 3 negative cells, the antibody has been identified, otherwise select additional set of panel.
   b) Patient should be negative for the antigen corresponds to the identified antibody, unless the patient has been transfused in the past 3 months.
5. Reaction phase:
   a) LS. phase indicate the presence of cold Ab as Anti M.; N; P or Lewis
   b) 37°C and AHG phases indicate the presence of warm Ab as Anti S, s; Rh; kell; duffy or kidd.
6. Auto Control:
   a) If the serum reacts with both the panel cells and autologous cells, this suggests the presence of autoantibody but does not exclude an alloantibody.
   b) If auto control is positive in the AHG phase, perform DAT and if positive perform eluate.
7. Dosage: Anti M, Anti c and Anti JK^a react only with cells homozygous for same antigens (show dosage).
8. Antibody to high frequency antigen: reactivity with all cells except auto control cells may indicate reactivity to high frequency antigen.
9. Panagglutination
   a) Reactivity with all cells including autocontrol cells, indicates non specific panagglutination.
   b) Perform autoadsorption or wash panel cells to rule out alloantibodies or reactivity to preservative solution
10. Phase of reactivity
    a) Each antibody has optimum phase of reactivity, e.g. anti-Fya react well in AHG phase.
    b) Do not consider the antibody as the first choice if it reacts in another phase different than its optimum one.

Extended workup

1. Perform a selected cell panel by choosing additional cells to include cells both positive (homozygous) and negative for each of those antigens not excluded.
2. If the pattern is not specific to any antibody, there may be combination of antibodies present.
   a) Antigen types the patients, to rule out antibodies against its own antigens.
   b) Use Ficin panel, to detect antibodies masked by Anti-Fya or Antiy Fyb if one of them is present.
   c) Neutralize anti-P, anti-Lea, anti-Le-b or Anti-Sda with group specific substances if one of them is present to remove one or more antibodies to identify others.

d) Perform adsorption or adsorption elution to separate specific antibodies.

3. Weak reactions can be enhanced by:
   a) Increase incubation time
   b) Increase serum to cell ration
   c) Perform dry button technique by washing each panel cell and decant to dry button, then add 2 drops of serum to dry button of cells.
   d) Test serum against Ficin panel
   e) Use PEG of NHance as enhancement reagents.
4. Perform prewartned panel or autologous adsorption to avoid antibodies react in room temperature (non-clinically significant) which mask the presence of clinically significant antibodies.
5. If anti-M, N, or P1 is identified at AHG phase, perform pre-warm testing:
   a) If disappear, report it as non clinically significant.
   b) If remain, report it as clinically significant.

Previously identified antibody

1. Select panel which are antigen negative to the previously identified antibody but positive for all remaining clinically significant antigens.
2. Perform auto control
3. Previously identified Ab need not be re identified.

Exclusions using polyethylene glycol (PEG)

PEG can be used as an alternative to LISS but not use both to perform exclusion on the same panel, expect:

1. Rh exclusions in Rh negative (d-) individual using PEG to rule out anti C, anti E and antiD.
2. If homozygous cell is not available using LISS, test 2 heterozygous cells with PEG, to rule out certain antibody.
3. If in the above two situations, an Ab detected with PEG, test the same cells with LISS as a comparison. If same Ab (ies) is non reactive with LISS, the other exclusions must be repeated using PEG.

Reporting results

1. Complete immunohematology report form with the type of identified antibody, together with all screening cell antigrams, panel sheet and antigen typing form.
2. For patients with previously identified antibodies, report the result as no additional antibodies, if the panel of selected cells is negative.

Procedure notes

1. Grade reactions and record on panel sheet
2. Use two heterozygous cells if homozygous cells is not available, to perform exclusions.
3. Clinically significant red cell antibody is one that associated with hemolytic transfusion reaction or HDN, as Rh antibodies, Anti-S, -S, -U, Lub, K, K-, Kpa, Kpb, Jsa, Jsb, Fya, Fyb, Jka, JKb, Dia, Dib, Doa, Dob, Coa and occasionally anti-M or Yta.
4. If new sample is required, perform an ABO/Rh, and repeat one more positive cell to ensure that it has the same result.
5. If a new antibody is detected in obstetric patient, perform and report antibody titration without waiting for titration order.

[III] DIRECT ANTIGLOBULIN TEST (DAT)

Principle:

The DAT detects antibodies bound to RBC's in vivo and help in diagnosis of

a) Hemolytic diseases of newborns (HDN)
b) Autoimmune hemolytic anemia.
c) Transfusion reaction.

d) Other clinical conditions.

Policy:

According to AABB standards DAT will be performed on:

a) All cord blood samples

b) All Transfusion reaction investigation

c) Blood samples which show positive auto control

Specimen:

Whole blood from patient in lavender top (EDTA) tube, 5 ml.

Materials:

a) 12mm x 75mm disposable glass tubes

b) Normal saline, polyspecific AHG

c) Monospecific (IgG, C3d) AHG

d) Automatic cell washer or serofuge, microscope

e) IgG sensitized cells (C.C.C,)

f) Complement Coombs Control Cells

Safety:

a) All blood and its products must be treated as if potentially infectious. Follow laboratory safety manual.

b) Pick up broken glass with dustpan or other scooping implement, but not with fingers and dispose it in sharps disposal container.

Quality control:

Do Q.C. for polyspecific AHG, and monospecific (IgG, C3d) AHG with known positive cells.

Procedure:

1) Make a 3-5% suspension of patient cells in saline.

2) Add one drop of cell suspension to ech of three tubes labeled for polyspecific AHG, IgG and C3d.

3) Wash the cells four times.

4) Polyspecific DAT:

a) To the dry cell button, add 2 drops of polyspecific AHG to the appropriately labeled tube.

b) Mix well, centrifuge, resuspend and examine macroscopically and microscopically for agglutination and record results.

c) If negative, incubate at room temperature for 10 minutes, centrifuge, resuspend and read microscopically and record results.

d) To each negative test add one drop of C.C.C. and centrifuge, resuspend and examine microscopically and if negative repeat the test.

e) If polyspecific AHG test is positive, perform monospecific AHG test.

5) Monospecific DAT:

a) Add two drops of Anti - IgG monospecific AHG into the appropriately labeled tube. Add two drops of Anti-C3d monospecific AHG into the appropriately labeled tube.

b) Mix well, centrifuge, resuspend and examine macroscopically and microscopically for agglutination.

c) If the DAT is positive with Anti-IgG, Anti-0d and polyspeicific AHG, you must perform 6% albumin control to rule out the presence of spontaneous agglutination and record result.

d) If the anti - C3 d is negative, incubate 10 minutes at room temperature, spin, read microscopically and record result.

e) To any negative IgG tests add one drop of C.C.C. and centrifuge, resuspend and examine and if negative repeat the test.
f)To any negative C3d tests add one drop of complement control cells, incubate at RT for 5 minutes, centrifuge, examine for agglutination and if negative repeat the test.

Interpretation:

1) Presence or absence of agglutination macroscopically or microscopically referred to as positive or negative results respectively.

2) A patient who develops a positive DAT after blood transfusion may be undergoing a delayed hemolytic transfusion reaction and the treating doctor should be notified.

3) A newborn who has positive DAT, may be undergoing a hemolytic disease of a newborn and the treating doctor should be notified.

Procedure notes:

1.Some patients with autoimmune disease process have routine DAT to monitor treatment.

2. Make sure that all samples for DAT has ABO/RH blood group or other wise do it.

[IV] ACID-ELUTION STAIN (MODIFIED KLEIHAUER-BETKE)

PRINCIPLE:

Fetal cells resist acid elution and retain their haemoglobin, while adult (maternal) cells eluted and lose its haemoglobin. Fetomaternal hemorrhage can be calculated from the percentage of fetal red cells in the maternal blood film.

SPECIMEN:

Maternal whole blood sample (clot).

REAGENTS:

A. Commercially available kit.

B. In-House preparations:

1. Solution A. (O.1M of citric acid) 21gm in 1 liter distilled water. (store in refrigerator).
2. Solution B.(0.2M of sodium phosphate) 53.6 in 1 liter distilled water. (store in refrigerator).
3. Mcilvaines buffer, pH 3.2
4. Prepare fresh buffer mixture for each test from:
   Mcllvaines buffer + 75m1 sol. A. + 21m1 So1.B in room temperature or 37°C.
5. Eerythrosin B, 0.5% in water
6. Harris hematoxylin (filtered)
7. 80% ethyl alcohol
8. Positive control specimen: 10 parts adult blood + one part ABO compatible cord (fetal) blood (anticoagulated).
9. Negative control specimen: anticoagulated adult blood.

PROCEDURE:

1. Prepare very thin blood smears (blood diluted with equal volume of saline).
2. Air dry
3. Fix smears in 80% ethyl alcohol for 5 minutes.
4. Wash smears with distilled water
5. Immerse smears in MCllvaines buffer mixture for I 1 minutes in RT, or 5 minute in 37°C. 6. Wash in distilled water
7. immerse in erythrosin B for 5 minutes.
8. Wash in distilled water

9. Immerse in Harris hematoxylin for 5 minutes.
10. Wash with running tap water for 1 minute.
11. Examine dry using 40X magnification, count 2000 red cells and record number of fetal red cells.
12. Calculate % of fetal red cells in the total counted.

INTERPRETATION:

1. Fetal cells are bright pink and refractile, while adult cells appear pale ghosts.
2. Volume in ml. Fetomaternal hemorrhge = % of fetal red cells X 50.

NOTE:

l. Rh IG dose is determined according to volume of hemorrhage in ml.
2.1 vial of 300gg (1500 IU) is needed for each 15m1 fetal red cells or 30m1 fetal whole blood.

[V] QUALITY CONTROL OF BLOOD BANK SEROLOGY REAGENTS.

PRINCIPLE:

1- Testing reagents, procedures and equipments against known samples is an accepted method of daily quality assurance in blood bank.
2- If expected results are observed, this means the quality of reagents, procedures and equipments are good enough and test results may be reported are good enough and test results may be reported.
3- If unexpected results are observed, the problem may be due to improper test procedure, faulty equipments or reagents.

POLICY:

According to AABB standard 5.1.3, if unexpected results are observed in the daily quality control, the problem must be resolved before test results are reported.

MATERIALS:

1- Reagents Anti-Al, Anti-B, Anti-D and Rh-Control.
2- Reagents A1,B, weak D cells.
3- Screening cells.
4- Coombs control cells.
5- Anti-Human Globulin (AHG).
6- Water bath, Centrifuge, Cell washer, and Timer.
7- 12 x 75mm test tubes, Disposable transfer pipettes.
8- Isotonic saline solution.
9- Other Antisera as indicated
10- QC worksheet for blood bank serology reagents.

SAFETY:

All blood and blood products and reagents should be treated as potentially infectious.

PROCEDURES:

1- Check water bath temperature to ensure it is 37°c ± 2°c., and record it on the Q.C. worksheet.
2- Check all reagents for appearance (turbidity, hemolysis.) and record observation.
3- Check and record manufacturer, lot number and expiration date of reagents.
4- ABO grouping and Rh typing reagents:
   a) Set 6 Test tube in a rack.
   b) Put one drop from routine reagents, plus one or two drops from QC. Reagents as follows.

ROUTINE REAGENT	QC.REAGENT
Anti-A	A1 D-ve, cell
Anti-B	B D-ve, cell.
Anti-D	O D+ve cell.
Rh- control	O D+ve cell.
A1-cells	Anti A
B-cells	Anti B

c) Mix and centrifuge.

d) Resuspend and read macroscopically, and record the results in the Q.C worksheet.

5- Antibody screen reagents:

a) Label one test tube for Ab. Screening as QC for each screening cell Si, SII, SIII against two known antibody sera.

b) Add 2 drops of known Q.C reagent antibody, to 1 drop from each corresponding screening cells in the labeled Ab. Screening test tubes.

c) Centrifuge, resuspend test tubes and read, in room temperature, and record.

d) Incubate test tubes in 37°c for 30 minutes centrifuge, resuspend, read and record results.

e) Wash 3 time, add AHG (2drops), spin 15 seconds, resuspend, read and record results

f)Add coomb's control cells to the negative test result, spin 15 seconds, resuspend, read and record result in Q.C worksheet.

6- Weak D Test (Du Test.):

a) Set up two tubes for the weak D cells (Du positive) labeling one tube Rh. Control QC and the other tube WK D QC.

b) Add one drop of weak D cells to each tube.

c) Add one drop of Anti-D reagent to tube labeled (WKDQC).

d) Add one drop of Rh control reagent to tube labeled (Rh cont Q.C).

e) Mix, incubate at 37°c for 30 minutes, centrifuge, resuspend, read and record.

f)Wash the tubes 3 times, add two drops of AHG, Centrifuge, resuspend, read, and record the results on the QC worksheet.

g) Add one-drop coombs control cells to all negatives, centrifuge, read and record the result.

PROCEDURE NOTES:

1- Record results on daily Quality control of serology reagents worksheet.

2- Check all reagents for appearance and performance and mark either satisfactory or unsatisfactory.

3-Put notes on QC worksheet, if there are any changes in lot number or Expiration data.

4-Tests are initialed by those performing the test.

5-We should clearly mark outdated reagents in use, and QC results should be acceptable.

DAILY QUALITY CONTROL OF BLOOD BANKS SEROLOGY REAGENTS WORKSHEET

Date: / /

Reagents Check		Anti A	Anti B	Anti AB	Anti D	Rh Control	AHG	Screen Cells	A1 Cells	B Cells	C.C.C	O Cells	A_2 Cells		
Water bath temp.															
Manufacturer															
Lot Number															
Expiration date															
Appearance															
Cells Antisera		A1 Cells	A_2 Cells	B Cells	ORh +ve Cells	WeakD Cells	SI	SII	SIII	C.C.C.			Satis-factory	Unsatisf actory	BBTech. Initial
Anti-A with															
Anti-B with Anti AB with															
Anti D with Rh-Control with															
AHG with															
Anti ( ) With	I.S.														
	37C														
	AHG														
Anti ( ) With	I.S. 37°C														
	AHG														

PART THREE: CLINICAL CONSIDERATIONS IN TRANSFUSION PRACTICE

CHAPTER ONE: GUIDELINE OF BLOOD TRANSFUSION PRACTICE

General Points of Blood and Blood Components

Blood and Blood Components Stock Monitoring:

- Daily stock for the available blood & components units is prepared by the Technologist in the processing section and is checked by the section incharge and approved by the director of BTS.

- The stock sheet will show : tested , untested , and issued units. Units that are for repeat testing or are discarded after repeated positive or repeated doubtful results are also shown.

Issuing of Blood and Blood Components:

1- There must be a clear and signed request for the blood component sent from the hospital .

2- Look to the blood record and the blood supplying book and/or your computer system to see the quantity available from the component requested .

3- Oldest blood must be issued first (according to the expiratory Date) . Fresh Blood (aged one week and less) is recommended for infants Thalassemic patients and some patients with electrolyte disturbances .

4- Check the unit number , blood group , expiratory date and signs of deterioration of the unit before issuing the blood : inspect the Red Blood cells for abnormal color or other abnormal appearance and the platelets concentrate should be inspected for presence of grossly visible aggregates while FFP. and Cryoprecipitate should be inspected for evidence of thawing and re freezing .

5- All units issued must be registered in the blood supplying book with the date of issuing the unit , the hospital name , blood group and unit number .

6- All units must be negative for all screening markers before issuing .

7- Always keep O negative blood units for emergencies .

8- Be sure that the supplied blood component will be transported in a proper way (using ice box ,do not allow direct contact of the component with the ice).

Protocol For Issuing Blood & It's Components From the Central Blood Transfusion Center to Governmental & Private Hospitals

1- It is the duty of the central blood banks to have enough stock of blood units and it's components , from different blood groups to cover the needs of the hospitals.

2- Governmental hospitals has the priority in having their full requirements from the blood and it's components.

3- Distance hospitals from the central BTS., has the priority in having enough stock from the different blood groups that they can cover their needs, especially in cases of emergency, where blood and components should be available in considerable amount in their blood banks.

4- The Central Blood Transfusion Services staff who is responsible for issuing should :

a. Issue the full quantity of blood and it's components if requested by governmental hospital and if the requested quantity is available.

b. In case the requested quantity is not available at that time in the central BTS., the staff in the central BTS. should look for the required units from other blood banks and the doctor in the central BTS. should be contact the blood bank who is requesting the blood or it's components to check the urgency of the request in order to arrange the requested units from near by blood banks.

c. Issuing of blood and it's components to private hospitals will be done routinely, unless the quantity requested is very high or the stock is low, then the doctor in the central BTS. should be consulted in order to control the stock.

d. Once the stock for blood units reaches 15 units for any positive blood group or 2 units for any negative blood group, then issuing of blood units to private hospitals to be postponeded and BTS. doctor to be consulted, in order to cover the emergencies in the governmental hospitals.

e. Once the available stock of tested blood units available in the CBTS. is reduced below the minimal recommended number, the doctor incharg in CBTS. should be informed to arrange things with the Director of BTS. without delay.

5- The ministry of health is considering the private sector as a part of the medical services in the country and that is why the different blood component is issued to the private sector in the cost price but they have to pay to M.O.H. immediately once they receive the units.

6- The price of different blood components has been decided by the directorate of medical laboratories and blood transfusion services after having different studies on the real coast of the different blood components.

7- It is important to clarify that any unit of blood or it's component dispatched to the private hospitals cannot be returned back at all for any reason. This is to avoid returning blood units which might be distorted due to bad storage or any other reason in the private sector. And to avoid these problems , returning blood units and it's components is not accepted at all even if it is for replacing blood group.

8- It is not permitted for any private hospital to put additional amount of money on the price of the blood unit and its component requested from Ministry of Health, so the same amount of money must be taken from the patient for that unit by the private hospital. The private hospital has the right to in charge the patient for the X-matching test performed according to value decided by the authorized sector in Ministry of Health.

1. WHOLE BLOOD (WB)

Introduction: Limitation of uses:

1. Whole blood should be strictly avoided in the availability of PRBC's and only for the very limited indications.

2.Whole blood should be separated into its components to benefit from them for each particular indication rather than overloading patients with unnecessary volume and to avoid sensitization to different elements of blood components.

Description:

1. Very low F. V and F. VIII.
2. No platelets.
3. Maintain FI,II,VII,IX.
4. 02. Carrying Capacity.
5. Blood volume expansion capacity.

Indications:

1. Patients of active bleeding and loss of >30% of blood volume. (Massive bleeding Ten blood units)
2. Neonatal Exchange Transfusion
   a. Fresh blood
   b. Compatible blood group

Contraindication: Chronic Anemia States.

Dose:

1. One unit of WB increases Hb by lgm/dl. And HCT by 3%
2. Pediatric: 8m1/kg. Increase Hb 1 gm/dl.

Administration:

l. Through 150-280 micron filter administration set.
2. Must complete within 4 hours.

2. FRESH BLOOD

Description:

1. Less than 7 days old.
2. Maintain Coagulation Factors and 2,3, DPG enzyme level.
3. Less K, uric acid, citrate, phosphates and lactate.

Indications:

1. Neonatal Exchange Transfusion.
2. Infants with Complex Cardiac Surgery (48 hours old blood)

3. PACKED RED BLOOD CELLS (PRBCs)

Indications:

I. In acute blod loss: (100% blood volume = 5000 ml of blood).

A. Amount of blood loss: Transfuse if blood loss is:

1. 15% with severe cardiac or respiratory disease.
2. From 15%-30% with preexisting anemia or with continous bleeding.
3. > 30% blood loss.

B. Hemoglobin concentration

1. If Hb < 7 gm/dl
2. If Hb < 8 gm/dl in elderly with cardiovascular or respiratory disease.

Maximum Blood Usage Table

Procedure	Blood
I- General Surgery	
1. Breast biopsy.	T / S.
2. Colon resection.	2
3. Laparotomy exploration	T / S.
4. Gasterectomy.	2
5. Mastectomy, radical.	T / S.
6. Pancreatectomy	4
7. Splenectomy	2
8. Thyroidectomy	2.
9. Parathyroidectomy	T/S
10. Hasab operation	4
11. Hepatobiliary	2
II- Vascular:	
1. Aortic bypass graft	4
2. Endarterectomy	T / S.
3. Femoral-popliteal bypass graft.	2
III- OB-GYN:	
1. Abdominal perineal repair or D.&.C.	T / S
2. Emergency or Elective C/S	2
3. Autepartum or post-partum hemorrhage	4
4. missd abortion	2
5. rupture uterus	4
6. placenta previa	T / S
7. myomectomy	2
8. Simple abdominal or laparoscopic hysterectomy	2
9. Radical hysterectomy	4
10. Ectopic pregnancy	2
11. Cystectomy	2
IV- Urology	
1. Trans urethral U.B. resection or prostatectomy	1
2. Radical Nephrectomy	3
3. Radical perineal prostatectomy	2
4. Renal Trans plant	2
5. Radical cystectomy	3
V. ENT:	
Laryngectomy	2
VI. Thoracic surgery:	
I. Lung biopsy	T/S
2. Lobectomy or bilobectory	2
3. Thoracotomy	2
4. Thoracotomy with decortication	4
5. Pneumonectomy	4
6. Rigid Thoracoscopy	T/S
7. Oesophagectomy	4
8. Pericardiectomy	2

9. Mediastinoscopy	T/ S
VII. Cardiac surgery:	
l. Aneurism resection	6
2. Coronary bypass (Redo)	4
3. Coronary bypass (primary)	2
VIII. Plastic surgery:	
1. Hemangioma	2
2. Cleft palate	T/S
3. Pharyngioplasty	T/S
4. Rhinoplasty and septoplasty	1
5. Abdominoplasty	2
6. Mamoplasty	2
7. Lipo suction	1-2
8. Bed sores	1-2
9. Burn	1-4
10. Head and neck	1-2
IX. Neurosurgery:	
1. Craniectomy:	
a. Extra or subdural Hematoma	2
b. Tumour excision	2
2. Lumbar dissectomy	1
3. Cervical dissectomy	1
4. Spinal tumour exicision	2
X. Orthopedic surgery:	
l. Arthroscopoy, laminectomy or knee replacements	T/S
2. Cervical spine fusion and fixation	2
3. Thoraco lumbar spine fusion and fixation	2-4
4. Shoulder,, Humerus or elbow surgery	2
5. Total Hip replacement	3-6
6. Austin moore	1
7. DHS	2-4
8. Femur shaft (plate or nail)	2-4
9. Tibia (plate or nail)	2
10. Debridment	2
11. Peodiatric CDH or Femur plating	1
XI. Dental Surgery:	
1. Reduction, fixation, biopsy, or Cyst	T/S
2. Coronidectomy	1
3. Condylectomy	2
4. Osteotomy	2
5. Genioplasty	1
6. Tumor Resection with reconstruction	4

4. WASHED RBC'S

Indications:

1. Prevent recurrent or severe allergic reactions (1 gA)
2. Neonatal or intrauterine tran sfusion.

5. PLATELET CONCENTRATES. (P.C.)

Description:

1. Volume:

a) Random: 50-70 ml.

b) Apheresis: 200-350 ml.

2. Platelet content:

a. Random: 5.5x1010 platelet. / unit.

b. Apheresis: $3x10^{11}$ platelet / unit.

3. PH: > 6.2.

4. Storage: 20-24 C for 5 days.

Indications:

1. Chronic stable thrombocytopenia (No bleeding) with platelet count <10,000/µl)

2. Thrombocytopenia with active bleeding and platelet count <50,000/µl.

3. Patients with bone marrow failure due to disease, chemotherapy or irradiation with platelet count <20,000/µl.

4. Prophylactic transfusion:

a. Emergency surgery with platelet count <50,000/µl

b. Brain or eye surgery with platelet count <I00,000/µl.

c. Cardiopulmonary bypass with:

Platelet count <50,000/µl

Excessive post operative bleeding.

d. Liver transplant surgery use thromboelastograph (TEG) to guide the need for platelet transfusion.

5. Acute leukemia patients without fever or bleeding with platelet count <10,000/µl.

6. Acute promyelocytic leukemia with platelet count < 20,000/µl.

7. Hematopoietic stem cell transplantation with platelet count <10,000/µl.

8. NICU infants with platelet count <I00,000/µl

9. Congenital or acquired platelet functional disorders.

a. Alternative measures:

- **Withdraw anti platelet drugs**
- **Correct the cause of dysfunction**
- **Use erythropoietin to increase HCt > 30% in patient with renal failure.**
- **Use desmopressin in patients with storage pool disease.**

b. Platelets concentrate transfusion if above fails.

- **Should be HLA matched**
- **Preoperative in patients with glanzmanns thrombasthenia.**

10. Massive blood transfusion:

a) In patients with acute bleeding and platelet count < 50,000/µl.

b) In patients with multiple trauma or CNS surgery with platelet count < 100,000/µl.

11. Disseminated intravascular coagulopathy (DIC):

a. First treat the cause of DIC.

b. No platelet in chronic DIC without bleeding

c. Platelet concentrate can be given to DIC patients with bleeding and platelet count < 50,000/µl.

12. Immune Thrombocytopenias Transfuse platelet concentrates in:

a. Autoimmune thrombocytopenia with life threatening bleeding with support of methylprednisone and IVIG.

b. Neonatal alloimmune thrombocytopenia (NAIT) Transfuse compatible platelet as soon as

possible, with HPA-la negative and HPA 5b negative.

c. Post transfusion purpura with bleeding with support of IVIG.

Contraindications:

1. TTP, ITP, Post-transfusion purpura without bleeding or heparin- induced thrombocytopenia.
2. Untreated DIC, thrombocytopenia, caused by Septicemia or hyperspleenism, unless there is active bleeding and must be under clinical monitoring.

Dose:

1. One unit random platelet will increase platelet count by 5000-10,000 / µl.
2. One platelet pheresis unit will increase platelet count by 30,000-60,000 / µl.
3. Corrected Count Increment (CCL)

$$= \frac{\text{(Post. transfusion Plt. C.- Pre-trans. plt. C.) X BSA}}{\text{Number of Plt. Transfused X}10^{11}}$$

4. Adult dose:
   a. One P.C / 10 kg (6-8 units)
   b. One platelet pheresis unit.
5. Pediatric dose: One P.C / 7-10 kg. Body weight

Administration:

1. Via 150-280 filter administration set.
2. Transfused within 4 hours.
3. ABO-matched platelets if RBC's> 2m1.

6. LEUCOCYTES REDUCED BLOOD CELLULAR COMPONENTS.

Benefits: To Prevent:

1. Febrile Non Hemolytic Transfusion Reaction (FNHTR)
2. Alloimmunization to HLA antigens and platelet refractoriness.
3. CMV, EBV and HTLV I / II Transfusion Transmitted Diseases (TTD).
4. Acute Lung Injury, and adult Respiratory Syndrome.

Indications:

1. Multiple PRBC's transfusion patients:
   a. Sickle cell disease.
   b. B-Thalassemia major.
   c. Leukemia.
   d. Cancer.
   e. Hematopoietic stem cell transplant
2. Patients need CMV negative blood:
   a. Infants born to negative CMV mothers.
   b. Open Heart Surgery.
   c. Premature babies.
   d. Seronegative CMV organ transplant.
3. Multiple pregnancy females who need blood.
4. Multiple platelet transfusion patients.
5. Patients with immune suppression:
   a. Organ transplants.
   b. AIDS.

7. FRESH FROZEN PLASMA (FFP.)

Indications:

1. Patients with active bleeding with prothrombin ratio >1.2.
2. Patients of liver disease with fibringen <100mg / dl
3. Massive transfusion with abnormal bleeding.
4. Therapeutic Plasmapheresis for:

a. Thrombotic Thrombo Cytopenic Purpura (TTP.)
b. Hemolytic Uremic Syndrome.

5. Rapid Reversal of Warfarin or Coumarin overdosage (to avoid delay of action of vit. K.
which need 8-12 hour to reverse Coumarin overdose.)
6. Liver transplant.
7. Acute DIC.
8. Congenital or Acquired Coagulopathies with PT >18 sec., APTT> 60 sec., or coagulation
factor assay <25%
9. Hyper coagulopathies:
a. Protein C. and Protein S. deficiency.
b. Antithrombin III Deficiency.

Contraindications:

1. Volume expanders.
2. Nutrition.
3. Wound Healing.

Dose:

1. 15-30m1 / kg. Body weight.
2. One FFP unit reduces PT and a PTT to hemostatic normal range.

Administration:

1. Via administration set with filter as blood.
2. Infusion Rae 1-2 ml. / minute.
3. Compatible FFP to ABO antigens.

8. CRYOPRECIPITATES.

Description: One unit of cryoprecipitate contain:

1. Fibrinogen, 150-250mg.
2. F. VIII, 80-150 units (2 Iu)
3. Von-Willibrand Factor (VWF.), 80-120 units (40-70%)
4. F.XIIL 20-30%
5. Fibrinectin 20-30%

Indications:

1. Fibrinogen deficiency (<100 mg / dl.)
a. Congenital: Dysfibrinogenemia, or deficiency.
b. Acquired: - Abruptio placenta.
- Amniotic fluid embolism.
- Intrauterine fetal death.
- DIC.
2. Uremic and Hepatic Patients to control bleeding.
3. Fibrin glue during surgery (Fibrinogen+ thrombin+(a.)
4. Hemophilia A.:
a. Minor decrease: Desmopressin
b. Severe decrease: Cryoprecipitate or Fractionated or recombinant F.VIII.
c. With inhibitors: Prothrombin concentrates or Anti inhibitor coagulation complex.
5. Von Willebrand disease (Desmopressin, but not II B)
6. Factor XIII deficiency.

Dose:

1. Normal levels of F.VIII = 2880u = 80 Iu = 100%
2. Adult dose: pool of 10 bags.
3. Pediatric dose: One cryo unit/ 7-10 kg. Body weight.
4. One unit of cryo. Will raise:
   a. Fibrinogen: 150-250 mg.
   b. F.VIIL: 80-150 units (2 Iu)
   c. VWF.:40-70%
   d. F.XIII:20-30%
   e. Fibrinectin.:20-30%
5. C'''oagulation improvement measured by PT, a PTT and Factor assay.

Administration:

1. Via administration set as blood.
2. Infusion rate 1-2m1 / minute.

9. GRANULOCYTES

Indications:

1. Neonates with severe neutropenia with gram-ve sepsis not respond to 48 hours antibiotics. 2. Neutrophil dysfunctions.

Dose:

1-2 x 10^9 granulocytes / kg. Body weight.

Administration:

1. Via administration set as blood.
2. Infusion rate 1-2m1 / minute.

10. IRRADIATED CELLULAR BLOOD PRODUCTS.

Benefits:

Prevent Graft vs. Host Disease.

Irradiation Dose:

Gamma irradiation 2500 cGY midpoint or 1500 cGY at any point.

Cellular blood products:

RBC's, platelets and WBC's.

Indications:

1. Severe immuno compromised patients,
2. After transplantation of B.M. or peripheral stem cells.
3. Fetal intrauterine transfusion.
4. Blood relative transfusion.
5. HLA matched platelets.
6. Hematological Diseases transfusions:
   a. Hodgkin disease.
   b. Non-Hodgkin lymphoma.
   c. Multiple Myeloma.
   d. All types of leukemia.
7. Severe combined immune deficiency syndrome.
8. HIV patients.
9. Neuroblastoma under chemotherapy.
10. Bone marrow depression under antilymphocytic treatment.
11. Organ transplant patients under immune suppression treatment.

11. MASSIVE BLOOD TRANSFUSION.

Definition:

1. Is transfusion of one or more blood volumes within 24 hours (Oesophageal varices)
2. Blood volume is 75m1 / kg. Or about SOOOmI in 70kg. Adult (10 units or more of whole blood.)

Emergency issue:

1. In trauma cases and hemorrhagic emergencies.
2. Strict identification of obtained patient sample, prior to transfusion.
3. Quick ABO and Rh. Typing, and antibody screening.
4. O-RBC's should be issued in response to signed (STAT) emergency release request for blood, without waiting for ABO and Rh. Typing or antibody screening.
5. O-RBC's Rh negative issued to females in childbearing period.
6. Crystalloid and / or colloids can be given as volume expanders.
7. If patient is B-negative woman we switch blood group to O- negative, before switching to B-positive blood.

Coagulation Support:

1. Poor tissue perfusion will release pro-coagulant materials leading to DIC, lactic acidosis and acidemia with poor myocardial function.
2. Platelet count <50,000 / µ1 and fibrinogen <100mg and marked prolongation of PT and APTT, or factor assay of <20-30% is indication for FFP, cryoprecipitate, AHF and platelet transfusion.
3. Within 3-8 hours after transfusion of stored blood, 50% of 2,3 DPG will be regenerated.

Hypothermia:

Avoid hypothermia in massive transfusion by warming the patient, crystalloids and blood.

12. PLASMA DERIVATIVES AND SYNTHETIC VOLUME EXPANDERS

I. Factor VIII concentrate:

Sources:

1. Human by fractionation of human plasma
2. Recombinant factor VIII

Half Life:

Indications:

1. Hemophilia A
2. Humate-P products used to treat von willebrand disease (contain VWF)

Dose:

1. Each unit of F. VIII/Kgm give rise to 2% to F. VIII (0.021U/ml).
2. Satisfactory level 30%-100%
3. Calculation

PV ml (plasma volume) = 40m1/kg X body weight (kg)

$$\text{Desired units of F. VIII} = \frac{\text{PV X (desired level \%-initial level \%)}}{100}$$

II. Factor IX concentrate:

Sources:

1. Human plasma fractionation product.
2. Recombinant

Half life:

Indications:

1. Hemophilia B.
2. F. IX complex (contain F II, VII, IX, X) used in treatment of inhibitors.

1. Patients with DIC in liver disease
2. Myocardial infraction
3. Vasomotor reaction

Dose:

1. Each unit of F. IX/kgm give rise to 1% to F IX
2. Satisfactory level is 50-100%]
3. Calculation: same as F. VIII

III. Other plasma protein derivatives:

1. Antithormbin concentrate (AT).

Action:

1. Inhibit coagulation (inhibit thrombin, F. IX, X, XI and XII)
2. Inhibition will increase in presence of heparin.

Indication:

Hereditary AT deficiency with thrombotic disease or as prophylaxis for surgery.

2. Recombinanat Factor VII a:

Action:

Activation of F. X and thrombin generation which increase thrombin activated platelet surfaces.

Indications:

Hemophiliacs with inhibitors.

3. Protein C. concentrate:

Action:

In presence of protein-S, inhibit coagulation (inhibit factor V and VIII), Vit. K dependent

Indication:

Hereditary protein C deficiency with venous thrombosis, purpura and DIC.

4. CI -Esterase inhibitor:

Action:

Regulate complement

Indications:

Hereditary angioedema-(deficiency of C1-estrate inhibitor).

5. Alpha 1-Proteinase inhibitor (alpha l-antitrypsin)

Action:

Serine proteinase inhibitor (alphal-antitrypsin).

Indications:

API deficiency with pulmonary emphysema and liver disease.

6. Fibrin glue (biological glue):

Source:

1-2 units of cryoprecipitate + thrombicin + Ca

Indication:

Stop bleeding in surgical sites.

7. Factor XIII-Concentrate:

Source:

Fibrogammin P is plasma derived F. XIII.

Indication:

Treat patients with F-XIII deficiency.

IV. Albumin and plasma protein fraction (PPF):

Sources:

1. Albumin obtained by plasma fractionation (95% albumin +4% globulin), available in 5% and 25%.
2. PPF obtained by plasma fractionation (83% albumin + 17% globulin), available in 5%.

Half life:

Indications: In hypovolemic and hypoproteinemic states:

1. Schock
2. Thermal injury
3. Nephrotic syndrome
4. Burns

Precautions:

1. 25% albumin diluted with normal saline.
2. Infusion rate 2m1/minute.
3. Not for long term therapy

V. Immune Globulin:

Sources:

Plasma fractionation

Types:

Intramuscular (IMIG), and intravenous (IVIG) preparation.

Half life:

18-32 days

Contents:

>90% IgG and traces of IgA and IgM.

Indications:

1. Passive immunization
   a. HBV
   b. Tetanus
2. Replacement therapy:
   a. Wiskott Aldrich syndrome
   b. Combined immunodeficiency
3. Immunomodulation:
   In acute and chronic TTP.
4. Treatment of:
   a. HIV related thrombocytopenia.
   b. Post transfusion purpura.
   c. Neonatal alloimmune thrombocytopenia.
   d. Guilliain -Barre syndrome.
5. Prophylaxis against Graft vs-host disease in bone marrow transplants.
6. Specific immune globulin:
   a.Tetanus toxoids
   b. RhIG Precautions:
      1. Hypersensitivity and anaplyactic reactions may occur.
      2. Using solvent/detergents give HCV free IVIG products.
      3. Passive transfer of ABO antibodies may cause hemolysis.

VI. Rh Immune Globulin:

Sources:

Pooled human plasma from Rh-D negative sensitized individuals.

Types:

TV and IM preparations.

Doses:

300-µg and 120 µg

Contents:

Ig Anti-D

Indications:

a. Antepartum: For D-Negative females in case of:

- **Abortion**
- **Miscarriage**
- **Ectopic pregnancy termination**
- **Amniocentesis or chronic villus sampling.**
- **Antepartum prophylaxis at 28 weeks of gestation.**

b. Post partum: For D- negative females who deliver Rh-D positive infant.

c. Special situations: - Rh.D negative individuals who receive Rh-D positive blood components (platelet contain >2ml / Uunit)Rh-D positive, nonsplenectomized patients with immune thrombocytopenia (ITP).

Administration Dosage:

1.Time: Within 72 hours of procedure.

2. Calculation: In case of fetomaternal hemorhage mother sample within 1 hour of delivery quantified by Kleihaure - Betke test or flow cytometry.

3.300 µg of IM Rh IG protects against 15m1 of Rh-D positive blood

4.In ITP treatment, we use 25-50 µg/kg IV Rh IG.

VII. Synthetic volume expanders:

Products:

1. Crystalloids:

a.Normal saline: contains Nacl.

b. Ringer lactate: contains Ca, K and lactate.

2. Colloids:

a.Dextran: Contain polysaccharide of glucose with 6% or10% concentration.

b. Hydroxy ethyl starch (HES).

Half life:

6 hours for Dextran and 24 hours for HES.

Indications:

1. Hemorrhage

2. Burns: Crystalloid in 1^{st} 24 hours 3 volumes of crystalloids for one volume of lost plasma.

3. Schock.

Advantages:

1. **Nontoxic and inexpensive.**
2. **Stored in RT.**
3. **Free from TTD.**
4. **No compatibility testing required.**

Disadvantages:

1. Fluid overload

2. Dextran may produce anaphylactic reaction, bleeding tendencies, renal failure and interference with blood typing.

3.HES may increase PT and PTT, and cause pruritis.

13. PHARMACOLOGIC ALTERNATIVES TO BLOOD TRANSFUSION

1. Recombinant growth factors:

a. Erythropoietin:

Function: Stimulate RBC production by BM.

Dose: 300-600 U/Kg, subcutaneous injection.

Indication:

1. **End stage renal disease.**
2. **Anemia in AIDS patients.**

3. Anemia with chronic disease.
4. Anemia with BM suppressor medications.

b. Granulocyte colony stimulating factor (GCSF):

Function: Stimulate granulocyte production by BM.

Indication:

1. Chemotherapy induced neutropenia.
2. Granulocyte and progenitor cell collection.
3. Patients with chronic neutropenia.

c. Interlenkin -11:

Function: Stimulate platelet production.

Indication: Cancer patients with thrombocytopenia.

l. Release VWF from vascular endothelium.
2. Increase F. VIII

Indications:

1. Mild cases of Hemophilia A.
2. Von Willebrand disease.
3. Platelet function disorders.

Dose: 0.3 - 0.4 µg/kg, single IV injection.

3. Fibrinolytic inhibitors: Aprotinin:

Function:

Inhibit proteinases (plasmin, kallikrin, trypsin and urokinase), so help coagulation and stop bleeding.

Indications:

1. Cardiac surgery, prostatectomy and liver transplant.
2. Mucosal lesions in mouth and GIT.
3. Dental extraction in hemophilia.

CHAPTER TWO: BLOOD TRANSFUSION ADVERSE REACTIONS

[A] ACUTE TRANSFUSSION REACTIONS (WITHIN 24 HOURS)

1. Acute intravascular haemolytic reaction:

Causes:

1. ABO incompatilibility:
   a. Clerical errors during
      i. Sample labeling ii. Transfusion
   b. IgM and IgG complement fixing (Anti A or Anti B) will cause RBC hemolysis.
2. Immune mediated.
3. Complement will release anaphylatoxin.
4. Activation of cytokines (IL-1, 6,8) and (TNF-cc).
5. Incidence: 1:38000-1:70,000

Signs and Symptoms:

1. Haemoglobinemia, Haemoglobinuria, Flank pain, fever, chills, shock, DIC, Dyspnea,chest pain, oliguria.
2. Occur within minutes of start of transfusion.
3. Confirm diagnosis:
   a. Increase BUN, creatinine.
   b. Urinary hemoglobinuria.
   c. Increase PT, PTT.
   d. Increase LDH, bilirubin.
   e. Decrease fibrinogen, platelet count, haptoglobin, HCT, Hb.
   f. Hemoglobinemia in plasma.

Management:

1. Stop transfusion
2. Verify correct unit to correct patient.
3. Maintain IV access with crystalloids or colloids.
4. Support BP., pulse respiration, renal function.
5. Treat chock and DIC.
6. Notify BB and send:
   a. Transfusion reaction report.
   b. Samples of blood and urine.
   c. Blood bag and administration Set.
7. Blood Bank workup:
   a. Clerical check for blood group, correct unit, correct patient ID.
   b. DAT
   c. Test plasma for hemoglobinemia.
   d. Test urine for hemoglobinurea e. Repeat ABO/Rh and crossmatch.
8. Consult with B.B. physician before further transfusion.

Prevention:

Ensure proper sample and recipient identification.

2. Acute Extravascular haemolytic reaction:

Causes:

1. IgG non-complement fixing antibody.
2. Coated RBC's with IgG will be cleared and destroyed in spleen.

Signs and Symptoms:

1. Fever, malaise.
2. Indirect hyper bilirubinemia.
3. Increase LDH.

4. Increase urine urobilinogen.
5. Falling HCT, and Hb.

Management:

1. Monitor HCT.
2. Monitor renal and hepatic function.
3. Monitor coagulation.
4. No acute treatment required.

Prevention:

1. Ensure proper sample and recipient identification.
2. Give antigen negative blood to multiple transfusion patients (sickle cell disease).
3. High dose of IVIG.

3. Febrile nonhemolytic transfusion reaction (FNHTR):

Causes:

1. Recipient antibodies against donor leukocytes or platelets.
2. Ag and Ab reaction stimulate phagocytes to release pyrogens.
3. Pyrogenic cytokines (IL 1,6) and TNF.oc, through prostaglandin E2 stimulate hypothalamus. 4. Sometime donor plasma contain cytokines from stored blood or platelets. (passive cytokines).
5. Bacterial contamination.
6. Patient underlying condition.

Signs and Symptoms:

1. Fever > 1'C or 1.8F
2. Chills
3. Frequency:
   a. 1 % of transfusions.
   b. 10% infrequent transfusion patients (Thalassemia)

Management:

1. Stop transfusion.
2. Antipyretics:
   a. Acetaminophen.
   b. Non steroidal anti inflammatory agents.
3. Meperidine 25-SOmg IV or IM, to adults for Rigors.

Prevention:

1. Pretransfusion antipyretics.
2. Leukocyte reduced blood components.

4. Allergic reaction:

Causes:

1. Antibodies to plasma proteins (Mild) (1.3%).
2. Rarely antibodies to IgA (severe), haptoglobin, C4 (Chido/Rogers blood group antigens) which cause anaphylaxis (1:50,000).

Signs and Symptoms:

1. Urticaria (hives).
2. Dyspnea.
3. Wheezing, throat tightening.
4. Rarely hypotension or anaphylaxis.

Management:

1. Stop transfusion.
2. Give antihistamine orally or IM. (25-SOmg diphenhydramine).
3. 100mg hydrocortisone IV or epinephrine if severe.

4. Secure IV acces for fluids.
5. Intubation for anaphylaxis.

Prevention:

1. Recurrent cases:
   a. 60 minutes pretransfusion give antihistamine and steroids.
   b. Give washed RBC's
2. For IgA deficient patient, give IgA deficient plasma from donors of rare donor register.

5. Hypotensive reaction:

Causes:

l. Plasma contact with artificial surfaces specially negatively charged bedside leukocyte filter will activate kinin pathway to generate bradykinin.
2. Bradykinin is a potent vasodilator.
3. Angiotensisn converting enzyme (ACE) inhibitor medications will inhibit degradation of bradykinin and exacerbate the condition.

Signs and Symptoms:

1. Hypotension. 2. Tachycardia. 3. Abdominal pain. 4.facial flushing .

Management:

1. Stop transfusion.
2. Usually resolve within minutes of stopping transfusion.
3. Secure IV access for fluids.
4.Trendeleberg position.
5. DD with:

a.Vasovagal attach. b. AHTR.
c. Bacterial contamination. d. Anaphylaxis.

Prevention:

1. Discontinue ACE inhibitor medication.
2. Avoid negatively charged bedside leukocyte filters.
3.Use prestorage leukocyte filters.

6. Hypervolemia (circulatory overload):

Causes:

1.Too rapid and/or excessive blood transfusion.
2. <1%

Signs and Symptoms:

1. Headache, dyspnea. 2. Pulmonary edema. 3. CHF. 4.Increase of systolic BP (>50 mmHg) above normal.

Management:

l. Stop transfusion.
2. Place patient in sitting position.
3. Give oxygen and diuretics.
4. Phlebotomy if needed.

Prevention:

1. Infuse blood at rate not faster than 4m1/kg/hour.
2.Aliquots from single unit within 4 hours.

7. Transfusion related acute lung injury (TRALI):

Causes:

1. Donor HLA or leukocyte antibodies transfused with plasma in component will attack recipient leukocytes.

2.This will activate recipient neutrophils in lung to release vasoactive mediators and capillary leakage.
3.Less common recipient antibodies to donor white cells.
4. Donor neutrophil lipid mediators released during storage will cause lung tissue injury in recipient.

Sings and Symptoms:
1. Respiratory distress.
2. Bilateral pulmonary oedema.
3. No heart failure.
4. Hypoxia.
5. Hypotension.
6. Fever.
7. Within 4 hours from transfusion or during transfusion.
8. Resolve within 48-72 hours.
9. Mortality rate 10%.

Management:
1. Stop transfusion.
2. Support blood pressure and respiration.
3. Oxygen therapy and intubation.
4. Steroids
5. Fluid support.
6. Send blood bag and administration set to BB for investigation (HLA antigen typing for donor and recipient).

Prevention:
1. Leukocyte reduced RBC's and platelet transfusion.
2. HLA matched components.

8. Bacterial contamination of blood component:

Causes:
1. Bacterial contamination due to:
a. Improper preparation of skin in venipuncture site in phlebotomy.
b. Improper preparation or handling of unit
c. Occult bacteremia in the donor.
2. Incidence: 0.3%
3. Common organisms:
a. Skin flora: Staphylococcus,
b. Gram negative rods: Acinetobacter, Klebsiella, Escherichia.
c.Yersinia and pseudomonas in RBC's units (low temperature and high iron).

Signs and Symptoms:
1. During or after transfusion patient may develop high fever.
2. Rigors.
3. Dspnea.
4. Hypotension.
5. Schock.

Management:
1. Stop transfusion.
2. Start broadspectrum antibiotics immediately.
3. Report reaction to blood bank.
4. Obtain blood culture of patient.

5. Return unit to BB for culture and Gram's stain.
6. Maintain circulation and urine output.
7. Revise antibiotic based on culture result.
8. Monitor for sings of DIC., renal failure and respiratory failure.

Prevention:

Care of:

1. Donor selection.
2. Blood collection and proper skin preparation for phlebotomy.
3. Component preparation and storage.

9. Drug Induced Hemolysis:

Causes:

1. Drug through hapten or immune complex or autoantibody formation cause hemolysis of RBC's

Signs and Symptoms:

1. It is not a transfusion reaction but can be confused with HTR.
2. DAT is positive.
3. S & S of hemolysis.

Management:

1. Discontinue drug.
2. Maintain supportive measures.
3. Maintain transfusion.

Pevention:

1.Avoid drugs like:

a. Cephalosporin.
b. Cefotetan
c. Ceftriaxone.

10. Non immune Hemolytic reaction: (Rare):

Causes:

1. Mechanical:

a. Artificial heart valves.
b. Extracorporial circulation.
c.Transfusion under pressure.

2. Hypotonic solutions transfusion in same line with blood:

a. 5% dextrose in water.
b. Distilled water.

3. Excessive heating by warmers.
4. Freezing due to refrigerator malfunction.

Signs and symptoms:

1. Hemoglobinemia.
2. Hemoglobinuria.
3. Hyperkalemia.
4.Transient renal failure.
5. Differentiate between AHTR as soon as possible.

Management:

1. Stop transfusion.
2. Maintain fluid support.
3. Maintain blood pressure, respiration and renal function.
4. Report reaction to blood bank.
5. Blood bank workup as AHTR.

Prevention:

Care of:

1. Patient clinical history.
2. No blood transfusion under pressure.
3. No infusions or medication with blood transfusion line.
4. QC for blood warmers and refrigerator.

11. Thermal reactions:

Causes:

Rapid infusion of cold blood, cause hypothermia leading to cardiac arrhythmia or arrest.

Signs and symptoms:

1. Cardiac arrhythmia.
2. Cardiac arrest.

Management:

1. Cardiopulmonary resuscitation.

Prevention:

l. Avoid rapid infusion of cold blood and use monitored warmers.

1. Citrate chelate Ca and decrease Ca. level.
2. K. increase in old stored blood.
3. Alkalosis from large volume of blood transfusion.

Signs and symptoms:

1. Circumoral paresthesia and fingers.
2. Hyperkalemia.
3. Hypokalemia.

Management:

1. Watch for subside.
2. Maintain renal function.
3. Administer potassium in Hypokalemia.

Prevention:

1. Washed RBC's or fresh blood less than 7 days is given to neonates during exchange transfusion, cardiac surgery or patients with renal failure.

(12) Air embolism:

Causes:

Air infusion via line in case of: a.Blood pumps b.Apheresis machines.

Signs and symptoms:

Sudden shortness of breath,acute cyanosis,pain,cough,hypotension,cardiac arrhythmia.

Management:

Place patient head down on left side to dislodge the air bubble from pulmonary valve.

Prevention:

Care of and monitor the patient on apheresis machine or on using blood infusion devices.

[B] DELAYED TRANSFUSION REACTIONS

(1) Delayed haemolytic transfusion reaction: (DHTR).

Causes:

1. Incidence 1%
2. Alloimmunization to RBC's antigens (Rh, kell, duffy, kid)
   a. Anamnestic response within days.
   b. Primary response within weeks.
3. Antibodies to RBC's antigens will coat RBC's (IgG) and stimulate phagocytosis in

spleen (Extravascualr and produce cytokines less than in AHTR).

4. Can be found in ABO/Rh. Mismatched hematopoietic cells and solid organs transplant.

Sings and symptoms:

1. Slight fever.
2. Anemia.
3. Indirect hyperbilirubinemia.
4. Elevated reticulocyte count.
5. Increase LDH.
6. Decrease haptoglobin.
7. Positive DAT.
8. Hemolytic disease of newborn.
9. Positive antibody screen.

Management:

1. Rarely life threatening.
2. If severe follow treatment described in AHTR.
3. High dose IVIG (400mg/kg) before transfusion may prevent DHTR, if compatible blood not available.

Prevention:

1. Avoid unnecessary transfusion.
2. Transfuse compatible blood as needed.
3. Identify antibody and give negative antigen.
4. Phenotype multiple transferred patients.
5. RhIG to Rh. Negative mothers within 72 hrs from delivery or abortion.

(2) Delayed Alloimmunization to platelet HLA antigen:

Causes:

1. Alloimmunization to platelet HLA antigen.
2. Antibodies to HLA and platelet antigens 28 days after primary exposure and 4 days in secondary exposure.

Sings and Symptoms:

Platelet refractoriness which can be determined by:

a. Corrected count increment.
b. Lack of response to 3 platelet transfusions within 2 weeks time.
c. Platelet antibody screen.
d. Lymphocytoxocity test.

Management:

Give HLA matching platelet or by crossmatching.

Prevention:

1. Use leukocyte filters for pooled platelet transfusion.
2. Use apheresis leukoreduced platelet.

(3) Post transfusion purpura:

Causes:

Recipient platelet antibodies (alloantibodies to HPA1) will destroy autologous platelets 1-3 weeks after transfusion.

Signs & symptoms:

1. Thrombocytopenic purpura.
2. Bleeding tendencies.
3. Platelet antibody screen and identification.

Management:

If there is risk of hemorrhage give:

a. High dose IVIG (400mg/kg).
b. Plasmapheresis to remove antibodies.
c. Platelet transfusion.

Prevention:

Avoid unnecessary transfusions.

(4) Graft-vs-host disease:

Causes:

Donor lymphocytes attack host tissues.

Signs and symptoms:

Erythroderma, maculopapular rash, anorexia, nausea, vomiting, diarrhea, hepatitis, fever, pancytopenia.

Management:

Treat with:

a. Methotrexate. b. Corticosteroids.

Prevention:

1. Irradiation with 2500 cGy to all components from:
a. HLA matched.
b. Blood relatives.
2. Give irradiated cellular products to an immuno-suppressed patients.

(5) Immune modulation:

Causes:

Interaction of donor WBC's or plasma factors with recipient immune system.

Signs and symptoms:

Increase in:

a. Renal graft survival.
b. Post resection tumor recurrence.
c. Infection rate.

Management:

Non specific.

Prevention:

1. Avoid unnecessary transfusions.
2. Autologous transfusion.
3. Leukocyte reduced RBC's and platelets.

(6) Hemosiderosis (Iron overload):

Causes:

1. One unit of PRBC's contain 250mg iron.
2. In chronic anemia patient who receive multiple transfusion, iron will accumulate in heart, liver and pancreas.

Signs and Symptoms:

Diabetes, liver cirrhosis and cardiomopathy.

Management:

1. Check serum iron and iron binding capacity.
2. Treat with:
a. Iron chelator (desferioxamine).
b. Red cell exchange by Apheresis in sickle cell disease.

Prevention:

Desferioxamine to patient receving chronic red cell therapy.

(7). Transfusion transmitted disease (TTD):

1. Hepatisis:

a. Hepatitis C. virus

Causes :

1. Risk of HCV infection by transfusion is 1:1600,000
2. Window period 16-32 days.

Signs and symptoms:

1. 20% acute infection: Hepatitis with jaundice, nausea, vomiting, fatigue, dark urine, and elevation of liver enzymes.
2. 80% chronic carriers without treatment:
   a. 50% develop cirrhosis within 20 years
   b. 20% develop carcinoma within 30 years.

Prevention:

To prevent test donor blood for:

a. Anti HCV screening by Elisea.
b. Repeat screening in dublicate if positive.
c. Confirm by HCV RIBA blot.

b. Hepatitis B. virus:

Causes:

1. Risk of HBV infection by transfusion in 1:63,000
2. window period 60-150 days.

Signs and symptoms:

1. 30-50% symptomatic with acute hepatitis infection.
2. Chronic carriers represent 5% of adults and 90% of children before immunizaiton.
3. 15% of chronic carriers develop cirrhosis and hepatocellular carcinoma, if not treated.

Prevention:

To prevent test donor blood for:

a. HBsAg EIA screening.
b. Repeat positive screening in duplicate.
c. Confirm by neutralization testing for HBsAg.
d. Screening for Anti HBC, if positive test for Anti HBs, and if positive use blood (Immune) and NAT,HBV,DNA.

c. HAV virus:

Causes:

Risk of HAV infection by blood (Plasma derivatives-e.g. F. VIII) is 1:1,000,000.

Signs and symptoms:

Very mild hepatitis and develop immunity.

Management:

No testing required.

d. HEV, HGV (GBVC), TTV and SEN-V:

Causes:

Lack clinical disease significance.

2. HIV I/II (Human deficiency virus disease):

Causes:

1. Risk of transmission is 1:1,900,000
2. Window period with Elisa screen 22 days, came to 16 days by using HIV p24 Ag testing.
3. HIV-1 group O variant.

Signs and symptoms:

1. HIV I/II viruses infect T. helper cells (CD4 + T. lymphocyte) in Lymph node (LN).
2. Viremia in plasma after 3 weeks.
3. 60% of acute infection fever, LN+, sorethroat, rash, joint and muscle. pain, diarrhea, headache and vomiting.
4. Infection without symptoms may last for 10-12 years.
5. Sharp decline in CD4+.
6. Immuno suppression lead to opportunistic infections.

Prevention:

To prevent HIV I & II TTD test donor blood with:

a. Anti HIV I /II EIA which can detect HIV I group O. variant.
b. NAT,HIV,RNA.
c. Repeat positive results in duplicate.
d. Positive screen confirmed by HIV I/II western blot test.

3. HTLV I /II human T. cell lymphotropic virus disease:

Causes:

Risk of transmission via blood transfusion is 1:641,000

Signs and symptoms:

HTLV 1/11 can cuase:

a. T. Cell lymphoma / leukaemia (ATL).
b. Peripheral neuropathy.
c. HTLV-associated myelopathy.

Prevention:

To prevent HTLV LII TTD we must test donors for:

a. Anti HTLV I/II EIA screening test.
b. Repeat positive screen in duplicate.
c. Confirm positive with HTLV I/II western blot.

4. CMV:

Causes:

Latent CMV infection is common among blood donors.

Signs and symptoms:

CMV infection is a major concern in immune suppressed patients.

Prevention:

1. Transfuse seronegative CMV blood.
2. Transfuse leukocyte reduced blood components.

5. B 19 parvovirus:

Causes:

Risk of transfusion transmission is (1:40,000-1:3,300)

Signs and symptoms:

Benign or transient nature.

Management:

Effective treatment by IGIV

Prevention:

Leukocyte reduced blood components.

6. Other viruses: Epstein-Barr virus, human herpes viruses 6 and 8:

Causes:

Risk of clinical disease transmission has not been demonstrated.

Prevention:

Leukocyte reduced blood components.

7. Syphilis:

Causes:

1. Risk of disease transmission by blood transfusion is exceedingly rare.
2. Causative agents treponema pallidum.

Prevention:

1. Prevented by VDRL or RPR testing for blood donors.
2. All positive cases confirmed by TPHA testing.

8. Malaria:

Causes:

l. Most frequently plasmodium falciparum.
2. Risk rate is <1:1,000,000

Signs and symptoms:

Of malaria infection.

Management:

Antimalaria medication.

Prevention:

1. Exclusion and deferral of high risk donors during donor selection.
2. Testing thick blood film for malaria for all blood donors. Or use more sensitive tests for detection of malaria if available.

9. Babesiosis:

Causes:

Risk factor <1: 1,000,000

Signs and symptoms:

Babesiosis

Prevention:

Defer donors from endemic areas or those who have previous infection.

10. Chagas disease:

Causes:

1. Risk factor is 1:42,000
2. Infectious agent is Trypanosoma cruzi.

Prevention:

Defer donors from endemic areas or those who have previous infection.

11. Creutzfeldt Jacob disease:

Causes:

1. Only theoretical risk of transmission via blood transfusion
2. Causative agents are the prions (CJD and variant CJD) through B lymphocytes and denderitic cells.

Signs and symptoms:

Spongiform encephalopathy.

Prevention:

1. Use leukoredcued blood component.
2. Defer donors with family history of (CJD), exposure to risk factors, or residence in endemic areas with (CJD), for more than 6 months (e.g. UK).

CHAPTER THREE: GENERAL RULES FOR PREVENTION OF TRANSFUSION TRANSMITTED DISEASES (TTD).

1. Proper selection of blood donor .

2. Screen all blood donors for the following tests :

a. Anti HCV.	**b. HBsAg.**	**c. Anti HBc.**	**d. Anti HBs.**
e. Anti HIV I/II.	**f. HIV RNA**	**g. Anti HTLV I/II**	**h. HBV DNA**
i. HCV RNA	**j. RPR for syphilis.**		

k. Thick blood Film for malaria or any other, available sensitive test.

3. Secure all unprocessed blood in separate storage places until TTD results.

4. Quarantine all positive blood units to the 1st screen positive test even if become negative by repeat testing, or by confirmation.

5. Follow the proper indication for blood transfusion and avoid unnecessary transfusions.

6. Use leukocyte filters in indicated situations, or to all transfused cellular components if applicable (universal leukoreduction).

TRANSFUSION REACTION INVESTIGATION FORM

Patient	Location:				BLOOD COMPONENT		
Name:				□Whole	□Red cells	□ Other	
				Unit number:			
Age:	Sex:						
				Time & date		Time apparent	
Doctor:						reaction begin:	Amount remaining in bag:
SYMPTOMS:						Before	At start of
□ Fever	□Rigors		Pain				
□Hypotension	□Dyspnea	□	Urticaria	Temperature			
□Oedema	□Nausea	□	Vomiting	Pulse			
□Oliguria	□ Anaphylaxixs	□	Other	Blood			
Time reported to blood bank:				Nurse Signature:			

BLOOD BANK

Immediate Investigations:

1. Have reached the identity of the patient, donor unit(s) labels, forms and records, and find no discrepancy. **Signature and date:----------**

2. Visual inspection of patient serum:		
Haemolysis / Jaundice	□ Pretransfusion	□Post transfusion.
3. Positive Direct Anti-globulin test:	□ Pretransfusion	□Post transfusion.
4. Bacteriological studies done on container	□ Positive	□Negative
5. First voided fresh specimen of urine, Hemolysis	□YES	□NO
6. Hb. And HCT:		
7. Haptoglobin level:		

CONFIRMATION OF ABO AND RH TYPE								
		ANTI SERUM				CELLS		BLOOD GROUP & Rh
		A	B	A, B	D	A	B	
RECIPIENT	PRE-TRANSFUSION							
	POST-TRANSFUSION							
DONOR	SEGMENT							

RECONFIRMATION OF CROSSMATCH					
	IS	37C	AHG	COMPATIBLE	CCC
Pt. serum + Donor (pre)					
Pt. serum + Donor (post)					
Conclusion:					
Technician signature:				Date:	

DATE: **PATHOLOGIST:**

COPY DISTRIBUTION

Patient File:

Laboratory File:

PART FOUR: QUALITY SYSTEM ELEMENTS IN BLOOD BANKS

A. INTRODUCTION:

Continuous quality improvement (CQI):

Synonyms: = Continuous improvement (CI)
= Quality improvement (QI)
= Total quality management (TMQ)
= Process improvement (PI)

Definition:

Continuous improvement of processes of health care services to meet standard, by adjusting and improving the system, instead of getting rid of "bad apples".

Quality Assurance (QA):

Definition:

Activities to determine the quality of care and improve it if it does not meet standards by examing performance of providers and get rid of "bad apples".

Quality control (QC):

Definition:

Measurements of actual performance, and make corrective actions for any unacceptable results.

Process control (PC):

Definition:

Sum of actions for assurance that the performance is within the policies, process and procedures.

Regulatory bodies:

1. Ministry of Health of K.S. A.
   Deputy Ministry of Laboratories and Blood Banks, Blood Bank Directorate.
2. Guidelines of:
   a) (FDA) Food and Drug Administration of USA.
   b) (CE) Council of Europe.

Voluntary Standards:

1. AABB standards and practical manuals.
   Quality system essentials (10 elements).
2. International organization for standardization (ISO)
   Focuses on broader processes and system, rather than details (20 clauses)

B. QUALITY SYSTEM ELEMENTS:

1. Organization.
2. Personnel.
3. Equipments.
4. Supplier issues.
5. Process control.
6. Documents and records.
7. Incidents, errors and accidents.
8. Assessments.
9. Process improvements.
10. Safety.

I. ORGANIZATION

Management: Structure: Organizational chart

Responsibilities:

1. Mission statement.
2. Review and approval of polices and procedures.
3. Active support of objectives and policies.
4. Compliance with local regulations and AABB standards.
5. Coordinate QA.
6. Facilitate QA.
7. Monitor QA.

QA Persons:

1. Work with blood bank.
2. Responsible for several areas.
3. Be part of management.
4. Committees for: Education, safety, policy, procedures, QC and inventory.

QA Functions:

1. Review and approval of:
   - S.O.Ps.
   - Training programs.
   - Documents and record control.
   - Lot release of supplies and BB products.
   - Assessments.
   - Suppliers.
   - Product specification.
   - Adverse reaction and corrective and preventive actions.
   - Deviations in procedures.
2. Criteria of evaluations.
3. Problems surveillance.
4. Yearly reports for finding and corrective and preventive actions.
5. Responsible persons to maintain and retrieve records.

ORGANIZATIONAL CHART

MANAGEMENT

B.B. ADMINISTRATIVE DIRECTOR

I. B.B. MEDICAL DIRECTOR

- Blood Bank Physicians
- BB medical specialists
- BB consultants

II. Q.A. UNIT HEAD

- Educational Committee
- Safety committee
- Policv and Procedures committee
- QC committee
- Inventory committee
- Document and record committee

III. B.B. SUPERVISOR

1. Clerk Supervisor Supervisor

- Secretary
- Typewriters
- Messengers

2. Immunohematology Supervisor

- Lab. Tech.

3.TTD serology

- Lab. Tech.

4. Component preparation Supervisor

- Lab. Tech.

5. Plasmapheresis Head nurse

- Nurses
- Lab. Tech.

6. Blood donation Head nurse

- Nurses
- Phlebotomist

7. Donor selection Supervisor

- Nurses
- Lab. Tech

2. PERSONNEL

1. Personnel Qualification
   - Licensure.
   - Training.
   - Experience.
2. Job description.

3. Orientation program.
4. Competency evaluation:
    - 6 months after hire, and annually thereafter.
    - 6 months after new procedure, and manually thereafter.
    - Competency program:
        i. Written evaluation.
        ii. Review of work records.
        iii. Testing unknown samples.
        iv. Problem solving skills.
    - Documentation of competency reports.
5. Training and retaining programs.
6. Recommendations.

3. EQUIPMENTS

1. Establishments of equipments:
    - Validation.
    - Calibration.
    - Preventive maintenance.
2. Schedules for equipment:
    - Monitoring.
    – Calibration.
    - Maintenance.
3. SOP for new equipment:
    - Installation.
    - Calibration.
    - Validation.
    - Problem documentation.
    - Follow up reports.
4. List of all critical equipments.
5. Recommendations for managements for replacement of defective equipments.
6. Maintenance and periodic revival of repairs and calibration reports.

4. SUPPLIER ISSUES

a. Supplier Qualification

1. List of suppliers.
2. Specifications of supplies.
3. Documentation of suppliers:
    - Ability.
    - Inability.
    - Failures.
    - Notifications.
4. Quarantine or replacement of defective supplies.
5. Tracking suppliers commitment to quality.

b. Agreement review:

1. Review contracts for:
    - Price and time of delivery.
    - Specifications, services and training.
    - Responsible party during shipping.
    - Changes notification.
2. Continued surveillance of materials.
3. Safety measures.

c. Receipt, Inspection and testing of supplies:

1. Approve specifications and supplies.
2. Supplies must meet or exceed FDA or local regulations.
3. Receipt: SOP must define:
   - Acceptance criteria.
   - Package.
   - Shipping.
   - Labeling.
   - Storage requirements.
4. Corrective action:
   - Return of Products.
   - Destruction of products.
5. Tracking receipt and inspection records.
6. List of materials require testing:
   - ABO & Rh reagents.
   - Infectious disease markers testing.

5. PROCESS CONTROL, FINAL INSPECTION AND HANDLING:

A. PROCESS CONTROL:

1. Elements:

a. SOPs development and use.
b. Proficiency testing for testing systems.
c. Monitoring production.
d. Qc., schedules, policies and procedures.
e. Process:
   i. For accepting software.
   ii. For supplier qualification.
   iii. To control non confirming products.

2. Validation:

a. Plan:
   i. System description.
   ii. Risk assessment.
   iii. Validation procedures.
   iv. Approval signatures.
   v. Times line.
   vi. Objectives.
   vii. Responsibilities.
   viii. Acceptance criteria.
   ix. Documentation.

b. Computer Validation:
   i. Computer system: Hardware, peripheral devices, personal and documentation.
   ii. Types:
      1.Custom development.
      2. Vendor-Development.
      3. Hybrid

3. Blood products label control:

a. Suitable units and aliquoted or pooled units.
b. Appropriate temperature during labeling.
c. Label review for accuracy: Facility name, address, product name, ABO and Rh, Collected date and time, expiration date, non reactive to infectious disease markers.

d. Q.A.

i. Audit.
ii. Discrepancies resolution.
iii. Compliance with regulation.

4. Proficiency Testing:

a. Aim: Test methods does meet standards.
b. SOP: Cover sample from receipt through testing and reports.
c. Documentation of corrective actions.
d. External or internal proficiency testing programs.

5. Quality control:

a. Key steps: Where required:
i. Reagents.
ii. Equipments.
iii. Blood components.
Review and approve procedures.
Q.C testing
Results evaluation:
i. Acceptable: continue.
ii. Unacceptable: stop and corrective actions.
iii. Retest and evaluate the fix. iv. File records.

b. Q.C. program flow chart:
c. Statistical analysis:
- Statistical tools:
i. X and R charts.
ii. Histograms.
iii. Pareto diagrams.
iv. Run charts.
- Purpose: Evaluation of:
i. Services.
ii. Demands.
iii. Production.
iv. Adequacy of personnel, test methods and equipments.
v. Inventory control.
- Identity problems.
- Early detection of trends to develop early corrective action.

d. Q.C records:
- Staff LD.
- Reagents:
i. Manufacturer.
ii. Lot number.
iii. Expiration date.
- Testing:
i. Date and time.
ii. Results.
iii. Interpretation and review.
- Corrective action.
- Frequency of testing.

Quality control testing intervals	
Equipment/ reagents/testing	**Frequency**
I. Refrigerators / freezers / platelets incubators	
A. Refrigerators:	
1. Recorder	Daily
2. Manual temperature	Daily
3. Alarm system board (if applicable)	Daily
4. Temperature charts (review daily)	Weekly
5. Alarm activation	Quarterly
B. Freezers	
1. Recorder	Daily
2. Manual temperature	Daily
3. Alarm system board (if applicable)	Daily
4. Temperature charts (review daily)	Weekly
5. Alarm activation	Quarterly
C. Platelet incubators	
1. Recorder	Daily
2. Manual temperature	Daily
3. Temperature charts (review daily)	Weekly
4. Alarm activation	Quarterly
D. Ambient platelet storage area	Every 4 hours
ll-Laboratory Equipments:	
A. Centrifuge/ cell washers	
1. Tube fill level (serologic)	Daily
2. saline fill volume	Weekly
3. Speed	Quarterly
4. Timer	Quarterly
5. Function	Yearly
6. Temperature of refrigerated centrifuge (record daily)	Monthly
7. Volume of antihuman globulin dispensed by cell washer	Monthly
B. Heating blocks/water baths	
1. Temperature	Day of use
2. Quadrant/area checks	Periodically
C. View boxes (temperature)	Day of use
D. Component thawing devices	Day of use
E. pH meters	Day of use
F. Blood irradiators	
1. Calibration	Yearly
2. Turntable	Yearly
3. Timer	Monthly/ quarterly
4. Source decay	Dependent on
5. Leak test	source type
6. Dose delivery verification	Biannually
a. Cesiuim – 137	Yearly

b. Cobalt-60	Biannually
G. Thermometers (vs NIST-certified)	Yearly
H. Timers/ clock	Yearly
I. Pipette recalibration	Yearly
J. Sterile connecting device	
1. Weld check	Each use
2. Function	Yearly
K. Blood warmers	
1. Effluent temperature	Quarterly
2. Heater temperature	Quarterly
3. Alarm activation	Quarterly
III. Blood collection equipment:	
A. Whole blood equipment	
1. Agitators	Day of use
2. Balances/ scales	Day of use
3. Gram weight (Vs NIST-certified)	Yearly
B. Microhematocrit centrifuge 1.calibration	Yearly
2.Centrifuge timer check	Quarterly
C. Cell counters/ hemoglobinometers	Day of use
D. Blood pressure cuffs	Periodically
E. Apheresis equipment	
1. Checklist requirements	As specified by manufacurer
2. Photophersis	Monthly
IV. Reagents	
A. Red cells	Day of use
B. Antisera	Day of use
C. Antiglobulin serum	Day of use
V. Blood products and components:	
A. RBC's	
Hematocrite <80%	Monthly/test at least 1% of components produced or 4 units, whichever is more
B. Cryoprecipitated AHF	
> 80 IU/unit in 100% of units tested	Monthly/test at least 1% of components produced or 4 units, whichever is more
> 150 mg fibrinogen in 100% of units tested	
C. Platelets	
> $5.5x10^{10}$ plt / bag in 75% of units tested	Monthly/test at least 1% of components produced or 4 units, whichever is more
All units tested ≥ 6.2 pH at max. storage.	
D. Platelets Pheresis	
> $3x10^{11}$ plt / bag in 75% of units tested	Monthly/test at least 1% of

All units tested $\geq$ 6.2 pH at max. storage.	components produced or 4 units, whichever is more
E. Granulocytes.	
> 1.Ox 10^{10} granulocytes Pheresis, and $1x10^{9}$ granulocytes in buffy coat in 75% of units .	Monthly/test at least 1% of components produced or 4 units, whichever is more
F. Whole blood, Leukocytes Reduced:	
Residual leukocyte count of <5 x 10^{6} in each red cell at container tested, and retain a minimum of 85% of original red cells in the component.	Monthly/test at least 1% of components produced or 4 units, whichever is more
G. Red blood cells, Leukocytes reduced:	
Residual leukocyte count of <5 x 10^{6} in each red cell at container tested , and retain a minimum of 85% of original red cells in the component.	Monthly/test at least 1% of components produced or 4 units, whichever is more
H. Platelets, Leukocytes reduced:	
Residual leukocytes:<8.3 X 10^{5} in random units tested and retain a minimum of 85% of original platelets in the component.	Monthly/test at least 1% of components produced or 4 units, whichever is more
I. Platelets, pheresis, Leukocytes reduced:	
Residual leukocyte count of $<5x10^{6}$ in each container tested and retain a minimum of 85% of original platelets in the component.	Monthly/test at least 1% of components produced or 4 units, whichever is more
VI. Miscellaneous:	
A. Copper sulphate specific gravity	Day of use
B. Shipping containers for blood transport (usually at temperature extremes).	Biannually

6. Identification and traceability:

a. Trace unit from donor to final disposition.

b. In cases of adverse events or lock-back.

c. Maintain records.

d. Hard copy of records can be supplied during inspection.

B. FINAL INSPECTION AND TESTING:

Before issue review records of:

a. Preparation.

b. Testing.

c. Q.C records.

C. HANDLING,STORAGE, DISTRIBUTION AND TRANSPORT:

a. Storage: Documentation of critical storage temperatures.

b. Shipping: Q.C. for:

i. Blood product.

ii. Transport containers.

c. Returned blood:

i. Storage temperatures.

ii. Date and time received.
iii. Inspection on receive.

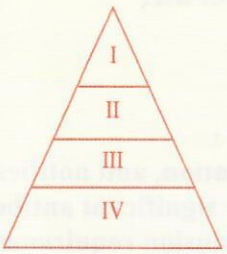
6. DOCUMENTS AND RECRODS

A. Documents:

1. ISO Documentation Hierarchy:

(NCCLS) and (AABB) depend on ISO 9001 model for maintaining documents

Policies
Processes
Procedures



Forms, records data.

Level I: Policies:

a. What to do?
b. Time independent.
c. Brief but conclusive.
d. Mission statement.
e. Written by management.

Level II: Process:

How it happens?
Example: Action: Supplier issues
Responsible party: Purchasing department.
Document: Contract.

Level III: Procedures:

How to do it?
Example: SOP:
Detailed but easy
For testing, QC, other operation
Available in very work place

Level IV:

Blank form, data sheets
IF filled, become records

2. Document development:

a. Develop SOP for:
   i. SOP.
   ii. SOP review.
   iii. Procedures changes.
b. Procedures for record review, maintenance and disposition.
c. QA unit management review and approve procedures.

3. Review and revision of procedures:

a. SOP-new regulation.
b. Control for SOP.

B. Records:

1. Archived or microfiched.
2. Confidentiality.
3. Easily retrieved.

4. Altered or corrected, remain legible.

5. Record storage:

a. Protected from damage.
b. Accessibility.
c. Adequate space.
d.Viewer for microfiched products.
e. Original color coded records.

6. Computer record storage:

a. Accuracy.
b. Security: User LD., Signature, date of use.
c. Backup disc or tape.
d. Periodical check.

7. Record retention policy:

a. Indefinite retention:

1. Indefinitely deferred donors information, and notification.
2.Blood group discrepancies, clinically significant antibodies and adverse Reactions to transfusion,special transfusion requirements and resolution of discrepancies.

b. 10 Years Retention:

1. Complete Blood donor information including and not limited to educational material, parental permission, and donor consent.
2. Complete records and information for all collected Blood and prepared Components either by manual or apheresis techniques.
3. Facility information who prepare components or perform testing.
4. Final disposition of each unit and ID of recipient.
5. Notification to hospitals about transfused blood which found and confirmed Positive to TTD.
6. Documents of quarantine of positive units to TTD.
7.Notification of recipients of potential exposure to TTD.
8. Names, ID and signatures of authorized personnel to collect, separate,test, issue,transport , receive ,carantine or review reports and records.
9. ABO group and Rh type and antibody screening and identification for all donors and recipients .
10.Inspection of incoming blood and components.
11.All Physician requests for therapeutic phelebotomy,autologus donation,blood or components without crossmatching or TTD screening .
12. Traceability of blood and blood components.
13.Unique identification of each blood unit.
14.Notification of donor of significant abnormal findings.
15.Apheresis complete records.
16.Inspection report of weld for component preparation.
17. Verification of irradiation dose delivery for cellular componenets.
18. Quality control report for testing methods, acceptability criteria, quarantined units, equipment qualification , equipment validation, equipment maintenance, and temperature monitoring charts.
19. Donors and recipients adverse events and lock back investigations.
20. Serologic crossmatching,ABO incompatibility,Two determination of recipients ABO grouping, Computer crossmatching, ABO/Rh for neonates with

significant antibodies.

21. Final inspection of blood and components before issue and records of transfusion service.
22. Inspection records prior to shipping.

C. 5 Years Retention :

1. Medical approval of autologous blood to allogenic recipient.
2. Complete information about therapeutic apheresis.
3. All Physician requests for blood and /or blood componenets.
4. Quality control reports for the method of testing.
5. Patient previous (last 12 month) ABO/Rh. And resolution of discripancies if any.
6. Patient complete medical records , identification and consent.
7. Suspected transfusion reactions,laboratory evaluation and interpretation.
8. Personnel records as job description, qualification ,training and competency.
9. Quality control records review , exception to or new policy,selection of suppliers, agreement review , inspection of supplies ,validation of new procedures and proficiency testing.
10. Annual review of policies, nonconformances, assessment results, Peer review,corrective actions, preventive actions.
11. Safety records for biologicals, chemicals, and irradiation.

d. 2 Year Retention:

1. Records of Implementaion of Information Technology System (after system retirement).
2. Records of Validation of Information Technology System (after system retirement).

e. Temporary retention:

1. Temporary deferred donors for deferral period.

7 - INCIDENTS, ERRORS AND ACCIDENTS

1. Investigation, classification and documentation:

a. Prameters to identify if it is reportable or not.
b.One time event or frequent.
c. Affect patient safety or not

2. CQI Team:

a.Brain storming.
b.Causing and effect diagram, root cause analysis
Fish bone diagram
i-Manpower ii-Machinery
iii-Monitors iv- Materials v- Methods
c. Corrective action.
d. Monitoring.

3. Examples:

a. Blood product fail QC.
b. Improper computer function.
c. Medical adverse events:
i.Transfusion transmitted diseases.
Ii. Hemolytic transfusion reaction due to ABO incompatibility.

4. Report of incident:

1. By telephone or email within one working day of incident.

2. Written report within 7 days.
3. Include new procedure to avoid reoccurrence.

8 - ASSESSMENTS

Definition:

Systematic, independent evaluation at sufficient frequency to prove the efficiency of procedures for achievement of the objectives. (Either external or internal).

1. External assessment:

a. Who: FDA, AABB, CAP or MOH .
b. How:
i.Field visit to facility.
ii.Check list which cover all areas.
iii. Facility procedures:
- Greet assessors.
- Responsible person to accompany assessors.
- What information, in which form can be given.
- Delineation of staff responsibilities.
iv. Assessment report discussed in an open meeting with facility staff.

2. Internal assessment:

a. Who: QA unit, blood usage review committee, peer review.
b. How:
i.Check list, and productivity measurement.
ii.Guidelines for blood product utilization.
iii. Maximum surgical blood ordering schedule.
iv. Transfusion audit:
a.30 cases or 5% of total transfusion.
b. Evaluate usage:
- Blood request: Measurable
- Clinical assessment: individual judgment
c. Transfusion reactions.
d. Policies and procedures for handling, use and administration.
e. Blood ordering practices.
f. Meet patients needs.
g. Look back for HIV transmission.
h. Follow blood unit from issue to transfusion.
v. Document and submit report to management.

9 - PROCESS IMPROVEMENT

l. Review of:
a. External or internal assessment reports.
b. Customer complaints.
c. Error or accidents reports.
d. Training programs for personnel.
2. Data collection and analysis by statistical tools.
3. Identification of inappropriate practice.
4. Discussion with:
a. Hospital administration.

b. Legal office.
c. Medical staff.
d. Blood bank staff.

5. Corrective action:
   a.Educational not punitive.
   b.Time frame.
   c. Reports:
      - Patient, physician or BB staff name.
      - (or) Anonymous statistical summary.
   d.Written notice to responsible person or party.
   e. Encourage response from responsible party.

6. Yearly report Form QA unit to management which include:
   a. Error analysis.
   b. Adverse reactions.
   c. QC records.
   d. Proficiency test results.
   e.Corrective and preventive actions.
   f. Recommendations for improvements.

10-SAFETY

A. General safety principles:

1. Safety program: Procedures for all safety issues.
2. Workplace:
   i. Design.
   ii. House keeping.
   iii. Restricted areas.
3. Safety training.
4. Personal protective equipments:
   i. Lab coats.
   ii. Gloves, masks, goggles, shields.
   iii. Biological safety cabinets.
   iv. Fume hoods.
   v. Hand washing, eye washers and showers.
5. Hepatitis B vaccination.
6. Accidents and injuries: Reports - Follow up.
7. c GMP requirements:
   i. Adequate place and ventilation.
   ii. Sanitation and trash disposal.
   iii. Control of:
      -Air pressure.
      - Humidity.
      - Temperature.
   iv. Water system.
   v. Toilets and hand- washing facilities.

B. Biosafetv:

a. Biosafety in BB. Classified as Biosafety level 2.
b. Biosafety level 2 precautions:
   1. Segregation between high and low risk areas.
   2. Bench top covered with absorbent papers.

3. Closable doors and sinks.
4. Use of safety protection equipment.
5. No mouth pipetting, eating, smoking, drinking, cosmetic applications or contact lenses manipulation.
6. Blood Spills:
    i. Evacuate area for 30 minutes.
    ii. Wear gowns and gloves.
    iii. Clean with detergents.
    iv. Flood the area with disinfectant.
    vi. Dispose all materials as a biological hazardous waste.
7. Bio-hazardous waste disposal:
    i. Red plastic bags or containers with biohazard symbol.
    ii. Double bag during storage or transport.
    iii. Rigid, puncture lead proof containers for sharps.
    iv. Infectious or medical waste:
        - Not compacted.
        - Decontaminated before disposal.
        - Disposal by:
            1. Autoclaving.
            2. (or) Incineration.
8. Secure closing of centrifuge or containers lids.
9. Decontaminate equipments.
10. Treat all blood samples as potentially infectious. 11. Report incidents immediately.

C. Chemical Safety:

1. List of hazardous chemicals and their specific hazard.
2. Proper labeling.
3. Store in chemical cabinets.
4. Transport by hand truks.
5. Check leaks with soapy water.
6. Liquid nitrogen tanks securely supported.
7. Liquid nitrogen: Use Goggles, boots and leather gloves.

D. Radiation safety: For BB Irradiators.

1. Manufacturer responsibility for:
    i. Transportation.
    ii. Installation.
    iii. Validation.
2. SOP availability.
3. Location:
    - Limited access.
    - Fire detection alarm system.
4. No threat to users.
5. Q.C.

E. Fire safety:

I. Fire protection systems:

a. Alarms.
b. Smoke detectors.
c. Sprinklers.

2. Fire extinguising system:
   a. Fire hoses.
   b.Fire extinguishers.
3. Secondary exits.
4. Fire walls, and fire doors.
5. Inspection of fire equipments.
6. Training:
   i. Use of fire hoses and extinguishers.
   ii. Report of unsafe situation.
   iii. Stop, drop and roll in clothing fire.
   iv. Evacuation policy.
   v. Annual fire drills.

F. Disaster plan:

1. Communication: notify supervisor immediately.
2. Evacuation.
3. Safety.
4. Safety minded employees and awareness.

PART FIVE: BLOOD BANKS AND BLOOD TRANSFUSION RELATED COMMITTEES

1. The National Committee for Blood Transfusion:

Most countries have organized a high standard committee under the name of the National Committee of Blood Banks and Blood Transfusion. The president of this Committee is usually a well recognized person in the country especially if this person has a very good reputation in participating in different humanitarian acts related to the medical field. This committee must include in its membership the following: -

- One representing the Ministry of Health.
- Director of Blood Transfusion & Research Services in ministry of health.
- Director of the Department of Health and Medical Services in ministry of health.
- Medical Directors of each University hospital blood banks.
- One representing The Red Crescent Society.
- One representing Military blood banks.
- One representing Police blood banks.
- One representing Private sector of blood banks.
- One representing Ministry of Media and Culture.
- One representing Ministry of Justice and Islamic Affair.

The Job Description For This Committee Includes:

1. Proper health education about blood transfusion to the public.
2. To create a relation between the blood bank centers and the people of different categories .
3. To establish the regulations for proper and healthy blood donation act taking the religious aspect and the governmental rules in consideration.
4. To establish a uniformed regulatory system for blood donation and blood transfusion for the different blood bank centers which are under different directorates such as Ministry of Health, Army Forces.

5. To unify the followed policy for detecting and screening the transmittable diseases in the different directorates and to be regarded as a national policy to be followed in the country.
6. To work hardly for supporting and arranging a highly standard blood donation campaigns.
7. Exchange medical and technical experiences in the field of blood banking and transfusion between the different directorates; to offer the best medical services to the public.
8. To establish Medical Researches and studies in which the different directorates are participating.
9. Uniform the scientific guidelines for blood transfusion in the hospitals related to the directorates that are participating in this committee.

2. Blood Transfusion Committee in Hospitals

Each hospital in the country has to organize a committee called The Blood Transfusion Committee. This Committee will be under the supervision of the Medical Director of the hospital, and should include the following members: -

1. Head of the Anesthesia Department.
2. Head of Laboratory (A Hematologist doctor).
3. Head of Emergency Department.
4. Head of Surgical Department.
5. Head of Internal Medicine Department.
6. Head of Nursing Department.
7. Head of Quality Control.
8. In -charge of Blood Bank in the hospital.

The Job Description For this Committee is: -

1. To establish the guidelines for the proper transfusion of blood and its components (they should be keen to follow the guidelines given by the directorate of Blood Banks).
2. To observe the blood transfusion practice in the hospital for the different medical cases and to look for the indications for ordering and transfusing blood to each patient.
3. To minimize the use of blood and its components and try to find substitutions as blood transfusion should be the last line for treatment.
4. Create and support the relation between the doctors and the nursing staff of the hospital and between the relatives and friends of the patients, by encouraging them to donate blood for the patient with the cooperation of the blood bank section in the hospital
5. To encourage the Autologus blood donation system for the patients with the cooperation of directorate of blood banks and blood transfusion services.
6. Try to establish a vital and active system for having enough blood stock is to cover the emergencies in the hospitals.
7. Supervise and revise (blood transfusion request form) frequently. This can be done by reviewing the patients files and checking the information written in the blood transfusion request form to be sure of the real need of the patient for the transfusion.
8. To avoid transfusing whole blood unit to the patients unless it is indicated and should encourage doctors to use the blood components instead according to the case. Leukocytes filtered units should be used when they are indicated.
9. To supervise the work in the blood bank section in the hospital, to ensure that this section is following the rules and regulations and the correct information are registered in the records. Also, to supervise the quality assurance and quality control system in

this section specially for the chemical reagents , equipment , and the medical instruments used according to the instructions of the directorate of blood banks .

10. To perform regular meetings weekly or every 15 days to follow up the work in the blood transfusion section in the hospital trying to offer the best services to the patients.
11. To have a vital and direct coordination between the committee and the blood banks directorate , and to inform about any emergency or any medical mistake immediately related to blood transfusion to the directorate of blood banks without any delay or hesitation .
12. Do a comparison between the number of cross match requests and the number of the transfused units. This rate must not exceed 2 to 1, and if it is more than that, so the requests for cross matching test must be controlled.
13. Register the comments of every meeting of the committee and send a copy of it to the
14. department of blood banks for consideration and follow up.

3. Quality Assurance Committees

Most countries have organized a committee entitled the quality control committee to supervise the blood banks in the hospitals. The president of this committee is the Director of Blood Banks Directorate in that country and the members includes the Following: -

1. Specialist in the field of pathology and blood transfusion.
2. The chief technician in the National blood bank center.
3. Head of laboratory in the National blood bank center.
4. In-charge of Quality Control in the directorate of blood banks.
5. In-charge of Mobile blood bank in the Directorate of Blood Banks.

The Job Description for this Committee include:-

1. Regular visit (every 3 months) to all the branches of blood bank centers in the country to observe work in these blood banks.
2. To supervise the quality assurance and quality control system in each blood bank, and to see to what limits they following the Federal guidelines for the Quality Assurance that has been established by the directorate of blood banks.
3. Give the instructions and advise to correct the mistakes which might be noticed by the committee during their visit to the blood banks.
4. To control the measures taken for preventing infectious diseases in the blood bank.
5. To discuss with the In-charge of blood bank different subjects regarding the work in the blood bank and how it is going, and to evaluate his abilities in managing the work and improving it forward .
6. Supervise the records and the information registered in the computer system which is related to the blood donors and blood units and the results of the tests, these information should be registered accurately.
7. To choose randomly any of the (cross matching request form for blood transfusion) to check if the information in it are correct and complete .
8. Supervise assess the blood transfusion in the hospital and do a comparison between the cross matching requests and the rate of blood units transfused , the rate must not exceed 2 to 1 . If the rate is more than that, the requests for cross-matching which is not needed must be reduced by the coordination between the In-charge of blood bank and the blood transfusion committee in the hospital.
9. Create a good connection between this committee and the blood bank
10. Committee in the hospital concerning the scientific blood transfusion rules.

11. **Supervise the work out of the machines and medical equipment, its efficiency and regular maintenance.**
12. **Specify the needs of each blood bank for equipment and medical items to improve the services of this blood bank to the best, and send these requests to the head of the committee.**
13. **Write a report of every visit, and handle it to the head of the committee. A copy of it must be send to the director of the hospital, to the In-charge of the blood bank and a copy to be saved in the file of the inspection committee.**

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