DBBT

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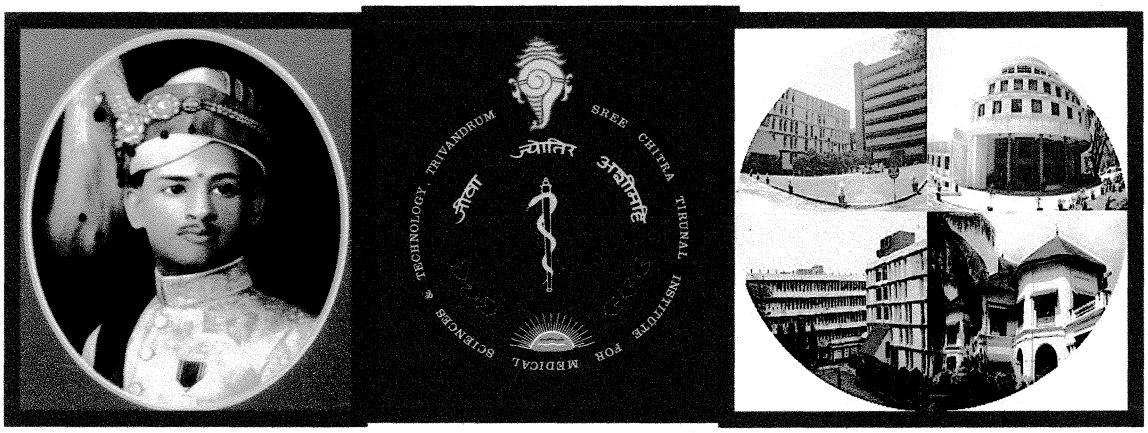

DIPLOMA IN BLOOD BANKING TECHNOLOGY

Work book submitted by:

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SCTIMST
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and junior DBBT students for their co-operation.

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

CERTIFICATE

I Mr. **SHIBIN.C.V** here by declare that I have actually performed all the procedures listed under the course of Diploma in Blood Banking Technology (DBBT)

Signature
Name SHIBIN.C.V

Place : Thiruvananthapuram
Date : 01/12/06

Signature,

Head
Dept of Blood Transfusion Services

AKNOWLEDGEMENT

First and most I would like o thank my Head of the Department Dr. Jaisy Mathi and Dr Sulochana PV, Dr.Sathyabhama.S and all other members who had guided me through the different phases of my studies, encouraged and helped me on all aspects of my training

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I extend my heartful thanks to Philomina Augustion the Chief Technician and all other staff of blood bank and I am thankful to the donors who where the core medium of my study

At last I would like to acknowledge my sincere thanks to senior and junior DBBT students for their co- operation at department and in studies

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HAEMOGLOBIN TESTING BY COPPER SULPHATE METHOD

SPECIFIC GRAVITY OF BLOOD

Specific gravity of blood is defined as the ratio of the weight of the blood to the weight of the same volume of water at a temperature 4°C.

Normal specific gravity is-	1052 to1063
Average for men-	1057
Average for women-	1053

Specific gravity of serum is- 1026 to 1031

Specific gravity of red cell is- 1092 to1095

There is a normal variation of 0.003, being lower in the afternoon and higher at night; specific gravity is lower after meals and higher after exercise

Specific gravity depends upon

1. The quantity of hemoglobin present in the red cells
2. The plasma protein levels

DETERMINATION OF SPECIFIC GRAVITY

COPPERSULPHATE METHOD

The method is based on specific gravity and is a reasonably reliable method for determining the hemoglobin of the blood donor, it is indirect measure of hemoglobin value.

The procedure consists of letting a drop of blood fall in to a graded series of copper sulphate solution of varying strength (known specific gravity) and noting whether the drop falls or rise in the solution. If the drop of blood is lighter than the solution it will rise perhaps only for a few seconds and then sink down. If it is of the same specific gravity, it will become stationary for this interval and then fall. If the drop is heavier, it will continue to fall during this interval

Each drop of blood on entering the copper sulphate become encased in a sac of copper proteinate and remains as discrete drop with out change of specific gravity for 15 to 20 seconds. So the behavior of the drop at this 15 to 20 seconds is considered.

TECHNIQUE

Preparation of stock solution

159.63 grams of copper sulphate crystals is added to distilled water, shaken vigorously for 5 minutes, and made-up to one liter. The solution is decanted off and filtered through a filter paper in to a clean and dry bottle. This is the stock solution. It must be kept at 4°C can be stored up to 6 months to 1 year. From this stock solution which has a specific gravity 1100, solutions of varying specific gravity can be prepared.

For determinating of specific gravity of blood a series of tube containing the solution varying in specific gravity of 0.002 may used A drop blood is dropped one centimeter above the column of solution and the behavior of the drop in the 15 to 20 seconds is noted. The solution in which the drop become stationary at this time corresponds to the specific gravity of the blood

FOR SCREENING OF BLOOD DONORS

Since the specific gravity of blood is dependent upon the haemoglobin content of the red cell, the determination of Hb% by copper sulphate solution technique has been advised by Van slyke and Philip et al to determine the suitability of the blood donors for donation.

PHILIP, VANSLYKES COPPER SULPHATE TECHNIQUE

The stock solution prepared as detailed above is used to 522.5ml of stock solution and 477.5ml distilled water. This corresponds to 1053 specific gravity and12.5 gram Hbpercentage. Male = 522.5cs + 477.5dw 1000 1053 12.5 gram%

Since the male volunteers can be accepted for donation if they have a Hb% 12.5 gram and above, this solution can be used for screening them. A drop of blood taken by finger prick is allowed to fall in to the solution and its behavior is noted. If the specific gravity is lesser than the solution, the donor can be rejected.

Since the females have a lower Hb% and hence lesser specific gravity, the minimum set Hb% acceptable for blood donation is also 12.5 gram that corresponds to the specific gravity 1053.

Advantage of this method in mass screening

1. This method is simple
2. The temperature coefficient of expansion of $CuSO_4$ and blood are equal, so there is no need for separate temperature regulation.
3. The substance used are not toxic and do not give explosive vapors.
4. Dispersion of blood in copper sulphate solution is low and visualization is easy
5. The copper sulphate solution cleans automatically by itself.

6 After a few minutes, the drop settles to the bottom and it can be removed and the solution can be used again

Quality control

The test method is inaccurate but it is-

1. Fast
2. Safe

The method is very good as long as person performing the technique is meticulous in technique

The first drop of blood from the finger must be wiped as it may contain the detergents, the second drop must be used for test.

The capillary tube should be filled with out air bubbles.

The drop must be dropped from a height of 1 cm above the surface the solution, if it is dropped from too high, it may break up to small fragments or if dropped from low it may not pass down the surface at all

The copper sulphate solution should be kept tightly capped before and after use and should be changed frequently while being used so that it does not have a chance to evaporate and concentrate and there by causing the donors to rejected unnecessary

A solution at 2.0l, kept in working bottles should be used only for 30-50 tests

All bottles should be emptied twice daily and the blood discarded and the solution replaced.

The specific gravity of solution should be verified after preparation. It can be done by using a specific gravity hydrometer or weighing a measured volume of solution and from that calculating the specific gravity.

Sl No	Date	Name of donor	Age	Sex	Hemoglobin
1	1/8/06	Beena	37	F	Hb 7/12.5gm
2	1/8/06	Sujatha	36	F	Hb 12.5dl
3	8/8/06	Sonilal	19	M	Hb > 12.5g
4	9/8/06	Joseph Henry	40	F	Hb < 12.5dl
5	10/8/06	Soman	55	F	Hb > 12.5dl
6	13/8/06	Sabna	23	F	Hb > 12.5dl
7	14/8/06	Binder	22	F	Hb > 12.5gm
8	15/8/06	Fazal Abbas	29	M	Hb > 12.5gm
9	15/8/06	Manjulal. J	29	F	Hb > 12.5dl
10	16/8/06	Swapna	22	F	Hb > 12.5dl

HAEMOGLOBIN ESTIMATION BY COLOUR SCALE

The haemoglobin colour scale is a simple device for estimating haemoglobin,it is intended for use when laboratory haemoglobinometry is not available The colour scale consist of book let containing a set of six shades of red and a pack of special absorbent test strips
The represent arrange of haemoglobin values from 4 to 14 gm /dl by matching the colour of one drop blood on test strip with one of the shade of stripes

Using of haemogl obin colour scale

- Add a drop of blood to one end of test strip – just enough to completely cover the aperture of the scale
- Wait about 30 seconds then read immediately by comparing of blood stain in the colour scale to find the best colour matched
- Keep the test strip close to the back of the colour scale
- Avoid direct sun light
- Avoid marked shade
- If the blood stain matches one of the shades of red exactly re the haemoglobimn value ,colour lies two values, record the mid value iscard test strip after use

HEMOGLOBIN ESTIMATION BY CYANMETHMOGLOBIN METHOD

PRINCIPLE

The basic principle of the method is dilution of blood in a solution containing potassium cyanide and potassium ferricyanide. Hb, Hi and HbCo are all converted to HICN (cyanmethmoglobin). The absorbance of the solution is then measured in a photoelectric calorimeter at a wave length of 540nm ±15nm.

BLOOD SAMPLE:

1) Venous blood or free flowing capillary blood added to any solid anticoagulant (1 mg EDTA/ml) can be used. Measurement can be carried on blood, which has been stored at 4°c.
2) Fresh capillary blood can be used if added immediately to reagent solution.

Reagent (diluent)

Modified drabkins reagent

Potassium ferric cyanide.....200 mg

Potassium cyanide..... 50 mg

Potassium dihydrogen phosphate .....140 ml

Nomidate PHO ..... 1 ml

Distilled water .....1 L

The reagent should be clear and pale yellow in colou.r

When measured as blank in photoelectric calorimeter at a wavelength of 540 nm the absorbance must read zero.

REAGENT PREPARATION

Dilute 50 ml drabkins solution up to 100 ml with distilled water, mix well. The prepared reagent is stable for at least 2 months at room temperature. Protect prepared reagent from light.

METHOD:

1) Switch on photoelectric calorimeter and wait for 15 to 20 minutes to warm before use.
2) Add 0.02 ml of blood to 5 ml of diluent (drabkins). Stopper the tube containing solution and invert it several times. Allow it to stand at room temperature for 5 to 10 minutes to ensure the completion of the reaction. The solution of HICN is ready to be compared with standard.
3) Select filter of wave length 540 nm.
4) Set the colorimeter at zero against blank
5) Measure the absorbance of standard solution in the calorimeter against the blank.
6) Observe the absorbance value of the test solution prepared as in step 2

INTERPRETATION:

1. Record the absorbance value directly in the calorimeter calibrated for the direct reading of Hb in gm/dl.
2. If the calorimeter not meant for taking direct reading of hemoglobin g/dl record the optical density reading and hemoglobin can be calculated from the following formula

$$\text{Hb g/dl} = \frac{\text{od the test}}{\text{OD of the standard}} \times \text{conof the standard solution}$$

PRECAUTIONS

1. Blood should not be clotted
2. The reagent should be discarded if it become turbid
3. The mixture of blood and reagent should be clear. Turbidity is due to contamination and gives false result.

4. Pipette should be accurate to take 20 micro liter blood.
5. Standard solution should be discarded at the end of the day on which ampule is opened

Hb in Cyanmethemoglobin method

Sl no	Date	Name	Age	Sex	Hemoglobin gm/dl
1	4/1/05	Seena	43	F	12.2 g/dl
2	4/1/05	Mariya Jacob	44	F	11.4 g/dl
3	4/1/05	Sumathi	27	F	11.5 g/dl
4	4/1/05	Ajith	26	M	13.2 g/dl
5	6/1/05	Mansoor	22	M	15.5 g/dl
6	6/1/05	Soosan Thomas	47	M	12.7 g/dl
7	12/1/05	R. J. Thomas	48	M	11.4 g/dl
8	19/1/05	Divya.	19	F	12.7 g/dl
9	21/1/05	Riyas	26	M	14.2 g/dl
10	22/1/05	Sreesa	22	F	13.5 g/dl

BLOOD COLLECTION

METHOD

The collection of blood from the donor shall be by aseptic method using a sterile closed system by single venipuncture.

MATERIALS

1.blood collection bags 350 to 450 ml capacity (single, double, triple, quadruple) containing CPD or CPDA-1 as anticoagulant preservative solution
2.sphygmomanometer
3.stethescope
4.test tubes for sample collection
5. Test tube racks
6.cotton wool swabs
7.scales for weighing the blood collected in the blood bag
8. Blood collection monitor
9. Tube sealer
10.artery forceps and scissors
11.antiseptic solutions
12.band-aids
13.syringes and needles
14emergency drugs
15labels

PROCEDURE TO COLLECT BLOOD

Inspection

The blood containers (blood bags) should be inspected for defects prior to as well as after blood collection, these include expiry date on label, appearance of anti coagulant solution, abnormal moisture, or discoloration of the bag or on the label which suggests a leak, kinking of tube etc. if one or more abnormally damp in that package should be rejected

Labeling

The identity of the donor should be checked with that donor record form sticker labels with unit number are pasted on donor card and blood bag.

Preparation of the phlebotomy site

Cleaning of the phlebotomy site is done with betadine first and then with the surgical spirit. Cleaning using sterile cotton is done in circles away from the center and allowing the antiseptic solution dry completely before venipuncure. At no time should fingers of the phlebotomist touch the sterile application prepared area of the skin or the sterile needle

Venipuncture

Pressure inflated and pressure is kept at 60 to 70 mm hg .A single Venipuncture and minimum trauma to the tissues should be ensured to prevent partial activation of coagulation system.
As soon as blood starts flowing it must immediately come in contact with the anticoagulant. The gentle inverting the bag continuously must ensure proper mixing of the blood with the anticoagulant. Automated blood shakers can be used for this purpose. Blood should be collected maintaining uninterrupted flow with a period of 8 to 10 minutes.
When adequate amount of blood is collected in the bag, putting a tight knot in the tube interrupts flow and tube is cut between clamps. Bp cuff pressure is released and the needle is removed from the vein the donor is asked to fold the elbow keeping the sterile gauze at the venipuncture site. The blood remaining in the tube is transferred to pilot tubes, which are labeled Clotted and anticoagulant samples. The needle is cut from the tube and discarded in to a puncture proof container containing disinfectant
The tubing attached to the bag is stripped and refilled with anticoagulant blood .the tubing is sealed by tube sealer. Then the bag is placed in a refrigerator intended for blood storage with temperature monitoring

DONOR REACTION AND MANAGEMENT

SYNCOPE: (VASOVAGAL SYNDROME):

This may be caused by physiological factors like sight of blood, excitement or other unexpected reasons or a neuropsychological response to blood donation. Symptoms such include dizziness, weakness, nausea, sweating, vomiting, pallor or in extreme cases loss of consciousness and involuntary passage of urine and feces.

In the lying down position, a syncopeal attack may be missed. So ask the donor for a feeling of dizziness or tingling sensation of the fingers, the skin feels cold and blood pressure falls. Sometimes the systolic level being as low as 50mmHg. The pulse rate is weak often slows significantly in a vasovagal attack.

MANAGEMENT:

Management includes raising foot and lowers head end. Loosening tight clothing to ensure adequate air way. Checking pulse and blood pressure applying cold compressor to forehead.

Delayed syncopial attacks may occur as late as 30minutes to 1 hour after donation, usually after donor has left the blood bank. A donor who gives history of such attacks more than twice should be permanently deferred.

Occasionally donor may vomit after a syncope attack and is subsides by its own. For hyperventilation and neuromuscular excitability like tetany, donor should be asked to breath into a paper bag.

Local complication due phlebotomy .eg:bleeding and sub cutaniouse haematoma are taken care of by firm pressure with thumb,raising arm above theleval of heart and applying ice .if haematoma is seen latter as a blackish discoloration around phlebotomy site after 24 hrs . it is painless and self limiting apply thrombophob and reassure the donor.

POST DONATION CARE OF DONORS

Donor should be kept under continues observation during and after donation for at least 15 minutes the donor should be laid down for at least 10 minutes after donation after he or she may allowed to sit, watch out for signs of postural hypotension like dizziness ,swetting,pallor ,etc .generally 3 to 4 minutes of manual pressure suffices bleeding after which a medicated plaster is applied at venipancture site

Post donation instructions

1) Ask the donor to avoid strenuous exercise for few hours
2) Ask the donor to Take more oral fluids
3) Ask the donor to remove the plaster after six hours

ABO GROUPING AND Rh TYPING

FUNCTIONS:

To test the antigens antibodies in the sample

SCOPE AND APPLICATION:

For safe transfusion

PROCEDURE:

Cell grouping:

1.take 3 test tubes labeled as A,B and AB

2.place one drop of corresponding anti serum in to each tube

3.add one drop of 2 to 5%cell suspension from test sample either in saline or plasma .mix well keep at room temperature

4.read after 15 –30 minutes agglutination or haemolysis indicate positive reaction and free cell suspension indicates negative results.

SERUM GROUPING:

ABO cell reagents are prepared by pooling 2 samples of each group and washing them in normal saline for three times to make at 2 to 5 %cell suspension

Procedure

1 Place one drop of test serum in to A,B and O labeled test tubes.

2.Put one drop of A, Band o cells corresponding tubes.

3.Mix the tubes and keep at room temperature. Read after 15-30 minutes.

4.Examine the tubes; hemolysis or agglutination indicates positive results .

(Reactions-cell grouping)

Anti-A	Anti-B	Anti-AB	Results
+	O	+	A
O	+	+	B
O	O	O	O

(Reactions – serum grouping)

A Cell	B Cell	OCell	Results
0	+	0	A
+	0	0	B
+	+	O	O
+	+	+	Oh

Grading of results

One solid aggregate red cells ------- +4

Several large aggregate red cells --- +3

Medium sized aggregate of cells--- +2

Small aggregates of cells with reddish back ground ---+

Very small aggregates seen microscopically----+w

No aggregates---------- 0

Rh GROUPING

1. Place one drop of anti-D serum a clean test tube
2. Add one drop of 2- 5% cell suspension from test tubes
3. Mix and incubate at room temperature for 30 minutes and then read, agglutination shows positive results .If the test is negative, it should tested for weak positive

Du TESTING

1. Incubate one drop of test rbc (2 to 5%) in a clean labeled test tube along with polyclonal or blend anti D at 37^0C for 30- 60 minutes
2. After incubation read microscopically for agglutination
3. If negative, wash, decant the saline completely Repeat the procedure thrice
4. After final wash, decant the saline completely
5. Then add one drop of poly specific AHG mix gently
6. Centrifuge at 1000 rpm for one minute , read microscopically
6. If the results is negative, add one drop of IgG coated cells and centrifuge. Positive results indicate correct procedure, if not repeat the tests.

Cell Grouping

Anti -A	Anti-B	Anti-D	INCUBATION	AHG
+3	+2	0	0	+2

A CELL	B CELL	O CELL
O	O	O

Serum grouping –AB weak +ve

ANTI HUMAN GLOBULUIN TEST

The antiglobulin test popularly known as coombs test

PRINCIPLE

Red blood cell coated with IgG or C3d component of compliment eitherin vivo as in DAT or in vitro as in IAT will be agglutinated by anti- human globulin (AHG) reagent binding to the IgG antibodies coating the cells.

Two types of anti globulin reagents are available

1)polyspecific AHG reagent (contain IgG and C3d)

2)monospecific AHG reagent(contains either IgG or compliment component)

Preparation of IgG coated positive control cells

Dilute Anti- D 1:50 ratio in saline and mix to equal quality of 5% pooled washed O cell suspension in saline .Incubate at 37 0 C FOR one hour . Wash three times in excess saline and suspended in saline.

IN DIRECT ANTI GLOBULIN TEST

In direct anti globulin test is done for the following purposes :

1) Compatibility test
2) Screening and identification of un expected anti body in serum of Patient
3) Detection of red cell antigen using specific antibody reaction only in antiglobulin test that is FY^a ,FY^b,K,JK^a,JK^b,and for weak positive testing.

METHOD

1) Take two drops of serum to be tested in a labeled tube.
2) Add one drop 2 –n 4 % suspension of reagent O cells.
3) Incubate at 37oc for 45 to 60 minutes.
4) Look for haemolysis or agglutination. Agglutination or haemolysis in this stage presence of saline reacting antibody.

5)If no agglutination is seen wash the cell three times in the saline and decant the last wash completely.
6) Add one drop of AHG reagent to the washed cell and mix.
7) Centrifuge the tube at 1000 rpm for one minute.

8) Gently shake tube and look for agglutination using optiocal lens.Microscopically
9)Record the results.
10) If negative add one drop of IgG coated cells.
11) Mix and centrifuge at 1000,rpm for one minute.
12) Look for agglutination if no agglutination is seen test is in valid and the whole Procedure is repeated.

CONTROLS

For positive control one drop diluted Anti D + one drop O positive washed cell keep at 37^0 C for one hour. After one hour wash three times and proceed to AHG phase

For negative control one drop diluted Anti D + one drop O neg washed cell (2 to 5 %) cells keep in 37^0 C for one hour wash three times and proceed for
AHG phase

PREPARATIONS OF COMPONENTS

RED CELL CONCENTRATE

DEFINITION

A component obtaining by partial removal of plasma from WB with out further processing.

FUNCTION

As blood cells, plasma and platelets have different specific gravities, they can be separated from one another by centrifugation at different centrifugal force for variable time or red cell settle down when anti coagulated blood is kept un disturbed enabling separation of plasma and cells.

Scope and application

Symptomatic anemia, acute or chronic for restoration of oxygen carrying capacity, less blood group antibodies in packed cell, less plasma proteins with packed cells. So there is minimum anaphylactic reaction.

PROCEDURE

Prepare either by sedimentation or centrifugation

1) **Sedimentation**: The blood is kept at 4^0C in an up right position undisturbed, separate the plasma when clear in to a satellite bag.The red cells have a pcv of 60 to 70%.

2) **Centrifugation**: a) Centrifuge the collected blood in a refrigerated centrifuge at 3000 rpm or 10 minutes at 4 to 6^oC.
b) Express approximately ¾ of plasma in to the satellite bag.
c) Double seal the tube between primary and satellite bag with pcv not higher than 80%.
d) Label the plasma and kept at -40^0C r below .
e) Expiry of red cell concentrate as same as that of whole blood .

LEUCOCYTE POOR RBC CONCENTRATE

FUNCTION

As blood cells, plasma and platelets have different specific gravity, they can be separated from one another by centrifugation at different centrifugal forces for variable time or red cells settle down when anti coagulated blood is kept un disturbed enabling separation of plasma and cells.

Scope and application

Symptomatic anemia, indicated in patients with repeated non hemolytic febrile transfusion reaction due to leucocytes as in transfusion dependant patients and multiparous women.

PROCEDURE

There are many procedures for preparing leucocytes poor red cells, all having varying effectiveness.

1) Centrifugation and removal of buffy coat.
2) Centrifugation and washing of red cells.
3) Spin and filtration.
4) Freezing and deglyceralisation .(not routinely performed)

Centrifugation and removal of buffy coat

Inverted spin:

1) Whole blood or rbc centrifuged in and inverted position at 3000 rpm for 10 minutes at 4^0c.
2) Suspend the bag in an inverted position on an IV stand and release the seal.
3) Collect the leucocyte poor red cell in the second satellite bag leaving about 70 to 90 ml buffycoat layer mixed with RBC and plasma in primary bag.
4) Double seal and label the bag.

Upright spin :

1. Whole blood unit is centrifuged in an upright position at 3000 rpm for 10 minutes at 4^0C .
2) Plasma buffycoat layer and 10 to 20 ml of red cells at the top are expressed in to satellite bag.
3) 3) Double seal and label the bag.

Washed RBCs

It reduces the incidence of febrile, urticarial and anaphylactic transfusion reactions.

1) RBCs after centrifugation of bag as above is washed with saline and packed (during each wash the supernatant is discarded, filled with fresh saline and centrifuged. Repeat the procedure thrice).
2) As this become an open procedure, cells to be used within 24 hours stored at 2 to 4^0c.

Spin and filtration

Filtration of blood to remove leucocyte can be done using specific leucocyte depleting filter before storage, after storage at the blood center or at bed side. It has a high efficiency of >99% of leucocyte removal.

GRANULOCYTE CONCENTRATE

DEFINITION

A component containing primarily of granulocyte suspended in small volumeof plasma prepared from random single donor or by leucopheresis using cell separator.

Scope and application

In neutropenic patients with absolute count of <500/cumm with bacterial sepsis unresponsive to 48 to 72 hrs of antibiotic therapy. Patients with qualitative defects are also candidates for granulocyte transfusion

Separation from single random units:

1. Blood is collected and kept at room temperature before processing which should be done with in 4 hours of collection, centrifuge at 3000 rpm for 10 minutes at 20 -24^{0}c.
2. Express the supernatant plasma into the satellite bag leaving 20 ml plasma above the cell layer. Double seal and separate the plasma.
3. Express the 20ml plasma and the upper 20-25 ml of cell layer rich in white cells into another satellite bag, leaving leucopoor red cell with the primary bag.
4. Label, store at 20^{0}C and to be used within 24 hours.

FRESH FROZEN PLASMA(FFP)

DEFINITION

Plasma separated from whole blood frozen with in six hours of collection and stored at -20^0C or below.

FUNCTION

Fresh frozen plasma contains both stable and labile clotting factors.

scope and application

For multiple coagulation factor deficiency, for the preparation of cryoprecipitate and fibrin glue, reversal of comarin drug effect, use in anti thrombin deficiency, immunodeficiency syndromes, in open heart surgery.

PROCEDURE

Centrifuge the bag at 3000 rpm for 10 minutes at 4^OC express ¾ plasma in to the satellite bag. Double seal and separate the bag label and freeze the plasma immediately.

Shelf life - 1 year if kept at -30^Oc or below. Coagulant activity – 1ml plasma contain 1 unit of coagulant activity.

CRYOPRECIPITATE

FUNCTION

Cryoprecipitate are precipitated proteins of plasma rich in factor VIII and factor XIII and fibrinogen obtained from a single unit of fresh plasma by rapid freezing with in 6 hours of collection, subsequent thawing at 4- 6 ^{0}c and the removal of supernatant.

scope and application

Factor VIII deficiency states hemophilia and von will brand disease, dissaeminated intravascular coagulation, Fibrinogen defects,
Preparation of fibrin glue

PROCEDURE

Collect blood in triple bag and prepare FFP and freeze the plasma immediately, hang the frozen plasma at 4^{0}c in the cold room in an inverted position keeping the second satallite bag lower down. The thawed plasma flows in to satellite bag. Seal the tubing when 10 –15 ml plasma remains with cryoprecipitate. Label the bags and store at –30^{0}c or lower, cryo poor plasma can be frozen as SDP.

PLATELET RICH PLASMA (PRP)

FUNCTION

Platelet concentrate prepared from a single donor unit of blood can temporarily elevate the Platelet Count 5000-10000, mm/BSA in patient whose thrombocytopenia is not due to increased destruction.

scope and application

Bleeding due ton thrombocytopenia
Defect in platelet function
Disseminated intravascular coagulation (DIC)
Viral disease associated with thrombocytopenia (eg: dengue)

PROCEDURE

Collect blood in a double bag and keep at room temperature for one hour. Centrifuge the blood bag at 20-24^{O}C at 1300 rpm for 9 minutes (or 1700rpm for 7 minutes) with in 8 hours of collection. Express the platelet rich plasma in to thesatellite bag with out contamination of white cell layer close to red cells .

Double seal the tubes, label and separate the bags keep at room temperature for another one hour with out disturbing and store the PRP in a horizontal platelet agitator at 22^{0}C at an rpm 70 stokes / minutes.

PLATELET CONCENTRATE

FUNCTION

Platelet in PRP is sediment by hard spin centrifugation and supernatant platelet poor plasma is removed leaving 50- 70 ml of it with the platelet. Platelet button is resuspended gently.

scope and application

For prevention of hemorrhage are disorders of bone marrow failure and bone marrow suppression, platelet qualitative disorders ,acquired platelet dysfunctions management of DIC and ITP. Patients on CPB and aspirin.

PROCEDURE

1) Prepare PRP, centrifuge the PRP at 20- 24 ^{0}c at 3000rpm for 10 minutes.
4) Express the supernatant plasma in to the satellite bag leaving approximately 50 – 70 ml of plasma with platelets.
5) Keep the bag stationary for 1 hour and place in an agitator for gentle agitation during ambient temperature storage.
4) Platelet poor plasma can be frozen as FFP.

SINGLE DONOR PLASMA(SDP)

FUNCTION

As red cells and plasma have different specific gravity ,the cells settle down when anticoagulant blood is kept un disturbed enabling the separation of plasma and cells

scope and application:

Used in volume deficient and in hypo protenemia. Pooled plasma is used for fractionation of plasma proteins like albumin and immunoglobulins.

PROCEDURE:

Blood is collected and kept at 4^0c in an up right position .un disturbed. Separated the plasma when clear in to A transfer or satellite bag on or before the fifth day after expiry of WB label and keep at -30^0c or below expiry –5 years.

SCREENING OF TRANSFUSION TRANSMITTED DISEASES

SCREENING HUMEN BLOOD FOR SURFACE ANTIGEN OF HEPATITIS B VIRUS

SCOPE AND APPLICATION

To ensure safe blood transfusion, blood donors are screened for HbsAg.each blood unit found negative is released for transfusion.

PRINCIPLE

Enzyme linked Immuno Sorbent Assay is the most widely used test for detection of HbsAg, HIV,HCV and ELISA test is used to improve the sensitivity and specificity of the test. Principle of ELISA micro wells coated with antigen or antibodies. The test serum and controls are added to the micro wells and incubate with conjugate (anti IgG) solution which contain an enzyme HRP (Horse Raddish Peroxidase).this forms antibody-antigen-Anti IgG enzyme complex. This complex reacts with substrate (colouring solution) to produce the colour.

PROCEDURE

Test Components

1) Anti-HbsAg coated microplate
2) Negative and Positive controls
3) Conjugate-Anti IgGlinked with an enzymeHRP
4) Substrate-colour giving solution (TMB/OPD)
5) Stop solution
6) Wash solution concentrate

STORAGE AND SHELFLIFE

Store at 2-8 ^{o}C.Shelf life approximately 12 months from the date of manufacturing date.

SPECIMEN COLLECTION

Specimen should be serum or plasma.Do not use heat treated specimens.The specimen may be stored at 2-8 C for upto one week or should be frozen at –10 Cor lower for long term storage.

Cutoff Report

Analysis Date : Oct 30,2006/16:19:14
Protocol File Name : qualisa hbsag.prt
Raw data file : C:\CODA\eia751.raw
Protocol Name :qualisa hbsag
Plate ID : Plate1
Regression:Cutoff Analysis
Kit Lot Number : b97ke

Cutoff method : Cutoff Formula

Cutoff value : 0.130 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.052		
Negative Control		-	1	0.030		
Positive Control		+	2	1.032		
92530		-	1	0.032		
92531		-	2	0.002		
92532		-	3	0.035		
92533		-	4	0.076		
92534		-	5	0.003		
92535		-	6	0.021		
92536		-	7	0.037		
92537		-	8	0.013		
92538		-	9	0.057		
92539		-	10	0.008		
92540		-	11	0.046		
92541		-	12	0.075		
92542		-	13	0.000		
92543		-	14	0.008		
92544		-	15	0.009		
92545		-	16	0.011		
92546		-	17	0.057		
92537 dup		-	18	0.000		
92523 rpt		-	19	0.041		
92523 rpt		-	20	0.071		
weak +ve		+	21	0.195		

REAGENT PREPARATION

Bring all reagents to room temperature for 15-20 min before beginning the assay.
Wash Buffer; Dilute wash buffer concentrate as by the direction.

ASSAY PROCEDURE

1) Fix appropriate number of strips to the microplate frame.
2) Add required amount of conjugate to each well.
3)Pipette out required amount of negative and positive control into each wells.then pipette out required amt of specimen to remaining wells.
4) Then tap the frame gently to mix completely.Then incubate at 37 C for 60 min.
5)Aspirate the contents from all wells and wash each well 5times with at least 300 micro liters prepared wash buffer per well for each wash.
6) Invert the plate and trap if dry on absorbent paper.Pipette required amt of subsrate into each well and incubate for 30 min at R.T after mixing with a gentle tap.
6) After incubation pipette required amt of stop solution in each well and shake well.
8)Within 30 min read the absorbence of NC, PC and specimens

INTERPRETATION

1) Specimens with absorbence value less than the cutoff value are considered as non reactive.
2) Specimens with cutoff value greater than or equal to the cut-off are considered as reactive.
3) Initially reactive specimens that do not react with either of the duplicate repeat tests are considered to be non reactive.
4) Initially reactive specimens that react with either of the duplicate repeat test
are considered as positive.
5) Sample OD values in the gray zone (10%below cutoff) are repeated.

SCREENING OF HUMAN BLOOD FOR ANTIBODIES TO HEPATITIS C VIRUS (HCV)

SCOPE AND APPLICATION

To ensure safe blood transfusion blood donors are screened for HCV. Each blood unit found negative is released for transfusion.

PROCEDURE

a) Antigen coated plates.
b) Negative and positive controls.
c) Sample diluent.
d) Conjugate
e) Substrate
f) Stop solution
g) Wash solution concentrate

STORAGE AND SHELF LIFE

Store at 2-8C. Shelf life- approximately 12 months from the date of manufacture.

SPECIMEN COLLECTION

The specimen should be serum or plasma. Do not use heat-treated specimens. The specimens should be stored at 2-8C for up to 1week or should be frozen at –10C or lower for long term storage.

REAGENT PREPARATION

Bring all reagents to R.T for 15-20 min before beginning the assay.
Wash buffer: Dilute wash solution concentrate as per the kit insert.
Conjugate: dilute conjugate concentrate with conjugate diluent.
Substrate: Dilute the substrate concentrate with the substrate diluent.

ASSAY PROCEDURE

a) Fix required number of strips to the micro plate frame.
b) Pipette required amount of sample diluent into each well. Then pipette out required amount of negative control, positive control and specimen into each well. Mix for 10sec using a micro plate shaker at 1000rpm.
c) Incubate at 37 c for 30 min after sealing the plate with plastic cover provided.

d) Aspirate the content from each of the wells and wash each well 5 times with at least 300 micro litre of wash solution.
e) Add required amount of conjugate to each well.
f) Incubate at 37 c for 30min after sealing the plate with plastic cover provided.
g) Aspirate the contents from all the wells and wash each one 5 times with wash buffer.
h) Invert the plate and tap it on absorbent paper to remove the remaining wash solution.
i) Add adequate amount of substrate to each well and incubate at R.Tfor 30 min.avoid exposure to sunlight.
j) Pipette out 100 microlitre of stop solution to each well and tap the plate gently to mix contents.
k) Read the absorbance at 450nm against air blank within 30 min of adding the stop solution.

INTERPRETATION

a) Specimens with absorbance values less than cut-off values are considered to be non-reactive.
b) Specimens with absorbance values equal or greater than cut-off are considered to be positive.
c) Initially reactive specimens that do not react with repeat tests are considered to be non-reactive.
d) Initially reactive specimens that react with repeat tests are considered as positive.
e) Sample OD values in the gray zone (10% below cut –off) are repeated.

Cutoff Report

Analysis Date : Oct 30,2006/16:00:55
Protocol File Name : qualisa hcv.prt
Raw data file : C:\CODA\eia748.raw
Protocol Name :qualisa HCV
Plate ID : Plate2
Regression:Cutoff Analysis
Kit Lot Number : 499

Cutoff method : Cutoff Formula

Cutoff value : 0.200 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.052		
Negative Control		-	1	0.000		
Positive Control		+	2	2.942		
92530		-	1	0.034		
92531		-	2	0.002		
92532		-	3	0.047		
92533		-	4	0.033		
92534		-	5	-0.004		
92535		-	6	0.050		
92536		-	7	0.029		
92537		-	8	0.020		
92538		-	9	0.024		
92539		-	10	0.012		
92540		-	11	0.032		
92541		-	12	0.130		
92542		-	13	0.000		
92543		-	14	0.022		
92544		-	15	0.011		
92545		-	16	0.010		
92546		-	17	0.023		
92537 dup		-	18	0.005		
92536 dup		-	19	0.029		
92544 dup		-	20	0.042		
weak +ve		+	21	0.405		

SCREENING HUMAN BLOOD FOR ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS (ANTI- HIV)

FUNCTION

Immunoassay which employs an immunosorbent consisting of recombinant hiv-1proteins and HIV- 2 antigen coated to the wells of the microplate.antigen react with antibodies in specimens together with anti igG enzyme (conjugate0to form antigen-antibody-anti-igG enzyme complex) This complex reacts with TMB to produce colour.

SCOPE AND APPLICATION

To ensure safe blood transfusion blood donors are screened for HIV. Each blood unit found negative is released for transfusion.

PROCEDURES

Test components;

a) Antigen coated plate
b) Negative and positive controls.
c) Sample diluent
d) Conjugate
e) Substrate
f Wash solution concentrate
g) Stop solution

Storage and shelf life

Store at 2-8c. Shelf life –approximately 12 months from the manufacturing date.

Specimen collection

The specimen should be serum or plasma. Do not use heat-treated specimens. The specimen may be stored at 2-8°C for up to 1week or should be frozen at –10°C or lower for long term storage.
Reagent preparation
a) Wash solution; prepare wash solution as per the kit insert.

Cutoff Report

Analysis Date : Oct 30,2006/15:57:11
Protocol File Name : qualisa hiv.prt
Raw data file : C:\CODA\eia747.raw
Protocol Name :qualisa hiv
Plate ID : Plate3
Regression:Cutoff Analysis
Kit Lot Number : 499

Cutoff method : Cutoff Formula

Cutoff value : 0.192 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.065		
Negative Control		-	1	-0.008		
Positive Control		+	2	* 3.04		
92530		-	1	0.039		
92531		-	2	-0.007		
92532		-	3	0.035		
92533		-	4	0.056		
92534		-	5	-0.016		
92535		-	6	0.008		
92536		-	7	0.015		
92537		-	8	-0.018		
92538		-	9	0.055		
92539		-	10	-0.012		
92540		-	11	0.038		
92541		-	12	0.039		
92542		-	13	-0.021		
92543		-	14	0.016		
92544		-	15	0.002		
92545		-	16	-0.008		
92546		-	17	0.021		
92537 dup		-	18	-0.006		
92536 dup		-	19	0.025		
92539 dup		-	20	0.077		
weak +ve		+	21	0.594		

Assay procedure

a) Fix required number of strips to the micro plate frame.
b) Pipette required amount of sample diluent into each well. Then pipette out required amount of negative control, positive control and specimen into each well. Mix for 10sec using a micro plate shaker at 1000rpm.

c) Incubate at 37°C for I hour after sealing the plate with plastic cover provided.
d) Aspirate the content from each of the wells and wash each well 5 times with at least 300 microlitre of wash solution.
e) Add required amt conjugate to each well.
f) Incubate at 37 c for 30min after sealing the plate with plastic cover provided.
g) Aspirate the contents from all the wells and wash each one 5 times with wash buffer.
h) Invert the plate and tap it on absorbent paper to remove the remaining wash solution.
i) Add adequate amount of substrate to each well and incubate at R.T for 30 min. Avoid exposure to sunlight.
j) Pipette out 100 microlitre of stop solution to each well and tap the plate gently to mix contents.
k) Read the absorbance at 450nm against air blank within 30 min of adding the stop solution.

Interpretation

a) Specimens with absorbance values less than cut-off values are considered as non-reactive.
b) Specimens with absorbance values equal or greater than cut-off are considered to be positive.
c) Initially reactive specimens that do not react with repeat tests are considered to be non-reactive.
d) Initially reactive specimens that react with repeat tests are considered as positive.
e) Sample OD values in the gray zone (10% below cut –off) are repeated.

SYPHILIS TESTING IN BLOOD DONORS

FUNCTION

Carbogen reagent is a particulate carbon suspension coated with lipid complexes. Carbogen detects antilipoidal antibodies in serum or plasma. These antibodies are traditionally referred as 'Reagins'. During test procedure, the specimen, serum or plasma mixed with the Carbogen reagent and allowed for react for 8 min.if antilipoidal antibodies are present in the specimen they react with the carbogen forming visible black floccules. If antilipoidal antibodies are not present in the specimen there will be no flocculation.

SCOPE AND APPLICATION

RPR assay is intended as a screening test to prevent transmission of Treponemal pallidium that causes syphilis. Screening for spirochetes helps to exclude donors who are at high-risk group for HIV infection also.

PROCEDURE

Test components

a) Carbogen antigen
b) Positive and negative controls
c) Disposable slides.
d) Disposable sample or control dispensing pipette.
e) Mixing sticks
F) Needle dropper for dispensing carbogen antigen.

SAMPLE COLLECTION

Fresh serum or plasma should be used for testing.

STORAGE AND STABILITY

Store the reagent at 2-8^0 C.
Bring all reagent and samples to RT before testing.
Thoroughly mix the carbogen reagent suspension by gentle agitation.

QUALITATIVE METHOD

a) Place I drop of test sample, positive and negative controls on to separate reaction circles of the disposable slide using a sample-dispensing pipette.
b) Add I drop of well-mixed carbogen reagent to the test sample. Positive and negative control by using a needle dropper provided with the kit.
c) Using a mixing stick mix the sample and carbogen reagent thoroughly spreading uniformly over the entire reaction circle.
d) Adjust the time on mechanical rotor for 8 min.
e) Rotate the slide gently and continuously on the mechanical rotor at 100 RPM.
f) Observe for flocculation.

INTERPRETATION

Flocculation is a positive test result and indicates the presence of antilipoidal antibodies in the test sample.

1) Large and medium black floccules against white background-reactive.
3) Small black floccules against white back ground-weakly reactive.
4) No floccules-Non reactive

QUANTITATIVE METHOD

If a sample is found positive in qualitative method the serial dilution of the test sample is done to find out they titer value of antilipoidal antibodies.

Method:

1) Label the reaction circles of the disposable slides as 1:2,1:4,1:8,1:16,1:32,1:64,1:128 and so on.
2) Put a drop of saline in all circles.
3) Place one drop of positive test sample in reaction circle labeled 1:2,mix it well and transfer 1 drop to reaction circle labeled 1:4 and the remaining diluted sample kept in reaction circle labeled 1:2.mix well the contents in reaction circle labeled 1:4 and transfer up to last reaction circle and discard 1 drop from the last reaction circle.
4) Add 1 drop of carbogen antigen in each circle.
5) Spread it with mixing stick uniformly over the entire reaction circle.
6) Rotate the slide in a mechanical rotator for 8 min.
7) Observe flocculation microscopically.

The titer value is reported as the reciprocal of the highest dilution, which shows a positive test result.

QUALITY CONTROL

QUALITY CONTROL OF BLOOD COMPONENTS

FUNCTION

Primary goal of Q.A in transfusion medicine is safe and effective transfusion. it should be ensured that methods function as expected and comply with standards.
QA should include invitro assay to document effective collection of specific element or coagulation factor

SCOPE AND APPLICATION

Since blood components are used to correct a known deficient, each product must be subjected to strict product QC .to ensure availability of quality products which are safe pure and efficacious for transfusion. Transfusion of components will also avoid possible side effective from infusion of un wanted constituents

PROCEDURE

Criteria of donor selection, volume of blood drawn, accuracy of seals and anticoagulant volume ,apply to component preparation as well as whole blood collection

GENERAL CONSIDERATION

1) Donor weight 60 Kg and above
2) Blood is collected to double or triple bags of 450 ml with 63ml CPDA and 350 ml with 49 ml CPDA
3) To prevent activation of coagulation system blood must be collected with in 10 minutes with minimum tissue trauma with single venipuncture
4) Gentle frequent mixing with anti coagulant FFP should be prepared with in 6-8 hrs of collection. ideally PRPshould be kept for one hour before centrifugation
5) Centrifuge contents in opposing buckets must be equal in weight and balanced with rubber materials only
6) Blood bags must be placed straight with broader end facing towards the out side .the outer side they must be wrapped with cover
7) Run time RPM and temperature to be programmed each components
8) Manuel braking should not be applied in between the run
9) All satellite bags must be correctly identified numbered and labeled

10) Each product to be stored at optimal temperature till expiry
11) Platelet after preparation must be kept undisturbed for one hour before putting on agitator
12) When freezing plasma, rate of cooling must be as rapid as possible with in 60 minutes
13) frozen units must be handled with care since the bags may become brittle .integrity of pack should be verified before and after thawing to exclude defects or leaks

I..CALIBRATING CENTRIFUGE FOR PLATELET SEPARATION

Once in a year or plate let yield is not constant
Calculate the number of platelet in one unit of WB or an EDTA sample

1.) Platelet count /microlitre X 1000 X vol of WB (In ml)= number of platelet in WB
2) Perform platelet count on PRP sample
3 platelet count /microlitre X 1000 X of PRP = no of platelet in PRP

Calculate % of yield
no of platelet in PRP X 100 / platelet in WB = percentage of yield

QC FOR FROZEN PLSMA

To known if it has been thawed and returned
1.frozen plasma flat and store in up right .Air bubble formed on the side of the bag during freezing will move to top of bag if thawing has taken place
2.Place a rubber band around the middle of the bag of plasma before freezing which will leave an indentation that disappears on thawing

Bacterial examination

1) For components prepared by open method and issued only after the report is received
2) Returned plasma units redesigned as SDP
3) For leucopoor red cell prepared by centrifugation and washing
4) Returned and and out dated units
5) AT blood after washing
6) Pediatric units or cc prepared from single bag should be discarded after 24 hours

Warmed blood can be cross matched for another patient if not used to be discarded

Transportation of blood components

During transport frozen component must be maintained at or below the required storage temperature in well insulated containers with dry ice in house transport

Periodic check of temperature must be done and documented. Place a thermometer between two components between two number and secure them with a rubber band check temperature after 60 seconds
for minimum requirements of documentation on labeling. Component preparation, storage, inspection of blood components

Collection of samples for component units of QC

1) Strip tubing of the component bag four times so that the contents are well mixed
2) Sealed a 3 inch segment distal top collection bag, double seal end of tubing next to component bag and detach segment
3) Empty contents of segment of segments in to suitable labeled tube for counting

QUALITY CONTROL OF CRYO PRECIPITATE BAG

Parameter	Quality requirements	Frequency of control
Volume	10-20 ml	
Factor VIII	80 units	
Von –will brand factor	40-70% of the original yield	To be checked
FactorXIII	20-30%of original levels of factorXIII	
Fibrinogen	150-250mg	

GRANULOCYTE PREPARED FROM SINGLE UNITS OF BLOOD

parameter	requirment	Frequency of control
Count Red cell contamination	$1X10^{10}$(leucopheresis) 25-50ml	Whenever prepared

PARAMETER	REQUIREMENT	FREQUENCY.OF CONTROL
Volume	40- 50ml	1% of all units
Platelet count	>5.5 X 10^{10}/ unit	1% of all units
pH	> 6(6.0 – 7.4)	1% of all units
Residualleucocytes and red cells	< 0.05-1x 10^9/L 0.2-1 x 10^9	1% of all units

NO	COMPONENT	RUN TIME (MINUTE)	RUN SPEED	TEMPARATURE
1	RED.CELL CONCENTRATE	10	3000	4 – 6^{O}C
2	BUFFY COAT	7	3880	20-24^{O}C
3	FFP	10	3000	4—6^{O}C
4	PRP	7	1700	20—24^{O}C
5	PC FROM PRP	10	3200	20- 24OC
6	CRYO	ONE HOURE	4000	4^{0}C

PARAMETER	REQUIRMENT	FREQUENCY OF CONTROL
VOLUME	200- 250 ml of plasma	1% of FFP
Factor VIII	.07 units in ml	To be checked periodically atTRU
residual cells platelets RBC	< 0.1x 10^9/L < 5X 10^9 /L < 5 ML	

RBC

PARAMETER	REQUIREMENT	FREQUENCY OF CONTROL
VOLUME	250 +/- 50 ml	When ever prepared
HCT	0.65- 0.75	When ever prepared
White cells	<1.0 X 10^9 (70% white cells removed)	When ever prepared
Red cell	Retain 80% of red cells	When ever prepared
Sterility testing	No growth	When ever prepared

NO	PAREMETER	REQUIRMENT	FREQUENCY OF CONTROL
1	HbsAg	Neg by ELISA	All units
2	HIV	Neg by ELISA	All units
3	HCV	Neg by ELISA	All units
4	VDRL	Neg by ELISA	All units
5	volume excluding anticoag;ant	350/450 +/- 10%	All units
6	% of hemolysis at the end of storage	<0.8% of red cell mass	1 % units
7	Sterility of growth	Sterility of growth	1 % units

QC OF REAGENTS

PROCEDURE

prior to receipt of any reagent to check

Name of anti sera, volume, Date of manufacture & date of expiry, Batch no, storage instructions, production License Number, preservatives and standard

colors used, Tested negative for HIV ,HBsAg & HCV.

1. Appearance: Observed for turbidity, suspended particles, precipitates and discoloration.
2. Reactivity or Avidity: It is the rapidity at which the antisera react with the specific antigen.

Method

1. Place a drop of 50 % cell suspension on a slide.
2. Add corresponding antisera.
3. Mix and start the stopwatch immediately.
4. Stop the stopwatch, as soon as the agglutination is visible and note the time taken.

Anti sera	Cells	Avidity
Anti A	A1 cells A2 cells A2b cells	10 seconds 15 to 18seconds 15 to 18 seconds
Anti B	B cells A1B cells	10 seconds 10 seconds
Anti AB	A1 cells B cells A2B cells	10seconds 10 seconds 15 to 18 seconds
Anti D (IgG- IgM blend)	D + CELLS WEAK D CELLS	10 to 15 seconds

3.Specificity on receipt: It is the ability of the reagents to react specifically with the Corresponding antibody and antigen (with the known + ve and – ve controls). The

antisera should be free of all other types of agglutinins and hemolysins.

Specificity of pooled cells (A, B, O)

1.Label and dispense one drop of each of antisera and 2 –5 % pooled cells are directed .

2.Keeps the tubes at room temperature for 30 minutes.

3.Spin at 1000 rpm for one minute.

4.Agglutination should be observed as above. If any unecxpected reaction is seen, test should be repeated.

Sensitivity / Titre (anti A/B/AB/D/H/A1- sera)

Titration is semi quantitative technique for measuring the conc. of antibody in the serum. The titre of antibody is usually determined by testing two fold serial dilution of serum in saline again select red cells.

METHOD

1.Label a row of 12 tubes according to the serum dilution (1 in 1,1 in 2,etc)

2.Add 100 microlitre of saline to all except 1st tube (To 1 add undiluted serum.)

3.Add 100 microlitre of antisera to test tubes 1 & 2.

4.Mix the tube well without forming air bubbles and transfer 100 microlitre to tube.

5.Continue the same till the last tube & discard 100 microlitre from the last.

7.Add 100 microlitres of 2-5 % washed saline suspended appropriate red cells to each tube.

8.Mix well and incubate for 30 to 40 minutes at room temperature.

9.At the end of incubation time, centrifuge the tubes at 1000 rpm for 1 minute.

10.Gently dislodge the cell button.

11.examine the test tubes macroscopically, commence reading the tube containing the most diluted serum and proceed to most conc. sample grade and record the reaction

12. Last tube which gives a reaction grater than or equal to +1, the titre of the antisera

QC OF ANTI HUMAN GLOBULIN REAGENT

Reactivity and specificity

1. Reaction of diluted AHG with 2-5% sensitized cells
2. Reaction of undiluted AHG with IgG coated cells sensitized with dilutions of Anti-D

Method- 1

(a) Label a row of 10 test tubes. Two test tubes are labeled positive and negative controls.
(b) Add 50 microlitre of saline to all tubes except the first.
(c) Add microlitre of AHG to test tube 1 & 2.
(d) Mix the tube well without forming air bubbles and transfer microlitre from test tube 2-3.
(e) Continue the same till the last tube and discard 50microlitre from that.
(f) Add 50 microlitre 2-5 % IgG coated red cells to each tube.
(g) Centrifuge at 1000 rpm for 1 minute.
(h) Check the positive and negative control. Positive control gives a positive test result and negative control should give a negative result. If it fails the whole test should be repeated.
(i) Last tubes which shows agglutination is considered as the titre of AHG.

Expected titre

Anti IgG	1: 64
Anti IgM	1: 4
Anti C3/C4	1: 8

NOTES

1. Non specific reaction ruled out with A,B and O cells (unsensitized)
2. Validity of test result should be confirmed by positive results with IgG coated cells
3. The reagent on visual inspection must be crystal clear and must not show prozone and haemolysis on testing

Method 2

1) Label test tubes
2) Add 100 microlitre of saline in all tubes
3) Add 100 microlitre of anti D (IgG antiD) in the first
4) Mix without forming bubles and transfer 100 microlitre to the next tube
5) Continue the same till the last and discard the 100 micro litre from the Last tube
6) Add 100 microlitre of 2- 5% O + ve cells to all the tubes
8) Mix and incubate the tubes at 370C for one hour
9) Wash thwe tubes three times with saline
10) To all the cell button in each tube add AHG
11) Centrifuge the tubes with 1000 rpm
12) Last tube which gives positive reaction is considered as the titre of AHG

DAILY QC OF AHG

Specificity

1) Sensitised O positive cells for positive control.
2) O negative cells or O + cells with out adding anti-D for negative control.
3) Take 50 microlitre of 5% cells to both positive and negative controls.
4) Add 50 microlitre of AHG to both the tubes.
5) Centrifuge at 1000 rpm for 1 minute.
6) The positive control should give agglutination and negative control tube should not give agglutination.

SPECIFICITY OF SALINE, ALBUMIN&AHG (DAILY)

1) Take 2 drops of 'AB' serum in a test tube.
2) Add 2 drops of O pooled cells.
3) Mix and incubate at 37C for 15 min.
4) Look for agglutination
5) If it is negative, centrifuge and discard the supernatant
6) Add 2 drops of albumin in and incubate at 370C for 45 minutes
7) Centrifuge and look for agglutination (if there is a negative albumin is not working)

8) If it is negative wash three times with saline and decant the saline completely
9) Add two drops of AHG and centrifuge at 1000 rpm for one minute
10) Look for agglutination
11) If it is positive AHG or saline is not working. If negative AHG and saline are working

QC OF BOVINE ALBUMIN

SPECIFICITY AND REACTIVITY

Positive control-

One drop diluted anti-D and one drop 3 to 5 % O+ve cells and bovine albumin

Negative control

Bovine albumin + [illegible] + one drop IgG anti D + one drop 5% RhD negative cells incubate at 37^0C. for one hour

Qc of reagents prepared daily

Saline: it should be prepared with fresh distilled water (ph 6-7) to a Con0.9g %.it should be stored in glass free silicate or plastic allowing no toxicsubstance to leach in to saline. It should be well stopped to prevent evaporation. Container should be washed at frequent intervals and rinsed thoroughly since detergents can interfere with the tests. Label the container

note

Mildly acidic water (6.5 ph) will enhance red cell agglutination. Alkaline of 9 or greater is inhibitory

Pooled cells

Pooled cells for antibody detection should be prepared from anti coagulated blood not more than five days old. Records of reactivity and specificity must be maintained. Should be prepared daily and inspected for evidence of hemolysis before using. When not In use they should be refrigerated

IgG coated cells

IgG coated cells are prepared by sensitizing O cells with IgG antibodies. Cells must be lightly coated with IgG antibodies to give no greater than +2 macro reaction. If heavily coated, cells will not detect loss

of IgG activity. As these cells are coated with antibody, it is possible for the antibody used for sensitisation , to eluate , as the cells stand storage

jerated when not in use

Note :-

If the cells are over-sensitized , cell may agglutinate with out AHG being added. To test this, centrifuge a drop of IgG coated cells and examine for agglutination.

ENZYME

Each time a stock solution is prepared, its reactivity must be tested and incubation period standardized form optimum effectiveness. . Store papain solution at – 20^0C
in small aliquots. The fresh aliquots for each day's use.

NOTE :-
False positive results may be obtained due to clinically insignificant cold agglutinins by raising their thermal amplitude . Prewarming technique may avoid this .

STADARDISATION OF PAPAIN CYSTEIN SOLUTION

Standardisation consists of titration of Anti-D with old papain and new papain

PROCEDURE

1.Arrange 10 tubes of 2 rows. Mark first row as 'new papain' and second row as 'old papain'
2.Make a serial dilution of Anti-D in first and second row
3.In the first row add new papain and in the second row add papain one drop each.
4.Add 2-5% O+ cells in both the rows.
5.Keep at 37^0C for 45 minutes and take the reading

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
New Papain	+4	+4	+3	+3	+2	+1	+	-
Old Papain	+4	+4	+3	+3	+2	+1	-	-

New papain should give a titre of 64
The pH of papain should be 6.2-6.4

Specificity

1. Take ABO cells, which contain both positive and negative cells.
2. Arrange 8 tubes in 2 rows. Mark first row as test and second row as negative control.
3. In the first row add one drop each of Anti-D +papain + 2-5% known cells.
4. To the second row add one drop each of papain cystein and 2-5% known cells.
5. Keep at 37^0C for 45 minutes and take the reading

impression:

Papain cystein solution prepared is specific

PREPARATION OF PAPAIN CYSTEIN

scope and application

Papain cystein enzyme is used in both antibody detection tests and crossmatching Procedure. Papain cystein is useful for the identification of warm reacting IgG or compliment
binding antibody. The papain technique serves to enhance the reaction of Rh, Lewis and Kidd antibodies and for detection of delayed hemolytic transfusion reaction

FUNCTION

Proteolytic enzymes like papain modify RBC antigens to enhance the reactivity of some antigen- antibody system by reducing Zeta potential (Rh, JK, Le, P)and abolishes other (M, N, S, and Duffy).Papain reduces the net negative charge on the surface of the red cell by cleaving sialoglycoprotien from the cell surface .This reduces intercellular distance; expose crypt antigen and increase mobility allowing clustering.

REAGENT

Papain powder : 1 gm
Cystein hydrochloride : 0.480 gm
5N NaOH(20 gm NaOH to 100ml distilled water)

1) Buffer KH_2PO_4 (9.078 gm potassium dihydrogen phosphate in 1 L of distilledwater.).....80ml
2) Na_2PO_4 (11.876 gm disodium hydrogen phosphate in 1 L of distilled water)..... 20ml.

Mix one and two and adjust the pH to 6.2 to 6.4 with 5 N NaOH.Take 10 ml prepared buffer separately and dissolve 0.480 gm cysein hydrochloride In 90 ml buffer , dissolve one gram papain powder . Centrifuge and filter .Mix the papain cystein hydrochloride. Adjust the pH at 6.2 to 6.4

. Incubate at 37^0C for one hour see the pH. dispensen small quantities and freeze at- 20^0C

PREPARATION OF PAPANISED 'O' CELLS

(By using LIQUIPAP)

PROCEDURE

Washed packed (50%) pooled O Rh +ve cells are used. One part of 'Liquipap-A commercially available product) is added to 3 parts of washed packed RBCs, incubate at 37^0C for 8 to 10 minutes. The red cells are then washed thrice with an excess of saline and resuspended in saline as 2-5 %. Papanised cells have to be prepared daily and stored at 4-6^0C when not in use.

QUALITY CONTROL

Diluted IgG Anti D (1:16) is tested with papanised cells as positive control. IgG Anti D and O negative papanised cells is used as negative control.

Each time a stock solution is prepared, its reactivity must be tested and incubation period standardized for optimum effectiveness. Store papain solution at-20^0C in small aliquots for each days use.

NOTE

False positive result may be obtained due to clinically insignificant cold agglutinins by raising their thermal amplitude.Prewarming technique may avoid this.

LOW IONIC STRENGTH SOLUTION (LISS)

NON- SEROLOGICAL

1. pH should be with in the range 6.65- 6.85.
2. Conductivity should be 3.6 – 3.7 mm bo /cm at 23^0C
3. Osmolarity 270- 285 m mol.

SEROLOGICAL

A weak IgG Anti-D (0.25 iu/ml) should give a + /+2 reaction with R_1r red cells by routine LISS_AHG test. This should be carried out in parallel with testsusing the current batch of LISS.

QC OF TEST KITS

Each day's run should be validated for run acceptance criteria . Along with kit positive and negative controls, 'in-house' external controls should be assayed as this will detect changes in lot to lot sensitivity, since they remain constant while batches of test controls are lot specific only. Pooled diluted kit positive controls or in-house controls close to cut off (border line reactor) can be used as external controls. Another method to monitor quality using internal and external control is to repeat one of them in triplicate in the same run and compare the results . (Intra run reproducibility) or controls put on threre consecutive days to evaluate Inter run reproducibility . By either of these methods, variation should not exceed 10%.

PREPARATION OF IgG COATED CELLS

SCOPE AND APPLICATION

To ensure the activity of coombs reagent which is used for antigendetection, antibody detection and identification

Procedure

Dilute IgG anti D 1/60 in saline. Add 19 drops of diluted anti D to one drop of washed pooled O positive cells. Mix well and incubate at 37^0C for 30 to 60 minutes. Wash three times in excess saline and re suspended to 2 to5 %. To be prepared daily.

Quality control

Add one drop of AHG to one drop IgG coated cells. Spin 1000 rpm for one minuteand look for agglutination IgG coated cells are prepared by sensitizing O cells with IgG antibodies.Cells must be lightly coated with IgG antibodies to give no greater than +2 macro reaction.if heavily coated, cells will not detect loss of IgG activity .

Note

If the cells are over sensitized, cell maybe agglutinate with out AHG being added. To test this, centrifuge a drop of IgG coated cells and examine for agglutination

PREPARATION OF EDTA

SCOPE AND APPLICATION

EDTA is used for the collection of blood samples for haemogram

PROCEDURE

Prepared 4gm % EDTA as stock solution. From the stock solution ,take 10 microlitre in small vial for the collection of 2ml of blood. Dry it in hot air oven at 160^0C then switch off it, Keep the EDTA bottles in it till they get dried up

PREPARATION OF PHOSPHATE BUFFER

SCOPE AND APPLICATION

Prepare phosphate buffered saline (PBS) at neutral PH which can then to used as diluent in serological tests

Function

Mixture of acids and bases can be prepared at specific PH values used to buffer other solutions to that buffer

Reagents

1) Prepare acidic stock solution (solution A) by dissolving 22.16 gm/L Na H2PO4.H2O. In one liter of distilled water. This 0.06 m solution of monobasic phosphate salt (monohydrate) has a Ph of 5.
2) Prepare alkaline stock solution (solution B) by dissolving 17.2 gm/L of NaHPO4 in / L distilled water. This .16 m solution of the dibasic phosphate salt has PH 9

Procedure

1) Prepare working buffer solution of the desired P^H by mixing appropriate volume of two solution
2) Check P^H of working solution before using it .Add small volumes of asid Solution acid solution A or alkaline sol B to achieve desired PH
3) To prepare PBS of desired P^H,add one drop a vol of phosphate buffer ,at that P^H to a volume of normal solution

Ref .AABB Technical manuel

CRYOPRESERVATION OF RED CELLS AND DEGLYCERALISATION

SCOPE AND APPLICATION

Preservation of red cells samples for longer period of storage

FUNCTION

Glycerol prevents freezing injury in red cells by limiting ice formation and providing a liquid phase in which salts are distributed As cooling proceeds excessive hypertonicity
is also provided

PROCEDURE

Reagents

Buffered potassium citrate, it contains,

1) Tripotassiumcitrate ($K_3C_6H_2O7H_2O$)-3.25 %
2) Potassium dihydrogen phosphate (KH_2PO_4)-0.47%
3) Dipotassium Hydrogen phosphate (KH_2PO_4)

The laying down solution

This is a 50% or 40 % which gives about a 30% concentration of glycerol when mixed with equal quantity of packed cells. 40 ml of glycerol well mixed with 60 ml
Of the buffered reagent is a convenient quantity.

Laying down process

The blood is to be stored is taken to ACD / CPD anticoagulant and v/v laying down solution is added gradually to the packed cells, mix well so that non of the cells are subjected to excessive concentration of glycerol. The mixture is frozen at –40 to 60^0 C.

recovery of cells

The frozen cell mixture is allowed to thaw at room temperature. Then centrifuged and removed the supernatant. The packed cells are then washed with each of recovery solution in turn beginning with the 16% and ending with 2% followed by buffered tripotassium citrate and then twice with saline.

preparation of the recovery solution

There are 16%, 8%, 4%, 2%, W/V glycerol to buffered citrate owing to high viscosity of glycerol it is best to prepare 16% solution and prepare others by doubling solution.
200 ml of 16% prepared by adding 25.6 ml of glycerol to 174.4 ml of buffered tripotassium citrate and take care to mix well.

PRE TRANSFUSION WORKUP

SCOPE AND APPLICATION

Compatibility testing are done prior to blood transfusion to ensure that units of blood are safely transfused. It should detect clinically significant antibodies.

The main functions of compatibility testing are:

1) It is a final check up of ABO compatibility between donor and recipient.
2) It may detect the presence of an antibody in the donors and recipients serum that was not detected in antibody screening.
3) It should ensure maximum safety to prevent alloimmunisation & transfusion reactions.

Compatibility testing procedures can be divided in to two parts: -

1) Major compatibility (major matching)
2) Minor cross match (minor matching)

We do major compatibility test only, as all donor units are screened for atypical antibodies.

Procedure:

No single method fulfills all the requirement. Methods used must demonstrate both IgM and IgG antibodies

Major compatibility (saline and albumin) method:-

1) Place two drops of each recipient's serum in two pre labeled tubes - saline and albumin.
2) Put one drop of washed 2- 5% suspension of donor cells in saline in both tubes .
3) To tube II (A) add one drop of bovine albumin 22%.
4) Both tubes are incubated at 37^0c for 30 – 40 mm

5) Check for hemolysis and agglutination (macro and microscopic).
6) Wash the test tube I (S) with normal saline 3 times taking care to decant completely the supernatant and to the cell button add one drop of polyspecific AHG.
7) Spin at 1000 rpm for one minute and read result microscopically.
   If the test is negative, add one-drop IgG coated cells to validate the test procedure and reagent used. Routinely compatibility is done by saline and albumin method. When there is a history of previous transfusion or pregnancy, anti globulin method (IAT) is also done

Enzyme technique

This can be done by the one and two stage methods. The two-stage technique (red cell pre treated with enzyme and then tested with the recipients serum) is more sensitive. One stage technique (enzyme, recipients serum and donor cells Incubated together) is convenient to apply in the cross match but it is not sensitive. The major limitation is that it does not detect certain anti body in the MNS and Duffy blood group system. Enzyme technique is mainly used for antibody identification and in delayed haemolytic transfusion reactions.

CROSS MATCH FOR NON SPECIFIC BLOOD GROUP TRANSFUSION

normally group specifc transfusion are to be carried out .but in expceptional circumstancesABO non specific group transfusions are to be done .as oa life savingh measure .A and B group recipient can receive O group red cells and AB group recipient can receive A or B group red cells .usually A group will be preferred because the titr e of anti B will less than antyi A in group B personsin this situation , apart from major cross match minor match is essential titre less than 100 is chosen to avoid hogh titre antibodiedsas seen in dangerouse O groupO donors .removal of plasma can reduce the amount of anti bodies

procedure

1) Dilute donor serum 1/ 100 (tom one drop serum, add 9 dropes normal saline to make 1/10 dilution .add 98 drops of normal saline to make 1/ 100)
2) Place 2 drops serum 1/ 100 dilution) each in labeled test tubes .add one drop of 2- 5 % patient cells. Put one drop bovine albumin 9 22 % in the test tube II
3) Incubate at 37^0C
4) Observe the hemolysis/ agglutination
5) Proceed to AHG with tubes

INVESTIGATION OF TRANSFUSION REACTION

SCOPE AND APPLICATION

investigation of transfusion reaction is essential for the management of patients

PROCEDURE

An acute HTR is a medical emergency and can occur with any blood component. Un toward effects may vary from mild to severe reaction. in can result of

1) Clerical
2) Technical
3) Storage problem and due to faulty administration

Samples required for serological investigation

1) Pre transfusion blood sample of the patient
2) Implicated donor unit sample
3) Clotted sample obtained from the patient immediately and about 24 hrs after transfusion
4) An EDTA sample of the patient in the immediate post transfusion period
5) Remains of the donor blood with administrator set
6) Post transfusion urine sample

outline of lab investigation of suspected HTR

* Check pre transfusion records of patients and donor details (hospital no, blood group, antibody status)
* Clerical error involving one patient may involve other cross matched at the same time. Hence it is essential to check sample, donor units and record of all cross matched at the same time
* Compare patient's pre post transfusion serum or plasma for pink or red discoloration, for the presence of free hemoglobin. Yellow or brown discoloration in a sample drawn 5 – 7 hrs after transfusion indicate recent haemolysis
* Perform DAT on post transfusion sample. if in compatible transfused cells are not immediately destroyed DAT will positive with an mixed field (MF) appearance

* Repeat ABO and Rh typing on patients pre and post transfusion sample, donor unit transfused at the time of reaction and other prior, units, when available a mixed field pattern on microscopic reading suggests presence of in compatible donor cells
* Repeat antibody detection on patients pre and post transfusion samples and on donor units. Test donor unit to see if there is corresponding antigen .if the donor blood has previously over looked antibody, do a minor cross match against patient pre transfusion sample or type patient for the suspected antigen
*Do an extended cross match of all red cell units administrated with pre and post transfusion sera against donor sample from blood bag tubing and pilot bottles. If cross match is compatible with pre transfusion sample, suspect a anmnestic reaction or passive administration of an antibody present in transfused component
* Do a smear study and culture on blood remaining in the bad at 4^0C ,20 0 c and 37^0C .if infected ,blood proved to the cause of reaction , investigate thoroughly all proceedings involved in collection ,processing ,storage and transport andf blood components
* Perform biochemical tests like plasma Hb and serum, bilirubin and haptoglobulin level
post transfusion urin sample for free Hb

DELAYED HAEMOLYTIC TRANSFUSIONREACTION(DHTR)

*Due to primary alloimmunisation (several weeks after transfusion)

*Due to anamnestic response (with in 7-10 days after transfusion)

FINDINGS

Reduced Hb,hct

*DAT may be positive

*Appearance of atypical antibodies and cross match incompatibility.

*Free Hb in urine,haemosiderin

Special tests to diagnose red cell incompatibility

*Perform antibody detection tests and cross match with more sensitive techniques by using LISS or enzyme or by increasing serum to cell ratio.

*DAT and antibody detection tests on several post transfusion samples at frequent intervals

*If antibody is identified, phenotype patients pre-transfusion cells to ascertain that the patients lacks the corresponding antigen.

INVESTIGATION ON NON-IMMUNE CAUSES OF HAEMOLYSIS

*Mechanical trauma from extra corporeal roller pumps in CPB,blood transfusion pumps, small bore needles.

*Thermal over heating or freezing.

*Osmotic (by infusion of hypnotic solution or drugs)

*Examine supernatant plasma from the donor unit for the presence of free Hb as a result of improper storage.

*Examine blood remaining in administration set for lysis.If set has been used for hypnotic solution ,there could be haemolysis in tubing not in bag

PRETRANSFUSION TESTING OF NEONATES
(Less than 4 months old infants)

PROCEDURE:

Pretransfusion in neonates should be restricted to cell grouping only. Antibody screening to be done on baby's / maternal serum. IgG antibody screen is negative; transfuse red cells, which are ABO identical or compatible with mother and baby. If unexpected antibody is detected in the serum, crossmatch upto AHG is necessary as long as maternal antibody is present in infant's serum.

Emergency crossmatching Test:

Occasionally the patient may need an urgent transfusion compatibility test. Under such circumstances, the incubation periode for compatibility testing can be reduced to 15 mts and results read after centrifugation(light spin only). Clinician is informed accordingly and blood is issued. However crossmatching tests should be continued for the periode of incubation and any descripancy should be informed. Modified or abbreviated crossmatch tube containing saline suspended donor cells and serum is spin and read result if antibody screen is negative. no incubation is needed.

LISS Compatibility test(Low Ionic Strength Solition):

LISS solution increases the rate of antibody uptake and reduces the electrostatic barrier surrounding the red cells.

Incubation time is reduced in LISS solution.

Procedure:

1) Wash the red cells twice in saline and once in LISS and suspend the red cells in LISS to make 2 - 5 % suspension.
2) Take two drops o patient serum in a labelled test tube.
3) Add 2 drops of LISS suspended red cells to the tube.
4) Incubate at 37 for 10 - 15 mts.
5) Centrifuge and read for agglutination / haemolysis.
6) If there is no agglutination, wash the tubes 3 times in saline.
7) Perform AHG test and read
8) Add IgG coated test cells if negative.
9) Centrifuge and check for agglutination.

INVESTIGATION OF HDN AND NEONATAL TRANSFUSION

Scope and application:

Maternal IgG cross the placenta and enter the fetal circulation leading to hemolytic complication in newborns, Very small blood volume of the newborn and the antibodies in the transfused blood pose a serious problem faced by the transfusion center.

PROCEDURE:

Neonatal period - period from birth to 4 months
Premature child - 100ml/Kg
Blood volume of mature child - 85ml/Kg
Transfusion dose - 10 ml/Kg
Exchange transfusion - double volume.

General guidelines in neonatal transfusion

Blood should be as fresh as possible, not more than 5 days. If mother and baby are of same ABO group identical group blood should be selected.
If both are ABO incompatible, O group blood with low titre anti A & anti B free from haemolysin should be selected. It is a good practice to give concentrated red cells with 1/3 volume of fresh AB plasma - first choice or A/B plasma as appropriate (If baby is A group, suspend O group red cell in A group plasma and if baby is B group, suspend O group cell in B group plasma)

Situation leading to transfusion in New borne

1) HDN leading to anaemia and hyperbilirubinemia –
   ABO HDN
   Rh HDN,
   HDN due to other blood group systems.
2) Surgical requirements:
3) Other causes:

Because the immune system of infant is immature and relatively unresponsive to antigenic stimulations during the first 4 months of life, the standards of compatibility testing for neonates are different from those of adults. The antibodies present in newborn plasma are passively transferred from the mother through the placenta. Since repeat blood bank testing cause demonstrable harm through blood loss AABB standards

permits reduction in pretransfusion serological testing for neonates. For the initial ABO and Rh testing new born sample must be used and for the rest of the testing maternal sample can be used.

Investigation of suspected HDN

Maternal and cord blood should be tested.

Maternal blood

Blood group: ABO and Rh, Weak D if Rh Negative, ICT for antibody detection and Identification of antibody if present.

Cord blood

Blood group: ABO and Rh, Weak D if Rh Negative, DCT,Elute antibody from cord cells & if DCT +ve, antibody detection should can be done by elution.

Suspecting ABO HDN

1) Clinical history of jaundice immediately after delivery.
Blood group of mother - 'O'
ICT - Neg
Cord blood/baby's blood
Blood group - A / B
DCT -Weak + / neg

Proceed to the following tests to confirm ABO HDN:-

IgM / IgG anti A/anti B titre in maternal sample significant if >1:128 / 1:32

Cord or baby's sample - IgG anti A / B titre (optional)
Elute from cord / baby's cells tested with A / B cells in ICT +ve

Blood Selection

O with lowest anti A / B titre. Rh as that of the newborn(eg- if child is A+, select O+ blood & if child is negative, select Onegative blood).

Confirming Rh HDN

Mother's blood
Group : Rh D Negative
ICT : Positive

Confirmation of antibody : +ve reaction in O+ cells, -ve reaction in Oneg cells.

Cord blood:

Blood group Rh D positive

DCT - strong +ve
ICT - + / -
Elute +ve with O+ cells, neg with oneg cells

Selection of blood in HDN

All units selected should be Rh D negative
ABO system can be selected as per the table.

Baby 's blood group	Mother's blood group	Blood selected
A	**A**	**A / O**
	B	**O**
	AB	**A / O**
	O	**O**
B	**A**	**O**
	B	**B / O**
	AB	**B / O**
	O	**O**
AB	**A**	**A / O**
	B	**B / O**
	AB	**AB / A / O**
O	**A**	**O**
	B	**O**
	O	**O**

Blocked D phenomenon:

When all the Rh D sites are blocked by anti D, cord cells will not be giving agglutination in anti D & will be grouped as Rh D neg. Confirmation of blood group can be done with eluted red cells.

HDN due to minor blood group antigens:

Investigation: same as Rh HDN

Confirmation: Elute from cord blood can be tested with panel red cells reagents if antibody confirmation is not possible. Crossmatch with maternal serum more no of units than required and find out ICT compatible units.

Elute can be tested with pateral red cells to find whether the antibody is directed against the paternal antigen.

Compatibility for neonatal transfusion

Do major x match with maternal serum up to IAT. If O group or ABO nonidentical group blood is selected, titre of the donor unit should be done and units having lowest titre unit can be selected.test for th haemolysin in the donor serum

Transfusion

Either semi packed RBC or RBC suspended in AB plasma (A/B plasma as appropriate)

Dose: 10ml/kg for correction of anemia

Double blood volume for exchange transfusion.

Transfusion in other condition:

Investigation in baby's sample - ABO &Rh determination. Antibody screening and compatibility testing with maternal sample.

Selection of blood:

Blood group compatible with both mother and baby.

HAEMOLYSIN TEST

Scope and application:

O group donor samples with strong haeomolytic sera(+3,+2) are likely to have high levels of IgG antiA/anti B. Haemolysin test is done when O group blood is selected for patients of other ABO blood groups especially for HDN.

PROCEDURE

Clear serum obtained from clotted samples within 12hrs for the presence of anti A and anti B haemolysins. Fresh AB group serum can be used as a source of complement when serum to be tested is more than 10 days old.

1) Put 2 drops of serum under test into each of two tubes.
2) Add 2 drops fresh AB serum.
3) Add 1 drop of 5% A cell suspension into one of the tube & 1 drop B cell into other
4) Observe the colour of supernatant serum. A pink or red colour indicates haemolysis. Haemolysis in the tube containing groupA red cells indicates the presence of antiA haemolysin and haemolysis in the group B indicates anti B haemolysin.

Grading of Haemolysis:

3+	Complete Haemolysis.
2+	Partial (>50% but not complete) haemolysis
1+	Trace haemolysis
Neg	No haemolysis

3+ & 2+ are considered clinically significant.

CONVERSION OF PLASMA TO SERUM

SCOPE AND APPLICATION

To preserve rare samples for experimental studies

PROCEDURE

1) prepare 2.7% solution of $CaCl_2$ (2.7 gm of unhydrus Ca Cl2 in 100 ml distilled water)
2) add 0.1 ml of CaCl2 solution to .9 ml plasma
3) incubate serum for one hour at 37^0C
4) recover serum from clot
5) centrifuge serum at 3000 RPM for 10m minutes to remove particles if necessary
6) specimen if to be used with in two days store at 2 to 8° C if long storage indicated specimen should be frozen

9 ml plasma + $CaCl_2$ one ml (2.7%)

ISSUE AND RETURN OF BLOOD UNITS

SCOPE AND APPLICATION

Blood components are issued for surgical requirements and post operative maintenance of blood volume, oxygen carrying capacity, leucocyte function and for haemostasis.

GENERAL CONSIDERATIONS

1. Group specific blood is issued according to patients requirements, after full pretransfusion work up.
2. Units are issued attached with compatibility label and reaction forms.
3. Before issue , inspect the unit for hemolysis, clots, leaking etc, recheck unit number and patient identity and compatibility label.
4. Blood is issued in insulated containers with instructions for storage at proper temperature, if not transfused immediately.
5. When there is urgent request for release of blood, it issued with an abbreviated crossmatch. "Emergency cross match only done" is marked on the reaction form. Extended crossmatch is done once blood is issued and incompatibility reported if any.
6. Plasma and platelets are issued group specific.
7. Cases not requiring blood routinely are put under "T" and "S" only if antibody test is negative with 2 or 3 panel cells . Blood is not cross matched .In case there is a need, blood is issued within 10-15 minutes with just a saline spin of the crossmatching tubes to look for ABO errors.
8. Request for stand by procedures are also put under "T & S" (Only grouping and antibody screening done)
9. If ABO group specific blood is not available. O group packed cells are given. A written request from the physician is needed before issue.
10. If Rh negative blood is not available, Rh positive blood is given in in life saving situations, taking into account, Patients age, Sex Child bearing potential. A written request from physician is obtained to that effect before issue.
11. Final identification of the recipient and donor unit is done by the trasfusionist.
12. Reaction forms are received back daily filled and signed, and is preserved with request form of each patient.

SPECIAL CONSIDERATIONS

1) Children weighing <25 kgs are given pediatric units.
2) Number of units issued initially are as per guidelines set by HTC

RETURN OF BLOOD AND COMPONENTS

1) Blood is received back and taken into inventory if returned with in 24-48 hrs and maintained with in acceptable temperature and no abnormality detected on inspection. Returned units are quarantined for 24-48 hrs and then taken into stock if there is no abnormality .ICU . Wards are advised to return the units at the earliest if not used.
2) Returned units are issued at the earliest.
3) FFP thawed, if received back is labeled as SDP only
4) Opened units if not used are discarded after 24 hrs

THERAPEUTIC PLASMA PHERESIS

FUNCTION

In small volume plasma exchange, whole blood collected from a patient is separated into cellular and plasma components by centrifugation. Cells are returned to patient with saline or plasma as a replacement fluid.

1.Scope and application

It is used in neurological diseases like GBS,CIDP and Mysthenia Gravis.the rationate for plasma exchange in these diseases is to remove antibody (an IgG auto antibody in most cases)

2.Procedure

1. Receive properly labeled whole blood from the patient in 450ml blood bag.for children use 350 ml collection bag.
2. Seal and weigh the bag ,record the total volume of blood collected.
3. Centrifuge the bags at 3000rpm for 10 minutes at 4^0C after balancing properly.
4. Express plasma into a transfer bag with aseptic precaution and discard.
5. Reinfuse equal volume of saline (vol. Equal to the amount of plasma removed from bag) or group specific / compatible plasma to the cellular constituents. Mix well and seal the bag properly and issue to patient for immediate transfusion.

AUTOLOGOUS TRANSFUSION

FUNCTION

Intra operative blood salvage in cardiac surgery refers to the collection and return of blood , recovered from extra corporal circuit . collected blood units are washed and reinfused to the same patient. Washing with normal saline decrease the concentration of free Hb, heparin ,potassium, cellular debries.

PROCEDURE

1. Blood units are collected and labelled in 450ml CPDA bags are received at blood banks.
2. Seal and centrifuge at 3000 rpm for 10 minute at 4^0C.
3. Express the supernatant fluid into blood bags and discard.
4. Reinfuse IV 0.9% normal saline solution to the concentrated cells till the bag is full and mix well .
5. Seal the bag, balance and centrifuge at 3000rpm for 10 minutes at 4^0C.repeat steps 3 to 5 again.
6. Express the supernatant to transfer bag and discard.
7. Mix well with small volume (30 to 50 ml) of saline and seal the bag.
8. Issue for transfusion as CC. It is an open procedure, blood should be used as early as possible, not longer than 4hours.

RECORD KEEPING

Scope and Application

1. To provide permanent references of implementation and maintenance of quality system.
2. To train personnel.
3. For product inventory.
4. For application of audit criteria to improve transfusion services.
5. Manual errors are avoided and system is made nearly false proof. It allows for computer check of datas and thus leads to patient safety.
6. Data from Blood transfusion services can be made available for hospital network and vice versa. Complete back up of data in any form , in a limited time frame work is an added advantage of computer system.

Record of Donors

1. Donor's registration card.
2. Donor register.
3. Blood component's register.

Record of Patients

1. Blood group register
2. Cross-match transfusion register.
3. Issue register.
4. Transfusion reaction investigation register.

PREPARATION OF POTTASSIUM DICHROMATE SLOUTION AND CLEANING OF GLASSWARE

SCOPE AND APPLICATION

Dirty glassware often gives rise to false positive results due to residual contamination of cells and serum. This may affect test in the lab.

PROCEDURE

After tests are completed and before drying, glassware (Test tubes, slides and sample bottle) are immersed in large amount of water containing bleach.

preparation of pottassium dichromate solution

Potassium dichromate -1 kg

Concentrated h2so4 - -1litre

Make up volume up to 10 liters with distilled water keeping 10-liter jar in a container with 3/4water to prevent explosion due to con.h2so4.

Always add acid to water

Glassware is immersed in dichromate solution for an hour or overnight if required and the solution is drained off. It is then washed with a jet of tap water, shaking out the water after each rinse. After several cleaning with tap water, they are rinsed with distilled water and shaken well to remove trace of water. They are kept in wire baskets with mouth down and dried in hot air oven at 150 c for 2 hours.

Chromic acid solution may be kept and repeatedly used several times.