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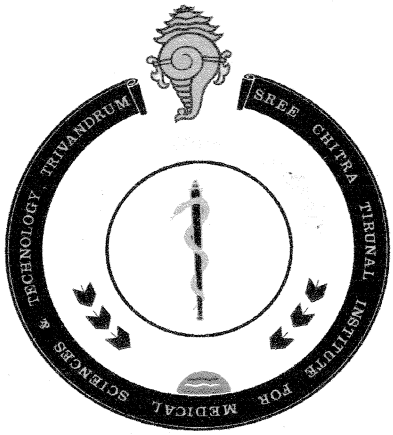


THIRUVANANTHAPURAM – 695 011

SUBMITTED BY :

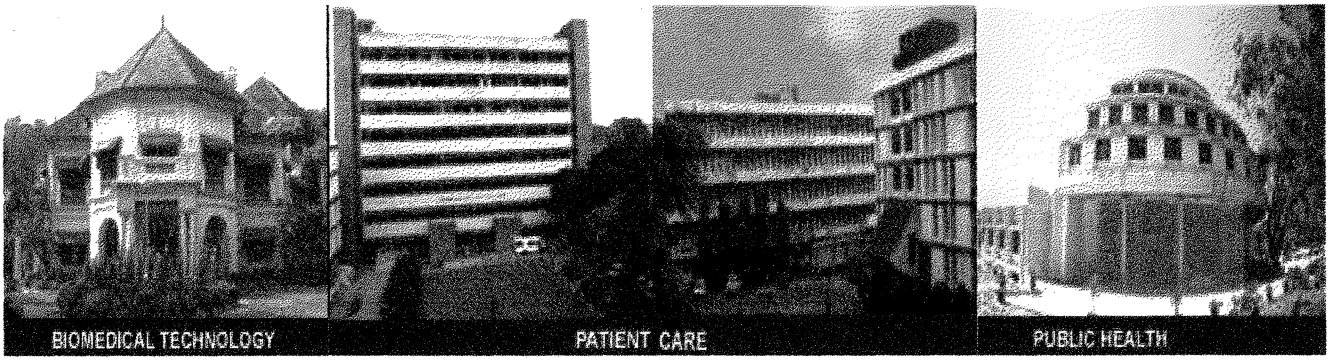
VIMAL RAJ.R

DIPLOMA IN BLOOD BANKING TECHNOLOGY

(DBBT)

MONTH AND YEAR OF SUBMISSION – DECEMBER 2005

THE INSTITUTE



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CERTIFICATE

I, Mr.....Vimal Raj.R.....hereby declare that I have actually performed all the procedure listed/carried out the project under report.

PLACE: Thiruvananthapuram
DATE: 01/12/2005

Vimal Raj. R

Forwarded she/he has carried out the minimum requirements of procedures etc.

PLACE: Thiruvananthapuram
DATE:

Head of The Department
Blood Bank

ACKNOWLEDGEMENT

First of all I would like to thank the almighty God, without his help I can't complete this Log Book. Next I would like to thank our Head of the Department Dr. Jaisy Mathai, who has been our chief tutor and instructor. She had given proper guidance to each and every aspect of my training. Then I would like to thank Dr. Sulochana and Dr. Sathyabhama, Blood transfusion Officers who had taken most of our lessons. They gave a kind attention towards me. I would also thank Mrs. Philomina Augustine, Chief Blood Transfusion Assistant and Smt. Usha Kandaswamy, Medico Social Worker of Blood Bank.

I thank the Director of the Institute Dr. K. Mohandas, Medical Superintendent Dr. Douglas Linsby, Dean Dr. Radhakrishnan and Registrar Dr. A.V. George, for their advice and kind attention towards me. I extend my heart full thanks to all Staff Members of our Blood Bank. I extend my heart full thanks to all Staff Members of other departments for their help during my training period in this Institute.

Vimal Raj. R

Sl. No	Contents	
1	Haemoglobin estimation by Copper Sulphate Methode.	
2	Haemoglobin estimation by Cyanmethaemoglobin	
3	Blood Collection.	
4	ABO grouping and Rh typing.	
5	Subgroups of A and AB.	
6	Antiglobulin Test.	
7	Preparation of Components a) Red cell concentrate b) Leucocyte poor RBC concentrate c) Granulocyte Concentrate d) Fresh Frozen Plasma e) Cryoprecipitate f) Platelet Rich Plasma g) Platelet Concentrate h) Single Donor Plasma	
8	Screening of Human blood for a) HBsAg b) HCV c) HIV e) Syphilis	
9	Quality Control of Components.	
10	Quality Control of Reagents.	
11	Preparation of Papain Cystein.	
12	Preparation of Papanised O Cells.	

13	Preparation of IgG coated cells.	
14	Preparation of EDTA.	
15	Preparation of Phosphate Buffer	
16	Cryopreservation of Red Cells and deglyceralisation.	
17	Pretransfusion workup.	
18	Pretransfusion testing of neonates.	
19	Issue and Return of Units.	
20	Investigation of Transfusion Reactions.	
21	Investigation of HDN and Neonatal transfusion.	
22	Haemolysin Test.	
23	Therapeutic Plasma pheresis.	
24	Autologous Transfusion.	
25	Record Keeping.	
26	Cleaning of Glassware.	

HAEMOGLOBIN TESTING BY COPPER SULPHATE METHOD

SPECIFIC GRAVITY OF BLOOD

Specific gravity of blood is defined as the ratio of the weight of the same volume of water at 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 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

DETRMINATION 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 1053 specific gravity and noting whether the drop falls or rise in the solution. If the drop of blood ifs lighter than the solution it will sink first, perhaps only for a few seconds and then rise. If it is of the same specific gravity, it will become stationary for this interval and then continue to 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 and 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 4^0C 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 determining 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 behavior of the drop 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 et and Philip et al to determine the suitability of the blood donors for donation

PHILIP, VANSLYKES COPPER SULPHATE TECHNIQUE

Mixing 522.5ml of stock solution and 477.5ml-distilled water modifies the stock solution prepared. This corresponds to 1053 specific gravity and12.5 gram Hb%

Male =522.5c.s +477.5d.w 1000 1053 12.5 gram Hb

Since the male volunteers can be accepted for donation if they have got an Hb% 12.5 gram and above, the solution can be used to 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 female s 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 method in the mass screening

1.This method is simple

2.The temperature coefficient of expansion of CuSO and blood are equal, so here is no need for separate temperature regulation.

3.the substance used are not toxic and do not give explosive vapors.

4.Dispersion of copper sulphate solution is low and visualization is easy

5.The copper sulphate solution cleans automatically itself

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

This test method is not a quantitative one but it is: -

1. Fast
2. Large no of donors can be screened in minimal time.
3. The method is very good as long as person performing the technique is meticulous in technique
4. The first drop of blood from the finger must be wiped as it may contain the detergents dropped from a height the second drop must be used for test.
5. Do not squeeze the finger, as this may dilute the drop of blood with tissue fluid and give falsely low results.
6. The capillary tube should be filled with out air bubbles.

7. 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
8. The copper sulphate solution kept tightly capped before and after use and should be changed frequently or after 25 tests while being used so that it does not have a chance to evaporate and concentrate and there by causing the donors to rejected unnecessarily.
9. All bottles should be emptied twice daily and the blood discarded and the solution replaced.
10. 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. Low values can be confirmed with a more accurate test for Hb.

Hemoglobin estimation by Copper Sulphate method

Sl No	Date	Name	Age	Sex	Hb g/dL
1		Suma	24	F	>12.5gm%
2.		Santhosh. G	29	M	>12.5
3.		Sudha. C.K	37	F	>12.5
4		Sunithakumari.	28	F	<12.5
5		Salim. A	32	M	>12.5
6.		Bijukumar. S	32	M	>12.5
7		Swapna. R	28	F	<12.5
8.		Praveen.	22	M	>12.5
9.		Preetha.	35	F	>12.5
10.		Ajithomas	29	F	>12.5
11.		Remya	20	F	>12.5
12.		Divya	25	F	>12.5
13.		Chandramathy	41	F	<12.5
14		Sreelekha	22	F	>12.5
15.		Neethu Naresh	18	F	>12.5

HEMOGLOBIN ESTIMATION BY CYANMETHMOGLOBIN METHOD

PRICIPLE

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) Venoues 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

Nomidet P-40 ..... 1 ml

Distilled water .....1 L

The reagent should be clear and pale yellow in colour,

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

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

PRACAUTIONS

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 give 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 ampoule is opened.

Sl No	Date	Name	Age	Sex	Hb gm/dl
1		Naseera. T	38	F	14.15
2.		Sudharma	43	F	13.69
3.		Baba	18	F	14.16
4.		Rupini	18	F	12.78
5.		Vinod . P.R	31	M	15.97
6.		Sudhakumar	38	M	13.6
7.		Sainaba	35	F	14.60
8.		Ruby Mathew	50	F	14.6
9.		Jainy Mary Titus	47	F	12.7
10.		Deepak. P	28	M	16.8
11.		Valsa John.	42	F	10.95
12.		Subaraj. L	34	M.	12.32
13.		Chandrika.	52	F	14.4
14.		Kavitha	30	F	13.69
15.		Rehana Albert	39	F	11.32

BLOOD COLLECTION

METHOD

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

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

14.Emergency drugs

15.Labels

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 venipancture and with 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 shaaker4 s 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. Flow is interrupted by putting a tight knot in the tube and tube is cut between clamps .BP cuff pressure is released and the needle is removed from the vein the donor is asked tom fold the elbow keeping the sterile gauze at the venipancture site .the blood remaining in the tube is transferred to pilot tubes. Which are labeled clotted and anticoagulant sample .the needle is cut from the tube and discarded in to a puncture proof container containing disinfectant.

The tubing attached to the bags 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,fits or in extreme cases loss of consciousness and involuntary passage of urine and feces.

In the lying down position, a syncope 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 with lowering of 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 syncopial 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 cutanious haematoma are taken care of by firm pressure with thumb, raising arm above the level of heart and applying ice. Haematoma seen later as

ablackishdiscolorationaround phlebotomy site after 24 hrs, is painless and self-limiting. Apply thrombophob and reassure the donor.

POST DONATION CARE OF DONORS

Donor should be kept under continuous observation during and after donation. For at least 15 minutes the donor should lie down, after donation after he or she may allowed to sit, look for signs of postural hypotension like dizziness, sweating, 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 and 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 for agglutination or haemolysis indicates positive reaction and a 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

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

(Reactions – serum grouping)

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 Du.

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 37Cfor 30- 60 minutes.

2.After incubation read microscopically for agglutination.

3.If negative, wash, decant the saline completely.

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 minutes, read microscopically.

7.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

Serum grouping

A CELL	B CELL	O CELL
O	O	O

SUB GROUPS OF A AND AB

A and AB are divided in to subgroups like A1, A1B, A2and A2B depending upon the reaction with Anti- A1.

PROCEDURE

1. Place one drop of anti A1 in a clean-labeled slide.
2. Add the test cells, mix and keep at room temperature. Positive results (agglutination) indicates the sample is A1. Negative results indicate the sample is A2.

SCREENING FOR ATYPICAL ANTIBODIES

For screening of atypical antibodies two techniques are commonly used. Enzyme method for donors screening and IAT for patients.

1) Enzyme technique (papain two stage method)

a) Take one clean-labeled test tube.

b) Place two drops of test serum into the tube.

c) Add 1 drop of papanised O cells into the tube.

d) Mix well and incubate at 37 C for 1 hour.

e) Read microscopically after 1 hour. Agglutinntion or hemolysis indicates positive reaction.

Negative result must be checked microscopically.

ANTI HUMAN GLOBULUIN TEST

The antiglobulin test is popularly known as Coomb's test.

PRICIPLE

Red blood cell wanted with IgG or C3d component of complement either in 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 complement component)

Preparation of igG coated positive control cells

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

IN DIRECT ANTI GLOBULIN TEST

Indirect anti globulin test is done for the following purpose

1) Compatibility test

2) Screening and identification of unexpected antibody 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 forDu testing

METHOD

1) Take two drops of serum to be tested in a labeled tube

2) Add one drop 2 – 4 % suspension of reagent O cell

3) Incubate at 37°C for 45 to 60 minutes

4) Look for haemolysis or agglutination. Agglutination or haemolysis in this stage indicates presence of saline reacting antibody.

5) If no agglutination is seen wash the cell three times in the saline and decant the last wash comp[lely

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 microscopically.

9) Record the results

10) If negative add one drop of IgG coated cells

11) Mix and centrifuge at 1000 rpm rpm one minute.

12) look for agglutination, if no agglutination is seen test is invalid and the whole procedure is repeated.

CONTROLS

For positive control: one drop diluted Anti D + one drop O positive washed cell keep at 36 ^{0}C for one hour. After 1 hour, wash 3 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.

INDIRECT ANTI GLOBULIN TEST

1) Take one clean, labeled test tube.

2) Place two drops of test serum in to the tube.

3) Add one drop of pooled O cell in to the tube.

4) Mix well and incubate at 37^0c for 30 to 60 minutes.

5) After 30 minutes examine for agglutination or haemolysis.

6) If there is no haemolysis or agglutination wash the cell 3-4 times with large volume of normal saline, decant each wash completely.

7) Add one drop of AHG serum in to the test tube mix well and centrifuge at 1000 rpm for one minute.

8) Gently shake the tube to dislodge the cell button and read. Agglutination indicates positive reaction. Negative results should be checked microscopically.

9) If the result is a negative, add one drop of IgG coated cells as control and centrifuge at1000 rpm for one minute. Examine for agglutination if the result is negative whole procedure should be repeated.

Control: - positive control – diluted Anti D with pooled O +ve cells

Negative control :-diluted Anti D with pooled Oneg cells

RED CELL CONCENTRATE

DEFINITION

A component obtained by partial removal of plasma from whole blood 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 and speed or red cell settle down when anticoagulated blood is kept undisturbed enabling separation of plasma and cells.

SCOPE AND APPLICATION

Symptomatic anemia acute or chronic for restoration of oxygen carrying capacity, less in high titre blood group antibodies, less plasma proteins with packed cells, there are minimum anaphylactic reactions.

PROCEDURE

Prepare either by sedimentation or centrifugation.

1.Sedimentation:

The blood is kept at 4°C in an up right position undisturbed ,separate the plasma when clear in to a satellite bag the red cells have a PCV of 70%.

2. Centrifugation:

1. Centrifuge the collected blood in a refrigerated centrifuge at 3000 rpm or 10 minutes at 4 -6 °C.

2. Express approximately ¾ of plasma in to the satellite bag.

3. Double seal the tube between primary and satellite bag with PCV not higher than 80%.

4.Llabel the plasma and kept at-30 °C or below.

5 Expiry of red cell concentrate is 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 undisturbed enabling separation of plasma and cells.

Scope and apllication

Symptomatic anemia indicated in patients with repeated nonhaemolytic febrile transfusion reaction due to leucocytes as in transfusion dependant patients and multi parous women.

Procedure:

There are many procedures for preparing leucocyte 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 an inverted position at 3000 rpm for 10 minutes at $4^{o}c$

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 $4^{O}c$

2) plasma buffy coat layer and 10 to 20 ml of red cells at top are expressed in to satellite bag

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 24hrs when stored at 2 - 6^{0} C

Spin and Filtration:

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

GRANULOCYTE CONCENTRATE

Definition

A component containing primarily of granulocyte suspended in small volume –

-Of 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 also indicated in aplastic anemia and bone marrow suppression as in hematological malignancy

Separation from single random units

1.blood is collected and kept at room temperature before processing which should be done within 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 Willebrand's disease,
disseminated 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 $5 - 10 * 10^9$ L/BSA in patient whose thrombocytopenia is not due to increased destruction.

Scope and application

Bleeding due to 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°c at 1300rpm for 9 minutes (or 1700rpm for 7 minutes) with in 8 hours of collection. Express the Platelet Rich Plasma into the satellite bag without contamination of white cell layer close to red cells.

Double seal the tubes, label and separate the bags and keep at room temperature for another one hour without disturbing and store the PRP in a horizontal platelet agitator at 22°C at an RPM of 70 strokes per minute.

PLATELET CONCENTRATE

FUNCTION

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

SCOPE AND APPLICATION

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

PROCEDURE

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

Platelet poor plasma can be frozen as FFP.

SINGLE DONOR PLASMA

FUNCTION

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

SCOPE AND APPLICATION

Used in volume deficient and in hypoprotenimia. Pooled plasma is used for preparation of plasma proteins like albumin and immunoglobulins.

PROCEDURE

Blood is collected and kept at 4°C in an up right position, undisturbed. 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 C or below.

Expiry –5 years

SCREENING OF

TRANSFUSION TRANSMITTED DISEASES

SCREENING HUMAN 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(ELISA) is the most widely used test for detection of HbsAg, HIV, HCV.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 micro plate.
2) Negative and Positive controls.
3) Conjugate-Anti IgG linked with an enzyme HRP.
4) Substrate-colour giving solution (TMB/OPD).
5) Stop solution.
6) Wash solution concentrate.

STORAGE AND SHELFLIFE

Store at 2-8^{0}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^{0}C for up to one week or should be frozen at –10 C or lower for longer term storage.

REAGENT PREPARATION

Bring all reagents to room temperature for 15-20 min before beginning the assay.

Wash Buffer: Dilute wash buffer concentrate as per the manufacturer's instruction.

ASSAY PROCEDURE

1) Fix appropriate number of strips to the micro plate 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 substrate 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 absorbance of NC, PC and specimens.

INTERPRETATION

1) Specimens with absorbance 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.

Cutoff Report

Analysis Date : Aug 22,2005/15:02:59
Protocol File Name : qualisa hbsag.prt
Raw data file : C:\CODA\eia950.raw
Protocol Name :qualisa hbsag
Plate ID : Plate1
Regression:Cutoff Analysis
Kit Lot Number : 61010

Cutoff method : Cutoff Formula

Cutoff value : 0.087 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.082		
Negative Control		-	1	0.037		
Positive Control		+	2	* 3.26		
85364		-	1	0.003		
85365		-	2	0.021		
85366		-	3	0.001		
85367		-	4	-0.002		
85368		-	5	0.000		
85369		-	6	0.030		
85370		-	7	0.003		
85371		-	8	-0.004		
85372		-	9	0.001		
85373		-	10	0.010		
85374		-	11	0.004		
85375		-	12	0.024		
85376		-	13	0.003		
85377		-	14	0.059		
85378		-	15	0.032		
85379		-	16	0.070		
85380		-	17	0.032		
85381		-	18	0.013		
85382		-	19	0.025		
82353		-	20	0.032		
weak +ve		+	21	0.530		

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 2-8^{0}C. 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 2-8^{0}C for up to 1week or should be frozen at –10^{0}C 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 37^0c 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 37^0c 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 sun light.

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. Sample OD values in the gray zone (10% below cut –off) are repeated

Cutoff Report

Analysis Date : Aug 20,2005/17:24:14
Protocol File Name : qualisa hcv.prt
Raw data file : C:\CODA\eia948.raw
Protocol Name :qualisa HCV
Plate ID : Plate2
Regression:Cutoff Analysis
Kit Lot Number : 61010

Cutoff method : Cutoff Formula

Cutoff value : 3.329 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.032		
Negative Control		-	1	0.009		
Positive Control		-	2	* 3.13		
85342		-	1	0.024		
85343		-	2	0.061		
85344		-	3	0.025		
85345		-	4	0.017		
85346		-	5	0.016		
85347		-	6	0.055		
85348		-	7	0.037		
85349		-	8	0.016		
85350		-	9	0.028		
85354		-	10	0.024		
85355		-	11	0.097		
85356		-	12	0.047		
85357		-	13	0.125		
85358		-	14	0.058		
85359		-	15	0.021		
85360		-	16	0.030		
85361		-	17	0.044		
85362		-	18	0.035		
85363		-	19	0.036		
85341 rpt		-	20	0.146		
wk+		-	21	0.260		

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.

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.

Cutoff Report

Analysis Date : Aug 22,2005/15:13:53
Protocol File Name : hivase hiv.prt
Raw data file : C:\CODA\eia951.raw
Protocol Name :Hivase HIV
Plate ID : Plate1
Regression:Cutoff Analysis
Kit Lot Number : D44A312P

Cutoff method : Cutoff Formula

Cutoff value : 0.097 (O.D.)

Patient ID	Name	+/-	Num.	Abs.	SD	CV%
Blank			1	0.074		
Negative Control		-	1	-0.003		
Positive Control		+	2	2.276		
85364		-	1	0.013		
85365		-	2	0.054		
85366		-	3	-0.007		
85367		-	4	0.012		
85368		-	5	0.003		
85369		-	6	-0.017		
85370		-	7	-0.008		
85371		-	8	-0.020		
85372		-	9	-0.012		
85373		-	10	-0.012		
85374		-	11	-0.014		
85375		-	12	0.007		
85376		-	13	-0.017		
85378		-	14	-0.020		
85380		-	15	-0.014		
85382		-	16	-0.020		
85351		-	17	-0.012		
85352		-	18	-0.017		
85353		-	19	-0.011		
85354 rpt		-	20	-0.016		
weak +ve		+	21	0.658		

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.

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 OF BLOOD COMPONENTS

FUNCTION

Primary goal of Quality Assurance 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 elements or coagulation factors.

SCOPE AND APPLICATION

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

PROCEDURE

Criteria of donor selection, volume of blood drown, accuracy of seals and anticoagulant volume, applicable to component preparation as well as whole blood collection.

GENERAL CONSIDERATION

1) Donor weight 60 Kg and above

2) Blood is collected in 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 venipancture.

4) FFP should be prepared with in 6-8 hrs of collection ideally PRP should 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.

7) Run time RPM and temperature to be programmed each components

8) Manual break 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, if plasma is intended for cryo.

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

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*1000 *Volume of WB (in ml)= number of platelets in WB

2. Peform platelet count on prpr sample

3. Platelet count /microlitre* 1000 of PRP=no of platelet in PRP

4.calculate Percentage of yield

Platelet in PRP*100 / Platelet in WB = Percentage of yield

Quality Control for Frozen Plasma

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 on inspection, there is abnormal appearance and color of units.

2) For platelet nearing out dating and returned plasma units redesigned.

3) For leucopoor red cell prepared by centrifugation and washing

4) Returned and out dated units

5) At blood after washing

IV Pediatric units or cell concentrates 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

For transportation of whole blood and red cell components, maintain temperature with wet ice in leak proof containers. 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

and secure them with a rubber band check temperature after 60 seconds.

IV .for minimum requirements of documentation on labeling. Component preparation, storage, inspection of blood components

VII 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

No	Name of Component	Storage Temp	Expiry
1	Red Cell Concentrate (RBC)	4 – 6^{0}C	35 days.
2	Byffy coat	20 – 24 ^{0}C	24 hrs
3	FFP	-20^{0}C	1 year
4	PRP	20-24^{0}C	72 hrs
5	PC(from PRP)	20-24^{0}C	72 hrs

No	Component	Run time(mts)	Run speed(RPM)	Temperature
1	Red Cell concentrate	10	3000	4-6
2	Buffy coat	10	3000	20-24
3	FFP	10	3000	4-6
4	PRP	7	1300	20-24
5	Pc from PRP	10	3000	20-24

QC of Whole Blood / Component

No	Parameter	Requirement	Frequency of Ctrl
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 RPR	All units
5	Volume excluding anticoagulant	350/450 +/-10%	All units
6	% of hemolysis at the end o f storage	<.8% of red cell mass	1% of units
7	Sterility of growth	No growth	1% of units

QC OF LEUCOCYTES – POOR RED CELL (BUFFY COAT REMOVED)

PARAMETER	REQUIRMENT	FREQUENCY OF CONTROL
Volume	250 ± 50 ml	Whenever prepared
HCT	0.65-0.75	"
White cells	<1.0* 10^9 (70% white cell removed)	"
Red cell	Retain 80 %of red cells	"
Sterility testing	No growth	"

QC OF PC PREPARED FROM ONE UNIT WB (450 ML)

PARAMETER	REQUIRMENT	FREQUENCY OF CONTROL
Volume	40 – 50 ml	1 % OF ALL UNITS
Platelet count	≥ 5.5 * 10^{10}/unit	"
Ph	>6 (6.0 – 7.4)	"
Residual leukocytes and red cells	<0.05 - 1*10^9/l 0.2 – 1*10^9	"

QUALITY CONTROL OF FFP

PARAMETER	REQUIRMENT	FREQUENCY OF CONTROL
Volume	200 - 250 ml of plasma	1 % of ffp
FactorVIII C	.07 units in ml	To be checked periodically at
Residuel cells	< 0.1* 109/L	
Platelets	< 50 * 109/L	
RBC	<5 ML	

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	$1*10^{10}$(leucopheresis)	Whenever prepared
Red cell contamination	25-50ml	

Q C OF REAGENTS

Procedure

QC of reagent on Receipt:

Prior to receipt of any reagent, check-

Name of antisera, 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.

Antisera	Cells	Avidity
Anti A	A1 cells A2 cells A2B Cells	10 Sec 15 – 18 secs 15-18 secs
Anti B	B cell A1B cells	10 secs 10 secs
Anti AB	A1 cells B cells A2B cells	10 sec 10 sec 15 – 18 secs
Anti D (IgM-IgG blend)	D+ cells Weak D cells	10-15 sec

3. Specificity on receipt: - It is the ability of the reagent 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 antiasera and 2-5%pooled cells as directed.
   2. Keep the tubes at room temperature for 30 minutes.
   3. Spin at 1000rpm for one minute
   4. Agglutination should be observed as above. If any unexpected reaction is seen, test should be repeated

4. Sensitivity/Titre (Anti A/B/AB/D/H/A1 – sera): Titration is a semi quantitative technique of measuring the con. 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 and 2.
   4. Mix the tubes 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.
   6. Add 100 microlitres of 2-5% washed saline suspended appropriate red cells to each tube.
   7. Mix well and incubate for 30 to 40mts at room temperature.

8. At the end of incubation time, centrifuge the tubes at 1000rpm for 1 min.
9. Gently dislodge the cell button.
10. 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.
11. Last tube which gives a reaction grater than or equal to +1, is the titre of the antibody.

QC OF ANTI HUMAN GLOBULIN REAGENT (on receipt)

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 labelled positive and negative controls.

(b) Add 50 microlitre of saline to all tubes except the first.

(c) Add 50 microlitre of AHG to test tubes 1 & 2.

(d) Mix the tube well without forming air bubbles and transfer 50 microlitre from test tube 2 to 3.

(e) Continue the same till the last tube and discard 50 microlitre 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 should give 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 C_3/C_4	1 : 8

Notes

1. Non specific reaction ruled out with A, B & 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

(a) Label 10 test tubes.

(b) Add 100 microlitre of saline in all tubes.

(c) Add 100 microlitre anti-D (IgG anti –D) in the first.

(d) Mix without forming bubbles and transfer 100 microlitre to the next tube.

(e) Continue the same till the last tube and discard 100 microlitre from the last tube.

(f) Add 100 microlitre of 2-5 % O+ cells to all the tubes.

(g) Mix and incubate the tubes at 37°C for 1 hour.

(h) Wash the tubes three times with saline.

(i) To the cell button in each tube add AHG

(j) Centrifuge the tubes at 1000rpm for 1 minute.

(k) Last tube which gives positive reaction is considered as the titre of AHG.

DAILY QC OF AHG

Specificity

1. Sensitize O positive cells for positive control
2. O negative cells or O+ cells without 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 37°C for 15 min.
4. Look for agglutination (If there is agglutination saline is not working)
5. If it is negative, centrifuge and discard the supernatant.
6. Add 2 drops of albumin and incubate at 37°c for 45 minutes.
7. Centrifuge and look for agglutination. (If there is agglutination albumin is not working).
8. If it is negative, wash 3 times with saline and decant the saline completely.
9. Add 2 drops of AHG and centrifuge at 1000 rpm for 1 minute.
10. Look for agglutination.
11. If it is positive AHG or saline is not working. If negative AHG & saline are working.

QC OF BOVINE ALBUMIN 22%

Specificity and reactivity

Positive Control - 1 drop of diluted anti D & 1 drop 3-5% O Rh +cells.

+ Bovine albumin.

Incubate at 37°C 45 minute. Centrifuge at 1000 rpm for 1 minute.

Negative Control

1) Bovine Albumin + 3.5% unsensitized Rh positive cells +one drop IgG anti- D + 1 drop 5% Rh D –negative cells

Incubate at 37°C for 1 hr.

QC of reagents prepared locally

Saline : It should be prepared with fresh distilled water (pH 6-7) to a conc. of 0.9 g%. It should be stored in glass free of silicate or plastic allowing no toxic substance to leach into saline. It should be well stoppered to present 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 (pH 6.5) will enhance red cell agglutination. Alkaline of 9 or greater is inhibitory.

Pooled Cells :

Pooled cells for antibody detection should be prepared from anticoagulated blood not more than 5 days old. Records of reactivity and specificity must be maintained. Should be prepared daily and inspected for evidence of haemolysis 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 sensitization, to eluate, as the cells stand on storage.

Note : If the cells are over-sensitized, cell may agglutinate without 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 for optimum effectiveness. (Page 561, AABB Technical Manual, 10th Edition 1990). Store papain solution at -20°C in small aliquots. Thaw 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.

Standardization of papain – cystein solution

Standardization 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 old papin one drop each.

4. Add 2-5% O+ cells in both the rows.
5. Keep at 37°C 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 Papaine	$+_4$	$+_4$	$+_3$	$+_3$	$+_2$	$+_1$	_	_

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°C for 45 minutes and take the reading.

	A+	A - ve	B+	B–ve	O+	O -ve	AB+	AB-ve
Anti-D+new papain + known cells	+	-	+	-	+	-	+	-
New Papain + Known cells	-	-	-	-	-	-	-	-

Impression : Papain cystein solution prepared is specific.

LOW IONIC STRENGTH SOLUTION (LISS)

Non – serological

1. pH should be within the range 6.65 – 6.85.
2. conductivity should be 3.6 – 3.7 mmbo/cm at 23°C
3. Osmolarity 270-285 mmol.

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 tests using 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 three consecutive days to evaluate Inter run reproducibility. By either of these methods, variation should not exceed 10%.

Copy of format in use : Nil

End of the document

PREPARATION OF PAPAIN CYSTEIN

SCOPE AND APPLICATION

Papain cystein enzyme is used both in antibody detection tests and cross matching 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 sialoglycoprotein from the cell surface. This reduces intercellular distance; expose crypt antigen and increase mobility allowing clustering.

REAGENTS

Papain powder : 1 gm .

Cystein hydrochloride : 0.480gm

5N NaOH(20 gm NaOH to 100 ml distilled water)

1) Buffer KH_2PO_4 (9.078GM potassium dihydrogen phosphate in 1 L of distilled water)------ 80 ml
2) Na_3PO4 (11.876 gm disodium hydrogen phosphate in 1 L of distilled water ----------20 ml

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 cystein hydrochloride. In 90 ml buffer, dissolve 1gm 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. Dispose in small quantities and freeze at 20^0c.

PREPARATION OF PAPANISED O CELLS

(By using LIQUIPAP)

Procdure:

Washed packed (50%) pooled O Rh +ve cells are used. One part of 'liquipap'(A commercially available product)is added to three part of washed packed RBCs, incubate at 37°c 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° C 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°c in small aliquots. Thaw fresh 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.

PREPARATION OF IgG COATED CELLS

Scope and Application:

To ensure the activity of Coombs reagent which is used for antigen detection, antibody detection and identification.

Procedure:

Dilute IgG anti-D 1/60 in saline. Add 19 drops of diluted anti D to 1 drop of washed pooled O positive cells. Mix well and incubate at 37°C for 30 to 60 minutes. Wash 3 times in excess saline and resuspend to 2-5%. To be prepared daily.

Quality Control:

Add 1 drop of AHG to I drop IgG coated cells. Spin at 1000rpm for 1minute and 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 +2macro reaction. If heavily coated, cells will not detect loss of IgG activity.

Note:

If the cells are oversensitized, cells may agglutinate without 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 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 2 ml of blood. Dry it in hot air oven. Preheat the oven at 160°C, 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 a neutral P^H , which can then be used as a diluent in serological tests.

Function:

Mixture of acids and bases can be prepared at specific P^H values used to buffer other solutions to that P^H.

Reagents:

1) Prepare Acidic Stock solution(Solution A) by dissolving 22.16gm/litre $NaH_2PO_4.H_2O$ in 1 liter of distilled water. This 0.16m solution of monobasic phosphate salt(monohydrate) has a P^H of 5.0
2) Prepare alkaline stock solution(Solution B) by dissolving 17.2gm/litre of $NaHPO_4$ in 1 liter of distilled water. This 0.16m solution of the dibasic phosphate salt has a P^H of 9.0

Procedure:

a) Prepare working buffer solution of the desired PH by mixing appropriate volume of two solutions.
b) Check P^H of working solution before using it. Add small volumes of Acid solution A or Alkaline solution B to achieve desired P^H.
c) To prepared PBS of desired P^H, add 1 volume of phosphate buffer, at that P^H to 9 volume of normal saline.

CRYOPRESERVATION OF RED CELLS AND DEGLYCERALISATION

SCOPE AND APPLICATION:

Preservation of red cell 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 hypertonocity is also avoided.

PROCEDURE:

Reagents:

Buffered tripotasium citrate, it contains,

1) Tripotassium citrate ($K_3C_6H_2O_7H_2O$) - 3.25%

2) Potassium dihydrogen phosphate(KH_2PO_4) - 0.47%

3) Dipotassium Hydrogen phosphate(KH_2PO_4) - 0.6%

It is convenient to make a liter of this stock solution.

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. 40ml of glycerol well mixed with 60ml of the buffered reagent is a convenient quantity.

Laying down process:

The blood is to be stored is taken into 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 paced 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 dilution. 200ml of 16% prepared by adding 25.6ml of glycerol to 174.4ml 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 requiremen. 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.

PRETRANSFUSION TESTING OF NEONATES

(Less than four months old infants)

Procedure:

Pretransfusion in neonates should be restricted to cell grouping only. Antibody screening to be done on baby's/maternal serum. If 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, cross match up to 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. Under such circumstances, the incubation period of compatibility testing can be reduced to 15mts and result read after centrifugation(light spin only). Clinician in informed accordingly and blood is issued. However crossmatch test should be continued for the period of incubation and any discrepancy should be informed. Tube containing saline suspended donor cells and serum is spin and read result if antibody screen is negative. No incubation is needed here.

LISS compatibility test(Low Ionic Strength Solution):

LISS solution increases the rate of antibody uptake and reduces the electrostatic barrier surrounding the red cells. Incubation time is reduced in LISS solution.

Pocedure:

1) Wash the red cells twice in saline and once in LISS and suspend the red cells in LISS to make up 2 – 5%suspension.
2) Take 2 drops of patient serum in a labeled test tube.
3) Add 2 drops of LISS suspended red cells to the tube.
4) Incubate at 37^0C for 10 to 15 minutes.

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) If tested negative, add IgG coated red cells.
9) Centrifuge and check for agglutination.

CROSSMATCH FOR NONSPECIFIC BLOOD GROUP TRANSFUSION.

Normally group specific transfusions are to be carried out. But in exceptional circumstances ABO nonspecific group transfusions are to be done. As a lifesaving 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 titre of anti B will be less than anti A in group B persons.

In this situation, apart from major crossmatch minor match is essential. Titre less than 100 is chosen to avoid high titre antibodies as seen in dangerous O group donors. Removal of plasma can reduce the amount of antibodies.

Procedure:

1. Dilute donor serum 1/100(i.e. to 1drop of serum, add 9 drops normal saline to make 1/10 dilution. To 1 drop of this diluted serum, add 9 drops of normal saline to make 1/100).
2. Place 2 drops serum (1/100 dilution) each in labeled test tube. Add one drop of 2-5% patient cells. Put one drop bovine albumin (22%) in the test tube II(A)
3. Incubate at 37^0C for I hour
4. Observe for hemolysis/agglutination.
5. Proceed to AHG with tube I(S).

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 haemolysis, clots, leaking etc, recheck unit no 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 crossmatch 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 mts 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 phycician is needed before issue.

10) If Rh negative blood is not available, Rh positive blood is given in life saving situations, taking into account, Patiens age, sex, Child bearing potential. A written request from the phycician is obtained to that effect before issue.

11) Final identifiation of the recepient and donor unit is done by the transfusionist.

12) Reaction forms are received back duly filled and signed, and is preserved with request form of each patient.

Special Considerations:

1) Children weighing <25 Kgs are given paediatric 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 within 24 - 36hrs and maintained with in acceptable temperature and no abnormality detected on inspection. Returned units are quarantined for 24 - 48hrs 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 labelled as SDP only.

4) Opened units if not used are discarded after 24hrs.

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. It can occur as a result of:-

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

Samples required for serological investigation

1) Pretransfusion 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 administration 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 affect other crossmatched units 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 incompatible transfused cells are not immediately destroyed DAT will be 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 incompatible donor cells

* Repeat antibody detection on patient's 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 anamnestic reaction or passive administration of an antibody present in transfused component

* Do a smear study and culture on blood remaining in the bag at 4^0c,20-24^0 C and 37^0c. If infected and blood proved to the cause of reaction , investigate thoroughly all proceedings involved in collection, processing, storage and transport of blood components

* Perform biochemical tests like plasma Hb and serum ,bilirubin, post transfusion urin sample for free Hb, etc

DELAYED HAEMOLYTIC TRANSFUSIONREACTION(DHTR)

*Due to primary alloimmunisation (several weeks after transfusion)

*Due toanamnestic 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

INVESTIGATION OF HDN AND NEONATAL TRANSFUSION

1) SCOPE AND APPLICATION:

Maternal IgG cross the placenta and enter the foetal circulation leading to haemolytic complications in newborns, Very small blood volume of the newborn and the antibodies in the transfused blood pose a serious problem faced by the transfusion centre.

2) 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 indented blood should be of same group 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 conc. 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 anemia 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 newborn 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
Elute tested against A,B & O using ICT.

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 positive

ICT : Positive

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

Selection of blood in HDN

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

Cord blood:

Blood group Rh D positive

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

Blocked D phenominon:

When all the Rh D sites are blocked by anti D, cord cells willnot 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 findout 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 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 cmpatibility testing with maternal sample.

Selection of blood:

Blood group compatible with both mother and baby.

INTERPRETATION

Absence of haemoglobinaemia and a negative DAT suggests that an acute immune HTR has not occurred. If the patients clinical condition strongly suggests an hemolytic reaction ,further investigation is warranted despite preliminary negative results.

HAEMOLYSIN TEST

1) 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.

2) Procedure:

Clear serum obtained from clotted samples within 12hrs of collection is tested for the presence of anti A and anti B haemolysins. Fresh AB group serum can be used as a sourse 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 and incubate for one hour.

4) Observe the color of supernatant serum. A pink or red color indicates haemolysis. Haemolysis in the tube containing group A red cells indicates the presence of anti A 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.

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 rationale for plasma exchange in these diseases is to remove antibody (an IgG auto antibody in most cases) and there by reduce tissue damage.

2) Procedure:

1) Receive properly labelled whole blood from the patient in 450 ml blood bag. For children use 350ml collection bag.

2) Seal and weigh the bag, record the total volume of blood collected.

3) Centrifuge the bags at 3000rpm for 10minutes at 4°c 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 & 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 the operation site or 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 labeled in 450ml CPDA bags are received at blood banks.

2. Seal and centrifuge at 3000rpm for 10min at 40c.

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 40C. Repeat the 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 4 hours.

RECORD KEEPING

Scope and Application

1. To provide permanent references for 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 the system is made nearly false proof. It allows for computer check of datas and thus leads to patient's 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 framework is an added advantage of computer system.

Record of Donors:

1. Donors Registration Card.
2. Donor Register.
3. Blood components register.

Record of Patients:

1. Blood group register.
2. Cross match-Transfusion Register.
3. Issue Register
4. Transfusion Reaction Investigation Register.

PREPORATION 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.

PREPORATION OF POTTASSIUM DICHROMATE SOLUTION

Potassium dichromate -1 kg

Concentrated H_2SO_4 -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. H_2SO_4

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.