

**SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL  
SCIENCE AND TECHNOLOGY**

*(An Institute of National Importance)*

**THIRUVANANTHAPURAM, KERALA, INDIA-695011**



***DIPLOMA IN BLOOD BANKING TECHNOLOGY***

***LOG BOOK***

***2014-2015***

***Submitted by***

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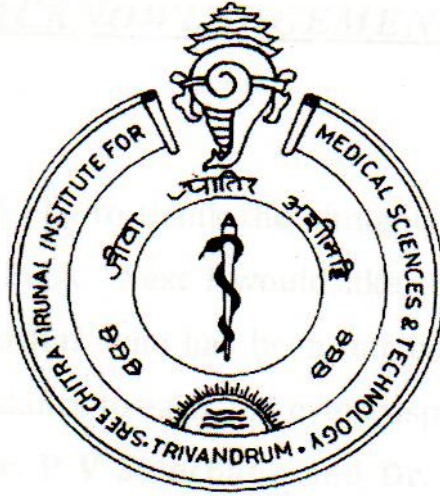
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I, **Ms. SAFEENA S** hereby declare that I have actually performed all the procedure listed/carried out the project under report.

Place: Thiruvananthapuram

Name .....

Date : .....

Signature

Forwarded. She has carried out the minimum requirements of procedures.

Place: Thiruvananthapuram

Head of the Department

Date :

Transfusion Medicine

## 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 Impartment 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. P V Sulochana and Dr. S Sathyabhama, Dr. Debasish Gupta Blood transfusion Officers who had taken most of our lessons. They gave a kind mention towards me.*

*I would also thank Mrs. K S Sheela Devi, Mrs. Sindhu P.N, Scientific officer and all other staff of Department of Transfusion Medicine.*

*I thank the Director of the Institute Dr. Asha Kishor, Medical Superintendent Dr. Sarada, Dean Dr. Suresh Nair Registrar Dr. A V George, for their advice and kind attention towards me. I extend my heartfelt thanks to all Staff members of other departments for their - r p during my training period in this Institute.*

**SAFEENA. S**

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# **HEAMOGLOBIN ESTIMATION METHODS**

# **HEAMOGLOBIN ESTIMATION 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 a temperature of 4° C

Normal specific gravity of blood is 1.052 to 1.063

Average for men is 1.057

Average for women is 1.053

Specific gravity of serum is 1.026 to 1.031

Specific gravity of red cell is 1.092 to 1.095

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 haemoglobin present in the red cells
2. The plasma protein levels

## **DETERMINATION OF SPECIFIC GRAVITY**

### **COPPER SULPHATE METHOD**

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

The procedure consists of letting a drop of blood to fall in to a graded series of  $\text{CuSO}_4$  (of varying strength) (known specific gravity) and whether the drop sinks or rise in the solution. If the specific gravity is lower than the solution it will rise 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 without change

of specific gravity for 15-20 seconds. So the behaviour of the drop at this 15-20 second is considered.

## **TECHNIQUE**

### **PREPARATION OF STOCK SOLUTION**

159.63gm of copper sulphate crystals is added to distilled water and shaken vigorously for 5 minutes, and made-up to one litre. 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, solution of varying specific gravity can be prepared.

For the determination of specific gravity of blood, a series of tubes containing the solution varying in specific gravity of 0.002 may used. A drop of blood is approximately 3 inches above the column of solution and the behaviour of the drop in the 15-20 second is noted. The solution in which the drop becomes stationary at this time corresponds to the specific gravity of 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 Vanslyke et al 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 and 12.5gm Hb%.

Male = 522.5CS + 477.5dw 1000 1053 12.5gm%

Since the male volunteers can be accepted for donation if they have a Hb% 12.5gm and above, this 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 behaviour is noted. If the specific gravity is lesser than the solution, the donor is rejected.

Since the female have a lower Hb% and hence lesser specific gravity, the minimum set Hb% acceptable for blood donation is also 12.5gm that corresponds to the specific gravity 1.053

### **PROCEDURE**

- Dispense a sufficient amount (at least 30 ml) of copper sulphate solution to allow the drop to fall approximately 3 inches into appropriately labelled, clean, dry tubes or bottles. Change solution daily or after 25 tests. Be sure the solution is adequately mixed before beginning each day's determinations.
- Clean the site of skin puncture thoroughly with antiseptic solution and wipe dry with sterile gauze.

- Using caution, puncture the finger firmly, near the end but slightly to the side, with a sterile, disposable lancet or spring-loaded, disposable needle system. A good free flow of blood is important. Do not squeeze finger repeatedly, as this may dilute the drop blood with excess tissue fluid and give falsely low results.
- Collect blood in an anticoagulated capillary tube without allowing air to enter the tube.
- Let one drop of blood fall gently from the tube at a height of about 1 cm above the surface of the  $\text{CuSO}_4$  solution.
- Observe for 15 seconds.
- Dispose of needles and blood-contaminated gauze in appropriate containers.

## **INTERPRETATION**

- If the drop of blood sinks, the donor's haemoglobin is at an acceptable level for blood donation.
- If the drop of blood does not sink, the donor's haemoglobin is not at an acceptable level for blood donation.
- 

## **ADVANTAGE OF THIS METHOD IN MASS SCREENING**

1. This method is simple.
2. The temperature coefficient of expansion of copper sulphate and blood are equal, so there is no need for separate temperature regulation.
3. The substance used is not toxic and does not give explosive vapours.

4. Dispersion of blood in copper sulphate solution is low and visualizations is easy.
5. The copper sulphate solution cleans automatically by itself.
6. After a few minutes, a drop settles to the bottom and it can be removed and the solution can be used again.

### **QUALITY CONTROL**

- The test method is not a qualitative test, but it is fast
- Large number of donor can be screened in minimal time
- 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
- Do not squeeze the finger, as this may dilute the drop of blood with tissue fluids and give falsely low result
- The capillary tube should be filled without air bubbles
- The drop must be dropped from a height of 1 cm above the surface of 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 and should be changed daily or after 25 tests while being used so that it does not have a change to evaporate and concentrate and thereby causing the donors to reject unnecessarily.
- A solution at 2.0L, kept in working bottles should be used only for 30 to 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.
- Mix solution thoroughly before beginning each day's determination.

Sl.no	Name of donor	Age	Sex	Haemoglobin value
1	Aswathy	29	F	<12.5gm/dl
2	Vijitha G Nair	24	F	<12.5gm/dl
3	Baby priyanka	21	F	<12.5gm/dl
4	Usha	48	F	>12.5gm/dl
5	Aravind	18	M	>12.5gm/dl
6	Mohanan p	42	M	>12.5gm/dl
7	vasantha	40	F	<12.5gm/dl
8	Aji sreekumar	35	M	>12.5gm/dl
9	AkkuAlex	22	F	>12.5gm/dl
10	Naseemabeevi	45	F	<12.5gm/dl

## **HEAMOGLOBIN ESTIMATION BY CYANMETHAEMOGLOBIN**

### **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 (cyanmethemoglobin). The absorbance of the solution is then measured in a photoelectric calorimeter at a wavelength of 540nm  $\pm$ 15nm.

## **BLOOD SAMPLE**

1. Venous blood or free flowing capillary blood added to any solid anticoagulant (1mg 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 Drabkin's reagent

Potassium ferri cyanide	200mg
Potassium cyanide	50mg
Potassium dihydrogen phosphate	140ml
Nomidet P40	1ml
Distilled water	11

The reagent should be clear and pale yellow in colour. When measured as blank in photoelectric calorimeter at a wavelength of 540nm  $\pm$ e absorbance must read zero.

## **REAGENT PREPARATION**

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

## **METHOD**

- Switch on photoelectric calorimeter and wait for 15-20minutes to warm before use
- Add 0.02ml of blood to 5ml of diluent. Stopper the containing solution and invert it several times .Allow it to stand at room temperature for 5-10 minutes to ensure the completion of the reaction. The solution of HICN is ready to be compared with standard.

- Select filter of wavelength 540 nm
- Set the calorimeter at zero against blank
- Measure the absorbance of standard solution in the calorimeter against the blank
- Observe the absorbance value of the test solution prepared as in step 2.

### **INTERPRETATION**

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

$$\text{Hb gm/dl} = \frac{\text{OP of the test} \times \text{conc. of the std. Solution}}{\text{OD of the standard}}$$

### **Hb ESTIMATION USING HEMOCUE**

The hemocue haemoglobin analyzer is a portable, rapid and accurate method of measuring haemoglobin.

The hemocue system consists of a disposable microcuvette, which contains reagents in dried form. The blood is placed in the microcuvette and a portable photometer determines the Hb. Whole blood is drawn up in to the microcuvette by capillary action and inserted in to the photometer. The light is passed through the sample and the absorbance of methhemoglobinazide is measured at 570nm and 880 nm to ensure automatic compensation for turbidity (due to lipemia or leukocytosis). Results are thus displayed after 45-60 seconds in gm/dl on a LCD display.

Each microcuvette has a volume of 10 micro litre and a short light path of 0.13nm between the parallel walls of the clear optical

windows. The microcuvette contains three reagents in dried form which convert the Hb into methemoglobinizide (H1N3).

Each microcuvette contains,

- Sodium deoxycholate
- sodium nitrate
- sodium azide
- Sodium deoxycholate haemolyses the red cells
- sodium nitrate convert haemoglobin (ferrous haemoglobin) to methaemoglobin(ferric haemoglobin)
- sodium azide converts methaemoglobin (HI) to methaemoglobin azide(H1N3)

After each measurement the photometer automatically zeros itself and check the intensity of the light source and the operation of the photocell.

Sl.no	Name	Hb value
1	Prasannakumar	14.4 gm/dl
2	Gopakumar	16.9gm/dl
3	Ajikumar	12.7gm/dl
4	Shaji	11.9gm/dl
5	Sajitha	13.1gm/dl
6	Sruthy	12.0gm/dl
7	Krishnasree	12.3gm/dl
8	Vyga	11.6gm/dl
9	Merlin	12.9gm/dl
10	Lekshmi	10.5gm/dl
11	Sreeparvathy	13.0gm/dl
12	Aneena	13.0gm/dl
13	Aparna	14.3gm/dl
14	Nikhitha	13.4gm/dl
15	Radhika	12.2gm/dl
16	Gayathri	11.2gm/dl
17	Chaithanya	13.9gm/dl
18	Bhagyalekshmi	11.4gm/dl
19	Nima	9.6gm/dl
20	Anjana rajesh	9.5gm/dl

## **COLOUR SCALE FOR HAEMOGLOBIN**

It is a simple and effective medical device for the accurate estimation of haemoglobin levels in blood. It comprises a small card with six shades of red that represent Hb level at 4, 6, 8, 10, 12 and 14 gm/dl respectively. The device is simple to use.

### **PRINCIPLE**

Colour scale for haemoglobin shall be based on the principle of comparing the colour of a drop of blood absorbed on a particular type of paper, against a printed scale of colour corresponding to different levels of Hb. The range of Hb shall be 4-14gm/dl.

### **TEST PROCEDURE**

1. Place a drop of blood on the test strip provided.
2. Wait about 30 seconds
3. Match immediately the colour of the blood spot against one of the shades on the scale. This will indicate whether the patient is anaemic and, if so, the severity of anaemia in clinical terms.

### **ADVANTAGE**

- Simple and inexpensive.
- It shall not require any additional chemical reagents, energy source etc. to perform the test.
- It shall be suitable for field work.

### **DISADVANTAGE**

- ❖ Unreliable, as the visual comparison of colour is subjective.
- ❖ Only 6 shades of colour with a scale difference of 2gm/dl given, accurate result cannot be obtained.

## **DONOR SELECTION**

Every blood transfusion service or hospital blood bank has a responsibility to ensure that blood donation does not harm either the donor or the recipient of the blood. The purpose of donor selection is to identify any factors that might make an individual unsuitable as a donor either temporarily or permanently. The process of donor selection includes.

- a. Medical history
- b. The health check

### **MEDICAL HISTORY**

It provides information needed to decide whether.

1. To accept the donor.
2. Defer the donor temporarily.
3. Exclude the donor permanently.

The simplest way of taking a medical history is to look into the standard medical history questionnaire filled by the donor each time they come to donate. It is important to explain the terms in simple words so that every donor can understand them and identify whether they have any of these conditions. Strict confidentiality must always be maintained about any information obtained.

The following criteria should be referred while evaluating the acceptance of a donor.

1. Diabetic: mild diabetic, diet controlled, donor is accepted. Diabetes controlled with oral hypoglycemic agents or insulin permanently unfit.
2. Heart disease: Acceptance depends on the nature of heart disease.

- ❖ A history of coronary heart disease or rheumatic heart disease with residual damage- Permanent deferral
  - ❖ Single episode of rheumatic fever or successful repair of a congenital defect -Donor is acceptable.
- 3. Malaria: Donor who have had malaria or taken anti-malarial prophylaxis must be deferred for 3 years after cessation.
- 4. Mental illness: Persons taking medication for psychiatric disorder or persons showing abnormal behavior are unacceptable as donors.
- 5. Drug addiction: Drug abuse of any nature, are cause of permanent deferral because intravenous drug abuse exposes the donor to parenteral transmission of transfusion transmitted disease and oral or inhaled drug abuse makes the donor unreliable about a proper history.
- 6. Prolonged bleeding from wounds: An abnormal bleeding tendency may be cause for permanent deferral. Individual with such a history may experience excessive bleeding at the site of venipuncture. Plasma from donor deficient in coagulation factors would not confer expected therapeutic benefit in a recipient who needs these factors.
- 7. Blood transfusion: Recipient of blood or blood products should be deferred for a period of 12 months.
- 8. Hospitalization: History of hospitalization gives a clue to the donor's medical history. Hospitalization for the medical management of diseases should be evaluated.
- 9. Allergy: Acceptable during symptom free period.
- 10. Hypertension: Known hypertensive on medication should be deferred. Nervous or anxious donor with blood pressure

reading ie. 140/90 at the first instance should be re evaluated after sometime.

11. Jaundice: Positive history of jaundice should lead to further interrogation to collect more information regarding duration of illness, intensity of illness.

### **PHYSICAL EXAMINATION**

1. General appearance: If the donor looks ill, appears to be under the influence of drugs or alcohol, or is excessively nervous, it is best to defer.
2. Weight: Donors weighing 50 kg or more may ordinarily give 525 ml. For donors weighing less than 50 kg, as little as 300ml may be drawn without reducing the amount of anticoagulant in the primary bag.
3. Temperature: The oral temperature must not exceed 37.5°C (99.5 F). Lower than normal temperatures are usually of no significance in healthy individuals.
4. Pulse: The pulse should be counted for at least 15 seconds. It should ordinarily exhibit no pathologic irregularity, and should be between 50 and 100 beats per minutes. If a prospective donor is an athlete with high exercise tolerance, a lower pulse rate may be acceptable.
5. Blood pressure: The BP should be no higher than 180mm Hg systolic and 100mm Hg diastolic.
6. Skin lesions: The skin lesions at the site of venipuncture must be free of lesions. Both arms must be examined for signs of intravenous drug abuse, especially multiple needle puncture marks or sclerotic veins.

## **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. Stethoscope
4. Test tube 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 solution
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 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 all bags in that package should be rejected.

## **LABELING**

The identity of the donor should be checked with that in the donor record form and 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 centre and allowing the antiseptic solution dry completely before venipuncture. 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 70mm 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 invert of the bag continuously must ensure proper mixing of the blood with 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, 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 to press the venipuncture site using sterile gauze. The blood remaining in the tube is transferred to pilot tubes, which are labelled 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 include dizziness, weakness, nausea, sweating, vomiting, pallor or in extreme cases loss of consciousness and involuntary passage of urine and faeces.

In the lying down position, a syncopal 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 and applying cold compressor to forehead.

Delayed syncopal attacks may occur as late as 30 minutes 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 referred.

Occasionally donor may vomit after a syncope attack and subsides by it's on. For hyperventilation and neuromuscular excitability like tetany, donor should be asked to breath into a paper bag.

Local complication due to phlebotomy, eg: - bleeding and utaneous haematoma are taken care of by firm pressure with thumb,

raising arm above the level of heart and applying ice. If haematoma is seen or as a blackish discolouration around phlebot.

Apply a bandage to the venipuncture site after 24 hours. It is painless and self limiting. Apply thromboprophylaxis to 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 he or she may be allowed to sit, watch out for signs of postural hypotension like dizziness, sweating, pallor etc. Generally 3-4 minutes of manual pressure suffices bleeding after which a medicated plaster is applied at venipuncture site.

### **POST DONATION INSTRUCTION**

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 & Rh TYPING**

### **FUNCTIONS**

To test the antigens and antibodies in the sample.

### **SCOPE AND APPLICATION**

For safe transfusion

### **CELL GROUPING**

### **PROCEDURE**

1. Take 3 test tubes labeled as A,B, and AB
2. Place one drop of corresponding anti-serum into each test tube.
3. Add one drop of 2 to 5% cell suspension from test sample either in saline or plasma. Mix well keeps at room temperature.
4. Read after 15-30 minutes for agglutination or haemolysis indicates positive reaction and a free cell suspension indicates negative results.

### **REACTION PATTERN □ CELL GROUPING**

<b>Anti A</b>	<b>Anti B</b>	<b>Anti AB</b>	<b>Results</b>
+	0	+	<b>A</b>
0	+	+	<b>B</b>
+	+	+	<b>AB</b>
0	0	0	0

### **SERUM GROUPING**

### **REAGENT CELLS PREPARATION**

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

**PROCEDURE**

1. Place one drop of test serum into A, B and 0 labeled test tubes.
2. Put one drop of A, B, and 0 cells corresponding tubes.
3. Mix the tubes and keep at room temperature. Read after 15-30 minutes.
4. Examine the tubes, haemolysis or agglutination indicates positive results.

**REACTION PATTERN □ SERUM GROUPING**

<b>A cell</b>	<b>B cell</b>	<b>0 cell</b>	<b>Results</b>
0	+	0	<b>A</b>
+	0	0	<b>B</b>
0	0	0	<b>AB</b>
+	+	0	<b>0</b>
+	+	+	<b>Oh</b>

**GRADING OF RESULTS**

- ❖ One solid aggregate of 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

### CELL GROUPING - DONORS

SI.No	Unit No.	Reaction With Anti A	Reaction With Anti B	Group
1	154373	0	0	O
2	154374	0	+3	B
3	154375	0	+3	B
4	154376	+3	0	A
5	154474	+3	0	A
6	154481	0	+3	B
7	154483	0	0	O
8	154485	0	+3	B
9	154486	0	0	O
10	131450	+3	+2	AB

### SERUM GROUPING

SI no	Unit No.	Reaction with A cell	Reaction with B cell	Group
1	131835	+L	+2L	0
2	131681	+2	+3	0
3	131824	0	0	AB
4	131828	0	+2	A
5	131900	+1	+2	0
6	13826	+2	0	B
7	131831	0	+L	A
8	131832	+2	0	B

9	131895	+2	+2	0
10	131837	+L	0	B
11	131772	0	0	AB
12	131738	0	0	AB
13	131839	+2	0	B
14	131840	+2	0	B
15	131841	0	+L	A

### **Rh GROUPING**

1. Place one drop anti-D serum in 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.

### **Rh GROUPING □ DONORS**

SLNo	Unit number	Reactions with			Rh
		Anti-D	Incubation	AHG	
1	154483	0	0	0	Neg
2	154485	0	0	0	-VE
3	125414	0	0	0	Neg
4	142000	0	0	+2	Du+
5	125416	0	0	0	Neg 1
6	131838	+2			+VE
7	126159	0	0	0	Neg

8	144441	0	0	+2	Du+
9	126211	0	0	0	Neg
10	131816	+3			+VE

### **Du TESTING**

1. Incubate one drop of red cells (2-5%) in a clean labeled test tube along with polyclonal or blend anti D at 37C for 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 1000rpm for 1 minute, read microscopically. If negative it is Rh neg, if positive it is Du pos.
7. If the result is negative, add one drop of IgG coated cells and centrifuge. Positive results indicate correct procedure, if not repeat the tests.

### **CELL GROUPING**

<b>Anti A</b>	<b>Anti B</b>	<b>Anti D</b>	<b>Incubation</b>	<b>AHG</b>
<b>+3</b>	<b>+2</b>	<b>0</b>	<b>0</b>	<b>+2</b>

### **SUB GROUPS OF A AND AB**

A and AB are divided into subgroups like A1, A1B, A2, 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.

### **RESULT**

- ❖ Positive results indicates the sample is A1
- ❖ Negative results indicates the sample is A2 or other subgroups.
- ❖

### **SCREENING FOR ATYPICAL ANTIBODIES**

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

#### **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 panned O cells into the tube
- (d) Mix well and incubate at 37°C for 15 minutes
- (e) Read microscopically after 1 hour, agglutination or haemolysis indicates positive reaction, negative results must be checked microscopically.

## ANTI HUMAN GLOBULIN TEST

The anti globulin test is popularly known as Coomb's Test.

### **PRINCIPLE**

Red blood cell coated with IgG or C3d component of complement either in vivo as in DAT or invitro as in IAT will be agglutinated by antihuman globulin (AHG) reagent binding to the IgG antibodies coating the cells.

Two types of anti globulin reagents are available.

1. Poly specific AHG reagent (contain IgG and C3d)
2. Mono specific AHG reagent (contains either IgG or complement component)

### **PREPARATION OF IGg COATED POSITIVE CONTROL CELLS**

Dilute Anti D 1:50 in saline and mix to equal quantity of 5% pooled washed O cell suspension in saline. Incubate at 37 C for 1 hour. Wash 3 times in excess saline and resuspend in saline.

### **INDIRECT ANTI GLOBULIN TEST**

IAT is used to detect the presence of incomplete antibodies and complement binding antibodies in the serum after coating red cells in vitro.

Indirect anti globulin test is done for the following purpose,

1. Compatibility testing
2. Screening and identification of unexpected antibodies in serum
3. Detection of red cell antigens using specific antibodies reacting only in antiglobulin test such as Fy<sup>a</sup>, Fy<sup>b</sup>, KJk<sup>a</sup>, Jk<sup>b</sup> etc.

### **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 37C 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 cells 3 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 1000rpm 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 1000rpm for 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 1/8 diluted IgG anti -D & one drop 0 positive washed cell kept at 37 C for one hour. After 1 hour, wash 3 times and proceed to AHG phase.

For negative control one 1/8 drop diluted anti D & one drop 0 negative washed cell (2-5%) kept in 37 C for 1 hour. Wash 3 times and proceed for AHG phase.

### **DIRECT ANTIGLOBULIN TEST**

DAT is used to detect in-vivo sensitization (coating) of red cells with immune antibody (IgG) or the complement component generally C3d.

### **INDICATIONS**

- ❖ Hemolytic disease of new born(HDN).
- ❖ Auto immune hemolytic anemia(AIHA).

- ❖ Drug induced red cell sensitization.
- ❖ Hemolytic transfusion reaction(HTR).

## **METHOD**

1. Place one drop of 2-5% cell suspension to be tested in a clean, pre- labelled test tube. Clotted sample should be fresh or taken in EDTA sample.
2. Wash the cells three times with saline and decant the final wash completely.
3. Add 1-2 drop of AHG reagent.
4. Mix and centrifuge at 1000 rpm for 1 minute.
5. Gently shake the tube and read the results using optical aid and record the result.
6. If the test is negative, add one drop of IgG coated cells.
7. Mix and centrifuge 1000 rpm for 1 minute. Look for agglutination. If no agglutination is seen the result is invalid. Repeat the test procedure.

## **CONTROLS**

For positive control: one drop 1/8 diluted IgG anti -D & one drop 0 positive washed cell kept at 37 C for one hour. After 1 hour, wash 3 times and proceed to AHG phase.

For negative control one 1/8 drop diluted anti D & one drop 0 negative washed cell (2-5%) kept in 37 C for 1 hour. Wash 3 times and proceed for AHG phase.

## **PREPARATION OF COMPONENTS**

## PREPARATION OF COMPONENTS

### **RED CELL CONCENTRATE (RBC)**

A component obtained by partial removal of plasma from whole blood without 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 anti coagulated blood is kept undisturbed enabling separation of plasma and cells.

### **PROCEDURE**

Prepare by either sedimentation or centrifugation.

- ❖ **Sedimentation:** Blood collected in CPDA anticoagulated 450ml or 350 ml double bag with is kept at 4°C in an upright position undisturbed, separate the plasma when clear into a satellite bag the red cells have a PCV of 70%.
- ❖ **Centrifugation:** Centrifuge the blood collected in CPDA anticoagulated 450ml or 350 ml double bag in a refrigerated centrifuge at 3550rpm or 15 minutes at 4-6°C. After centrifugation, express approximately 3A of plasma using plasma expresser into the satellite bag. Double seal the tubing between primary and satellite bag with PCV not higher than 80% red cell in the primary bag is label as packed red cells. Expiry of red cell concentrate in CPDA anticoagulant bag is 35 days from the date of collection and stored in 2- 4°C. In additive solutions, the expiry is 42 days.

## **INDICATIONS**

- ❖ Severe anaemia to reduce chance of circulatory overload.
- ❖ Haemolytic anaemia in aplastic crisis.
- ❖ Anaemia accompanying chronic renal disease.
- ❖ Less blood group antibodies in packed cells, so non-specific blood can be given to the patients.
- ❖ Less plasma proteins with packed cells, so there are minimum anaphylactic reactions.

## **LEUCOCYTE REDUCED RBC CONCENTRATE (LR-RBC)**

LR-RBC implies the removal of at least 70% of leucocytes, with a loss of less than 20% of red cells. A unit of donor whole blood contains 2- 3x10<sup>9</sup> leucocytes per ml. Leucocytes and platelets form a buffy coat in the stored blood and can be removed by several methods.

## **METHODS OF PREPARATION**

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

1. Centrifugation and removal of buffy coat by using top& bottom bag.
2. Centrifugation and washing of red cells.
3. Spin and filtration.
4. Freezing and deglyceralisation (not routinely performed]

## **CENTRIFUGATION AND REMOVAL OF BUFFY COAT**

On hard spin centrifugation of a unit of blood pack, the buffy coat layer is found between the red cell and plasma. Recently a semi-automated technique using top& bottom bag and an improved vaccumised extractor (optipress) has been introduced for preparation

of leucoreduced blood components by removing buffy coat. The efficiency of this method is up to 85%.

### **Inverted spin**

1. Whole blood or rbc centrifuged in an inverted position at 3000rpm for 10 minutes at 4°C
2. Suspend the bag in an inverted position on an IV stand and release the seal.
3. Collect the leukocyte poor red cells in the second satellite bag leaving about 70 to 90 ml buffy coat layer mixed with rbc and plasma in primary bag.
- (4) Double seal and label the bag as leukopoor RBC by centrifugation. Stored at 4 C, expiry date as same as RBC

### **Upright spin**

1. unit is centrifuged in an upright position at 3000rpm for 10 minutes at 4°C
2. Plasma buffy coat layer and 10 to 20 ml of red cells at top are expressed in to satellite bag. Double seal and label the bag.

### **WASHED RBC fw-RBC)**

Saline washing of red blood cells result in the removal of majority of leucocytes and platelets. Washing of RBC can be done either manually or by using machines.

- RBCs after centrifugation is washed with saline and packed (during wash the supernatant is discarded, filled with fresh saline and centrifuges. Repeat the procedure thrice.)
- As this become an open procedure, cells to be used within 24 hours when stored at 2-6°C.

## **SPIN AND FILTRATION**

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

## **IRRADIATED RBC**

Irradiated blood components have gained significance in severe immunosuppressed patients because of PT-GVHD due to transfused donor lymphocytes. Inactivation of transfused lymphocytes by irradiation of blood has proven most efficient for inhibiting lymphocyte blast transformation and mitotic activity.

## **INDICATIONS**

- Congenital immune-deficiency syndrome.
- Bone marrow transplant recipients.
- Pre-mature newborns.
- Patients with haematologic malignancies.
- patients receiving exchange and intrauterine transfusion.

## **PLATELET RICH PLASMA (PRP)**

### **FUNCTION**

Platelet concentrate prepared from a single donor unit of blood can temporarily elevate the platelet count  $5-10 \times 10^9$  in patient whose thrombocytopenia is due to increased destruction.

### **SCOPE AND APPLICATION**

1. Bleeding due to thrombocytopenia
2. Defect in Platelet function.
3. Disseminated intravascular coagulation (DIC)
4. Viral disease associated with thrombocytopenia (dengue)

## **PROCEDURE**

Collect blood in a double bag and kept at room temperature for one hour. Centrifuge the blood bag at 20-24°C at 1700rpm for 7 mts within 8 r.ours of collection. Express the PRP into the satellite bag without rontamination 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 (PC)**

### **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 resuspended 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°C at 3200rpm for 10 runutes. Express the supernatant plasma into 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 (SDP)**

### **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 hypoproteinemia. Pooled plasma is used for preparation of plasma proteins like albumin and immunoglobulins.

### **PROCEDURE**

Blood is collected and kept at 4 °C in an upright position, undisturbed separated the plasma when clear into a transfer or satellite bag on or before the fifth day after expiry of WB, label and keep at 30°C or I below. Expiry is 5 years from the date of collection.

## **FRESH FROZEN PLASMA (FFPI)**

### **DEFINITION**

Plasma separated from whole blood frozen within six hours of collection and stored at -20°C or below.

### **FUNCTION**

Fresh frozen plasma contains both stable and labile clotting factors.

### **SCOPE AND APPLICATION**

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

### **PROCEDURE**

Centrifuge the bag at 3550rpm for 15mts at 4°C express <sup>3</sup>A plasma into the satellite bag. Double seal and separate the bag, label and freeze the plasma immediately.

Shelf life-1 year if kept at -30°C or below.

Coagulant activity- 1ml plasma contains 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 hrs of collection, subsequent thawing at 4-6°C and the removal of supernatant.

#### **SCOPE AND APPLICATION**

Factor VIII deficiency states hemophilia and von Willebrand's disease, disseminated intra-vascular coagulation, fibrinogen defects, : reparation of fibrin glue.

## **PROCEDURE**

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

# **SCREENING OF HUMAN BLOOD**

## **SCREENING HUMAN BLOOD FOR HBsAg**

### **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.

Micro wells are 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 Radish Peroxide). This forms antibody-antigen-Anti IgG enzyme complex. This complex with substrate (coloring solution) to produce the color.

### **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-color giving solution (TMB+UP)
5. Stop solution
6. Wash solution concentrate.

#### **STORAGE AND SHELF LIFE.**

Store at 2-8 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 up to one week or I should be frozen at -10 C or lower for longer term storage.

Reagent preparation.

Bring all reagents to room temperature for 15-20minutes 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 amount of specimen to remaining wells,.
4. Then tap the frame gently to mix completely. Then incubate at 37C for 60 minutes.
5. Aspirate the contents from all wells and wash each well 5 times with at least 300 micro liters prepared wash buffer per well for each wash.
6. Invert the plate and trap it dry on absorbent paper. Pipette required amount of substrate into each well and incubate for 30 minutes at room temperature after mixing with a gentle tap.
7. After incubation, pipette required amount of stop solution in each well and shake well.
8. Within 30 minutes read the absorbance of NC, PC and specimens.

## **INTERPRETATION.**

1. Specimens with absorbance value less than the cut-off value are considered as non-reactive.
2. Specimens with cut off 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 as negative.
4. Initially reactive specimens that react with either of the duplicate repeat tests are considered as positive.
5. Sample OD value in the gray zone (10% below cut-off )are repeated.

## **SCREENING HUMAN BLOOD FOR ANTI -HIV**

### **FUNCTION**

Immunoassay which employs an immunosorbent consisting of recombinant hiv-1 proteins and HIV-2 antigen coated to the wells of the micro plate antigen react with antibodies in specimens together with anti IgG enzyme to form antigen-antibody-anti IgG enzyme complex. The complex react with TMB to produce color.

### **SCOPE AND APPLICATION.**

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

### **PROCEDURE**

#### **Test components**

- a. Antigen coated plate
- b. Negative & Positive controls
- c. Sample diluents
- d. Conjugate
- e. Substrate
- f. Wash solution concentrate
- g. Stop solution

### **STORAGE AND SHELF LIFE**

Store at 2-8°C .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 1 week or should be frozen at -10 °c or lower for long term storage.

## **REAGENT PREPARATION**

Wash solution: prepare wash solution as per the kit insert.

## **ASSAY PROCEDURE**

1. Fix required number of strips to the micro plate frame.
2. Pipette required amount of sample diluents into each well.
3. Pipette out required amount of negative control, positive control and specimen into each well. Mix for 10s using a micro plate shaker at 1000rpm.
4. Incubate at 37 °c for 1 hour after sealing the plate with plastic cover provided.
5. Aspirate the content from each of the wells and wash each well 5 times with at least 300 micro liter of wash solution.
6. Add required amount of conjugate to each well.
7. Incubate at 37 °c for 30 minutes after sealing the plate with plastic cover provided.
8. Aspirate the contents from all the wells and wash each one 5 times with wash buffer.
9. Invert the plate and tap it on absorbent paper to remove the remaining wash solution.
10. Add adequate amount of substrate to each well and incubate at room temperature for 30 minutes. Avoid exposure to sunlight.
11. Pipette out 100 micro liter of stop solution to each well and tap the plate gently to mix contents.
12. Read the absorbance at 450 nm against air blank within 30 minutes of adding the stop solution.

## **INTERPRETATION:**

- Specimens with absorbance values less than cut-off values are considered as non-reactive.
- Specimens with absorbance values equal or greater than cut-off are considered to be positive.
- Initially reactive specimens that do not react with repeat tests are considered to be non-reactive.
- 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.

## **SCREENING OF HUMAN BLOOD FOR ANTI-HCV**

### **SCOPE AND APPLICATION**

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

### **Test components**

1. Negative and positive controls
2. Sample diluents
3. Conjugate
4. Antigen coated plates.
5. Substrate
6. Stop solution
7. Wash solution concentrate

### **STORAGE AND SHELF LIFE**

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

### **PECIMEN COLLECTION**

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

### **REAGENT PREPARATION**

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

Wash buffer : dilute wash solution concentrate as per the kit insert.  
Conjugate : dilute conjugate concentrate with conjugate diluents.  
Substrate : mix TMB and UP as per kit insert.

## **ASSAY PROCEDURE**

- Fix required number of strips to the micro plate frame.
- Pipette required amount of sample diluents into each well. Then pipette out required amount of negative control, positive control and specimen into each well. Mix for 10 sec using a micro plate shaker at 1000rpm.
- Incubate at 37 C for 30minutes after sealing the plate with plastic cover provided.
- Aspirate the content from each of the wells and wash each well 5 times with at least 300 micro liter of wash solution.
- Add required amount of conjugate to each well.
- Incubate at 37 C for 30 minutes after sealing the plate with plastic cover provided.
- Aspirate the contents from all the wells and wash each one 5 times with wash buffer.
- Invert the plate and tap it on absorbent paper to remove the remaining wash solution.
- Add adequate amount of substrate to each well and incubate at room temperature for 30 minutes avoid exposure to sunlight.
- Pipette out 100 micro liter of stop solution to each well and tap the plate gently to mix contents.
- Read the absorbance at 450 nm against air blank within 30 minutes of adding the stop solution.

## **INTERPRETATION**

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

## **SCREENING FOR MALARIAL PARASITE**

### **FUNCTION**

Screening of donor blood for malarial parasite.

### **SCOPE AND APPLICATION**

It is an immunoassay based on the sandwich principle to prevent transfusion-transmitted malaria.

### **PRINCIPLE**

It is an immunoassay based on the sandwich principle. The method uses anti-pan specific pLDH (P Lactate Dehydrogenase) antibody conjugated to colloidal gold and another anti-pan specific pLDH antibody immobilized on a nitrocellulose strip in a thin line. The test sample is added in the sample well 'A' followed by addition of Assay buffer in Buffer Well B. If the sample contains the parasite, the colloidal gold conjugate complexes the pan specific pLDH in the lysed sample. This complex migrates through the nitro cellular strip by capillary action. When the complex meets the line of the immobilized antibody, the complex is trapped forming a pink purple band which confirm a reactive test result. Absence of a colored band in the test region indicates a negative result.

### **PROCEDURE**

1. Bring the complete kit and specimen to be tested to room temperature prior to testing.
2. Remove the test card and assay buffer from the foil pouch prior to use.
3. Label the card with the unit number.
4. Mix the anti-coagulated blood sample evenly by gentle swirling to make the homogenous before use. Dip the sample

loop into the sample and make sure that the loop is full of sample. Blot the blood onto the sample pad in the sample well A. Make sure that the blood from the sample loop has been completely transferred to the sample pad.

5. Add drops of Assay Buffer in the buffer well B.
6. Allow reaction to occur during the next 20 minutes.
7. Read results at 20 minutes.

### **INTERPRETATION**

Presence of a colored band both at the test region (T) and control region (C) indicates a positive result. The absence of a colored band at the test region (T) and presence of colored band at control region (C) indicates negative result.

## **SYPHILIS TESTING IN BLOOD DONOR**

### **FUNCTION**

Carbogen reagent is a particulate carbon suspension coated with lipid complexes. Carbogen detects anti-lipoidal antibodies in serum or plasma. These antibodies are traditionally referred as 'reagins'. During rest procedure, the specimen, serum or plasma mixed with the carbogen reagent and allowed for react for 8 minutes. If anti-lipoidal antibodies are present in the specimen they react with the carbogen forming visible black floccules. If anti-lipoidal 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 palladium that causes syphilis. Screening for spirochetes helps to exclude donors who are at high risks groups for HIV infection also.

### **PROCEDURE**

#### **1ST COMPONENTS.**

- Carbogen antigen
- Positive and negative controls
- Disposable slides
- Disposable sample or control dispensing pipette.
- Mixing sticks
- Needle dropper for dispensing carbogen antigen

#### **SAMPLE COLLECTION**

Fresh serum or plasma should be used for testing.

## **STORAGE & STABILITY**

1. Store the reagent at 2-8 C
2. Bring all reagent and samples to room temperature before testing.
3. Thoroughly mix the carbogen reagent suspension by gentle agitation.

## **QUALITATIVE METHOD**

1. Place 1 drop of test sample, positive and negative controls on to separate reaction circles of the disposable slide using a sample-dispensing pipette.
2. Add 1 drop of well- mixed carbogen reagent to the test sample. Positive and negative control by using a needle dropper provided with the kit.
3. Using a mixing stick mix the sample and carbogen reagent thoroughly spreading uniformly over the entire reaction circle.
4. Adjust the time on mechanical rotor for 8 minutes.
5. Rotate the slide gently and continuously on the mechanical rotor at 1000rpm.
6. Observe the 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.
2. Small black floccules against white background- weakly reactive.
3. 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 the titer value of anti-lipoidal 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 minutes.
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 element 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 drawn, accuracy of seals and anticoagulant volume, applicable to component preparation as well as .. hole blood collection.

#### **General consideration**

- Donor weight 60 kg and above.
- Blood is collected in double or triple bags of 450ml with 63 ml CPDA and 350ml with 49ml CPDA.
- To prevent activation of coagulation system blood must be collected within 10 minutes with minimum tissue trauma with single venipuncture.
- FFP should be prepared within 6-8 hours of collection ideally PRP should be kept for one hour before centrifugation.

- Centrifuge: contents in opposing buckets must be equal in weight and balanced with rubber materials only.
- Blood bags must be placed straight with broader end facing towards the outside.
- Runtime RPM and temperature to be programmed each component.
- Manual break should not be applied in between the run.
- All satellite bags must be correctly identified, numbered and labeled.
- Each product to be stored at optimal temperature until expiry.
- Platelet after preparation must be kept undisturbed for one hour before putting on agitator.
- When freezing plasma, rate of cooling must be as rapid as possible within 60 minutes, if plasma is intended for cryo.
- 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 platelet yield

**Calculate the number of platelet in one unit of WB or an EDTA sample.**

1. Platelet count /microliter x 1000 x volume of WB (ml)  
no. of platelets in WB.
2. Perform platelet count on proper sample.
3. Platelet count /microliter x 1000 of PRP  
= no. of platelet in PRP.
4. Calculate percentage of yield.
5. Platelet in PRP x 100/ platelet in WB = percentage of yield.

## **QUALITY CONTROL OF FROZEN PLASMA**

To know if it has been thawed and returned

1. Frozen plasma flat and store in up right. Air bubbled 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, their is abnormal appearance and color of units.
2. For platelet nearing out dating and returned plasma units redesigned.
3. For leuco-poor red cell prepared by centrifugation and washing.
4. Retuned and out dated units.
5. Autologous blood after washing.

Pediatric units or cell concentrates prepared from single bag by open method 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.

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 into suitable labeled tube for counting.

No	Name of component	Storage temp	Expiry
1	Red Cell Concentrate	4-6 C	35 days
2	Buffy Coat	20-24 C	24 hrs
3	FFP	-20 C	1 year

No	Name of Component	Run time (mtsl)	Run speed (RPM)	Temp
1	Red Cell Concentrate	10	3000	4-6 C
2	Buffy Coat	10	3000	20-24 C
3	FFP	10	3000	-20 C
4	PRP	7	1300	20-24 C
5	PC	10	3000	20-24 C

## QC OF REAGENTS

### **PROCEDURE**

QC of reagent on receipt.

Prior to receipt of any reagent, check name of antisera, volume, date of manufacture and date of expiry, batch number, storage instructions, production license number, preservatives and standard colors used. Tested negative for HIV, HBsAg and HCV

- ✓ Appearance: Observed for turbidity, suspended particles, precipitates and discoloration.
- ✓ Reactivity or Avidity: It is the rapidity at which the anti-sera 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.

### **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 haemolysins.

### **Specificity of pooled cells (A,B,0)**

1. Label and dispense one drop of each of antisera and 2-5% pooled cells are directed
2. Keep 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 unexpected reaction is seen, test should be repeated.

**Sensitivity / Titer (Anti A/B/AB/D/H/A1- sera)**

Titration is a semi-quantitative technique of measuring the concentration of antibody in the serum. The titer of antibody is usually determined by testing two fold serial dilution of serum in saline against select red cells.

**METHOD**

- a. Label a row of 12 tubes according to the serum dilution (line 1, line 2 etc)
- b. Add 100 micro liter of saline to all except 1<sup>st</sup> tube (to 1<sup>st</sup> add undiluted serum)
- c. Add 100 micro liter of antisera to test tubes 1 and 2
- d. Mix the tubes well without forming air bubbles and transfer 100 micro liter to tube
- e. Continue the same till the last tube and discard 100 micro liters from the last.
- f. Add 100 micro litres of 2-5 % washed saline suspended appropriate red cells to each tube.
- g. Mix well and incubate for 30 to 40 minutes at room temperature.
- h. At the incubation time, centrifuge the tubes at 1000 rpm for 1 minute
- i. Gently dislodge the cell button
- j. Examine the test tubes macroscopically, commence reading the tube containing the most diluted serum and proceed to most cone. Sample grade and record the reaction,
- k. Last tube, which gives a reaction greater than or equal to +1, is the titer of the antibody.

## PREPARATION OF PAPAIN CYSTEIN

### **FUNCTION**

Proteolytic enzymes like papain modify RBC antigens to enhance the reactivity of some antigen-antibody systems by reducing zeta potential (Rh & Jk, Le and P) and abolishes others (M,N, S & Duffy). Papain reduces the net negative charge on the surface of the red cells by cleaving sialoglycoprotein from the cell surface. This reduces intercellular distance, exposes crypt antigens and increase mobility allowing clustering.

### **SCOPE & APPLICATION:**

Papain cystein enzyme is used both in antibody detection tests and cross matching procedures. Papain cystein is useful for the screening of warm reacting IgG or complement binding antibodies. The papain technique serves to enhance the reaction of Rh, Lewis & Kidd antibodies and for antibody detection of delayed haemolytic transfusion reactions.

### **REAGENTS**

Papain powder : 1 gm

Cystein hydrochloride : 0.480 gm

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

### **Buffer**

1. KH<sub>2</sub>PO<sub>4</sub> (9.078 gm potassium dihydrogen phosphate in 1 liter of distilled water) 80ml
2. Na<sub>2</sub>P<sub>0</sub>4 (11.876 gm disodium hydrogen phosphate in 1 liter of distilled water)20ml

Mix 1 and 2 and adjust the pH to 6.2-6.4 with 5N NaOH.

Take 10ml prepared buffer separately and dissolve 0.480gm cysteine hydrochloride. In 90ml buffer dissolve 1gm papain powder.

Centrifuge and filter. Mix the papain cysteine hydrochloride. Adjust the pH at 6.2 to 6.4. Incubate at 37 C for one hour. See the pH.

Dispense in small quantities and freeze at -20°C.

## **PREPARATION OF PAPANISED 'O' CELLS**

### **SCOPE AND APPLICATION**

Detection of atypical antibody **PROCEDURE**

Papanised cells for 2 stage method.

Washed packed (50%) pooled 'O' Rh positive cells are used. One part of Low's papain is added to one part of washed packed RBCs, incubate at 37 C for 15 minutes. The red cells are then washed twice 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 (1/8 dilution) is tested with papanised cells as positive control. IgG anti D and 0 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 for each days use.

## **PREPARATION OF EDTA**

### **SCOPE AND APPLICATION**

EDTA is used for collection of blood samples for heamogram.

### **PROCEDURE**

Prepare 4gm% EDTA as stock solution. From the stock solution take 100ml in small vial for the collection of 2 micro litter of blood. Dry it in hot air oven ie. Preheat the oven at 150°C then switch it off; keep the EDTA bottles in it till they get dried up.

## PREPARATION OF PHOSPHATE BUFFER

### SCOPE AND APPLICATION

Prepare phosphate buffered saline (BS) at a neutral pH; which can be used as a diluent in serologic tests.

### REAGENTS

1. Prepare acidic stock solution (Solution A) by dissolving 22.16gm/L of  $\text{NaH}_2\text{PO}_4$ . In 1 liter of distilled water. This 0.16 m solution of the monobasic phosphate salt (monohydrate) has a pH of 5.0
2. Prepare alkaline stock solution (Solution B) by dissolving 22.16gm/L of  $\text{NaH}_2\text{PO}_4$ . In 1 liter of distilled water. This 0.16ml solution of the dibasic phosphate salt (monohydrate) has a pH of 9.0

### PROCEDURE

- a. Prepare working buffer solution of the desired pH by mixing appropriate volumes of the two solutions. A few examples are:

pH	SOLUTION A	SOLUTION B
5.5	94 ml	6 ml
7.3	16 ml	84 ml
7.7	7 ml	93ml

- b. Check pH of working solution before using it. Add small volumes of acid solution A or alkaline solution B to achieve desired pH.
- c. To prepare PBS of desired pH, add one volume of phosphate buffer at that pH to nine volume of normal saline.

## LOW IONIC STRENGTH SOLUTION (LISS)

### **EFFECT OF LISS SOLUTION**

- ✓ Reduces electro-static barrier surrounding red cells and antibody molecules.
- ✓ Increases the rate of antibody uptake 2-4 folds compared with normal saline and also increases the total antibody taken up.
- ✓ Titre of antibody increases
- ✓ Detection of antibody with low equilibrium constant
- ✓ Reduced incubation period.

### **PREPARATION OF LISS SOLUTION**

0.17M saline

0.15M phosphate buffer

0.3M sodium glycinate

### **METHOD**

1. 18 gm of glycine is dissolved in about 500 ml of distilled water.
2. The  $p^H$  is adjusted 6.7 by drop wise addition of 1N NaOH.
3. 20 ml of phosphate buffer  $p^H$  6.7 is added to the glycine solution.
4. 1.79 gm of NaCl dissolved in 100ml of distilled water is added to the solution.
5. The solution is made up to 1L with distilled water, mix thoroughly.
6. Adjust  $p^H$  to 6.7 with 1N normal NaOH.
7. Dispense into 100ml amounts.

## **QUALITY CONTROL**

### **NON-SEROLOGICAL**

1. pH should be within the range 6.65-6.85
2. Conductivity should be 3.6-3.7 mm at 23 C
3. Osmolarity 270-285 mmol

### **SEROLOGICAL**

A weak IgG anti- D (0.25 iu/ml) should give a +/+2 reaction with Rlr red cells by routine LISS-AIIG 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 control, 'in-house' external controls should be assayed as this will detect changes in lot specific only. Pooled diluted kit positive controls or in-house control 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%.

### **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 hyper tonicity is avoided.

### **SCOPE AND APPLICATION**

Preservation of cells for longer period of storage

### **PROCEDURE**

Reagent : Buffered tripotassium citrate

It contains,

1. Tripotassium citrate ( $K_3C_6H_5O_7 \cdot H_2O$ ) 3.25%
2. Potassium dihydrogen Phosphate ( $KH_2PO_4$ ) 0.47%
3. Dipotassium hydrogen phosphate ( $K_2HPO_4$ ) - 0.6%

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

### **LAYING-DOWN SOLUTION**

This is a 50% w/v or 40% v/v solutions which give 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 solution is a -Convenient quantity.

### **LAYING-DOWN PROCESS**

The blood taken into ACD/CPD anticoagulant is centrifuge and laying down solution is added gradually to the packed cells. Mix well so that none of the cells are subjected to excessive concentration of glycerol. The mixture is frozen at -40 C - '60 C in small aliquots.

## **RECOVERY OF CELLS**

The frozen cell mixture is allowed to thaw at room temperature. It is then centrifuged and the supernatant removed. 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**

Recovery solutions 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% is prepared by adding 25.6ml of glycerol to 174.4 ml of buffered tripotassium citrate. Take care to mix well.

## **PRE-TRANSFUSION TESTING**

Pre-transfusion testing refers to set of procedures required before blood is issued as being compatible. The purpose of pre-transfusion testing is to select blood and its components that will have:-

- Acceptable survival when transfused.
- Will not cause destruction of recipients red cells.

The procedure involves,

- Proper identification of patient's blood sample.
- Checking the patients previous records.
- ABO & Rh grouping of patient.
- Screening for irregular antibodies with identification.
- Selection of ABO & Rh compatible blood free from blood transmissible infections and irregular antibodies.

### **Screening for antibody identification**

The main purpose for using screening cell before doing cross matching is to select compatible blood before hand in those patients who have formed antibodies. The panel of cells for antibody screening may be obtained commercially or prepared in the blood bank. A set of two specially selected group O R1R1 and R2R2 cells are used. These cells must carry the main antigens of Rh, Kell, Kidd, Duffy, MNS, Lewis and Lutheran blood group systems.

## **COMPATIBILITY TESTING**

A cross match is only a part of compatibility test. Compatibility tests consist of following:

1. Review of patient's past blood bank history and records.
2. ABO & Rh typing of recipient & donor.
3. Antibody screening of recipient's and donor's serum.
4. Cross match.

The cross match test is carried out to ensure that there are no antibodies present in patient's serum that will react with donor cells when transfused.

The two main functions of cross match tests are:

- It is the final check of ABO compatibility between the donor and patient.
- It may detect the presence of an antibody in the patient's serum that will react with an antigen on donor red cells which was not detected in antibody screening because of the absence of corresponding antigens in screening cells.

### **TYPES OF CROSSMATCH**

Major cross match

Major cross match consist of mixing donor's red cells with patient's I serum.

Minor cross match

It consists of mixing patient's cells with donor plasma.

### **MAJOR CROSS MATCH TECHNIQUES**

- Immediate spin technique
- Saline room temperature technique
- Albumin addition technique at 37°C
- Indirect antiglobulin technique

### **Immediate spin technique**

Immediate spin technique or saline room temperature technique is inadequate for detection of clinically significant IgG type of antibodies.

Thus both these techniques are not good specially if antibody screening has not been carried out earlier.

### **Albumin addition technique**

This technique is also capable of detecting antibodies reactive at 37°C but is not as sensitive as IAT technique. It may be incorporated as an alternative technique to IAT.

### **Indirect antiglobulin technique**

This test is widely used in cross matching techniques as it detects majority of incomplete antibodies

Cross matching method

1. Put two drops of patient's serum in a pre-labelled glass test tube.
2. Add one drop of 2-4% of suspension of donor red cells.
3. Mix the contents and incubate 5-10 minutes for immediate spin method or 45-60 minutes for saline room temperature technique.
4. Centrifuge the tube at 1000 rpm for one minute (immediate spin method). While in case of saline room temperature technique, centrifugation is optional.
5. Examine the tube for haemolysis or agglutination.
6. If haemolysis or agglutination is present at this stage, the cross match is incompatible.

7. If negative (no haemolysis or agglutination) wash the cell 3 times with saline and decant the last wash completely. Add one drop of AHG reagent.
8. Centrifuge at the tube at 1000rpm for minute and look for haemolysis or agglutination.
9. Record the result.
10. If the test is negative, add 1 drop of control IgG coated cell. Centrifuge again at 1000 rpm for 1 minute.
11. Look for haemolysis or agglutination. If no agglutination, the test invalid. Repeat the procedure.

### **INTERPRETATION**

Haemolysis or agglutination at any stage of the test procedure except after adding control IgG coated red cells indicates incompatibility.

### **ORTHO BIOVIEW CROSS MATCHING PROCEDURE (GEL CARD METHOD) PRINCIPLE**

Microtubes in the form of cards, filled with buffed dextran gel are used which may be natural or impregnated with AHG. With negative reaction the red cell pass through the gel upon centrifugation where as in positive reaction the agglutinated red cells are trapped on top of the gel or suspended within it.

### **METHOD**

Wash red cells in normal saline and make following suspensions:

Saline cell volume	Packed red blood cell volume	Red cell concentration
1ml	40 micro lit.	3%
1ml	50 micro lit.	4%
1ml	60 micro lit.	5%

- 1. Add 50 micro litre ortho bliss to the appropriate wells.
- Add 10 micro litre 3-5% washed red cells to the wells.
- Add 40 micro litre patient's serum to the appropriate wells.
- Incubate 37°c for 10 minutes.
- Centrifuge for 5 minutes.
- Read and record.

## **COMPATIBILITY TESTING IN EMERGENCIES**

Blood can be issued in emergency situation after grouping for ABO & Rh for both patient and donor followed by crossmatch by immediate spin technique. However, it is advisable to complete the routine crossmatch procedure after issue of blood.

## **INVESTIGATION OF TRANSFUSION REACTION**

### **SCOPE AND APPLICATION**

Investigation of transfusion reaction is essential for the management of patients.

An HTR is a medical emergency and can occur with any blood component. Its effects may vary from mild to severe reaction. It can occur as a result of:

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

### **SAMPLE REQUIRED FOR SEROLOGICAL INVESTING**

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

### **PROCEDURE**

- Check identification of patient sample (pre & post) and the donor blood component. Search appropriate records to determine whether other patient sample or donor units have been involved. If no clerical error, trace each step of the transfusion.

- Compare the colour of the serum or plasma in the patient's pre & post transfusion samples. Pink or red discolouration in post transfusion sample but not in the pre-transfusion sample may indicate free Hb from destruction of donor RBC.
- Perform DAT on post transfusion EDTA sample. Absence of hemoglobinemia and negative DAT strongly suggest that an acute hemolytic reaction has not occurred.
- Repeat ABO & Rh test on pre & post samples and sample from blood bag. Test the post transfusion sample for ABO & Rh, a mixed field pattern on microscopic reading suggest the presence of incompatible donor cells.
- If the ABO & Rh typing on the patient's two samples do not agree, there has been an error in patient or sample identification or in the testing. If so, another patient's sample may have been drawn and incorrectly labeled, making it important to check records of all specimen received at approximately at the same time.
- If the donor blood is not of the ABO group affixed on the bag label, there has been an error in labeling and processing.
- Repeat antibody detection on pre & post transfusion sample.
- Repeat cross match including an antiglobulin phase testing with pre & post transfusion serum sample against RBC from bag.

### **INVESTIGATION OF NON- IMMUNE HAEMOLYSIS**

- Examine the blood units for colour and clot.
- Examine the supernatant donor plasma for free Hb.
- Examine the blood remaining in the administration tubing for free Hb.

## **INTERPRETATION**

Absence of haemoglobinemia and negative DAT suggest that an acute immune HTR has not occurred. If the patient's clinical condition strongly suggests a hemolytic reaction, further investigation is warranted despite preliminary negative result.

## **PRETRANSFUSION TESTING OF NEONATES**

### **PROCEDURE**

Pre-transfusion in neonates should be restricted to cell grouping only antibody screening to be done on baby's /maternal serum. If antibody screen negative, transfused 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 :n infant's serum.

### **EMERGENCY CROSS MATCHING TEST**

Occasionally the patient may need an urgent transfusion. Under such circumstances, the incubation period of compatibility testing can be reduced to 15 minutes and result read after centrifugation (light spin only). Clinician is informed accordingly and blood is issued. However cross match 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.

### **LISS COMPATIBILITY TEST**

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

### **METHOD**

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

- Centrifuge and read for agglutination/haemolysis.
- If there is no agglutination, wash the tubes 3 times in saline.
- Perform AHG test and read.
- If tested negative, add IgG coated red cells,
- Centrifuge and check for agglutination.

## **INVESTIGATION OF HDN AND NEONATAL TRANSFUSION**

### **SCOPE AND APPLICATION**

Maternal IgG cross the placenta and enter the foetal circulation leading to hemolytic complications in newborns, very small blood volume of the newborn and the antibodies in the transfused blood cause a serious problem faced by the transfusion center.

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

### **GENERAL GUIDELINES IN NEONATAL TRANSFUSION**

Blood should be as fresh as possible, not more than 5 days. If mother 2nd baby are of same ABO group indented blood should be of same group selected.

If both are ABO incompatible, 0 group blood with low titer anti -A anti B free from haemolysin should be selected. It is a good practice to give con. cells with 1/3 volume of fresh AB plasma- first choice or A/B plasma as appropriate (if baby is A group, suspend 0 group red cells in A group plasma and if baby is B group suspend 0 group cell in B group plasma)

### **SITUATION LEADING TO TRANSFUSION IN NEW BORN**

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 pre-transfusion 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.

### **SUSPECTING ABO HDN**

1) Clinical history of jaundice immediately after delivery

Blood group of mother -----0

ICT-----neg

Baby's blood

Blood group -----A/B

DCT -----weak+/neg.

Elute tested against A, B and 0 using ICT

Proceed to the following tests to confirm ABO HDN

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

Cord or baby's sample -IgG anti A/B titer (optional)

Elute from cord/baby's cell tested with A/B cells in ICT +ve

### **BLOOD SELECTION**

Group O with lowest anti A/B titer. Rh as that of the newborn (eg: if child is A+, select O+ blood and if child is negative, select O negative blood)

Confirming Rh HDN

Mother's blood Group : Rh D -ve

ICT : +ve

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

### **SELECTION OF BLOOD IN HDN**

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

Cord blood:

Blood group Rh D -ve

DCT..... strong +ve

ICT -----+/-

Elute .....+ve with O+ve cells, neg with O neg cells.

### **Blocked D phenomenon**

When all the Rh sites are blocked by anti D, cord cells will not be giving agglutinating in anti D and will be grouped as Rh 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 reagent if antibody confirmation is not possible. Cross match with maternal serum more number of units than required and find out ICT compatible units.

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

## **COMPATIBILITY FOR NEONATAL TRANSFUSION.**

Do major cross match with maternal serum up to IAT. If O group or ABO non-identical group blood is selected, titer of the donor unit should be done and units having lowest titer unit can be selected.

Transfusion

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

Dose: 10 ml / kg for correction of anemia double blood volume for exchange transfusion.

## **TRANSFUSION IN OTHER CONDITION**

### **Investigation in baby's sample**

- ABO and Rh determination
- Antibody screening and compatibility testing with maternal sample

### **Selection of blood**

Blood group compatibility with both mother and baby.

## **AUTO-IMMUNE HAEMOLYTIC ANAEMIA**

### **SCOPE AND APPLICATION**

To find the reason for incompatibility and to get compatible unit for transfusion.

### **PROCEDURE**

During routine cross matching if no blood units are found compatible the following procedures are followed.

- Get another clotted sample for cross matching.
- Repeat grouping and compatibility with more blood units. If no units are found compatible, do DCT and auto-control.
- If auto-control is positive, check reactivity at 4°C, room temperature, and 37°C to find out the thermal amplitude and type of auto antibody.
- Do auto adsorption using panned patient's RBC.
- Test the auto adsorbed serum for allo- antibody by IAT methods.
- If allo- antibody present, used auto adsorbed serum for compatibility.
- If no allo- antibody detected, do titration cross match with unadsorbed serum and find out least incompatible units.
- If allo and auto antibodies are present select allo compatible units which are least incompatible with auto antibody.
- Document the results

## **HEMOLYSIN TEST**

### **FUNCTION**

Transfusion of O group RBC to non O group patients safely.

### **SCOPE AND APPLICATION**

O group blood is considered as a universal donor and transfused but some donor samples are strongly hemolytic and are likely to have high levels of IgG anti A and anti B. Hemolysin test is done when O group blood is selected for patients of other ABO blood groups especially for HDN.

### **MATERIALS REQUIRED**

Serum to be tested, fresh AB serum, A and B reagent cells.

### **PRINCIPLE OF THE TEST**

Hemolysis of RBC takes place if the serum contains hemolysin in incubated with RBC in presence of complement.

### **PROCEDURE**

1. Clear serum obtained from clotted samples within 12 hours for the presence of Anti A and Anti B hemolysins. Fresh AB group serum can be used as a source of complement when serum to be tested is more than one day old.
2. Put two drops of serum under test into each of two tubes.
3. Add 2 drops of fresh AB serum.
4. Add one drop of 5% A cell suspension into one of the tubes and 1 drop of B cells to the other. Mix and incubate at 37 C for 1 hour. Observe the color of the supernatant serum. A pink or red colour indicates hemolysis. Hemolysis in tube containing group A red cells indicates presence of anti-A hemolysin and hemolysis in group B indicate anti B hemolysin.

### **GRADING HEMOSYSIS**

Complete hemolysis	-	+3
Partial hemolysis	-	+2
Trace hemolysis	-	+2
No hemolysis	-	Neg

+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  $\text{CaCl}_2$  (2.7 gm of anhydrous  $\text{CaCl}_2$  in 100ml distilled water).
2. Add 0.1ml of 2.7%  $\text{CaCl}_2$  solution to 0.9ml plasma.
3. Incubate serum to form clot.
4. Recover serum from clot.
5. Centrifuge serum at 3000rpm for 10 minutes to remove particles if necessary.
6. Specimen if to be used within two days store at 2-8° C. If long storage is indicated specimen should be frozen.

## **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**

- ❖ Group specific blood is issued according to patients requirements, after full pre transfusion work up.
- ❖ Units are issued attached with compatibility label and reaction forms.
- ❖ Before issue, inspect the unit for haemolysis, clots, leaking etc, recheck unit number and patient identity and compatibility label.
- ❖ Blood is issued in insulated containers with instructions for storage at proper temperature, if not transfused immediately.
- ❖ When there is urgent request for release of blood, it issued with an abbreviated cross match, "emergency cross match only done." is marked on the reaction form. Extended cross match is done once blood is issued and incompatibility reported if any.
- ❖ Plasma and platelets are issued group specific.
- ❖ 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 cross matching tubes to look for ABO errors
- ❖ Request for standby procedures are also put under 'T and S' (only grouping and antibody screening done).

- ❖ If ABO group specific blood is not available, O group packed cells are given. A written request from the physician is needed before issue.
- ❖ If Rh negative blood is not available, Rh positive blood is given in life saving situations, taking into account, patients age, sex, child bearing potential. A written request from the physicians is obtained to that effect before issue.
- ❖ Final identification of the recipient and donor unit is done by the transfusionist.
- ❖ Reaction forms are received back duly filled and signed, and is preserved with request form of each patient.

#### **SPECIAL CONSIDERATIONS**

- ❖ Children weighing <25kgs are given pediatric units.
- ❖ Numbers of units issued initially are as per guidelines set by HTC.

#### **RETURN OF BLOOD AND COMPONENTS**

- ❖ Blood is received back and taken into inventory if returned within 24-36 hours and maintained with in acceptable temperature and no abnormality detected on inspection.
- ❖ Returned units are quarantined for 24-48 hours and then taken into stock if there is no abnormality. ICU/Wards are advised to return the units at the earliest if not used.
- ❖ Returned units are issued at the earliest.
- ❖ FFP thawed; if received back is labeled as SDP only.
- ❖ Opened units if not used are discarded after 24 hours.

## **THERAPEUTIC PLASMAPHERESIS**

### **FUNCTION**

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

### **SCOPE AND APPLICATION.**

It is used in neurological diseases like GBs, CIDP and Myasthenia Gravis. The rationale for plasma exchange in their disease is to remove antibody (an IgG antibody in most cases and thereby reduce tissue damage.

### **PROCEDURE**

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

## AUTOLOGOUS TRANSFUSION

### **FUNCTION**

Intra operative blood salvage in the cardiac surgery refers to the collection and return of blood, recovered from the operation site or from the extra corporeal circuit. Collected blood units are washed and reinfused to the same patient. Washing with saline decreases the concentration of free Hb, heparin, potassium and cellular debris.

### **PROCEDURE**

1. Blood units are collected and labelled in 450 ml CPDA bags are received at blood bank.
2. Seal and centrifuge at 3000 rpm for 10 minute at 4°C.
3. Express the supernatant fluid into transfer bag 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 3000 rpm for 10 minutes at 4°C.
6. Repeat the steps 3-5.
7. Express the supernatant to transfer bag and discard.
8. Mix with small volume (30-50 ml) of saline and seal the bag.

It is an open procedure; blood should be used as early as possible, not longer than 4 hours.

## APHERESIS

### **SCOPE AND APPLICATION**

Apheresis platelets are prepared when there is a need to raise platelet count with minimal donor exposure.

### **PROCEDURE**

Apheresis using the *CS 3000 plus Baxter* cell separator is done only when there is a request for a pheresed product. The most commonly one procedure is plateletpheresis. It is a continuous 2 arm procedure. A routine procedure of plateletpheresis takes 90 minutes-2 hours. Platelet prepared by apheresis procedures provide the adequate dose form a single donor and as such the patient is exposed to less no. of donors, thus prevents the chances of refractoriness to a great extent. This platelet may be stored for 5 days on platelet agitator at 22°C. If the product is prepared in an open system, it must be transfused within 24 hours.

## **SPECIAL METHODS**

## **ADSORPTION AND ELUTION TECHNIQUE**

### **SCOPE AND APPLICATION**

This technique is used to confirm weak A or B subgroup.

### **PROCEDURE**

- Wash 1ml of cells to be tested atleast 3 times with saline. Discard the supernatant after last wash.
- Add 1 ml of anti - A to red cells if weak variant of A is suspected or 1ml of anti -B if weak variant of B is suspected.
- Mix the cells with antisera and incubate at 40°C for 1 hour.
- Centrifuge the mixture to pack RBCs and discard the supernatant antisera.
- Wash the remaining red cells for a minimum of 5 times with large volume of saline (10 ml or more). Save the supernatant of the 5<sup>th</sup> wash to test for free antibody.
- Add an equal volume of saline to the washed and packed cells and mix.
- Eluate the adsorbed antibody by placing the tube at 56°C water bath for 10 minutes and mix the red cell saline mixture atleast once during this period.
- Centrifuge and remove the cherry coloured supernatant eluate and discard the cells.

### **TESTING OF ELUATE**

- If anti-A is used, test the eluate against 3 different samples of Ai cells and 3 group 0 cells at room temperature, at 37°C and with AHG.

- If anti-B is used, test the eluate against 3 samples of B cells and 3 group O cells at room temperature, at 37°C and with AHG.
- Test the 5<sup>th</sup> saline wash in the same manner to show that washing has removed all antibodies, not bound to the RBCs.

## **INTERPRETATION**

If the eluate agglutinates or reacts with antiglobulin testing with specific A or B cells and does not react with O cells, the cells tested have active A or B antigen on their surface capable of binding with specific antibody. If the eluate also reacts with O cells, it indicates non-specific reactivity, and the results are not valid. If the 5<sup>th</sup> saline wash material is reactive with A and B cells, the results of the test made on eluate are not valid, because it indicates that active antibody was present in the medium unattached to the RBCs being tested.

## **SALIVA TEST FOR A, B & H SUBSTANCE**

### **SCOPE AND APPLICATION**

Determination of blood groups in case of discrepancy

### **MATERIALS REQUIRED**

Saliva to be tested, secretor A, B, and O saliva; antisera A, B and H, reagent RBCs

### **PRINCIPLE OF THE TEST**

'Se' gene governs the secretion of water-soluble ABH antigens in all body fluids. These secreted antigens can be demonstrated in saliva by inhibition tests with ABH antigens.

### **PROCEDURE**

Preparation of Antisera

Find the titre of antisera. For that prepare doubling dilutions of the appropriate blood group reagent (anti A, B and H). To one drop of reagent dilution add one drop of 2-5% saline suspension of A, B and O RBC. Centrifuge each tube and examine macroscopically for agglutination. Select the highest dilution that gives 2+ agglutination. If the final titre of antisera is 1:256 and the desired titre is 1:8, dilute the antisera 1:32.

#### **Preparation of saliva**

1. Collect the saliva in a test tube after rinsing the mouth.
2. Keep the test tube in a boiling water bath for 10 minutes to inactivate enzymes.
3. Collect the clear supernatant after centrifugation at high speed for 10 minutes.

## METHOD

Control A	Control B	Control H
1 drop saline +	1 drop saline +	1 drop saline +
1 drop dil. Anti-A	1 drop dil. Anti-B	1 drop dil. Anti-H

Test A	Test B	Test H
1 drop saliva +	1 drop saliva +	1 drop saliva +
1 drop dil. anti-A	1 drop dil. Anti-B	1 drop dil. anti-H

- Kept all the test tubes (test & control) at room temperature for 20 minutes.
- Add one drop of 2-5% saline suspending A cells to tube A, B cells to tube B and 0 cells to tube H.
- Mix and incubate all the tubes at room temperature for 30-60 minutes.
- Centrifuge all tubes at 1000 rpm for 1 minute.
- Record the result.

## RESULT

### Test

Tube A	Tube B	Tube H
+2	+1	0

### Control

Tube A	Tube B	Tube H
+2	+1	+1

## **INTERPRETATION**

No agglutination in the test sample and agglutination in the corresponding control tube indicate that the antiserum has been neutralised by the blood group specific substance A, B or H that is the individual is a secretor. Agglutination in all the test sample and control tube indicates the absence of blood group substance, that is a non-secretor or Bombay phenotype.

## **RECORD KEEPING**

### **FUNCTION**

An efficient blood bank will be able to monitor the records of specimens from the time the samples arrive until the time blood is issued. Uniformly and consistency in record keeping is possible through computer application.

### **SCOPE AND APPLICATION**

- To provide permanent references for implementation and maintenance of quality systems.
- To train personnel.
- For product inventory.
- For application of a criteria to improve transfusion services.
- Manual errors are avoided and the system is made nearly 'false proof. It allows for computer check of data and thus leads to patient's safety.
- 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 as an added advantage of computerized system.

Following records are maintained.

### **Donor records**

- Donor registration card.
- Donor registers.
- Blood components record register.
- Adverse donor reactions register.
- TTD screening register.

- Apheresis register.
- Discard register.
- Daily stock register.
- Blood grouping & Hb estimation register.
- Donor deferral register.

**Patient's records**

- Blood group register
- Cross-match transfusion register
- Issue register-inside & outside
- Transfusion reaction investigation register.

## **PREPARATION OF POTASSIUM DICHROMATE SOLUTION 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 tests in the lab.

### **PROCEDURE**

After tests are completed and before drying glassware are immersed in large amount of water containing bleach.

#### **Preparation of Dichromate solution**

Potassium dichromate - 1 kg

Cone. H<sub>2</sub>SO<sub>4</sub> - 1 Liter

Make up volume up to 10 liters with distilled water keeping 10 liter jar in a container with <sup>3</sup>A water to prevent explosion due to concentrate H<sub>2</sub>SO<sub>4</sub> add acid to water.

Glassware is immersed in dichromate solution for an hour or overnight if required and solution is drained off. It is then washed with a jet of tap water, shaking out the waste after each rinse. After several cleaning with tap water, glassware is 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.