

**SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL
SCIENCE AND TECHNOLOGY
(An Institute of National Importance)**

THIRUVANANTHAPURAM, KERALA, INDIA-695011



PG DIPLOMA IN BLOOD BANKING TECHNOLOGY

LOG BOOK

2015-2016

Submitted by

ASWATHY AS

CERTIFICATE



I, **ASWATHY AS** 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

Date:

Head of the Department
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 raining. 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. Kalliyana Krishnan Registrar Dr.A.V George, for their advice and kind attention towards me. I extend my heartfelt thanks to all Staff members of other department for their –r p during my training period in this Institute.

ASWATHY AS

TECHNICAL ASPECTS OF BLOOD DONATION.

DONOR SELECTION

Blood collection is the most essential function of a blood transfusion service and its organisation should be given proper attention. Blood donation must be made a present and rewarding experience for the blood donor.

MEDICAL HISTORY

It provides information needed to decide whether,

- To accept the donor
- Defer the donor temporarily
- Exclude the donor permanently

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

- Diabetic: - mild diabetic, diet controlled, donor is accepted. Diabetic controlled with oral hypoglycaemic agents or insulin permanently unfit.
- Heartdisease: - 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.
- Malaria: - donor who had malaria or taken anti- malarial prophylaxis must be deferred for three years after cessation.
- Mental illness: - persons taking medication for psychiatric disorders or person showing abnormal behaviour are unacceptable as donors.
- Drug addiction :- drug abuse of any nature are cause of permanent deferral because intravenous drug abuse exposes the donor to parenteral transfusion transmitted disease and oral or inhaled drug abuse makes the donor unreliable about a proper history.
- Prolonged bleeding from wounds: -an abnormal bleeding tendency may be cause for permanent deferral. Individual with such history may experience excessive bleeding at the site of venepuncture. Plasma from donor deficient in coagulation factors would not confer expected therapeutic benefit in recipient who needs this factors.

- Blood transfusion: - recipient of blood or blood products should be deferred for a period of twelve months.
- Hospitalisation :- history of hospitalisation gives a clue to the donors medical history. Hospitalisation for the medical management of diseases should be evaluated.
- Allergy :- acceptable during symptom free period.
- Hypertension :- known hypertensive on medication should be deferred. Nervous or anxious donor with blood pressure reading i.e. ; 140/90 at the first instance should be re-evaluated after some time.
- Jaundice :- positive history of jaundice should be lead to further interrogation to correct more information regarding duration of illness , intensity of illness.

DONOR PHYSICAL EXAMINATION

- A prospective donor should be in good health .
- Donors weight more than 45 kg (for 350 ml) and more than 50 kg (for 450 ml) are acceptable .
- Blood pressure should be within acceptable range for a particular age group with or without medication. Diastolic BP 60-90mmhg, systolic BP – 100-160 mmhg.
- Hb should not be less than 12.5g/dl.
- Pulse of the donor should observed for 1 minute and should be within 60-100 .any irregularity in the pulse or pulse defect more than 10 should be considered for deferral.
- Phlebotomy site is examined for any infective divisions and scars of needle pricks. Needle pricks indicative of intravenous drugs abuse or frequent blood donation.
- Systemic examination of heart , lungs and abdomen should be normal.
- There should be no generalised lymphadenopathy or epitrochlear nodes.

DONOR ROOM PROCEDURE

Blood donation complex should be located at a place which is easily accessible for general public and patient's relatives.

DONOR CARE AND SATISFACTION

- Everyone involved in interviewing and counselling should develop a friendly and tactful approach that encourage donor to be honest and accurate in their answers to questions about their medical history.
- The blood collection should be done in an area which is pleasant, convenient well ventilated, well lighted and preferably air conditioned. So that the donor feel comfortable and relaxed.
- It is essential that staff should always be smart and clean in appearance with high standard of hygiene.
- Be sensitive to the donor's feeling of fear previous health check or donor deferral.
- No chatting with other staff and ignoring the donor.
- Thanks donors with appreciation so that they are motivated to come again.

MATERIALS AND METHODS FOR BLOOD COLLECTION

MATERIAL REQUIRED

- Demethylated spirit
- Betadine
- Cotton and gauze swabs
- Artery forceps and scissors
- Test tubes for sample collection
- Test tube rack
- Gloves
- Tube stripper
- Electronic tube sealer
- Sphygmomanometer
- Blood collecting bags
- Blood collecting monitor (Blood bag mixer)
- Comfortable donor couch or chair
- Discard jar with 1% sodium hypochlorite
- Adhesive plaster
- Oxygen cylinder with accessories.

SELECTION OF BAGS

Each blood collection bag should be properly inspected for any leakage, defect and clarity of anti-coagulant solution. If found so reject the bag and do not use.

- The blood collection bag and pilot tube should be identified by specific donor registration number, so that it can be traced back to the donor and also to the recipient.
- The date of collection and expiry should be written on the label of bag.
- Different types of blood bag in use.
 1. Single
 2. Double
 3. Triple with additive solution eg; SAGM
 4. Quadruple / top and bottom
- Receive donor cordially as he/ she should feel that staff is caring, concerned and committed.
- After a thorough screening the donor is asked to lie down on the donor couch in a well lit and air conditioned environment.
- Identification of the donor must be re-checked by asking him / her to tell name and tally it with card , blood bag and sample tube and registration number.

PREPARATION OF PHLEBOTOMY SITE

- Select the suitable large firm vein in the antecubital area, after inspecting both the arm of the donor carefully and make the donor to lie down on donor couch accordingly.
- Apply the cuff of sphygmomanometer just above cubital fossa and inflate to 60-70 mm mercury.
- Asking the donor to make the fist usually helps to bring the vein into prominence.
- Examine and palpate the selected vein.
- Release the pressure cuff and prepared the venepuncture site.

DONOR REACTION AND MANAGEMENT

Untoward feeling by blood donor before, during or after blood donation is known as donor reaction. This may be caused by psychological factors like sight of blood, excitement or other unexpected reasons or a neuro psychological response to blood donation.

Type of vasovagal reaction

- Mild vasovagal reaction.

Symptoms :- pallor, perspiration, specifically on palms, forehead which may be generalised, sign or yawning, hyper ventilation, feeling of warmth or air hunger, dizziness, light headedness, nausea with or without vomiting.

- Moderate vasovagal reaction.

Symptoms :- there is progression of all the symptoms of mild reaction and additional symptoms such as bradycardia, shallow respiration, hypotension (systolic as low as 60mmhg) and quite anxiety may be present. There is prolonged recovery. Donor must be protected from injury if unconsciousness occurs.

- Severe vasovagal reaction.

Symptoms :- all symptoms of mild or moderate reaction along with any or all of the following – incontinence of urine or faeces, convulsions – focal or generalized.

- Tetany/muscular spasm
- Haematoma
- Convulsion
- Delayed syncope
- Accidental puncture of artery
- Problems with blood flow.

MANAGEMENT:-

- Discontinue donation.

- Immediately seal venepuncture site .
- Apply wet towels to donors forehead and ask donor to cough.
- Loosen any tight clothing and ensure clear air way.
- Raise knees with feet flat on the bed or couch.
- Talk to donor to assure that it is nothing serious and he or she will be perfectly all right.
- Never leave the donor alone.

POST DONATION CARE

Instruct the donors regarding the following.

- Take more fluids for next 4 hours.
- Do not smoke or drive for next half an hour.
- Do not drink alcohol for next 6 hours.
- If bleeding occurs from phlebotomy site , raise the arm and apply pressure on the venepuncture site.
- If donor is feeling dizzy, make him /her lie down with leg slightly raise above the head level and symptoms still persist consult blood bank clinician.
- Remove the adhesive band after 5-6 hours.
- Do not apply any medication on venipuncture site on your own.
- Avoid lifting heavy weight or strenuous exercise to prevent bleeding.
- Rest and refreshment should be given to all. Thank the donor for their valuable contribution and ask them to repeat the same for this noble cause. A thank you card goes a long way for the donor to come back as a repeat donor.

HEMOGLOBIN ESTIMATION BY DIFFERENT METHODS

Scope and Application

Adequacy of donor's Hb level is mandatory before blood donation. This screening test is designed to prevent taking blood from an anaemic donor; does not ensure that the donor has an adequate store of iron. Minimum level of Hb for acceptance 12.5 g/dl

HEMOGLOBIN ESTIMATION BY COPPER SULPHATE SOLUTION

PRINCIPLE

This is a quantitative test based on specific gravity and is a reasonably reliable method for determining the Haemoglobin of the donor. It is indirect measure of haemoglobin value.

PREPARATION OF COPPER SULPHATE SOLUTION

- Dissolve 159.63g of pure air dried crystal of copper sulphate in water and make up to exactly 1000 ml at 25°C
- The specific gravity of the solution must be 1100
- Add 52ml of prepared solution to 48ml of distilled water to make 100 ml of working solution.
- Check the specific gravity (1.053) by hydrometer.
- The solution should be stored at room temperature in tightly capped containers to prevent evaporation.

PROCEDURE

- Clean the fingertip thoroughly with a spirit swab and allowed to dry.
- Medial side of the ring finger of the donor is pricked using a sterile lancet.
- The first drop of the blood is wiped and next blood drop is allowed to fall into a beaker containing CuSO₄ solution of specific gravity 1.053 from a height of at least 1 cm.
- If blood drop sinks to the bottom of the solution then Hb is more than 12.5g/dl
- If the blood drop floats for more than 15 seconds then Hb is less than 12.5 g/dl.

Prepare standard copper sulphate solution of specific gravity 1.052 to 1.055

Specific gravity	Stock solution	Distilled water to make	Hb equivalent
1.052	51	49	12
1.053	52	48	12.5
1.054	53	47	13
1.055	54	46	13.5

Dispense 30 ml of copper sulphate solution (specific gravity 1.053) in to labelled clean , dry tubes or bottles. Change the solution daily or after 25 tests and be sure that the solution is properly mixed before performing tests daily.

Results

Date	Name of the donor	sex	age	Hb value (g/dl)
14.04.2015	Ajayan	male	33	>12.5
14.04.2015	Anitha	female	28	>12.5
18.05.2015	sreeja	Female	25	<12.5
18.05.2015	vinayan	male	36	>12.5
19.05.2015	deepak	male	42	>12.5
20.05.2015	yazhini	female	21	<12.5
20.05.2015	aarya	female	26	<12.5

Hb ESTIMATION BY PORTABLE DIGITAL HAEMOGLOBINOMETER

PRINCIPLE

This is a quantitative test. Sodium deoxycholate haemolyses the erythrocytes and releases haemoglobin. Sodium nitrate convert haemoglobin to cyanomethhaemoglobin which together with sodium azide gives azidemethhaemoglobin. The absorbance is measured at two wavelength 570 and 880nm in order to compensate for turbidity in the sample.

PROCEDURE

- The middle finger tip is cleaned thoroughly with spirit and allowed to dry.
- The middle finger is punctured firmly near the tip with sterile disposable lancet.
- The first drop of blood is wiped and then the drop of blood is allowed to fill the cuvette completely in once continues process.
- Wipe off excess of blood on the out side of cuvette. Make sure that no blood is with drawn out of the cuvette completely in this procedure.
- Place in the filled cuvette in to the cuvette holder immediately and push it in to the measuring position
- Results are displayed with n 15-4 sec as g/dl
- Dispose te cuvette and lancets n 1% sodium hypochlorite solution in puncture proof containers.

Results

Date	Name	Sex	Age	Hb value (gm/dl)
14.08.2015	Lekha	Female	22	12.3
14.8.2015	Aiswarya	Female	25	12.8
26.08.2015	Fathimasana	female	18	14.1

19.11.2015	Vijayakrishnan	Male	55	15.8
20.11.2015	Ajayan	Male	32	14.3
23.11.2015	Venugopal	male	41	12.9

ABO GROUPING AND Rh TYPING

SCOPE AND APPLICATION

To determine the correct ABO group of an individual and ensure the reliability of the result. This procedure describe the method of detection of ABO antigen on the red cell and reciprocal antibodies in the serum. It provides the guidance for the use of blood grouping reagents in order to detect weak variants acquired antigens, Bombay (Oh) blood group and irregular red cell antibodies.

PRINCIPLE

ABO system is the only system in which there is reciprocal relationship between the antigen on the red cell and the naturally occurring antibodies in the serum. Routine grouping of donors and patients must there for include both rbc and serum tests each serving as check on the other. The procedure is based on the principle of agglutination of antigen positive red cell in the presence of antibody directed toward the anigen.

CELL GROUPING (FORWARD GROUPING)

PRINCIPLE

Forward/ direct grouping is based on an agglutination reaction between A and B antigens present on RBCs with commercial anti-A and anti-B anisera respectively.

MATERIAL REQUIRED

- Test tubes
- Test tubes rack
- Marker pen

- Pipette
- Anti-A,anti-B,anti-D (IgM+IgG),IgM antisera
- Normal saline
- Centrifuge
- Microscope
- Control known Rh positive and Rh negative cells

PROCEDURE

- Label four tests tubes as anti-A, anti-B , anti-D (Igm+IgG),and IgM.
- Add one drop of antisera to each tube respectively.
- Add i drop of 2-5 % cell suspension to each tube.
- Mix and incubate at room temperature.
- Look for agglutination / haemolysis.
- Record the result immediately.

GRADING OF AGGLUTINATION

STRENGTH OF REACTION	APPERANCE
+4	A single large agglutinates. No free cells
+3	A number of large agglutinates. No free cells
+2	Medium or small size of agglutinates
+1	Many small agglutinates in the background of free cells
Trace or micro	Appears negative macroscopically
o	No agglutination

REACTIONS OF CELL GROUPING

ANTI-A	ANTI- B	RESULT

+	0	A
0	+	B
0	0	0
+	+	AB

INTERPRETATION

Agglutination or haemolysis indicates a positive reaction

A smooth suspension indicates a negative reaction

The positive control – presence of agglutination

The negative control – absence of agglutination

SERUM GROUPING

Reverse / indirect grouping is based on an agglutination reaction between naturally anti-A and anti-B antibodies in serum / plasma with reagent A or B red cells respectively.

PROCEDURE

- Label four clean and dry test tubes as A,B,O and panned O cell.
- Spin test sample to separate serum.
- Add two drop of serum to each tubes.
- Add 1 drop of 2-5% A cells, B cells,O cells and panned O cells to the corresponding tubes.
- Mix and incubate at room temperature for 30 minutes (1000 rpm for 1 min)
- Resuspend cell button by gently shaking the tubes and read against well- light background.
- Record results according to the grade of agglutination.

REACTION OF SERUM GROUPING

A CELL	B CELL	O CELL	PAPANISED O CELL	RESULTS
0	+	0	0	A
+	0	0	0	B
+	+	0	0	O
0	0	0	0	AB
+	+	+	+	Oh (-H should be neg)

INTERPRETATION

Agglutination or haemolysis indicates a positive reaction.

A smooth suspension indicates a negative reaction

SUB GROUP OF A AND AB

A and AB blood groups are divided into sub groups A1 , A1B ,A2B depending up on the reaction with anti-A1

PROCEDURE

- Place 1 drop of anti-A1 in a clean labelled test tube.
- Add the test cells with 2-5% cell suspension.
- Mix and keep at room temperature.
- Positive results (agglutination) indicate that sample is A1.
- And negative results indicate that sample is A1.

Rh GROUPING

SCOPE AD APPLICATION

It describes the method for detection of D antigen on the red cells and provide guidance for the use of anti-D blood grouping reagent.

OBJECTIVES

- Understand the various technique used for Rh (D) grouping.
- Familiarize with different types of anti-D reagent available and its use.
- Understand and solve the problems encountered in Rh grouping.
- Know the indication and techniques of Rh genotyping.

CLINICAL IMPORTANCE OF Rh

The Rh blood group system is important because

- Haemolytic disease of new-born (HDN) may occur in the Rh negative pregnant women with Rh positive foetus.
- Rh antibodies may develop in Rh negative patients if given Rh positive blood.

MATERIAL REQUIRED

- Test tube
- Anti-D antisera (monoclonal IgM or blend of IgM and IgG)
- Controls : known Rh positive and positive
- Centrifuge
- Normal saline
- Microscope
- Known IgG coated cells

PROCEDURE

- Label the test tube anti-D
- Add one drop of blend IgM and IgG in the labelled tube.
- Add 1 drop of 2-5% cell suspension.
- Look for agglutination, macroscopically
- If test is positive, record the sample as D positive.
- If test is negative ie; no agglutination in the test tube

- Incubate the test negative sample tube at 37°C for 45 mins. With blend IgM+IgG
- After incubation wash the cells thrice with normal saline. (3000 rpm/ 1min)
- Decant the last supernatant and add 1 drop of AHG (Coombs sera)
- Centrifuge at 1000 rpm for 1 min
- Gently resuspend and look for agglutination with macroscopically and microscopically.
- No agglutination indicates Rh D negative.
- All negative reaction should be confirmed by adding known IgG coated cells, re-centrifuge and look for agglutination.
- Presence of agglutination confirms the test results and no agglutination indicates invalid test.

RESULTS

Presence of agglutination indicates Rh D positive

Absence of agglutination indicates Rh D negative

WEAKER DU TESTING

It is defined as the weakened expression of the normal D antigen i.e.; there are than normal D antigens per red cell.

This is an inherited characteristic, there are two grades of Du.

High grade du and low grade Du.

High grade Du red cells are agglutinated by certain anti-D antisera while low grade Du are mostly detected by AHG test.

SIGNIFICANCE OF Du

In donor: Du blood is considered as D positive

In recipients: Du recipient are given only Rh negative blood.

PROCEDURE

- Take 1 drop of anti-D (blend of IgM and IgG) in a labelled test tube.
- Add 1 drop of 2-5% test cells which test D negative on routine testing with anti-D
- Incubate at 37°c for 45 mints
- Look for agglutination.
- Wash the cell thrice with normal saline.
- Decant the last supernatant and add 1 drop AHG (polyspecific coombs sera)
- Centrifuge at 1000 rpm for 1 mint
- Look for agglutination microscopically.

INTERPRETATION

If indirect antiglobulin test (IAT) is positive , the donor is Weak D positive (Du positive)

SCREENING FOR MALARIAL PARASITE

SCOPE AND APPLICATION

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

PRINCIPLE OF THE TEST

It is an immunoassay based on the sandwich principle .the method usesantipan specific pLDH

(p Lactate Dehydrogenase) antibody conjugated to colloidal gold and another anti pan specific pLDH antibody immobilised on a nitrocellulose strip in a thin line.

PROCEDURE

- Bring the complete kit and specimen to be tested to room temperature prior to testing.
- Remove the test card and assay buffer from the foil pouch prior to use.
- Label the card with the unit number.
- Mix the anti coagulated blood sample evenly by gentle swirling to mak it homogenous before use.
- Dip the sample loop into the sample and make sure that the loop is full of sample.
- Blot the blood on 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.
- Add two drops of buffer in the buffer well B.
- Allow reaction to occur during the next 30 minues
- Read results at 30 minutes.

INTERPRETATION

Presence of a coloured 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 coloured band at control region (C) indicates negative results.

SI no:	NAME	AGE/ SEX	DONOR ID	RESULT
1	VENKAT	36/M	158460	NEGATIVE
2	SREEJA	22/F	158461	NEGATIVE
3	VENU GOPAL	45/M	158462	NEGATIVE

4	AJAYAN	36/M	158463	NEGATIVE
5	SHAMSUDHEEN	49/M	158464	NEGATIVE

SCREENING FOR RAPID PLASMA REAGIN CARD TEST FOR SYPHILIS

TESTING

SCOPE AND APPLICATION

RPR is a microscopic non-treponemal flocculation test for the detection and quantitative analysis of antilipoidal antibodies. Quantitative method is used for routine screening.

SYPHILIS

Syphilis is basically a sexually transmitted disease caused by *Treponema pallidum*. *T. pallidum* can be transmitted through fresh blood and platelets. It is not transmitted by plasma products fractionated from pooled plasma such as factor VIII. Post transfusion syphilis was a big problem in the past, however as the incidence of syphilis has remarkably come down after the advent of antibiotics.

PROCEDURE

- Bring reagent and sample to room temperature before testing.
- Thoroughly mix the carbogen reagent suspension by gentle agitation before testing.
- Add one drop of serum in to the circle on the card. Spread the sample inside the circle.
- Add one drop of well mixed carbogen reagent to the test specimen using the reagent dropper provided with kit.
- Using the mixing stick, mix the test specimen and the carbogen reagent thoroughly spreading uniformly over the circle.
- Keep the card on the VDRL shaker and set for 8 minutes at an rpm of 180.
- Observe the flocculation macroscopically after procedure.

RESULT

SI NO:	NAME	AGE / SEX	DONOR ID	RESULT
1	JAYAKUMAR	45/M	150018	NON- REACTIVE
2	LEELA DEVI	38/F	150019	NON- REACTIVE
3	VISHNU	28/M	150020	NON- REACTIVE
4	SAROJAM	42/F	150021	NON- REACTIVE

SALIVA TEST FOR A,B AND H SUBSTANCES

PRINCIPLE

Saliva testing for determination of secretor status and ABO substances secreted in saliva is based on the principle of inhibition i.e; the prevention of antigen antibody reaction by neutralization of a specific antibody by soluble antigens present in saliva.

OBJECTIVE S OF THE STUDY

To solve problems in grouping (AIHA, acquired blood group substance and subgroups of A & B)

To check the secretor status of a person.

Understand the indications and procedure of antibody titration.

PROCEDURE

- Collect 3-5 ml of saliva in clean glass test tube after rinsing the mouth.
- Centrifuge 3000 rpm for 10 mints.
- Collect the supernatant after centrifugation.
- Collect the clear supernatant keep the container in a boiling waterbath for 10-15 mints to inactivate enzymes.
- Boiled saliva centrifuge 3000 rpm for 3 mints.
- Collect the clear supernatant after centrifuge.
- Prepared doubling dilution of the appropriate blood group reagent (anti-A, anti-B, and anti-H)
- Label three tubes as A,B and H
- Add two drops of diluted antisera in corresponding tubes.
- Add one drop of saliva in each tubes.
- Mix the tube contents and keep it 15 mints at room temperature.
- Add one drop of 2-5 % A,B,O cells suspension in corresponding test tubes.
- Mix the test content and keep 10 minutes at room temperature.
- Centrifuge each tube and inspect RBC button macroscopically.

PREPARATION OF ANTISERA

- Label 3 test tubes as Anti-A, anti-B and anti-H
- Add 1500 µl saline in labelled anti-A and anti-B test tubes
- Add 100µl antisera in corresponding test tubes for 1:16 dilution.
- Add 100µl saline in labelled anti-H and add 100µl anti-H and makes 1:2 dilution.

INTERPRETATION

Agglutination of indicator RBCs by antibody in tests containing saliva indicates absence of corresponding blood group substance antigen in the saliva.

No agglutination of indicator RBC by antibody in tests containing saliva indicates presence of the corresponding blood group substance in the saliva.

METHOD

TEST A	TEST B	TEST H
1 DROP OF SALIVA +2 DROPS OF DILUTED ANTI-A	1 DROP OF SLIVA + 2 DROPS OF DILUTED ANTI -B	1 DROP SALIVA + 2 DROPS DILUTED ANTI-H
CONTROL -A	CONTROL - B	CONTROL -H
2 DROPS SALINE + 2 DROPS DILUTED ANTI-A	2 DROPS OF SALINE + 2 DROPS OF DILUTED ANTI-B	2 DROPS SALINE + 2 DROPS OF DILUTED ANTI-H

RESULT

Agglutination:- test -A Test-B Test- H

+ + -

blood group:- O

PREPARATION OF ANTI-A1 FROM DOLICHOS BIFLORUS

SCOPE AND APPLICATION

To detect the sub group of A.

PRINCIPLE

They are the saline extract of seeds having agglutinating properly with specific antigens and thus make useful typing reagents eg; Dolichosbiflorus extract agglutinates A1 cells but not A2.

PROCEDURE

- Two grams of dolichosbiflorus is soaked over night in approximately 25 ml of normal saline.
- The seeds are granited using a grainter the extract is taken in a container.
- Again 25 ml of saline is added and the seeds are rinsed.
- The fluid is centrifuged at high rpm and the supernatant is collected .
- It is checked with A1 and A2 cells and it should be at a serial dilution which gives strong agglutination with A1 cells.

Specificity

A1 CELLS	+
A2 CELLS	○
A1 B CELLS	+
A2 B CELLS	○
B CELLS	○
O CELLS	○

TITRE :- 1/32

AVIDITY :- 3 SECONDS

SI NO:	CONTENT	PAGE NO:
1	HAEMOGLOBIN ESTIMATION	

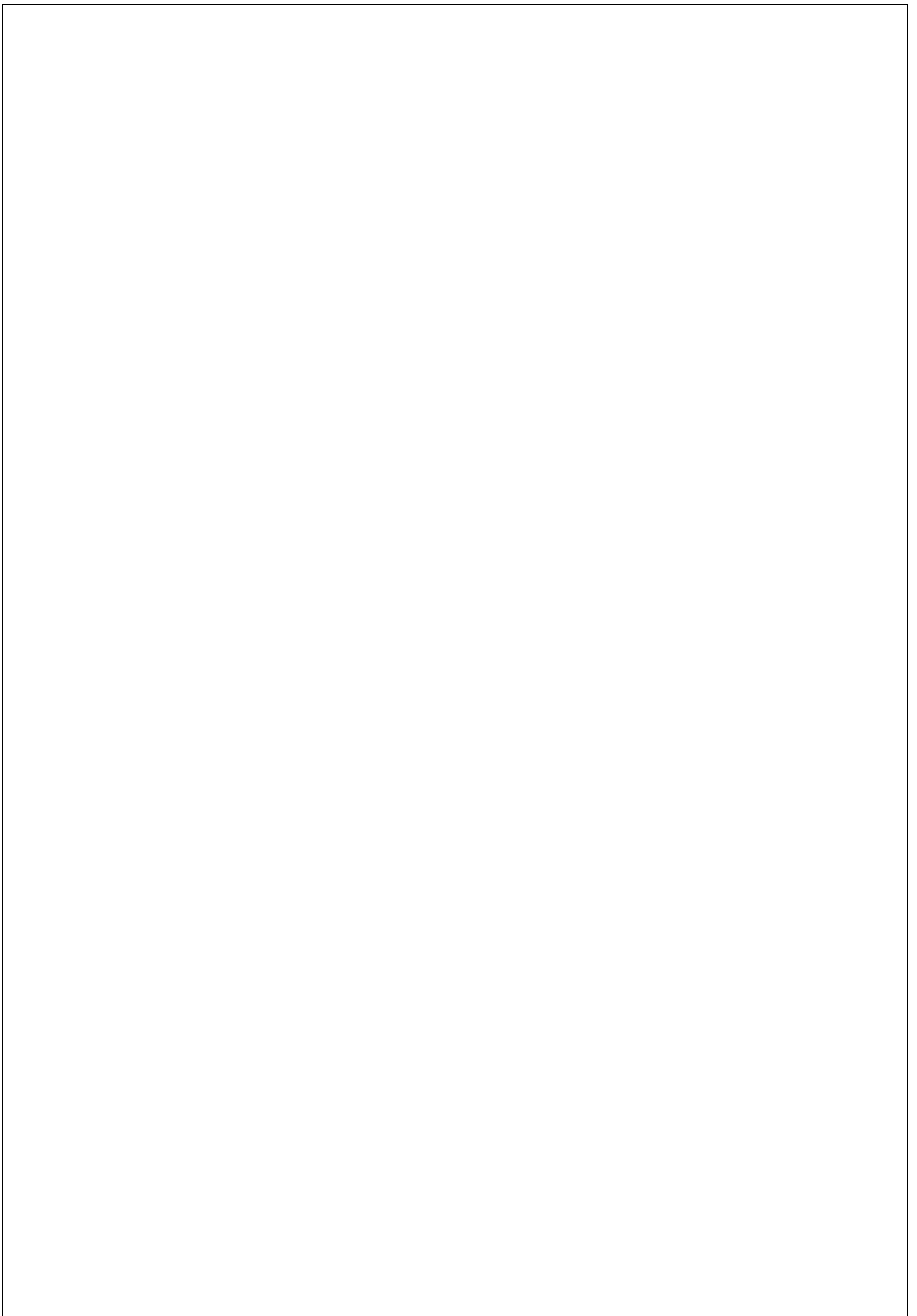
2	DONOR SELECTION	
3	BLOOD COLLECTION	
4	DONOR REACTION AND MANAGEMENT	
5	ABO GROUPING AND Rh TYPING	
6	ANTI HUMAN GLOBULIN TEST	
7	PREPARATION OF BLOOD COMPONENTS	
8	SCREENING FOR TRANSFUSION TRANSMITTED DISEASES	
9	QUALITY CONTROL OF BLOOD COMPONENTS	
10	QUALITY CONTROL OF REAGENTS	
11	PREPARATION OF PAPAINE CYSTEINE	
12	PREPARATION OF PAPANAISED O CELLS	
13	PREPARATION OF EDTA	
14	PREPARATION OF PHOSPHATE BUFFER	
15	PREPARATION OF LISS	
16	CRYO PRESERVATION OF RED CELLS AND DEGLYCERALSATION	
17	PRE TRANSFUSION TESTING	
18	COMPATIBILITY TESTING	
19	INVESTIGATION OF HDN AND NEONATAL TRANSFUSION	
20	PRE TRANSFUSION TESTING OF NEONATES	
21	AUTO IMMUNE HAEMOLYTIC ANEMIA	
22	HAEMOLYSIN TEST	
23	CONVERSION OF PLASMA TO SERUM	
24	ISSUE AND RETURN OF BLOOD COMPONENTS	

25	THERAPEUTIC PLASMA APHERESIS	
26	AUTOLOGUS TRANSFUSION	
27	APHERESIS	
28	ADSOBTION AND ELUTION	
29	SALIVA TEST FOR A,B,H SUBSTANCE	
30	PREPARATION OF ANTI A1 LECTIN	
31	RECORD KEEPING	
32	CLEENING OF GLASSWARES USING POTASSIUM DICHROMATE	

HAEMOGLOBIN ESTIMATION

BLOOD COMPONENTS

SCREENING FOR TTD



QUALITY CONTROL

SPECIAL METHODS

PREPARATION OF COMPONENTS

RED CELL CONCENTRATE

A component obtained by partial removal of plasma from whole blood with out further processing.

FUNCTION

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

PROCEDURE

Prepare by either sedimentation or centrifugation.

- **SEDIMENTATION** :- Blood collected in CPDA anticoagulant 450ml or 350ml 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 350ml double bag in a refrigerated centrifuge at 3550 rpm for 15 minutes at 4-6 °C. After centrifugation express 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 a primary bag is label as packed red cells. Expiry of red cell concert rate in 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 $2-3 \times 10^9$ leucocyte per ml.

Leucocytes and platelets from a buffy coat in the stored blood and can be removed by several methods.

METHODS OF PREPARATION

There are many procedures for preparing leucocyte poor red cell all having varying effectiveness.

- Centrifugation and removal of buffy coat by using top & bottom bag.
- Centrifugation and washing of red cells.
- Spin and filtration.
- Freezing and deglyceralization.

CETRIFUGATION 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 has been introduced for preparation of leucoreduced blood components by removing buffy coat. The efficiency of this method is up to 85%.

INVERT SPIN

- Whole blood or rbc centrifuged in an inverted position at 3000rpm for 10 minutes at 4°C.
- Suspend the bag in an inverted position on an IV stand and release the seal.
- Collect the leucocyte poor red cell in the second satellite bag leaving about 70 to 90 ml buffy coat layer mixed with rbc and plasma in primay bag.
- Double seal and labelled the bag as leucopoor RBC by centrifugation. Stored at 4°C , expiry date as same as RBC

UPRIGHT SPIN

Unit is centrifuged in an upright position at 3000rpm for 10 minutes at 4°C

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 (W-RBC)

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

- RBC 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 centre or at bedside. It has a high efficiency of >99% of leucocyte removal.

IRRADIATED RBC

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

INDICATION

- Congenital immune – deficiency syndrome.

- Bone marrow transplant recipients
- Pre mature new borns.
- Patients with hematologic 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

- Bleeding due to thrombocytopenia
- Defect in platelet function
- Disseminated intravascular coagulation (DIC)
- 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 1700 rpm for 7 minutes within 8 hours of collection.

Express the PRP into the satellite bag without contamination of white cell layer close to red cells.

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

PLATELET CONCENTRATE (PC)

FUNCTION

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

SCOPE AND APPLICATION

For prevention of haemorrhage 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 mins. 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

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 hypoproteinaemia. Pooled plasma is used for preparation of plasma proteins like albumin and immunoglobulins.

PROCEDURES

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 1°c below. Expiry is 5 years from the date of collection.

FRESH FROZEN PLASMA (FFP)

Plasma separated from whole blood frozen within six hours of collection and stored at -20°c or below. Fresh frozen plasma contains both stable and labile clotting factors.

SCOPE AND APPLICATION

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

PROCEDURE

Centrifuge the bag at 3550 rpm for 15 mins at 4°C express 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- 1 ml plasma contains 1 unit of coagulant activity

CRYOPRECIPITATE

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 six hours of collection, subsequent thawing at 4-6°C and the removal of supernatant.

SCOPE AND APPLICATION

Factor VIII deficiency states haemophilia and von Willebrand's disease, disseminated intravascular coagulation, fibrinogen defects, separation of fibrin glue.

PROCEDURE

Collect blood in triple bag and prepare FFP and freeze the plasma immediately, FFP is thawed in 4°C water bath, centrifuge the bag when the plasma is slushy, at 5000 x g for 5 minutes at 4°C. Then supernatant cryo-poor plasma is siphoned out in the satellite bag, leaving 10-15 ml plasma with cryoprecipitate. Seal the tubing and separate the bags. Label bags.

SCREENING HUMAN BLOOD FOR HBsAg

SCOPE AND APPLICATION

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

PRINCIPLE

Enzyme linked immune 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 (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

- Anti HBsAg coated microwell plate
- Negative and positive controls
- Conjugate-anti IgG linked with an enzyme HRP
- Substrate – color giving solution
- Stop solution
- Wash solution concentrate

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

SPECIMEN COLLECTION

Specimens should be serum or plasma. Do not use heat treated specimens. The specimens may be stored at 2-8°C for up to one week or it should be frozen at -10°C or lower for long term storage. For reagent preparation bring all reagents to room temperature for 15-20 minutes before beginning the assay.

Wash buffer :- dilute wash buffer concentrate as per the manufacturer's instruction.

ASSAY PROCEDURE

- Fix appropriate number of strips to the micro plate frame.
- Add required amount of conjugate to each well.
- Pipette out required amount of negative and positive control into each well. Then pipette out required amount of specimens to remaining wells.
- Then tap the frame gently to mix completely. Then incubate at 37°C for 60 minutes.
- Aspirate the contents from all wells and wash each well 5 times with at least 3000 micro litres prepared wash buffer per well for each wash.
- Invert the plate and trap it 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.
- After incubation, pipette required amount of stop solution in each well and shake well.
 - Within 30 minutes read the absorbance of NC, PC and specimens.

INTERPRETATION

- Specimens with absorbance value less than the cut- off value considered as non- reactive.
- Specimens with absorbance value greater than or equal to the cut-off are considered as reactive.
- Initially reactive specimens that do not react with either of the duplicate repeat tests are considered as negative.
- Initially reactive specimens that react with either of the duplicate repeat tests are considered as positive.
- 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-2 antigen coated to the wells of the micro plate 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 colour.

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

- Antigen coated plate
- Negative and positive control
- Sample diluents
- Conjugate
- Substrate
- Wash solution concentrate

- Stop solution

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 specimen. The specimen may be stored at 2-8°C for up to one week or should be frozen state at 10°C or lower for long term storage.

REAGENT PREPARATION

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

ASSAY PROCEDURE

- Fix required number of strips to micro plate frame.
- Pipette required amount of sample diluents into each well.
- Pipette out required amount of negative control, positive control and specimen into each well. Mix for 10s using a micro plate shaker at 1000rpm.
- Incubate at 37°C for 1 hour 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 litre 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 on 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 each well and incubate at room temperature for 30 minutes. Avoid exposure to sunlight.

- Pipette out 100 micro litre of stop solution to each well and tap the plate gently to mix contents.
- Read the absorbents at for 450 nm against air blank within 30 mins of adding the stop solution.

INTERPRETATION

- Specimens with absorbance value less than cut off value considered as non- reactive.
- Specimens with absorbance value 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 value 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 units found negative is released for transfusion.

PROCEDURE

Test components

- Antigen coated plate
- Negative and positive control
- Sample diluents
- Conjugate
- Substrate
- Wash solution concentrate
- Stop solution

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 specimen. The specimen may be stored at 2-8°C for up to one week or should be frozen state at 10°C or lower for long term storage.

REAGENT PREPARATION

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

ASSAY PROCEDURE

- Fix required number of strips to micro plate frame.
- Pipette required amount of sample diluents into each well.
- Pipette out required amount of negative control, positive control and specimen into each well. Mix for 10s using a micro plate shaker at 1000rpm.
- Incubate at 37°C for 1 hour 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 litre 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 on 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 each well and incubate at room temperature for 30 minutes. Avoid exposure to sunlight.
- Pipette out 100 micro litre of stop solution to each well and tap the plate gently to mix contents.
 - Read the absorbents at for 450 nm against air blank within 30 mins of adding the stop solution.

INTERPRETATION

- Specimens with absorbance value less than cut off value considered as non- reactive.
- Specimens with absorbance value 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 value in the gray zone (10% below cut-off) are repeated.

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 with standards. QA should include invitro assay to document effective collection of specific elements or coagulation factors.

SCOPE AND APPLICATION

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

PROCEDURE

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

GENERAL CONSIDERATION

- Donor weight should be 50 kg and above.
- Blood is collected in double or triple bags of 450 ml with 63 ml CPDA and 350 ml with 49 ml CPDA.

- To prevent activation of coagulation system blood must be collected within 10 minutes with minimum tissue trauma with single venepuncture.
- 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 for each component.
- Manual break should not be applied in between the run.
- All satellite bags must be correctly identified, numbered and labelled.
- 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 bag 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

- Platelet count/microliter *1000*volume of WB(ml)= no. of platelet in WB
- Perform platelet count on proper sample.
- Platelet count/microliter *1000of PRP= no. of platelet in PRP.
- Calculate percentage of yield.

- Platelet in PRP*100/platelet in WB = percentage of yield.

To known if it has been thawed and returned.

- Frozen plasma flat and store in upright. Air bubbled formed on the side of the bag during freezing will move to top of bag if thawing has taken place.
- Place a rubber band around the middle of the bag of plasma before freezing which will leave an indentation that disappear on thawing.

BACTERIAL EXAMINATION

- For components prepared by open method and on inspection, there is abnormal appearance and color of units.
- For platelet nearing out dating and returned plasma units redesigned.
- For leuco-poor red cell prepared by centrifugation and washing.
- Returned and out dated units.
- Autologous blood after washing.

Paediatric 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 components 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. Or minimum requirements of documentation on labelling, components preparation, storage inspection of blood components.

COLLECTION OF SAMPLE FOR COMPONENTS UNITS OF QC

- Strip tubing of the components bag four times so that the contents are well mixed.
- Sealed a 3 inch segment distal top collection bag , double seal end of tubing next to component bag and detach segment.
- Empty contents of segment into suitable labelled tube for counting.

SI NO:	NAME OF COMPONENTS	STORAGE TEMPERATURE	EXPIRY
1	RED CELL CONCENTRATE	4-6	35
2	BUFFY COAT	20-24	24
3	FFP	-20	1 YEAR

NO:	NAME OF COMPONENTS	RUN TIME(MTS)	RUN SPEED (RPM)	TEMPERATURE
1	RED CELL CONCENTRATE	10	3000	4-6
2	BUFFY COAT	10	3000	20-24
3	FFP	10	3000	-20
4	PRP	7	1300	20-24
5	PC	10	3000	20-24

QC OF REAGENT

PROCEDURE

QC OF REAGENT ON RECEIPT

Prior to receipt on any reagent , check the name of antisera , volume, date of manufacture and date of expiry, batch number, storage instructions, production license number, preservatives and standard color 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

- Place a drop of 50% cell suspension on a slide.
- Add corresponding antisera
- Mix and start the stopwatch immediately
- 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 agglutinin and haemolysins.

Specificity of pooled cell.(A,B and O cell)

- Label and dispense one drop of each of antisera and 2-5% pooled cells are directed.
- Keep the tubes at room temperature for 30 minutes.
- Spin at 1000 rpm for one minutes.
- Agglutination should be observed as below.

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

- Label a row of 12 tubes according to the serum dilution.
- Add 100 micro litre of saline to all except 1 st tube (to first add undiluted serum)

- Add 100 micro litre of antisera to test tube 1 and 2.
- Mix the tubes well without forming air bubbles and transfer 100 micro litre to tube.
- Continue the same till the last tube and discard 100 micro litres from the last.
- Add 100 micro litres of 2-5 % washed saline suspended appropriate red cells to each tube.
- Mix well and incubate for 30to 40 minutes at room temperature.
- At the incubation time , centrifuge the tube at 1000 rpm for 1 minutes.
- Gently dislodge the cell button.
- Examine the test tube macroscopically , commence reading the test tube containing the most diluted serum and proceed to most concentrated sample grade and grade and record the reaction.
- Last tube, which gives a reaction greater than or equal to +1 is the titre of the antibody.

PREPARATION OF PAPAIN CYSTEIN

FUNCTION

Proteolytic enzyme 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 other (M,N,S and Duffy). Papain reduces the net negative charge on the surface of the red cell by cleaning sialoglycoprotein from the cell surface. This reduce intercellular distance, exposes crypt antigens and increases mobility allowing clustering.

SCOPE AND 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 compliment binding antibodies. The papain technique serve to enhance the reaction of Rh, Lewis & Kidd antibodies and for antibody detection of delayed haemolytic transfusion reactions.

REAGENTS

Papain powder :- 1 gm

Cysteine hydrochloride :- 0.480 gm

5N NaOH (20 gm NaOH make upto 100ml with distilled water)

BUFFER

- KH_2PO_4 (9.078 gm potassium dihydrogen phosphate in 1 litre of distilled water) - 80ml
- Na_2HPO_4 (11.876 gm disodium hydrogen phosphate in 1 litre of distilled water) – 20ml

Mix 1 & 2 and adjust the pH to 6.2-6.4 with normal NaOH.

Take 10 ml prepared buffer separately and dissolve 0.480 gm cysteine hydrochloride. In 90 ml buffer dissolve 1 gm papain powder. Centrifuge and filter. Mix the papain cysteine hydrochloride. Adjust the pH at 6.2 – 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

Papainised cells for 2 stage method.

Washed packed (50%) pooled o Rh positive cells are used. 1 volume of papain is added to one part of washed packed RBC's 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

Dilute IgG anti D (1/8) dilution is tested with papanised cells as positive control. Ig G anti d ad O negative papanised cells is used as negative control.

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

PREPARATION OF EDTA

SCOPE AND APPLICATION

EDTA is used for collection of blood samples for heamogram.

PROCEDURE

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

PREPARATION OF PHOSPHATE BUFFER

SCOPE AND APPLICATION

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

REAGENTS

- Prepare acidic stock solution (solution A) by dissolving 22.16 gm /L of NaH_2PO_4 . In 1 liter of distilled water. This 0.16m solution of the monobasic phosphate salt (monohydrate) has a pH of 5.0
- Prepare alkaline stock solution (solution B) by dissolving 22.16 gm/L of NaH_2PO_4 . In 1 liter of distilled water. This 0.16 ml solution of the dibasic phosphate salt (monohydrate) has a pH of 9.0

PROCEDURE

- Prepare working buffer solution of the desire pH 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	93 ML

- Check pH of working solution before using it. Add small volume of acid solution A or alkaline solution B to achieve desired pH.

- 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.
- Increase 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 LISS SOLUTION

0.17 M saline

0.15 M phosphate buffer

0.3 M sodium glycinate

METHOD

- 18 gm of glycine is dissolved in about 500 ml of distilled water.
- The pH is adjusted 6.7 by drop wise addition of 1N NaOH.
- 20 ml of phosphate buffer pH 6.7 is added to the glycine solution.
- 1.79 gm of NaCl dissolved in 100 ml of distilled water is added to the solution.
- The solution is made up to 1 L with distilled water , mix thoroughly.

- Adjust pH to 6.7 with 1N normal NaOH.
- Dispense into 100ml amounts.

QUALITY CONTROL

NON- SEROLOGICAL

- pH should be within range 6.65 – 6.85
- conductivity should be 3.6 – 3.7 mm at 23 °C
- osmolarity 270 – 280 mmL

Serological

a weak IgG anti –D (0.25 iu/ml) should give a +/+2 reaction with red cells by routine LISS-AHG test. This should be carried out in parallel with tests using the currentbatch of LISS.

CRYOPRESERVATION F RED CELLS AND DEGLYCERALIZATION

FUNCTION

Glycerol prevent freezing injury in red cells by limiting ice formation and providing a liquid phase in which salt 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 3.25%
2. potassiumdihydro phosphate 0.47%
3. dipotassium hydrogen phosphate 0.6%

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

LAYING – DOWN SOLUTION

This is a 50% w/v or 40% 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.

JOYING 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 - the 40°C to -60°C in small aliquots.

RECORY 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 endingwith 2% followed by buffered tripotassium citrate and then twice with saline.

PREPARATION OF THE RECOVERY SOLUTION

Recovery solution 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 other by doubling dilution. 200 ml of 16% is prepared by adding 25.6 ml of glycerol to 174.4 ml of buffered tripottasium citrate. Take rare 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 resting is to select blood and its components that will have :-

- acceptable survival when transfused.
- Will not cause destruction of recipient red cells.
- The procedure involves
- Proper identification of patients 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 retching is to select compatible blood before hand in those patients who have formed antibodies. The panel of cells for antibody screening may be ictained commercially or prepared in the blood bank. A set of two specially selected group O R IRI and R2R2cells 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 consist of following:

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

The cross match test is carried out of ensure that there are no antibodies present in patient' 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 patients.
- 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 1 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 anti globulin technique

Immediate spin technique

Immediate spin technique or saline room temperature technique is inadequate for detection of clinically significant I g G type of antibodies. 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 this is not as sensitive as IAT technique

INDIRECT ANTIGLOBULIN TECHNIQUE

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

Cross matching method

1. Put two drops of patients serum in a pre-labelled glass test tubes.
2. Add one drop of 2-4% of suspension of donor red cell.
3. Mix the content 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 the tube at 1000rpm for 1 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 minutes.
11. Look for haemolysis or agglutination. If no agglutination, the test is invalid. Repeat the procedure.

INTERPRETATION

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

ORTHO BIOVIEW CROSS MATCHING PROEDURE (GEL CARD METHOD)

PRINCIPLE

Micro tubes in the form of cards , filled with buffed dextran gel are used which may be natural or impregnated with AHG. μ

With negative reaction the red cells 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 the red cells in normal saline and make following suspension

SALINE VOLUME	CELL	PACKED BLOOD VOLUME	RED CELL	RED CELL CONCENTRATION
1 ML		40 μ L		3 %
1 ML		50 μ L		4 %
1 ML		60 μ L		5%

- Add 50 micro lit. ortho bliss to the appropriate wells.
- Add 10 micro lit. 3-5% washed red cells to the wells.

- Add 40 micro lit. patients 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 effect 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 INVESTIGATION.

1. Pre transfusion blood sample of the patient.
2. Implicated donor unit sample.
3. Clotted sample obtained from the patient immediately and about 24 hours after transfusion.
4. An EDTA sample of the patient immediately 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 record to determine whether other patient sample or donor units have been involved. If no clerical error, trace each step of the transfusion.
- Compare the color of the serum or plasma in the patients pre and post transfusion samples. Pink or red discoloration in post transfusion sample but not in the pre transfusion sample may indicate free Hbdestruction of donor RBC.
- Perform DAT of post transfusion EDTA sample. Absence of haemoglobinemia and negative DAT strongly suggest that an acute haemolytic reaction has not occurred.
- Repeat ABO & Rh test on pre and post sample and sample from blood bag. Test the post transfusion sample ABO &Rh , a mixed field pattern microscopic reading suggest the presence of incompatible donor cells.
- If the ABO & Rh typing on the patients two samples do not agree, there has been are error in patient or sample identification or in the testing. If so , another patient sample may have been drawn and incorrectly labelled, making it important to check the records all specimens 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 the labelling and processing.
- Repeat antibody detection on pre and post transfusion sample.
- Repeat cross match including an AHG phase testing with pre and post transfusion serum sample against RBC from bag.

INVESTIGATION OF NON- IMMUNE HAEMOLYSIS

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

INTERPRETATION

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

INVESTIGATION OF HDN AND NEONATAL TRANSFUSION

SCOPE AND APPLICATION

Maternal IgG cross the placenta and enter the foetal circulation leading to haemolytic complication in new born, very small blood volume of the new born and antibodies in the transfused blood cause a serious problem by the transfusion centre.

INVESTIGATION OF SUSPECTED HDN

Maternal and cord blood should be tested.

Maternal blood

- Blood group – ABO & Rh , weak D if Rh negative , ICT for antibody detection and identification of antibody if present.

SUSPECTING ABO HDN

- Clinical history of jaundice immediately after delivery.
Blood group mother----- O
ICT----- Negative
Baby's blood group----- A or B
DCT----- weak +ve / negative
- Elute tested against A,B and O cells using ICT

Proceed to the following test to confirm ABO HDN

- IgM or IgG anti-A / anti –B titre in maternal sample significant if > 1: 128 / 1: 32
- Cord or baby's sample -- IgG anti- A / B titre (optional)

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

BLOOD SELECTION

- Group O with lowest -A/B titre .
- Rh as that of the new born (example child is A+, select O+ve blood and if child is Rh negative , select O negative blood)

Confirming Rh HDN

mothers blood group – RH D negative

- ICT - +ve
- Antibody conformation----- +ve reaction in O+ve cells
_ve reaction in O negative cells.

Cord blood :- blood group Rh d – ve

DCT ----- strong positive

ICT ----- +/-

Elute --- + ve with + vecell , negative with O negative cells.

Selection of blood in HDN

- All units selected should be Rh D negative. ABO system can be selected.

BLOCKED D PHENOMENON

When all the Rh sites are blocked by anti D , cord cells will not giving agglutination in anti D and will be grouped as Rh negative.confirmation of blood group can be done with luted red cells.

HDN DUE TO MINOR GROUP ANTIGEN

Investigation same as Rh HDN.

Conformation ----- elute from cord blood can be tested with panel red cells reagent if antibody conformation is not possible. Cross match with maternal serum more number of unit 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

- Due 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 can be selected.
- Transfusion either semipacked RBC or RBC suspended in AB plasma
- Dose – 10 ml / kg for correction of anaemia double blood volume for exchange transfusion.

AUTOIMMUNE HAEMOLYTIC ANAEMIA (AIHA)

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 s 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 papanised patients RBC.
- Test the auto adsorbed serum for allo antibodyby IAT methods.
- If allo antibody present, used auto adsorbed serum for compatibility.
- If no allo antibody detected , do titration cross match with un absorbed serum and find out least incompatible units.
- If allo& auto antibodies are present select allo compatible units which are least incompatible with auto antibody.
- Document the reults

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 haemolytic and likely to have high levels of IgG anti A and 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 2 drops of serum under test in each two tubes
- 3 Add 2 drops of AB serum

- 4 Add 1 drop 5% A cell suspension into one of the tubes and 1 drop of B cell suspension to the other
- 5 Mix and incubate at 37 c for 1 hour
- 6 Observe the colour of the supernatant serum
- 7 A pink or red colour indicated haemolysis
- 8 Hemolysis in tube containing group A red cells indicate of anti –A haemolysin and haemolysis in group B indicates anti B haemolysin

GRADING HEMOLYSIS

- 1 Complete haemolysis - +3
- 2 Partial haemolysis - +2
- 3 Trace haemolysis - +2
- 4 No haemolysis - Neg+3,+2 are considered as clinically significant

CONVERSION OF PLASMA TO SERUM

SCOPE AND APPLICATION

To preserve rare samples for experimental studies

PROCEDURE

- 1 Prepare 2.7% solution of CaCl₂ (2.7 gm of anhydrous CaCl₂ in 100 ml distilled water)
- 2 add 0.1 ml of 2.7% CaCl₂ solution to 0.9 ml plasma
- 3 Incubate serum to form to clot
- 4 Recover serum from clot
- 5 Centrifuge serum at 3000 rpm for 10 min to remove particles if necessary
- 6 Specimen if to be used within 2 days store at 2-8 C. If long storage is indicated specimen should be frozen

