GENERAL OBJECTIVE OF THE TRAINING

• Blood is the life of a person and to provide blood to the persons in grave emergencies is the responsibility of blood bank personnel

• The present manual will discuss in detail the collection, preservation, processing and supply of safe blood to the patients in need. Not only will this module emphasize on provision of safe blood but also judicious use of blood component instead of whole blood so that with a single unit of blood two, three or even four persons may be benefited.

• Subsequent discussions will also include method of storage preservation and transportation of blood to distant places without causing any harmful effects on blood.

• While collecting the blood from donors, the donors may face some adverse effects which will be discussed along with the method to deal with such adverse effects.

• The various type of blood group systems will be discussed with special emphasis on ABO and Rh blood group system.

• Any untoward effects of blood transfusion and methods to deal with such situations will also be covered in the following discussions.

• The ultimate aim of the subsequent discussions will be to provide safe and appropriate blood or component to the people who need them the most.
INTRODUCTION

Historical aspect

Ever since ancient times it has been realized that blood is essential for life. Many physicians tried experimenting with blood by transfusing dog to dog and then animal to man and finally man to man transfusion. Richard Lower in 1665 started his experiment at Oxford, by dog to dog transfusion and proceeded to animal to human in next 2 years. James Blundell began experimenting with transfusion on animals followed by human to human transfusion. By 1800 blood transfusion meant direct donor to patient transfusion, which frequently resulted in disastrous result.

In 1900 Landsteiner elucidated the ABO blood group system.

In 1907 Richard Weil alerted by his intern Renben ottenberg, who in 1908 was the first to perform ABO typing of patient and donor before blood transfusion and also was first to use compatibility testing before transfusion. In 1914 and 1915 the use of sodium citrate as an anticoagulant was proposed.

Rous and Tunner in 1916 were the first to introduce anticoagulant preservative. Later in December 1943 Louis and Mollison introduced acid citrate dextrose (ACD) as a preservative having the advantage of reduced volume of solution.

In 1940 Landsteiner and Weiner discovered the Rh system of blood grouping.

World war II led to many improvements in the blood transfusions.
TERMINOLOGY AND BASIC IMMUNOLOGY

Specific objective
To familiarise the staff with the basic terminology and immunology for better understanding of the principle of blood banking.

Mode of teaching: Lecture method.

In order to make a selection of the compatible donor it is essential to understand the immunological characteristics of the blood in so far as they apply to this area.

Some aspects of basic immunology needs to revised here before undertaking the study of blood groups.

Antigens - These are substances of high molecular weight, mostly protein but also carbohydrates and lipids which when injected in the blood lead to the production of antibodies. These react specifically with the corresponding antigens.

Agglutinogen - Any antigen which is demonstrated by agglutination reaction with the corresponding antibody.

Antibody - It is a gamma globulin (immunoglobulin Ig) produced in response to the administration of an antigen and reacts specifically in vitro and vivo with that antigen.

There are 5 classes of immunoglobulin viz IgG, IgM, IgA, IgD and IgE. IgM antibody has a very large molecular size and cannot cross the placental barrier from mother to foetus while IgG can cross it easily.

The antibodies are further classified as:

1. Iso-antibodies are naturally occuring antibodies. These antibodies develop in the body even without antigenic stimulation. In a person of blood group A, anti B, and in a person of blood group B anti A develop naturally. These antibodies are of class IgM. These IgM antibodies can cause agglutination in saline medium since these cannot cross the placental barrier the fetus of different blood group then its mother is protected from injury.
2. Immune antibodies - These are the classical IgG class of antibodies which develop in response to antigenic stimulation. These antibodies may cause agglutination in saline medium and need protein medium. Anti Rh antibodies do not develop when such a person gets transfusion of Rh positive blood. These can cross the placental barrier.

**Immunization** - The process by which a person or an animal develop antibodies against a foreign antigen introduced in the body deliberately or accidentally.

**Agglutinins** Antibodies which on combining with the corresponding antigen cause agglutination reaction (clumping of red cells).

**Hemolysins** - Certain antibodies do not cause agglutination but lead to hemolysis. This is mediated through the action of complement. In the absence of complement they can cause agglutination only.

**Complement** - It is naturally occurring protein in the blood and consists of many components. These get activated by antigen antibody reaction and bring about destruction of the antigen. Complement in thermo-labile and on storage gets progressively deteriorated. In blood bank work hemolytic antibodies cannot be detected in the absence of complement. In major cross matching if patient's sample is old fresh sample should be obtained.

**Adsorption** - when red cells are mixed with antibodies to their group there is rapid coating of the red cells by the antibody which is then followed by agglutination. The phenomenon of coating by the antibody is called absorption.

**Elution** : This is the process of removing the adsorbed antibodies from the red cells surface. It is used to get the antibody in pure form and find out its specificity.

**Sensitization** - some of the antibodies e.g. Rh antibodies, fail to cause agglutination in saline medium but these would be absorbed on the red cells. The phenomenon of absorption without agglutination is called sensitization. These sensitized cells are recognized by the coomb's test.
Gene The basic unit of heredity. It is made up of DNA.

Alleles - Alternative genes that may occupy a single locus on a chromosome e.g. A,B,O.

Linked genes - When two or more genes determining two or more characters are carried on the same chromosomes and are inherited together are called linked genes.

Blood group system: A group of antigen produced by allelic genes at a single locus on the chromosome. These may be linked genes.

Dominant, Recessive and Codominant genes. A gene which can express itself even in heterozygous state i.e. corresponding allele is different is a dominant gene. Recessive gene can express itself only in homozygous state. When both allelic genes can express themselves in heterozygous state they are called codominant gene. Blood group genes are codominant genes AB group is an example where both A&B allelic gene express themselves.

Genotype. The gene actually present on the chromosome at a given locus whether dominant or recessive. eg. AO, AB, BB, OO, BO AA.

Phenotype - observable character or trait determined by the genotype e.g. AO, AB are both A group phenotypes even though the genotypes are different.

In vitro - Outside the body e.g. in a test tube, petridish etc in vivo Inside the body.

Lectins - Extract of some seeds of certain plant possess antibody like character which can cause agglutination of certain red cells antigen e.g. A, and H.
BLOOD COLLECTION

Specific objective
1. To provide basic information of the types of donors.
2. To teach the staff the method of labelling and record keeping
3. Criteria for donor selection
4. To provide basic information for donor deferral

Mode of teaching
Lecture method, over head projector and practical demonstration.

Blood Donor
Blood donors are of various types
1. Voluntary donors- Donors who donate blood not for any particular person, but on their own free will.
2. Relatives and friends as donors.
3. A person who is none of the above, is a first time or not a frequent donor, who agrees to give blood under some pressure or promise of some reward (e.g. an employee who wants to please his employer donates blood for the employer or his relative.
4. Professional donors- These are people who have made it a profession to sell their blood, moving from one blood bank to another, donate as frequently as possible. Such donors should be strongly discouraged as they are very high source of infection. They also have a poor quality of blood, with less hemoglobin due to frequent donations.

Blood Banks anywhere uses as far as possible voluntary donors and relative donors.

A voluntary donor should be accorded highest courtesies as they are self motivated and can be greatest motivators.
A professional donor should never be accepted and bled. They are usually identified by needle prick marks on their arms.

Special donor categories

1. Therapeutic bleeding- Donation made for therapeutic reasons. These are mainly cases of polycythemia who undergo blood letting to reduce the red cell mass. These are very few.

2. Autologous donors- In this category donation is made by the same recipient (person) who serves as his own donor. In this case the blood may be required at a later date. This is the safest possible transfusion with no risk of transfusion transmitted infection and alloimmunization.

3. Recipient specific designated donations. In some situations there is a need for blood to be collected, processed and stored for a specific recipient e.g. in kidney transplant patient, for newborn with neonatal thrombocytopenia. Such transfusions are called donor specific transfusion and are made when a patient has an antibody to a high frequency antigens or a combination of antibodies that make it difficult to find compatible blood from random donors.

4. Directed donors - Due to the fear of AIDS and other transfusion transmitted disease sometimes a recipient wants to get blood from his relative or friend who he feels has safe blood. In these circumstances a request is made by the patient for blood from specific donor to be transfused. The decision of providing such a blood is entirely dependent in the individual blood bank and their rules and regulations.

Donor area : To encourage blood donation and volunteer donors for their continuous participation, the condition surrounding blood donation should be as pleasant, safe and convenient as possible. It should be well lighted, ventilated, clean and attractive. It should create a feeling of welcome to the donor. It should be open at convenient hours for donors.
Registration and identification of donor

1. Date of registration/ donation - registration number
2. Name
3. Father's / Husband's Name
4. Address
5. Telephone No.
6. Age & Sex.

Blood donor should be between the age of 17 and 65 years. Donor who are legal minors may be accepted only if written consent to donate blood has been obtained in accordance with the applicable state law. Persons over 66 years of age may be accepted as donors at the direction of blood bank physician.

Autologous donation : Has no age limit, but evaluate if it is safe to collect blood.

7. A record of reason for previous deferral.
8. Blood group if known
9. Date of last donation
10. Previous donor rejection if any

Selection of donor

Proper selection of the donor is vital to the safety of both donor and the patient. An apparently healthy donor on proper questioning and medical examination may reveal information showing that a blood donation will expose him to risk or to the recipient.

Selection involves

1. Physical examination
2. Proper medical history

Physical examination

The result of physical examination should be recorded. Any exception to the norms must be evaluated by the physicians and decision recorded in writing and signed.
1. Weight > 45 Kg for 350 ml of blood and > 55 Kg for 450 ml of blood
2. Temperature - should be between 36.5 and 37.5°C
3. Pulse : Should show no pathological irregularity and should be between 60-100 per minute.
4. Blood pressure - systolic between 90-160 mm and diastolic between 50-100 mm of Hg.
5. Donor skin - site of ventipuncture must be free of any lesion or scar of needle pricks indicative of addiction to narcotics or frequent blood donation (as in case of professional donors)
6. General appearance - A prospective donor should be in good health.
7. Haemoglobin- It should be at least 12gm/dl.

**Medical history**

The medical history should be carefully taken and the decision documented on the requisition form.

Following are the important questions that should not be missed while taking medical history.

Questions.

1. **Blood donation:** Have you ever donated blood? If yes date of last donation.
   *Decision:* The intervals between donations should be at least 8 weeks

2. **Rejection as donor:** Have you ever been rejected previously as a donor? If so when and whey?
   *Decision:* Evaluate according to the cause of rejection.

3. **Surgical procedure or major illness** have you had surgery or any long standing illness for at least 6 months? When? What type?
   *Are you under care of a doctor for any reason? Why?*
   *Decision:* Donors who have had major surgery be deferred for at least 6 months, minor surgery is disqualifying only till healing is complete.
   If it appears that the donor is not in good health, refer to blood bank physician.
4. **Malaria-** Have you had malaria? Have you taken any medicine to prevent malaria?

Decision: Donors who have had malaria or taken antimalarial treatment should be deferred for 3 years after stopping the treatment, provided they have been asymptomatic for 3 years.

Donations to be used for preparation of plasma, plasma components or fractions devoid for red blood cells are exempt from these restriction.

5. **Syphilis-** Have you had genital sores? or generalized skin rashes?

Decision: Best to reject such donors, but if accepted the blood should not be issued before at least 3 days of storage in refrigerator. The spirochetes do not survive refrigeration.

6. **Blood transfusion-** Have you ever received blood transfusion within last 6 months? Decision: Defer till 6 months are over.

7. **Vaccinations, Inoculations :** have you been vaccinated in the last 2 months: why? which vaccine did you receive?

Decision: Donors who have been recently immunized with toxoid or killed viral, bacterial or rickettsial vaccines are accepted provided they are symptom free and afebrile. Vaccination with measles, mumps, yellow fever, oral polio, rabies, and animal products have to be deferred for 2 weeks after the last dose. German measles donor has to deferred for 4 weeks after the last injection. Rabies vaccination given following bite by a rabid animal, the donor is deferred for 1 year after the bite. Hepatitis B vaccination- donor is not deferred unless it has been given for specific exposure, in such cases the donor is deferred for at least 6 months after last exposure. Hepatitis B immunoglobulin recipient, donor deferred for 12 months.

8. **Dental surgery-** Have you had tooth extraction or dental surgery in last 72 hours?

Decision: deferred for 72 hours.

9. **Do you have abnormal bleeding tendency?** Decision: Defer permanently subject to evaluation by blood bank physician.
10. Have you ever had epilepsy, convulsions of fainting? Decision- Reject the donor.

11. Do you have cancer?
   Decision: All donors with lymphoma, Leukemia must be permanently deferred. Minor skin or cervical cancers should be evaluated by the blood bank physician and then decided.

12. Do you have unexplained weightloss > 4.5 kg? Decision. It may be a cause of some serious illness hence may be deferred.

13. Pregnancy - Deferred during pregnancy and for 6 weeks following pregnancy.

14. Are you on drugs like tetracyclic, Isoniazids, oral antidiabetic drugs? Decision deferred all such persons.

15. Chronic illness like Hypertension, emphysema, heart disease, chronic bronchitis? Decision- Refer to blood bank physician for decision.

Following donors should be deferred permanently

- With a history of viral hepatitis after age of 10 years.
- Who is or has been an intravenous drug abuser. Check both arms for needle use.
- Patients with AIDS.

Deferred for 6 months

- Recipient of blood, blood component or derivatives eg factor II, VII, IX, X and antihemophilic factor.
- Recipient of skin allograft or tattoo.
- Donor who has had close contact with a person with viral hepatitis or AIDS.
- Recipient of blood transfusion.

**Collection of blood**

   Donation Premises- To encourage blood donation and to remove the fear of blood donation, it is important that the donor room is attractive, well lighted well-ventilated, preferably air-conditioned so that donors feel comfortable and relaxed.
Blood should be collected only by trained personnel, working under the direction of a qualified, licensed physician. The personnel should be friendly as well as professional and well trained. Blood collection must be by aseptic methods, using a sterile closed septum and a single venipuncture.

**Material and Equipment's**

(a) Blood containers must be pyrogene free sterile and should contain sufficient anticoagulant with a label to state the kind and amount of anticoagulant used.

All the items used for phlebotomy (blood collection) should be in sterile, single use, disposable form Plastic bags are preferred over bottles; as they are a closed system of single, double triple, quadruple bags.

- The bag expand when blood is being collected so there is no need for vacuum or airway.
- There is no danger of breakage and they occupy less space.
- No separate donor set is required as a long transparent tubing attached to the bag with needle at one end is used as a donor set.
- In situations for rapid transfusion. It can be done by squeezing the bag.
- Double or triple bags connected to it help in making a closed system for preparing blood components.
- Bag bears a unique number on the bleeding set tubing also in the short segments which can be sealed with blood in the tube, and these tubes can be used as pilot tube without any accident of mis labelling.

**Other instruments include**.

- Syphgmomanometer (B.P. instrument weighing scale, thermometer, blood weighing balance, clips, sealers, artery forceps, sterile disposable syringe with needles.
- Sterile cotton swabs, and band aids.
- Methyalted spirit, tincture of iodine, chlorhexidine hydrochloride (Savlon)
• Emergency drugs like, intravenous fluids, injections (adrenalin, dexamethasone, sodium phosphate, calcium gluconate, phenargan) oxygen cylinder with mask and regulator.

Identification

This is a very important step. A numeric or alphanumeric system must be used to identify and relate the donor record.
1. Identify the donor record by name of the donor.
2. Write the name of the patient for whom blood is given, registration number.
3. Attach identically numbered label to donor record, blood collection container (bags) and test tubes for donor blood samples.
4. Be sure that processing tubes are correctly numbered and they accompany the container during collection of blood to avoid any mistake.
5. Recheck all the numbers.

Method of phlebotomy

1. Choose the site of venipuncture in the anticubital area of the arm, check both the arms to choose the site. It is preferable to make venipuncture in the left hand, as this hand is less frequently used.
2. Apply tourniquet or B.P. Cuff.
3. Clean 4-5 cm area starting at the site of venipuncture and moving outwards in a concentric spirit or savlon.
4. Inflate the blood pressure cuff to 50-60 mm of Hg and check for the prominence of vein.
5. Inspect the bag for leakage or the other defects check the label on the bag.
6. Uncover the sterile needle and perform venipuncture immediately using aseptic procedures.
7. Make the donor open and close hand. If possible then by asking him to open and close hand.
8. Mix the blood and anticoagulant gently and periodically.
9. See that the blood flows freely. On an average one unit of blood collection takes 8-10 minutes.

10. Keep a constant watch on donor for nervousness or any sign for reaction. Keep the donor busy in talking by talking to him in a polite and pleasing manner.

11. After the required amount of blood has been collected clamp the tubing of the bag with artery forceps. Deflate the cuff. Place the sterile swab at the venipuncture site apply light pressure and withdraw the needle. Put the needle in pilot tube.

12. Apply pressure over the swab at venipuncture site and ask the donor to put the hand of other arm at the site.

13. Take the bag and pilot tubes to the processing table.

14. Loosen the artery forceps and apply light pressure on the bag to transfer 5-6 ml blood in the pilot tubes.

15. Now tighten the knot and cut the tube distal to the knot and separate the needle.

16. Keep the blood bag at 4-6°C in the refrigerator immediately after collection.

**Care of the donor after phlebotomy**

1. Check the arm and apply band-aid/bandage after the bleeding stops.

2. Ask the donor to remain on the bleeding couch for a few minutes under the observation of staff.

3. Allow the donor to sit up and go for refreshment, while sitting inquire as to how he feels.

4. Instruct him to drink more fluids than usual in next 4 hours.

5. Ask him not to smoke for half an hour.

6. Thank the donor for an important contribution and encourage repeat donation.
ADVERSE DONOR REACTIONS

Specific objective

To familiarise the blood bank staff with any untoward reaction that a donor may face and to teach the staff to manage such emergencies.

Mode of teaching, Lecture method, overhead projector and practical demonstration.

Most donors tolerate giving blood very well, but occasionally adverse reactions may occur which are usually harmless. The blood bank staff must learn to recognize and manage them. It would be desirable if the personnel are trained in cardiopulmonary resuscitation (CPR) the adverse donor reactions may be as follows.

1. Syncope (fainting or vasovagal syndrome) It may be due to sight of blood or due to donation. The symptoms include sweating, weakness, dizziness or unconsciousness. The skin is usually cold, blood pressure falls and the pulse becomes thready.
2. Hyperventilation or deep breathing it may cause the excited donor to loose excess carbondioxide which may result in hyperventillation, tetany, characterized by twitching and or muscular spasm.
3. Nausea and vomiting.
4. Hematoma
5. Convulsions.

Treatment

1. General- Remove the tourniquet and withdraw the needle from the arm. If possible remove the donor who experiences the adverse reaction to an area where they can be attended to in privacy.
2. Fainting
   a) Place the donor on his / her back and raise the feet above the level of the donor’s head.
   b) Loosen any tight clothing.
   c) Ensure adequate airway.
   d) Administer inhalation of aromatic spirit of ammonia
e) Check the blood pressure, pulse and respiration periodically until the donor recovers.

f) Apply cold compresses to the forehead and the back of neck

(3) Nausea and vomiting
(a) Make the donor as comfortable as possible.
(b) Ask the donor to breathe slowly and deeply.
(c) Turn the donor’s head to a side to avoid aspiration of vomits.
(d) If the donor vomits, provide suitable receptacle & towel or cleansing tissue.
(e) Apply cold compresses to donor’s head.
(f) Give water to clean or rinse his mouth.

(4) Twitching or muscular spasms
If the donor experiences the twitching or tingling sensation the donor is asked to breath into a paper-bag which brings prompt relief. Do not give oxygen.

(5) True convulsions are rare. In case they occur:
(a) Prevent the donor from injuring himself.
(b) Place tongue blade between the teeth to prevent him from biting the tongue.
(c) Ensure adequate airway.

(6) Haematoma
(a) Remove tourniquet or deflate the blood pressure cuff and withdraw the needle from the vein.
(b) Place 3 or 4 sterile gauge pieces or cotton swabs over the haematoma and apply digital pressure for 7-10 minutes with the donor’s arm held above the heart level.
(c) Apply ice to the area for 5 minutes, if desired.

(7) Cardiac or respiratory difficulty
(a) Call for medical aid immediately.
(b) If the donor is in cardiac arrest, begin cardiopulmonary resuscitation immediately and continue till the help arrives.
The nature and cause of all reaction should be recorded on the donor record or on any special form maintained for this purpose.

Processing of donor blood

1. Recheck the number of blood bags processing tube, and donor record before processing.

2. Also group must be determined on donor’s blood by testing the red cells with anti-A, anti B and anti AB sera and testing the serum with known A, B and O cells.

3. Test the donor’s blood for Rh group with anti D serum.

4. Test the blood for transmissibility which include
   
   (a) Hepatitis B surface antigen
   
   (b) HIV testing for AIDS
   
   (c) Test for syphilis
   
   (d) Hepatitis C virus testing
   
   (e) Blood must also be tested for malarial parasite.

   Once all these procedures are performed blood is now ready to be released.
STORAGE AND PRESERVATION OF BLOOD

Specific objectives
To emphasize upon the importance of storage and preservation blood.

Mode of teaching, Lecture method, overhead projector and practical demonstration of the blood bags and their constituents.

The main objectives of storage of blood are
1. To maintain the red cell viability and function of each constituent.
2. To prevent any physical changes which may be harmful to the constituents.
3. To prevent coagulation
4. To prevent bacterial proliferation

The red cells are living cells and require energy for their metabolism. During metabolism there is production of waste products in the body, so as anticoagulant preservative solution apart from preventing coagulation should also provide proper nutrients for continued metabolism of cells during storage, for RBCs to maintain their integrity there should be a balance between many biochemical materials like glucose, hydrogen ion (pH) and adenosine triphosphate (ATP) for this balance RBCs are best preserved at 1-6°C, this will slow down the metabolism as well as also prevent bacterial multiplication. DO NOT FREEZE THE BLOOD SOLID, AS IT WOULD CAUSE HEMOLYSIS OF THE RED CELLS.

The various types of anticoagulant preservative solutions are:

1. ACID CITRATE DEXTROSE (ACD)
   - Trisodium citrate (dihydrate) 22gm
   - citric acid (monohydrate) 8gm
   - dextrose 24.6gm.
   - Distilled water made to 1 liter.
   - pH 5.0-5.1
   - 15 ml of ACD solution is used for 100 ml of blood
Blood which is stored in ACD solution at 2-6° C for 21 days has more than 70% erythrocyte survival at 24 hour post transfusion, which is accepted as clinically satisfactory.

**Citrate** : Acts as anticoagulant by chelating calcium.

**Dextrose** : After 7 days of storage only 50% of red cells survive at 24 hours post transfusion. Addition of glucose to the anticoagulant solution increases the post transfusion survival of red cells by decreasing the rate of hydrolysis of ester phosphorus during storage and provides energy for synthesis of phosphorus compounds particularly diphosphoglycerates and adenosine triphosphate.

**Citric acid** : It prevents carmalization of glucose in citrate in citrate dextrose solution during autoclave. Citric acid is a weak acid and along with citrate it gives an optimal pH which has a least deleterious effect on red cells. Acid citrate dextrose solution has minimal effect on recipient acid base balance.

**Temperature** : The blood should be stored in the anticoagulant solution at a controlled constant temperature of 2-6°C.

- At lower temperature the rate of glycolysis in very solw.
- Low temperature minimizes the rate of proliferation of bacteria.
- At low temperature the rate of diffusion of electrolytes across the cell membrane is less.

During storage the cellular components of blood gravitate in defined layers on the basis of their weight, the heavier red cells sediment to the lowest level, followed by reticulocytes on top of mature cells, leucocytes and platelets as separate layers between the red cells and plasma.

On prolonged storage of blood at the lower limit of temperature for a long period, layer of micro aggregates of aged platelets leucocytes, fibrin strands, cold insoluble globulin and cellular debris in formed as the duration of storage increases their number and size increases proportionately. These
micro aggregates may pass through filter of the transfusion set, but can be remove by the micro aggregate filters.

Refrigerators used for storing blood should have a temperature control system, an internal fan to ensure uniform temperature throughout the cabinet along with battery operated alarm outside the refrigerator to warn for any abnormal temperature or power failure.

**Citrate Phosphate dextrose solution (CPD)**

In 1957, Gibson and his colleagues showed that by adding phosphate to the acid citrate dextrose solution the rate of survival in post transfused blood increases.

**Citrate phosphate dextrose (CPD) solution**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Trisodium citrate (dihydrate)</td>
<td>26.30 gm</td>
</tr>
<tr>
<td>Citric acid (monohydrate)</td>
<td>3.27 gm</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate (monohydrate)</td>
<td>2.28 gm</td>
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<tr>
<td>Dextrose</td>
<td>25.50 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
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</table>

pH 5.6 to 5.8

14 millilitres of the solution is used for 100 ml of blood

- The blood can be stored for 21 days and has about 80% erythrocyte survival at 24 hours post transfusion.
- Red cells 2.3 – diphosphoglycerate [2,3-DPG] is better maintained in CPD than in ACD solution.

**Use of Adenine in Blood preservation**

In 1962 Simon showed that in modified CPD solution if adenine is added then the 24 hour post transfusion survival of red cells is 80.5% ± 6.5% after 35 days of storage. The ATP level is 56.4±15.9% of the initial level at five weeks.
With CPD adenine adequate levels of 2,3-DPG can be maintained for 12-14 days.

The level of 2,3-DPG recovers in vivo within 3-8 hours after transfusion.

**Citrate Phosphate Dextrose Adenine solution (CPDA-1)**

<table>
<thead>
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<th>Ingredient</th>
<th>Quantity</th>
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</thead>
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<tr>
<td>Tri-sodium citrate (dihydrate)</td>
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<tr>
<td>Citric acid (monohydrate)</td>
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<tr>
<td>Sodium dihydrogen phosphate (monohydrate)</td>
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<tr>
<td>Dextrose (monohydrate)</td>
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<tr>
<td>Adenine</td>
<td>0.275 gm</td>
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<td>Distilled water</td>
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14 m of CPDA–1 is used for 100 ml of blood

Blood can be stored for 35 days

**Citrate phosphate dextrose adenine (CPDA-2) solution**

<table>
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<th>Quantity</th>
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<tr>
<td>Citric acid (monohydrate)</td>
<td>3.27 gm</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate (monohydrate)</td>
<td>2.22 gm</td>
</tr>
<tr>
<td>Dextrose (monohydrate)</td>
<td>44.6 gm</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.55 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 liter</td>
</tr>
</tbody>
</table>

In both CPDA-1 and CPDA–2 solutions the percentage of erythrocyte survival at 24 hours post transfusion is 100%.

**Heparin**

Heparin prevents coagulation by inactivating thrombin after complexing with anti thrombin III and thrombin. It also inactivates factors Xa, IXa, Xla, XIIa and plasmin.

Dose of heparin required for anticoagulation is 0.5-2.0 IU per ml i.e. ~ 350 IU for 350 ml of blood, although plastic bags contain 2250 IU of heparin for 500 ml of blood.

Heparinized blood should be used within 24 hr. of collection.
Circulating heparin can be neutralized by injecting protamine sulphate.

**Ethylene Diamino Tetra Acetic Acid [EDTA]**

Sodium salts of EDTA are powerful chelating agents and bind Ca$$^{++}$$
1.1 mM of EDTA is sufficient to prevent clotting of 500 ml of blood.

Although it is 10 times more patient than citrate, but it has no advantage over citrate. Moreover it is toxic and damages the platelets; therefore it is used for preserving blood samples only.

Additive solutions for preservation of red cells

**CPD-SAG and CPD-SAGM**

Hogman 1975 and Hogman et al in 1978 used four bags system in which one bag contained CPD another 100 ml of physiological saline with adenine (0.125 mM) and glucose (4.45 mM) called SAG solution (saline adenine glucose solution). This caused undesirable amount of hemolysis which was prevented by adding mannitol by Beutler in 1979.

The CPD-SAGM solution has mean 24 hour post transfusion survival of >80% and blood can be stored upto 42 days.

**CPD-SAGM solution**

It has four integrally connected plastic bag system.

One bag contains 63 ml CPD solution another bag contains SAGM additive solution.

Saline adenine glucose mannitol solution (SAGM)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>8.77 gm</td>
</tr>
<tr>
<td>Dextrose (monohydrate)</td>
<td>8.99 gm</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.16 gm</td>
</tr>
<tr>
<td>Mannitol</td>
<td>5.25 gm</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>1 liter</td>
</tr>
</tbody>
</table>
100 ml of this solution (pH 5.7) is added to packed red cells (200 ml) from 450 ml of blood.

**CPD – adsol system**
- It has higher concentrating of glucose, adenine & mannitol.
- Red cell has shelf life of 42 days or 49 days.

**Adenine dextrose solution (adsol)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>9.0 gm</td>
</tr>
<tr>
<td>Dextrose (monohydrate)</td>
<td>22.0 gm</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.27 gm</td>
</tr>
<tr>
<td>Mannitol</td>
<td>7.5 gm</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>1 liter</td>
</tr>
</tbody>
</table>

Blood is collected into the primary bag containing CPD solution. The system of bags is centrifuged. Plasma is expressed into one of the empty bags to prepare components. Now SAGM / adsol solution is transferred into the primary bag containing red cells within 24 hours after blood collection.

The red cells can be preserved in SAGM / adsol solution for 42 days even for 49 days in adsol.

The 24 hr. post transfusion survival in 73.3±6.6%.

**Storage of red cells in frozen state**

**Effect of freezing**
- Freezing damages the red cells due to intracellular ice formation and probably due to hypertonicity also.
- If glycerol (a cryoprotective agent) is added to the cells, they can be frozen and thawed without damage. Glycerol acts by limiting ice formation and provides liquid phase in which salts are distributed.
  - As cooling proceeds excessive hypertonicity is also avoided.
  - Glycerol permeates red cells fairly rapidly during freezing hence is most effective in protecting human red cells.
Method of freezing and preservation of red cells in frozen state

1. Glycerolized red cells which have 40% w/v of glycerol can be frozen at -80°C over a period of 30 minutes using mechanical refrigeration. These can be preserved at –40 to –50°C for 3 years.

2. Glycerolized red cells having a final concentration of 20% w/v of glycerol can be frozen at –190°C using liquid nitrogen for 2-3 minutes and can be preserved in gas phase of liquid nitrogen at –150°C for 3 years.

   Note the glycerolized blood has to be thawed and deglycerolized before use.

Indications for use for frozen red cells

1. Freezing rare blood groups
2. Storage of blood for patients with antibodies against high frequency antigens.
3. Storage of blood for autotransfusion for patients with rare blood groups.
4. Prevent of nonhemolytic febrile transfusion reactions in patients sensitized to leucocytes, platelets or plasma proteins.
5. Prevention of sensitization against HLA system in potential recipients of tissue transplants.
TRANSPORTATION OF BLOOD

Specific Objective To provide basic information as to how to transport the blood. To provide basic information of the changes in stored blood and the effects of transfusing stored and altered blood.

Criteria for re-issuance of blood and precautions of receiving back the blood.

Mode of teaching: Over head projector and lecture method.

During transport, the temperature of blood must be kept below 10°C. Sturdy well-insulated containers with adequate cooling material are suitable for this purpose. Broken pieces of wet commercial ice is best suited for it. Ice should preferably be above the blood to take advantage of cool air moving downwards. Blood containers and ice should be in plastic wraps. If the blood is to be taken long distance or weather is unusually hot, the ice may be packed in between and below the blood containers. The amount of ice should roughly be equal to the amount of blood.

Changes in the stored blood

The changes given below refer to the blood collected in ACD

1. Rise of lactic acid and ammonia in the plasma.
   Red cells use up dextrose and release lactic acid as a waste product. It reduces the pH. Ammonia is also released and tends to raise the pH.

2. Rise of potassium and fall of sodium in plasma
   ACD solution is slightly hypotonic and causes red cells to swell up increasing their permeability. It causes shift of sodium into the red cells and escape of potassium into the plasma leading to the rise of potassium and fall of sodium in the plasma. Plasma may come to contain 10 mEq/L after 10 days; 20 mEq/L after 20 days and 30 mEq/L after 28 days of storage.
   Donor blood even after 10 days of storage may be dangerous to newborn infants and cases of renal failure who already have a high potassium level, by causing cardiac embarrassment.

3. Rise of inorganic phosphates in the plasma (strong affinity to oxygen)
   High energy phosphates break down to inorganic phosphates.
4. Rise of plasma hemoglobin
Some red cells may hemolyse leading to rise in the plasma hemoglobin

5. Red blood cells
Blood stored for 14 days usually has 85% survival of the cells which comes down to 75% at the end of 21 days.

6. Leucocytes
These lose their viability within 6 hours. Fresh blood transfusion for raising the leucocyte count are not very successful.

7. Platelets:
Normal life span of platelets in vivo is 9 days. Platelets may be found in the stored blood even after 1 to 2 weeks. However these are functionally useless. Transfusions to thrombocytopenic patients for raising platelet count blood must be as fresh as one week. PRP (Platelet rich plasma) or platelet concentrates are a better choice. Ideally blood should have been collected in the plastic bag.

8. Coagulation factors
At least 50% of factor VIII (antihemophilic globulin) is destroyed within 24 hours at 4°C in the refrigerator. Factor V survives even less. As against it factors XI, X, IX, VII, II are more stable and sufficient amounts would be available in plasma after 14 days of storage. Fibrinogen is present in fair amount even after prolonged storage but in cases of hypofibrinogenemia concentrated lyophilized fibrinogen gives better results.

Factor XII (contact factor) on coming in contact with the glass gets activated. It also gets activated in the plastic bag but it takes as much as 48 hours. Activated form of this factor in turn activates factor IX. However other factors of intrinsic pathway are not activated due to the absence of ionic calcium. However on transfusion calcium becomes available and thrombin is formed which gets widely disseminated. In case of a few units of blood nothing happens as thrombin would be quickly neutralized by antithrombin substances. In case of massive transfusions where as many as 10 units are
transfused in 24 hours the thrombin may damage platelete causing serious thrombocytopenia.

All in all cases of coagulation factor deficiencies should be given as fresh blood as possible.

**Transfusion of stored blood**

Upon transfusion the body removes the dead red cells and viable cells are restored to their normal condition. High energy phosphates (2,3 DPG; ATP) are built up. Bodily systems restore the various biochemical parameters.

**Massive blood transfusion**

There are occasions when a person receives large amounts of bank blood in a relatively short time. There are certain hazards connected with it which should be kept in mind.

1. **Antigen antibody reactions**
   
   There is a greater possibility of development of immune antibodies. Another danger is of reactions occurring between the plasma and the red cell of the various donors. Normal cross match will not detected this possibility and inter donor cross matches have to be put up. In case hypothermia is contemplated these matches should also be checked at 4°C.

2. **Biochemical disorganizations**
   
   a. **Hyperkalemia**
      
      As discussed previously the danger of the rise in potassium level becomes greater.
   
   b. **Citrate toxicity**
      
      With large amount of bank blood, greater amount of citrate also enter the blood creating its own problems. Normally in the body excess citrates are metabolised by liver and through Kreb's cycle.
1. **Depression of calcium level of blood**: Cause increase muscular excitability e.g. tremors and tetany. It can be easily corrected by injection of calcium gluconate.

2. **Cardiac irregularities**: Excess citrate can cause cardiac irregularities which can be fatal particularly in newborns in adults with liver dysfunction. Hypocalcemia and hypercitremia can be doubly dangerous to such persons and they should receive either pack cells or heparinized blood.

3. **Thrombocytopenia**: It can lead to haemorrhages.

**Transfusion induced hypothermia**
Large volumes of stored blood at 4°C infused in a person can cause hypothermia leading to cardiac irregularities and death. Normally practice of warming the blood is to be strongly discouraged where only a few units are to transfused. In cases of massive transfusions cautious warming or part supply of fresh blood should be done.

**Issue of blood from the bank to the wards**
1. Blood is to be issued to responsible person of the ward and signature taken in the issue register.
2. Before releasing the blood check the bottle lables and the cross match tags to know that it is being issued to the proper patient. Check that it bears tag indicating that it is free of hepatitis B antigen or other diseases which are being tested in the laboratory.
3. Check that the supernatant plasma is clear and does not show hemolysis, murkiness etc. Hemolysis should be checked at the junction of the pack cells and plasma.
4. Check that it is still within dates of expiry.
Reissuance of blood

Blood is issued to the wards for transfusion. Sometimes for some reasons it is not transfused and is sent back to the blood bank. Whether such blood can be reissued has to be carefully decided, following guidelines should be adopted.

1. Check that the blood for remained continuously as $1^\circ$ to $8^\circ$C in the wards. Warming blood beyond this limit, even with subsequent cooling, tends to accelerate the metabolism, produce hemolysis and may permit bacterial growth.

2. Check that the container has not been penetrated. It is to ascertained that sterility has been maintained.

3. In case the pilot tube was sent with the unit is should be received back and should be clearly identifiable with the unit.

Records to show that the unit was received back and at the time of reissuance has been inspected and found normal.
BLOOD GROUP SYSTEM

Specific objectives

- To provide basic information of various blood group systems.
- To provide information of the types of blood systems in use.
- Performing ABO and Rh grouping.
- To provide information of the sources of errors in the grouping.

Mode of teaching, Lecture method, overhead projector and practical demonstration.

There are a wide variety of antigens in the red cells. As many as 300 have been identified on this basis various blood group system have been found eg. ABO, Rh, MN, Kell, Kidd, Lutheran, Duffy, P&Lewis

Of these most frequently used blood group system for cross matching are the ABO & Rh system.

ABO system of blood groups

This is the basic system of blood groups. Depending on the presence or absence of antigens on the red cell membrane, they are divided into four groups.

A, B, AB and O (O denotes absence of A or B antigens), in addition corresponding to antigens A and B, there are naturally occurring antibodies anti A and anti B in the plasma/serum of individuals whose red cells lack the corresponding antigen.

The ABO antigens and corresponding antibodies

<table>
<thead>
<tr>
<th>Antigen on RBC</th>
<th>Antibody in plasma / serum</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anti –B</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>Anti – A</td>
<td>B</td>
</tr>
<tr>
<td>AB</td>
<td>None</td>
<td>AB</td>
</tr>
<tr>
<td>None</td>
<td>Anti A and Anti B</td>
<td>O</td>
</tr>
</tbody>
</table>
These blood groups are inherited. New born infants can be typed from the cord blood, however care is needed as the reactions are not strong.

The reverse (serum) grouping cannot be done on newborns, as at birth antibodies are not present. These start appearing at the age of 3-4 months and keep on increasing till adolescence.

**H antigen**

Apart from antigens A & B there is yet another antigen called H antigen. This antigen is necessary for the development of antigens A and B. accordingly it is present on all A,B, AB and O antigens. There are a minority of persons who do not possess H antigen.

These persons may develop anti-H isoantibodies and cannot accept O group blood.

The A,B and H antigenicity is determined by specific sugars linked to the terminal portion of oligosaccharides (short chain sugars). These are present on glycoproteins or glycolipids in the red cell, membrane and in the plasma only glycolipids in soluble form are found. Cell membranes of endothelial and epithelial cells have both glycolipids and glycoproteins.

**Bombay Blood Group (Oh)**

This is a rare variant of O group. Normal O group does not have A & B antigen but has H antigen. Bombay group does not have H antigen also. These persons develop anti H isoantibodies. Because of this antibody they cannot accept blood from normal O group and can accept only blood from another Bombay group. This type of blood was first found in Bombay hence the name.

**Secretor status**

The blood group substances A,B and H exist in the red cell in alcohol soluble form i.e. not water soluble 80% of persons also produce these in water soluble form. The ability to secrete A,B and H substance is determined
by the presence of the secretor gene (Se), in either the homozygous SeSe or heterozygous Seee state, which is inherited independently of the ABO and Rh genes. Normally all secretors secrete H, in addition to A and or B substance.

**Secretion status**

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Substance secreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>H</td>
</tr>
<tr>
<td>A</td>
<td>A and H</td>
</tr>
<tr>
<td>B</td>
<td>B and H</td>
</tr>
<tr>
<td>AB</td>
<td>A, B &amp; H</td>
</tr>
<tr>
<td>*Oh</td>
<td>nil</td>
</tr>
</tbody>
</table>

* Bombay blood group

All individuals possessing the secretor gene (SeSe or Seee) secrete these antigens in all body fluids except the cerebrospinal fluid.

**Subgroups of ABO Groups**

Antigen A has two components A₁ and A₂. A₂ is a weakly reactive antigen found in a few individuals. These subgroups have been divided the A and AB groups into the following types A₁, A₁B, A₂ and A₂B depending on the reaction with the extract of the lectin dolichos biflorus seeds or human Anti A serum.

22% of persons with A antigens in the A or AB groups are A₂ or A₂B. Since anti A₁ antibody cannot react with A₂ antigen about 2% of A₂ persons show the presence of this isoantibody in their blood. These individuals have to be transfused with only A₂ blood.

A₂ is a weak Antigen, unless great care is taken, may show so weak agglutination so as to be missed with the result that an A₂ person may be reported as group O and A₂ B as B with disastrous results.

**Other Weak Subgroups of A**

Subgroups of weaker than A₂ occur infrequently. These are A₃, Am, Ax and A intermediate.
Antibodies of ABO system

In the ABO system once the antigen in absent from the cells, the corresponding antibody is present in the serum. The antibodies in ABO are usually naturally occurring and are mostly IgM. However IgG can also be seen. IgG anti A and anti B are found more commonly in group O individuals than in A or B individuals.

They bind the complement and are strongly reactive.

Anti A and Anti B are usually not produced in infant upto the age of 3-6 months. However, they reach a maximum titer by 5-10 years and then gradually become weaker as the individual ages. The antibodies formed in the serum of infants at birth are almost of maternal origin. The serum grouping of newborn is thus not recommended.

Rhesus blood group system

Landsteiner and Weiner (1940) injected red cells from Rhesus monkey into rabbits. The resulting antibodies against the antigens of the monkey cells also agglutinated human red cells in = 85% of population. This antigen found to be common between man and Rhesus monkey was called Rh antigen and later called D antigen. The antibody against this is called anti D antibody. Using this antibody, the entire population can be divided into Rh positive and negative. In the entire world, majority of people are Rh positive although the relative proportion is different, 95% of Indian population is Rh Positive.

The Rh blood group is the second most important group in practice of blood transfusion. Anti Rh antibody is not a naturally occurring antibody found in Rh negative individuals. It develops only after injection of Rh positive blood in Rh negative individuals, hence it is an immune antibody. This happens in 50-75% of individuals. Such persons if given a second, transfusion of Rh positive blood may develop fatal incompatible reaction.

An Rh negative mother with Rh positive husband may have Rh positive fetus. During delivery fetal red cells are likely to escape into mother's circulation leading to her sensitization and development of anti Rh
antibodies. If the second pregnancy is also Rh positive fetus, immune anti-Rh antibodies from mother cross through placenta leading to a condition of hemolytic disease of the newborn.

Rh antigen is a complex antigen and consists of 3 parts of antigens called D, d, C,c, E,e. Genes for these are linked. On each chromosomes one of the pair is present. Accordingly following combinations are possible on one of the chromosomes.
1. CDE, CDe, cDE, cDe.
2. CdE, Cde, cdE, cde.

The so called Rh positivity depends on the presence of D-antigen. All the persons in combinations given in 1 above are Rh positive. The antisera against each of the above antigens except anti-d are available with the help of which the exact genotype of the person can be found. Naturally occurring antibodies are not formed against any of these antigens. Rarely incompatible transfusion reactions can occur with them.

**Variant of D antigen**

Certain person have a weak form of D antigen. Red cells of these persons do not show agglutination with slide or tube test with anti D serum and can be mistakenly classified as Rh negative. However coomb's test is performed after sensitizing these cells with anti D serum shows agglutination. Accordingly final reporting of Rh negativity should not be done without coomb's test. This weak antigen is called Du antigen. Incidence of Du antigen is very low in Indian population.

**Other blood group systems**

Apart from ABO and Rh systems there are many more blood group systems, some of which are as follows: Blood groups with natural and immune antibodies Lewis (Le); P; MNS; Lutheran (Lu)

Blood groups with immune antibodies only.
Keil (k), Duffy (Fy); Kidd (JK)
These blood groups are not routinely tested except in cases of difficult cross matches and transfusion reactions, some of these may also lead to hemolytic disease of newborn.

**Miscellaneous antigens**

Apart from the blood group antigens described above, there are certain specific antigens on the surface of leucocytes, platelets and tissue-cells. These are called human leucocyte antigens or HLA since their matching is of paramount importance in tissue transplantation, they are also called transplant antigens. In cases given multiple transfusion anti leucocyte and/or antiplatelet antibodies can develop creating difficulty in transfusions.
ABO GROUPING

Specific Objective: To perform ABO grouping.

- To find out source of error in ABO grouping.

Mode of teaching- lecture method to teach basic principle of ABO grouping and practical demonstration.

1. Routine ABO grouping must include both cell and serum testing as each test serves as a check on other.
2. ABO grouping must be done at room temperature or lower, test at 37°C weakens the reaction.
3. Follow the instructions given with the ABO antisera product insert strictly. And store these at 4°C.
4. Tubes, slides and microplates should be labeled properly.
5. Serum should always be added before adding cells.
6. Tubes and slides should be clean and dry.
7. Optical aid should be used to examine reaction that appear negative by the naked eye.
8. Results should be recorded immediately after observations.

ABO typing in done by three basic techniques

1. Slide test.
2. Tube sedimentation.

The test in performed both on cells (Red blood) and serum.

Procedure

Specimen

1. Sterile plain tubes with clotted blood samples are best. The sample can be stored at 4°C and should be tested within 48 hours.
2. Clotted blood is centrifuged at 1000-3000 rpm for 3 minutes.
3. 1-2 ml of serum is pipetted into a pre labeled tube for serum grouping.
4. Take about 1ml of cells into pre labeled tube and add 0.9% normal saline and mix wash the cells thrice with normal saline and make 2-5% cell suspensions in normal saline.
CELL GROUPING

Slide method

1. Take a slide and divide it in two halves by marking it in the middle by a glass marking pencil. On one side mark A and pencil on one side mark A and on the other mark B.
2. Place a drop each of anti A and anti B sera on side A and B respectively, of the slide. Add 2 drops of 20% red cell suspension on each side.
3. Mix the cells suspension and antiserum with a clear stick or tooth pick. Spread it to form a 20 mm circle; tilt the slide back and forth to complete the mixing.
4. Leave the test for 2 minutes at room temperature (20-24°C). Rock the slide and look for agglutination. Protect the slides from drying particularly in hot weather.
5. Record the result of agglutination.

Tube Method

Tube method is preferred over slide method because it is easy to perform and advantageous because the centrifugation involved enhance the reactions, allowing weaker antigens/antibodies to be detected and because the contents can be protected from drying and smaller amounts of reagents are required.

1. Prepare a 5% cell suspension in normal saline. It is advisable to wash the cells 2-3 times in normal saline, especially in case of cord blood or specimens in the poor conditions.
2. Take 3 test tubes mark them A,B, and AB. Add 2 drops of anti A in tube labeled A, two drops of anti B in tube labeled B and two drops of anti AB in tube labeled AB.
3. Add 1 drop of test cell suspension in each tube.
4. Centrifuge at 1500 rpm for 1 minute (spin tube method) or incubate at room temperature for 30-60 minutes.
5. Observe the supernatant fluid for presence of hemolysis against a well lighted background.

6. Gently disperse the cell button and check for agglutination against a well lighted background.

7. Where no agglutination is seen macroscopically, examine the contents under an microscope.

8. Record the result immediately.

**Serum grouping - Preparation of reagent cells**

Collect blood in plain or EDTA vial from 3-5 donors of known A, B and O Rh positive donors.

Collect cells and wash them 3-5 times with saline. After final wash pool them according to their group. Prepare a 3-5% suspension in saline for tube test and 20% suspension in saline for slide test. Check with known antisera. Store in small aliquots. Store at 4°C. The cells are used for serum grouping and as control for checking on the antisera being used O Rh positive cells are also used for checking on the blood group antisera and also for antibody screening. These cells should be prepared daily.

**Slide Method (Serum grouping)**

1. Use A, B and O reagent cells. A 20% suspension is used.
2. Take a slide and divide it into three portions A, B and O add one drop of reference cells A, B and O in the respective areas.
3. Add 1 drops of test serum on the cell suspension and mix with a clean stick or toothpick spread in a 2cm circle. Rock and rotate for complete mixing.
4. Observe the agglutination and record.

**Tube method (Serum grouping)**

1. Take three test tubes and label them A, B and O.
2. Place 2 drops of test serum in each tested tube.
3. Add 1 drop of A cells in the tube labeled A, one drop of B cells in the tube labeled B and one drop of O cells to the tube labeled O.
4. Mix the contents of each slide by gentle shaking and leave at room temperature for 30-60 min. In urgent cases centrifuge at 1500 rpm (spin tube method for one minute).
5. Observe the supernatant fluid for the presence of hemolysis against well lighted background.
6. Gently disperse cell button and see for agglitination.
7. All negative results must be examined under microscope.
8. Record the results immediately.

**Results (ABO Grouping)**

<table>
<thead>
<tr>
<th>Reaction of test red cells with</th>
<th>Reaction of serum with pooled cells</th>
<th>Interpretation (Blood group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti A</td>
<td>Anti B</td>
<td>Anti AB</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
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</tr>
</tbody>
</table>

+=Agglutination - = no agglutination, H= Hemolysis

**Grouping of cord or infant blood**

Special precautions should be taken while testing cord or newborn infant blood since ABO antigens are not fully developed and alloagglutinins are usually absent. Cord red blood cells should be washed 3-4 times with saline to minimize error due to wharton's Jelly. Serum grouping is not recommended in new-born.

**Problem in ABO grouping**

Discrepancy between the results of cell and serum grouping may be due to one or more of the following.

1. An inaccurate centrifuge may lead of over/under centrifugation to give false. Positive or false negative results. Dirty glassware may give a false positive results.
2. Incorrect labeling of reagent or specimens, contamination or inactivation of cells or reagents also give false positive or negative results.

3. Incorrect cell to serum ratio, failure to add test serum may cause false negative results.

4. Careless reading or recording of observation may give false positive or negative results.

Failure to interpret hemolysis as positive reaction may give a false negative results.

**Problems with test red cells**

Rouleaux formation- This is an aggregation of red cells in the forms of piles of coins and it can be misinterpreted as agglutination. It occurs with the cells suspended in their own serum in conditions with abnormally high concentration of protein.

**Weakening or loss of antigens**

Due to age or diseases such as leukemas and other malignancies.

Acquired B-antigen like activity- Associated with gram negative septicemia, intestinal obstruction and carcinoma of colon or rectum. It may cause false-positive reaction.

Polyagglutinable cells- Red cells may have genetic or acquired surface abnormalities that render them polyagglutinable. Polyagglutinable red cells are agglutinated by almost all normal adult human sera and gives false positive reaction.

Antibody coated an red cells as in hemolytic disease of new born auto immune hemolytic anemia, incompatible blood transfusions- may mask the antigen on red cell surface and give a false negative results.

Presence of two separate cell populations as in a chimera eg a patient recently transfused group nonspecific blood (massive or exchange transfusions) bone marrow transplant, which leads to mixed reaction.
Problems with test serum
1. Rouleaux formation- May occur in paraproteinaemias and recipients of plasma expanders. These give false negative reactions.
2. Unexpected antibodies in serum like anti A₁, in A₂ or A₂B individuals, anti I, Anti H, or anti P₁, may cause problems.
3. Absence or loss of antibodies in serum may be seen in immunodeficiency, leukemia, malignancies, newborns, old age, marrow transplant recipients.
4. Hemolysis.
5. Auto agglutinuns.

Solving problems of discrepancies
Before additional investigations are carried out. Quality assurance of reagents, correct techniques, careful observation and interpretation of results resolve many problems.

Repeat Preliminary procedures
1. Obtain a fresh blood sample from donor unit or patient to rule out discrepancy due to contamination or unidentified samples.
2. Wash the cells 3-4 times in normal saline to rule out rouleaux formation and prepare 2-5% cell suspension.
3. Perform direct coomb’s test in cells.
4. Retest the cell with fresh and potent anti A, anti B, anti AB, and anti A₁, or anti H as appropriate for individual problem.
5. Test the serum against appropriate A₁, A₂, B and group O cells.
6. Use group O cord cells if anti I is suspected.
7. Keep the tests at room temperature and at 4°C for 30-45 min. and then interpret the results.

Rouleaux formation
1. Washing test cells 3-4 times with normal saline may rule out rouleaux formation.
2. Routleaux formation can be dispersed rapidly by adding a drop of saline to cell and serum mixture on slide while true agglutination persists.

3. Add 1-2 drops of saline to the tube after gently resuspending the centrifuged with, adding saline usually disperses rouleaux but not true agglutination.

If abnormalities persists then special investigation to relevant weak A₁B antigen.
- Acquired B antigen
- Poly agglutination.
- Antibody coated red cells.
- Mixed- Field agglutination as in chimera.
- Auto Anti I
- Hemolysis.
RH BLOOD GROUPING

Specific Objective
To perform Rh grouping.
To find out the problems in Rh grouping and solving the problems.

Mode of teaching Lecture Method and Practical Demonstration

The technique is similar to ABO typing but it has to be done in protein medium and preferably at body temperature. It is usually done along with ABO typing.

Reagent
1. Anti D typing serum
2. Coomb's serum
3. Normal saline

Procedure
Rh(D) typing should be performed according to the instructions given by the manufacturers of the antisera.

There are three methods
1. Slide test
2. Tube sedimentation

The blood sample should be collected with or without anticoagulant in a sterile tube and stored at 4°C. It should be tested within 48 hours. No sign of haemolysis should be there.

Slide method
This may be used to emergency RhD typing if centrifuge is not available. The slide test is not recommended for routine tests because it is not reliable especially for weakly reactive cells and it also has the disadvantage that drying of the reaction mixture can cause aggregation if the cells that may be misinterpreted as agglutination.
**Method**

1. Divide the glass slide in two halves by a glass marking pencil and label on half as 'T' (test) and other 'C' (control) warm the slide on the view box (surface temperature 40° to 46°C).
2. Place one drop of anti D on side 'T' and one drop of 22% Bovine albumin on side 'C'.
3. With the help of pastuer pipette prepare 40-45% cell suspension in patient's own serum. Place 2 drops each, of this suspension on both the sides.
4. Mix the cell suspension and reagent, using a clean stick for each slide and spread the mixture evenly on the slide over an area of 15mm diameter.
5. Tilt the slide gently and continuously for 2 minutes. observe for agglutination.

**Interpretation**

A positive test has agglutination with anti Rh (D) in the test and smooth suspension of the cells in the control. A negative test has a smooth suspension of cells in both the 'test' and 'control'. If there is agglutination in the control, the test result must be considered invalid and the test with saline reacting anti-D must be performed.

False positive result may seen in the following cases

1. Drying of reaction mixture may be confused with agglutination.
2. Small fibrin clot may give appearance of agglutination.
3. Incomplete anticoagulated blood may clot on the heated slide.

False negative result is interpreted in the following cases.

1. Saline suspended cells react poorly or not at all.
2. A weak suspension of cells may agglutinate poorly, 40-50% cells suspension must be used. Whole blood from a severely anaemic patient maybe concentrated by centrifugation or by removing plasma or serum.
3. Weakly active cells which normally take two full minutes to agglutinate are read before time.
4. Reagents may be identified incorrectly, resulting in the wrong reagent being used in place of anti-Rh (D).

**Test tube method**
1. Place 1 drop of anti Rh (D) serum in a tube labelled test
2. Place 1 drop of control diluting reagent or 22% albumin in a tube labelled control.
3. Add 1 drop of 2-5% cell suspension in plasma or serum in each tube.
4. Mix well and keep at 37°C for one hour (sedimentation method).
   In case of emergency incubate the tube for 10 min at 37°C and then centrifuge to at 1000 rpm for 1 minute (spin tube method)
5. Gently resuspend the cell button and observe for agglutination. All negative results must be confirmed under microscope.

**Interpretation**
Positive test : Agglutination with anti Rh (D) in the 'test' and smooth suspension of cells in control.
Negative test - smooth suspension of cells in both test and control.
If there is agglutination in the control the test result must be considered invalid and the test is performed in saline reactive anti Du.

False positive results is seen in following case.
1. The anti Rh(D) used may contain antibodies to other specificities.
2. If cells and antisera remain together for too long before the test is read, the high protein medium may produce rouleaux which resembles agglutination.
3. Poly agglutinable red cells may cause false agglutination with any reagent containing human serum.
4. Antisera and reagents may be contaminated with bacteria, foreign substance or another antiserum.
False negative test is interpreted in following cases
1. Inadvertent failure to add the antiserum.
2. Failure to properly identify reagents, resulting in wrong reagent being used in place of anti RhD.
3. Too heavy cells suspension in the tube test may result in poor agglutination.

**Anti Rh (D) sera for saline test tube technique**
Saline agglutinating anti Rh sera is used when the control of high protein anti-D gives a positive test even when washed red cells are used. Such cells giving a positive direct antiglobulin test can usually be tested with saline reacting anti-D serum.
DU TEST

Specific Objective
To understand weak D antigen and method to detect this antigen.

Mode of Teaching - Lecture Method and Practical Demonstration

Cells of low grade Du possess the D antigen but expressed so weakly that they are not directly agglutinated by most anti-D sera. Du can be detected by antiglobulin test.

Method
1. Take one drop of anti Rh(D) serum in a clean labeled test tube
2. Take one drop of appropriate control reagent in labeled tube.
3. Add 2-5% of cell suspension to be tested to both the tubes.
4. Mix and incubate both the tubes at 37°C for 15-30 min
5. Centrifuge at 1000 rpm for 1 min.
6. Gently resuspend the cell button and examine for agglutination. If there is strong agglutination of cells in test tube, then sample is Rh (D) positive and there is no need to proceed with antiglobulin phase of test.
7. If no agglutination or doubtful reaction is observed, wash the cell 3-4 times with saline and decant the last washing.
8. Add 1-2 drops of anti globulin reagent (Coomb's serum) mix gently and centrifuge at 1000 rpm for 1 min.
9. Resuspend the cell button gently and examine for agglutination and record the test result.
10. If the test is negative, the reaction may be confirmed by adding known IgG sensitized cells, recentrifuge and reexamine for agglutination. The presence of agglutination confirms the test result.

Interpretation

Agglutination in the test tube and no agglutination in the negative control tube constitutes a positive test result and the blood is accordingly labeled Du. If negative control test is positive no valid interpretation of Du test is made.
COMB’S TEST OR ANTIGLOBIN TEST

Specific Objective
To understand the principle of Coomb’s test for detecting incomplete antibody.
To understand the use of Coomb’s test.

Mode of Teaching Lecture method and Practical Demonstration

The antiglobulin test was discovered by Coomb’s et al in 1945 for detecting non agglutinating (incomplete) blood group antibodies in serum.

Anti human globulin reagent (AHG)
Anti human globulin reagent is prepared by immunizing animals usually rabbits, with whole human serum or with specific fractions of human globulin. Whole human serum is used for preparation of broad spectrum sera which contains antiglobulin antibodies (anti IgG) and anticomplement antibodies (anti C\textsubscript{3} and Anti C\textsubscript{4}).

Antiglobulin antibodies (Anti IgG)
Since vast majority of clinically significant alloantibodies are IgG so anti IgG is essential component of AHG reagent.

Anti complement antibodies
The roles of anti complement antibodies in AHG reagent are

1. To detect complement binding clinically significant antibodies of Kidd, Kell and Duffy system as they may not be picked up by anti IgG.
2. To enhance the reactions of complement binding antibodies.
3. To detect IgM antibodies which invariably bind to complement.

Thus an antiglobulin serum for use in the indirect antiglobulin test (IAT) contains anti C3b as well as anti IgG antibodies, an antiglobulin serum for direct antiglobulin test (DAT) contains anti C3d and anti C4d antibodies as well as anti IgG.
**Principle of antiglobulin test**

In any agglutination reaction there are two stages

1. The antibody coats the surface of the red cell bearing the corresponding antigen.
2. As the red cells hit against each other the second attaches to the other cell causing agglutination.

Sometimes the antibody coats the red cells but fails to agglutinate. Cells are sensitized but no visible reaction occurs. Problem is how to identify that sensitization has occurred? Coombs test is designed to detect this sensitization.

The incomplete antibodies (IgG) attach to the red cell membrane by the Fab portion of immunoglobulin molecule (IgG). The IgG molecule attached to red cells are unable to bridge the gap between sensitized red cells which are separated from each other by negative charge on their surface, thus the sensitized cells do not agglutinate. When AHG serum is added two the wasted sensitized cells the Fab portion of AHG molecule (Anti-IgG) react with the Fc portion of two adjacent IgG molecule thereby bridging the gap between sensitized red cells causing agglutination

There are two types of coombs's test

1. Direct Coomb's test
2. Indirect Coomb's test
Reagents
1. Anti human globulin serum (Coomb's serum)
2. Coomb's control cells
3. Isotonic saline.

Direct Antiglobulin test (DAT/ DCT) or direct Coomb's test
It is done to find out if cells under the test are already coated with antibody. It consists of simple washing the cells thoroughly in order to remove all traces of serum proteins, adding coomb's serum and centrifuging. Agglutination is then observed microscopically.

Procedure
1. Prepare a 4% suspension of test cells and known normal cells in isotonic saline.
2. Take two test tubes and label then T and C. (test and control)
3. Transfer one drop of test cells to tube T and normal cells to tube C.
4. Wash both tubes 3 to 4 times with isotonic saline. Discard the saline after the last wash.
5. Add 2 drops of anti human globulin serum to each tube.
6. Mix well and centrifuge for 1 minute at 1500 rpm.
7. Resuspend the cells by gentle agitation and examine microscopically for agglutination.
8. If the result is negative i.e. no agglutination proceed as follows for further confirmation and checking on the reagents before reporting the results.
   If agglutination is seen in the control tube the result are invalid.
   (a) Incubate for 5 to 10 minutes, centerfuge and read again. In case of slow reacting complement dependent antibodies, agglutination will occur now.
   (b) Add on drop of coomb's control cells. Mix, centrifuge and read again. If serum is reactive and the technique is correct, the agglutination should occur now.
If agglutination occurs in the test and none in the control coomb's test in positive.

**Indirect coomb’s test**

This test is done when the antibody is not coating the red cells but present free in the (serum) blood.

**Procedure**

1. Prepare a 4% suspension of fresh known O Rh positive and patient's cells in isotonic saline.
2. Take two test tube and mark them T (Test) and C (control)
3. Add two drops of patients serum in each tube.
4. Add one drop of O Rh positive cells suspension to tube T and one drop of patient cell suspension to tube C.
5. Mix and incubate for 30 to 60 min at 37°C.
6. Wash cells 4 times with large volume of saline and completely decant after the last wash.
7. Add 2 drops of anti human globulin serum mix well.
8. Centrifuge for 1 minute at 1500 rpm.
9. Re suspend the cells by gentle agitation and check macroscopically for agglutination.
10. Add one drop of coomb's control cells to the tube marked control. Recentrifuge and read again. It should be negative at the first reading and positive at the second reading.

**Error in Coomb's test**

False positive

1. Chemical contamination of glassware with detergents.
2. Dirty or scratched glassware.
3. Bacterial contamination of samples.
5. Plasma volume expanders to the patient.
False negative
1. Neutralization of coomb's serum by protein
   (a) Insufficient washing
   (b) Coomb's serum or its dropper coming in contact with serum
2. Lack of complement by the use old patient sample (Therefore always use fresh serum)
3. Old red blood cells
4. Too long or too short incubation

Application of direct coomb's test
1. Diagnosis of hemolytic disease of new born (HDN)
2. Diagnosis of autoimmune hemolytic anemias (AIHA)
3. Investigation of drug induced red cell sensitization
4. Investigation of hemolytic transfusion reactions.

Applications of indirect coomb's test
1. This is used in the coomb's cross match to find if the patient possess any antibody which could coat and hemolyse the donor cells.
2. Du test uses indirect coomb's test
3. For finding Rh antibody titre in expectant mothers.
COMPATIBILITY TESTING OR CROSS MATCHING

Specific Objective
To provide basic information of cross matching Principle and Procedure.
To find out sources of error in cross matching procedure.
To investigate for possible incompatible cross match.

Mode of Teaching Lecture method and practical demonstration
Before a patient can be given a blood transfusion, a compatibility test with the donor's blood has to be performed to prevent any hemolytic reaction (incompatible reaction) in the patient. Such a reaction can be fatal. It follows ABO grouping and Rh typing of patient and the donor.

There are two types of cross match
1. Major cross match
2. Minor cross match

Major cross match: It involves mixing of specified amounts of patient's serum and donors cells in different conditions. Any agglutination in any condition would indicate the presence of antibodies, natural or immune, in the patient's serum, capable of causing hemolytic reaction. It is most important cross match for a safe transfusion.

Minor cross match: It involves mixing of donor's serum and the patients cells in the same way as for major cross match. Any incompatibility in minor cross match may cause minor ill effects or reduced survival of donor's cells but is unlikely to cause severe hemolytic reaction. Ideally both cross matches should be performed the reason why incompatible minor cross match does not cause ill effect is the same why O group is treated as a universal donor. The serum of the donor is small in amount and get diluted in the patient's blood during transfusion and the antibodies in it are unable to damage the donor's cells.
Cross match procedure

It involves matches in three phases

1. Saline phase at room temperature for IgM antibodies e.g. ABO antibodies.
2. Protein phase at 37°C for IgG antibodies e.g. Rh antibodies.
3. AHG phase for irregular and incomplete antibodies.

If there is no agglutination in any of the phase donors are recipient blood are considered compatible.

Some important considerations in cross matching

Following points are applicable to any antigen antibody reaction including cross matching.

1. All reagent have to be of good quality and all glasswares scrupulously clean. Centrifuge should be properly calibrated.
2. The reactants have to be in equivalent proportions. In cross match procedure the percent cell suspensions and the number of drops have been strictly adhered to By the same pipette should deliver the same size of drop.
3. The Ag Ab reaction takes some time, so this time should be strictly adhered to.
4. Temperature- Different antibodies react at different temperature e.g. IgM (ABO antibodies) act at room temperature (20 to 23°C) white IgG (anti Rh antibodies) at 37°C. Certain antibodies is react at cold temperature e.g. 4°C. If care is not taken in this regard agglutination may be missed, leading to false negative results.
5. Medium - The reactants (antigen and antibody) react together only in presence of some medium, which may be water, saline, buffer or protein. For an antibody a specific medium is needed. ABO antibodies (IgM antibodies) act in saline white Rh antibodies (IgG antibodies) act in high protein medium.
6. Mixing - For a good reaction the antigen antibody should be well mixed which is brought about by shaking and centrifuging.
Centrifugation helps to push the red cells together so that agglutination can occur easily. The centrifuged deposit is shaken to resuspend the deposit and look for agglutination. Points to be considered here are as follows.

Centrifugation has to optimum. It should be neither more or less, high speed centrifugation for prolonged period can produce a false impression when re suspended. Excessive shaking to re suspended the deposit can break small agglutinates and give false negative results.

**Sample**
1. Serum and cells from the pilot tubes
2. Fresh clotted blood of patients from which serum and cells would be prepared.

**Procedure**
1. Label 4 tubes as follows:
   (a) Major saline (MaS)
   (b) Major albumin (MaA)
   (c) Minor saline (MiS)
   (d) Minor albumin (MiA)

   Mark the bag number and the patient's ID.
2. Prepare a 4% suspension of patient's and donor's cells in saline
3. Proceed as given below.

<table>
<thead>
<tr>
<th>Major cross match</th>
<th>Minor cross match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube No.</td>
<td>Drops of Pt. serum</td>
</tr>
<tr>
<td>MaS</td>
<td>Two</td>
</tr>
<tr>
<td>MaA</td>
<td>Two</td>
</tr>
<tr>
<td>MiS</td>
<td>-</td>
</tr>
<tr>
<td>MiA</td>
<td>-</td>
</tr>
</tbody>
</table>

a. Incubate the tube marked MaS and MiS at room temperature and the tube marked MaA and MiA at 37°C for 15-30 minutes.
b. Centrifuge all tubes at 1000 to 1500 rpm for 1 minute
c. Observes hemolysis/ agglutination in all tubes, first macroscopically and then microscopically.

If there is no agglutination in any of the tubes, proceed as follow for the coomb's cross match.

d. Wash the tube marked MaA three to four times with saline Decant the saline after the last wash and add two drops of coomb's serum, mix well and centrifuge at 1500 rpm for 1 minute.

Resuspend the cells after gentle agitation and observe microscopically for agglutination.

If there is no agglutination matching is complete. Use control cells for checking on the Coomb's serum.

**Sources of error in cross matching**

1. Clerical errors and technical faults
2. Errors in ABO grouping of patient or donor.
3. Errors in Rh typing of patient or donor
4. Presence of auto agglutinins and cold agglutinins in patient's or donor's serum
5. Presence of abnormal proteins and rouleaux formation in serum

The errors of cross matching may show false compatibility leading to transfusion of incompatible blood causing fatal hemolytic reaction.

Errors could also be of false incompatibility, so that an otherwise compatible donor is rejected. This would lead to wastage of times as cross matching would be done with other donor.

Of the reasons given above for errors in cross matching the clerical error accounts for more than 80% of incompatible transfusion all over the world.

**Clerical errors**

(a) Identifying wrong patient.
(b) Mislabeling, improper or incomplete labeling.
(c) Mixing of sample  
(d) Entering wrong name and/or number in the register, or on the cross match tags.  
(e) Mixing up donors - putting wrong label on the donor bottle  
(f) Mixing of pilot tube while bleeding the donors or while cross matching  
(g) Mixing up cells and serum of different patients when more than one patient is being cross matched at one time.  
(h) Putting wrong match tags on cross matched units of blood.  
(i) Releasing wrong units of blood  

Investigation of a possible incompatible cross match

Sometimes a patient shows an untoward reaction while being given transfusion and it is suspected that it may be due to mismatching of blood. Such case needs to be investigated properly to find out the reasons as reaction. One should proceed as given below.

1. Recheck to eliminate clinical errors  
   (a) Mixed specimens  
   (b) Incorrectly labeled specimen  

2. Recheck to eliminate technical errors  
   (a) Contaminated pilot tube  
   (b) Contaminated reagents  
   (c) Dirty glassware  

3. Recheck the ABO and Rh grouping of patients and the donor  
   (a) Check ABO grouping from original specimen.  
   (b) Repeat serum grouping with fresh known cells  
   (c) If possible collect fresh specimen from the patient and regroup.  

4. Recheck Rh group of the patient and donor  

5. Recheck patient's own cell suspended in saline with his own serum at 4°C, room temperature, 37°C by high protein method  

6. Check patient chart for previous transfusion, pregnancy, symptoms, diagnosis etc.  

7. Test for Rouleaux
(a) Observe microscopic characteristics of agglutination
(b) Check with saline, rouleaux beaks up while true agglutination will not.

8. Perform cross matching again upto Coomb's cross match. This should be negative

9. Test for cold agglutination

10. Perform direct comb's test on red cells

11. Other investigations of the patient is A, B, and AB
    (a) Subgroup patient and donor cells with anti A_1 serum or anti A_1 lectin and anti AB serum.
    (b) If possible test serum of the patient with A_1, A_2 or O

12. Other investigation if the cells patient is O
    (a) Test patient and donor cells with anti H lectin
    (b) Test patient and donor cells against fresh known O cells
**BLOOD TRANSFUSION REACTIONS**

- **Specific Objective** - To provide basic information regarding the blood transfusion reactions.
- To provide the basic information as to how to deal with such reactions.

**Mode of Teaching:** Over head projector and lecture demonstration.

Blood transfusion is sometimes attended with adverse reaction, some of which may be fatal. Below is given the various types of reactions encountered.

1. **Embolic reactions** - These may be due to
   a. Particles
   b. Air

   **Particles**, which most of the time are fragments of fibrin, can be prevented by the use of transfusion set containing a filter.

   **Air embolism** it can lead to fatality. It occurs:
   a. If the giving set is not completely filled with blood before venipuncture.
   b. If transfusion is completed and the needle is not removed in time.
   c. If the blood is given under pressure.

2. **Circulatory over load**

   This danger is in cases receiving blood for correction of anemia, that is they have no fluid loss. Elderly, debilitated persons, or persons with heart disease and infants may not be able to adjust to this sudden increase in blood volume and could go into heart failure and die.

   They usually show difficulty is breathing, cyanosis, cough with frothy sputum. Death could occur in moments if prompt action is not taken.

   **Actions to be taken:**
   a. Stop transfusion immediately.
   b. Apply tourniquet to all the four limbs so as not to stop the arterial flow but to stop the venous return.
c. An amount equal to the amount transfused should be withdrawn. Giving either pack cells or slow transfusion would prevent this type of reaction.

3. **Reaction due to lekoagglutinins**

Patients receiving multiple transfusions tend to get immunised against the leucocytes. Leucocyte antigens are different from red cells antigens. In subsequent transfusions if the donor's blood contains the offending antigens, patient suffers from chills and fever.

4. **Pyrogenic reactions**

Pyrogenic means any fever producing substance. Commonly such substances like dried blood, serum proteins, killed microorganisms and bacterial metabolic products are present in improperly cleaned and sterilized blood bottles and transfusion sets. Use of disposable sets prevents this problem.

5. **Allergic reaction**

Such reactions occur in patient's who may be allergic to some components of the donor's blood. Occasionally a patient may be passively sensitized by receiving blood characterized by urticaria, diffuse rash, oedema or even asthmatic attack.

6. **Bacteriogenic reaction**

This type of reaction is the result of transfusion of contaminated blood. Certain bacteria e.g pseudomonas and coliforms can even grow at refrigerator temperature. These organisms produce endotoxins which is injected in large enough dose can kill the patient.

   Proper cleaning of the donor arm and use of sterile precautions can prevent this danger. Further more blood in the bank should be inspected regularly for any evidence of contamination (Discolouration, cloudiness and appearance of fibrin clots). Blood bag or bottle should be critically inspected before issue.

   Bacteriogenic reaction present with chills, fever, pain and shock. There is marked erythema at the site of puncture. Transfusion has to be
stopped immediately, antibiotics started and shock managed. Some of the cases may end up with DIC.

7. **Hemolytic transfusion reaction**

This is caused by the transfusion of incompatible blood. Symptoms consist of chills, fever, low back pain, nausea and vomiting. Patient may then develop hypotension (fall in blood pressure) and go into shock.

Transfusion should be stopped immediately and shock treated. Even if the patient recovers there is a danger of renal shut down after a few days.

Red cell antigens combine with the antibody and activate complement leading to hemolysis of red cells. Liberated hemoglobin is passed in the urine giving it a pink colour. The antigen antibody complexes also damage the kidney and red cells are demonstrated in the urine.

This phenomenon of antigen antibody reaction with activation of complement can also induce disseminated intravascular coagulation (DIC).

Much the same types of phenomenas can occur with bacteriogenic reaction, the mechanism being different. In bacteriogenic reactions complement is activated directly.

It may be noted that the types of reactions mentioned in 3 to 7 above give almost identical symptoms. While 3, 4, and 5 are not serious, the types 6 and 7 are extremely dangerous. These need to be identified with certainly. Accordingly any request for transfusion reaction must be dealt on stat basis.

Since most of the incompatible transfusion is due to clerical error, technicians have to exercise extreme caution in identifying and patient and be particular in labeling the sample and the matching tubes. There can be no compromise on these counts.

8. **Haemorrhagic reactions**

These are caused due to severe drop in the platelet counts. This is likely to occur when a patient has lost a lot of his blood and platelets are lost with it. His blood is then replaced with bank blood which are poor in platelets.

Thrombocytopenia (loss of platelets) can also occur due to antibodies in the patient's serum which would destroy the transfused platelets.
9. **Citrate intoxication**

   This is likely to occur in cases where large amount of bank blood is transfused in a short time.

   Symptoms are due not only to increased amount of sodium citrate in the blood but also due to the following
   1. Increased Potassium content in the bank blood
   2. Increased ammonia content in the bank blood
   3. Low availability of ionic calcium in the bank blood
   4. Decreased pH of the blood

10. **Delayed hemolytic transfusion reactions**

   It is possible for a patient to develop hemolytic transfusion reaction a few days after transfusion. Person shows jaundice, nausea, vomiting, passage of dark urine and drop in hemoglobin.

   It is explained on the basis that the person is already immunised to a certain antigen by a previous transfusion. However the antibody titre is low. The very same antigen is present in the present blood but does not show in the cross match because of low titre. However with this transfusion the antibody titre is stimulated to rise and leads to hemolytic reaction. If direct Coomb's is done at this time, it would be found to be positive. After all the donor cells are destroyed the test would become negative.

11. **Drug incompatibility**

   No drug should be added to the blood bag or given through the same transfusion set, because some of these may adversely react with the blood and cause harm to the patient. If any drug is to be given it should be given by separate route.

12. **Two different group bloods**

   Sometimes a situation arises when a patient is being a compatible blood. However he may need more blood but his group blood is exhausted and he has to be given, say O group as a universal donor. In such situations the same transfusion set should not be used in the tubing and filters would react with it causing agglutination. A new set should be used.
HEMOLYTIC DISEASE OF NEWBORN (HDN)

Specific objective
- To provide information regarding the hemolytic disease of newborn
- To provide information regarding prevention of HDN in compatibility
- To provide information as to manage the hemolytic disease of newborn.

Mode of teaching: Overhead projector, lecture demonstration and practical demonstration.

Hemolytic disease of newborn (HDN) is a syndrome associated with hemolysis in the foetus either in utero and / or after delivery with consequent hyperbilirubinaemia. While it is commonly due to immune destruction of red cells of the foetus as a result of maternal IgG antibodies which transmit through placenta.

It is believed that during pregnancy, foetal cells at some stage find entrance into the maternal circulation. It may occur intermittently, but it occurs especially during delivery when placenta separates. If foetal cells carry an antigen, inherited from father and not present in mother, she would get immunised (i.e. produce antibodies against such an antigen) and these would be class IgG. These are not naturally occurring but have developed in response to antigenic stimulation.

Similarly mother's serum proteins gain access to foetus through placenta. If these proteins contain antibodies against foetal cells the latter can be damaged.

IgG antibodies having a smaller molecule can cross the placental barrier. Naturally occurring IgM antibodies against ABO antigens cannot cross the placental barrier.

Blood group incompatibility between mother and foetus can be on account of any of the group e.g.

1. ABO incompatibility - most common form of incompatibility between mother and foetus. ABO-HDN results from IgG anti A and anti B and most commonly occurs in A or B group babies born to O group mothers. The disease is usually mild and requires no treatment.
2. **Rh (D) incompatibility** - Rh(D) incompatibility occurs between mother (Rh D negative) and foetus (Rh D positive), resulting in formation of IgG anti D in mother and is responsible for Rh (D) HDN. This is moderate to severe and needs treatment. In frequency it is next to ABO-HDN. Jaundice is usually seen within 24 hours after delivery.

3. **Other incompatibilities** e.g. Kell, Duffy, Kidd etc.

HDN due to ABO incompatibility is very mild as the ABO antibodies are mainly IgG class which cannot cross placenta. It is only when IgG class antibodies form that ABO HDN develops.

**RH (D) HEMOLYTIC DISEASE OF NEWBORN**

Factors influencing the producing of Rh antibodies are

1. **Size of fetomaternal hemorrhage leak.**
   The amount of foetal red cells that cross the placental into maternal circulation in third trimester of pregnancy is usually small and insufficient to cause antibody production; therefore the first Rh incompatible foetus in unaffected. At delivery, a transplacental haemorrhage is not uncommon. The amount of this may be 1ml to 10 ml. Rh (D) positive foetal red cells stimulate the production of anti Rh (D). The antibody appears in the mother's blood within 6 months of delivery and mother become sensitized but do not have demonstrable levels of antibodies until they have a secondary stimulus during a subsequent pregnancy with 2\textsuperscript{nd} Rh (D) positive foetal cells. The foetal red cells crossing the placental from about 24th weeks of gestation either stimulate the sensitized mother to produce antibody or stimulate the existing antibody to higher titer.
   This is a secondary response and only a small amount of red cells are capable of producing antibody sufficient to cause HDN. The anti Rh(D) formed is IgG and can cross placental barrier and combines with foetal Rh(D) positive red cells leading to their destruction.

2. **Parity** - About 70% cases are seen in II\textsuperscript{nd} and III\textsuperscript{rd} pregnancy
3. ABO incompatibility between mother and foetus - If there is difference between ABO group of mother and foetus, any foetal cells gaining entrance into mother will be destroyed before they can immunise the mother against Rh antigen

4. Rh (D) negative who has been immunized by Rh (D) positive blood transfusion has a risk of delivering a baby with Rh HDN even in the first pregnancy.

5. Medical termination of pregnancy with Rh positive foetus may also immunize the Rh negative mother

**Prevention of HDN in Rh incompatibility**

In case of delivery of Rh positive child or abortion in a Rh negative mother, Rh immunoglobulin is injected in the mother within 24 to 48 hours. This is really anti D serum. Any cells of the foetus which may have entered the mother during delivery are coated by this antibody and rendered incapable of immunising the mother.

**Schedule for antenatal study on cases of suspected HDN**

Antenatal study for ABO incompatibility is of little value hence it is only directed for Rh incompatibility.

1. Mother's history is carefully taken.
2. Mother's and her husband's ABO Rh type is carefully determined.
3. If indicated from grouping and typing indirect coomb's test is done on the mother's serum. This should be done once in the third trimester and then at the 28th week and 36th week.
4. If at any time indirect coomb's test is found to be positive, antibody titer is done and further repeated monthly upon 28th week and thereafter every fort night.

**Investigations on suspected Hemolytic disease of newborns child.**

Normally cord blood is supplied such blood may get contaminated with Wharton's Jelly. Red cells must be thoroughly washed as presence of Wharton's jelly may give false positively of direct Comb's test.
1. Perform ABO grouping - only cell grouping should be done, as at this stage naturally occurring (iso-antibodies) are absent.

2. Perform Rh typing If the results indicate Rh negative, it cannot be relied upon as Rh positive cells coated with maternal antibody may fail to agglutinate with anti D typing sera. This problem would be resolved with Direct Coomb’s test.

3. Direct Coomb's test - cells must be very thoroughly washed due to possible contamination with Wharton's Jelly.
   If direct Coomb's test is positive it would establish that the infant's cells are coated with maternal antibody. It would also establish that a supposed Rh negative infant is really Rh positive.

4. Du test - If Rh typing shows negative and Coomb's test is negative perform Du test.

5. Bilirubin - Value greater than 3.0 gm/dl is high for cord blood

6. Hemoglobin - Normal cord blood hemoglobin is 14-20 gm/dl. Baby's heel prick blood may be up to 4 gm/dl higher than cord blood. Hence as a general rule, cord blood hemoglobin less than 12 gm/dl to be taken as definitely anemic.

Note - All cord blood sample to be tested stat.
ANTI RH ANTIBODY TITER

Specific objective
To provide information regarding the procedure of anti Rh antibody titer to prevent hemolytic disease of newborn.

Mode of teaching: Lecture method, overhead projector and practical demonstration.

Request for such investigation is made in suspected cases of HDN (Haemolytic disease of new born)
The Principle is to prepare serial twofold dilutions of the test serum, mix Rh positive O or ABO compatible cells and note the agglutination. Highest dilution upto which macroscopic agglutination can be observed is the titer of anti Rh antibody.

Primary aim of this test is to detect evidence of rising titre of anti-Rh antibodies. There are many variables in this test. This created difficulty in assessing rise in titre. Samples are being examined on different times - about a month apart. At these different times there may be differing reagents and personnel. To maintain identity of the conditions following precautions need be taken.

1. Follow up test on a particular patient should be done by the same technician.
2. As far as possible test cells should be taken from the same donor.
3. Patient's serum after the test must be stored frozen as control for the next test. Suppose it is 1/16, it is taken as the base line.
4. On the next turn this original sample is titred side by side with the fresh sample. A difference of two dilutions in the old and fresh sample is considered significant.

Procedure
1. Take eleven 75x10 cm tubes in a row. Lable first ten according to dilution, 1,2,4,8,16,32,64,128,256,512. Leave the last one blank.
2. Place 0.1 ml of saline in each tube except the first.
3. Add 0.1 ml of test serum to the tube 1 and 2. Mix From tube 2 take 0.1 ml of diluted serum and add to tube 3 and mix. In this way make doubling dilution upto tube 10 which will now contain 0.2ml of dilution serum (1/512). 0.1ml of diluted serum from tube 10 is placed in the unlabeled tube. This tube is kept in reverse in case testing is to be done in further higher dilutions. All the tube 1 to 10 will contain same volume of 0.1ml.

4. Add a drop of 22% Bovine albumin to each tube and mix.

5. Add 1 drop of 2.5% O Rh positive or Rh positive same group cells as the patient. Mix.

6. Incubate the tube for 30 mts as 37°C.

7. Centrifuge for 1 min at 1000 to 1500 rpm. Observe for agglutination macroscopically. Reciprocal of the dilution is the titer.

8. In case no or weak agglutination is observed, wash cells of each tube 4 times and perform coomb's test and report.

9. If each titer is more than 512, make further dilution from the unlabeled tube and proceed as before.

Blood type to be used for exchange transfusion of new born babies.

Principle

1. Blood must lack the red cells antigens corresponding to the maternal antibodies.

2. In Rh and "other" HDN it is desirable to transfuse with blood of the infant's own ABO group, provided the mother's serum is ABO compatible. There is no advantage of Rh Negative blood for an Rh positive baby unless HDN is caused by anti D antibodies.

3. In ABO HDN, group O cells of the same Rh type as the baby should be used.
<table>
<thead>
<tr>
<th>If Mother is</th>
<th>and Baby is</th>
<th>Donor should be</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. O Negative</td>
<td>A, B, or O Positive</td>
<td>O Negative</td>
</tr>
<tr>
<td>2. A Negative</td>
<td>A Positive</td>
<td>A Negative or O Negative</td>
</tr>
<tr>
<td>3. A Negative</td>
<td>B Positive</td>
<td>O Negative</td>
</tr>
<tr>
<td>4. A Negative</td>
<td>AB Positive</td>
<td>A Negative or O Negative</td>
</tr>
<tr>
<td>5. B Negative</td>
<td>B Positive</td>
<td>B Negative or O Negative</td>
</tr>
<tr>
<td>6. B Negative</td>
<td>A Positive</td>
<td>O Negative</td>
</tr>
<tr>
<td>7. B Negative</td>
<td>AB Positive</td>
<td>B Negative or O Negative</td>
</tr>
<tr>
<td>8. A or B Negative</td>
<td>O Positive</td>
<td>O Negative</td>
</tr>
<tr>
<td>9. AB Negative</td>
<td>AB Positive</td>
<td>Any negative group</td>
</tr>
<tr>
<td>10. AB Negative</td>
<td>A Positive</td>
<td>A Negative or O Negative</td>
</tr>
<tr>
<td>11. AB Negative</td>
<td>B Positive</td>
<td>B Negative or O Negative</td>
</tr>
<tr>
<td>12. O Positive</td>
<td>A, B or O Positive</td>
<td>O Positive</td>
</tr>
<tr>
<td>13. O Positive</td>
<td>A, B or O Negative</td>
<td>O Negative</td>
</tr>
<tr>
<td>14. A Positive</td>
<td>A Positive</td>
<td>A Negative or O Positive</td>
</tr>
<tr>
<td>15. A Positive</td>
<td>A Negative</td>
<td>A Negative or O Negative</td>
</tr>
<tr>
<td>16. A Positive</td>
<td>B Positive</td>
<td>O Positive</td>
</tr>
<tr>
<td>17. A Positive</td>
<td>B Negative</td>
<td>O Negative</td>
</tr>
<tr>
<td>18. B Positive</td>
<td>B Positive</td>
<td>B Negative or O Positive</td>
</tr>
<tr>
<td>19. B Positive</td>
<td>B Negative</td>
<td>B Negative or O Negative</td>
</tr>
<tr>
<td>20. B Positive</td>
<td>A Positive</td>
<td>O Positive</td>
</tr>
<tr>
<td>21. B Positive</td>
<td>A Negative</td>
<td>O Negative</td>
</tr>
<tr>
<td>22. A or B Positive</td>
<td>O Positive</td>
<td>O Positive</td>
</tr>
<tr>
<td>23. A or B Positive</td>
<td>O Negative</td>
<td>O Negative</td>
</tr>
<tr>
<td>24. AB Positive</td>
<td>AB Positive</td>
<td>Any positive group</td>
</tr>
<tr>
<td>25. AB Positive</td>
<td>A Positive</td>
<td>A Positive or O Positive</td>
</tr>
<tr>
<td>26. AB Positive</td>
<td>A Negative</td>
<td>A Negative or O Negative</td>
</tr>
<tr>
<td>27. AB Positive</td>
<td>B Positive</td>
<td>B Positive or O Positive</td>
</tr>
<tr>
<td>28. AB Positive</td>
<td>B Negative</td>
<td>B Negative or O Negative</td>
</tr>
<tr>
<td>29. AB Positive</td>
<td>AB Negative</td>
<td>Any negative group</td>
</tr>
</tbody>
</table>
TRANSFUSION OF NONGROUP SPECIFIC BLOOD
(Hetero-specific ABO Transfusion)

Specific objective
To provide information regarding alternative blood group transfusion.

Mode of teaching: Lecture method and overhead projector.

Sometimes a group specific blood is not available and due to emergency, pleaded by the physician, a nonspecific group compatible in the major cross match is desired. In other words a O group (Universal donor) to any person or any group to a patient of AB group (universal recipient).

1. As far possible such a practice is to be discouraged.
2. Situation may arise that blood has to be given. In such a case only packed cells by given and plasma be avoided as it is where the antibodies to the receipient's blood would be present.
3. Still there may arise situations where the very life of the patient depends on the supply of whole blood. In such a case the blood should be supplied after excluding the presence of high titre of saline antibodies in the donor’s blood.

Following table is recommended for giving packed cells of group nonspecific bloods.

<table>
<thead>
<tr>
<th>Patient's group</th>
<th>First choice of donor</th>
<th>First alternative</th>
<th>Second alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>O Group</td>
<td>O Group</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>A Group</td>
<td>A Group</td>
<td>O red cells</td>
<td>None</td>
</tr>
<tr>
<td>A2 containing anti A1 (37°C)</td>
<td>A2</td>
<td>O red cells</td>
<td>None</td>
</tr>
<tr>
<td>B group</td>
<td>B group</td>
<td>O red cells</td>
<td>None</td>
</tr>
<tr>
<td>AB group</td>
<td>AB group</td>
<td>A or B red cells</td>
<td>O red cells</td>
</tr>
<tr>
<td>A2B containing anti A1 (37°C)</td>
<td>A2B group</td>
<td>A2 or B red cells</td>
<td>O red cells</td>
</tr>
<tr>
<td>Oh Bombay</td>
<td>Oh Bombay</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Note: A fresh infusion sets should be used whenever units of different ABO groups are given subsequently.
In situations where A or B could be used, either one should be used only. Subsequently if need be group O cells be used.

Note: In any of the situations where non group specific, pack cells or whole blood has to be used. It should be done with the permission of the blood bank and attending physicians.

**Screening the nongroup specific donor for whole blood transfusion.**
In case it is essential that whole blood has to be given the donor's blood should be screened to see if there are:

1. High titres of anti A and/or anti B antibodies in 0 or other groups.
2. Presence of hemolysins.

Any of the two above will make the particular donor unsuitable for whole blood transfusion. Titre of anti A and anti B should be less than 1 in 50.

**Test for high titre saline antibodies**
1. Prepare a 1:50 dilution of the serum under the test. Mix 0.1 ml and 4.9 ml saline.
2. Take two 75x10 ml test tubes and label them A and B.
3. Place 0.2 ml of diluted serum in each of the tubes.
4. Add 0.1ml of 4% saline suspension of A1 cells in the tube marked A and similar suspension of B cell sin tube marked B.
5. Mix well and allow to stand at room temperature for 15 mts,
6. Centrifuge for 1 mt. at 1000 to 1500 rpm.
7. Gently dislodge the cell button and observe for macroscopic agglutination.

**Interpretation** - Agglutination of either A or B cells indicates high titre antibodies. Such a blood can only be given to its own group.

**Test for hemolysins**
1. Serum under the test should by fresh. Plasma or aged serum cannot be used as presence of complement is needed to detect the hemolysins.
2. Take two 75 x 10mm test tubes and mark them A and B.
3. Place 2 drops of serum in each tube.
4. Add 2 to 5% saline suspensions of A\textsubscript{1} and B cells in tubes marked A and B respectively.
5. Mix gently and incubated at 37\textdegree C for 10 to 15 min.
6. Centrifuge and examine the supernatant against well lighted white background. Any shade of pink or red will indicate the
7. Record result as positive or negative.
PREPARATION OF BLOOD COMPONENTS

Specific objective
- To provide information about various components of blood.
- To provide information about preparation of blood components
- To provide information about indications of transfusion of various blood components.

Mode of teaching: Lecture method, overhead projector and practical demonstration.

Various components commonly used are:
1. Pack cells washed or unwashed.
2. Platelet rich plasma.
3. Platelet concentrate.
4. Fresh frozen plasma.
5. Cryoprecipitate
6. Platelet poor plasma

Items 1 and 6 can be prepared in any good blood bank. Others need costly equipment's like refrigerated blood bank centrifuge and deep freeze preferably - 60°C.

With the supply of plastic bags in combinations of twos or threes the various components can be separated in a close system.

1. Pack cells
Red cells of the units lying in the refrigerator gradually settle at the bottom leaving clear plasma above. By gentle pressure the plasma above is transferred to the second bag leaving only the pack cells. Do not try to transfer the entire plasma, leave just a little. Pack cells are issued. The plasma duly labeled with the group, date of bleeding is transferred to a deep freeze to be issued as and when required.

2. Washed cells
If the recipient is allergic to plasma factors, red cells need to be free of all trace of plasma. Plasma is removed and sterile pyrogen free saline
introduced and gently mixed. Bag is centrifuged and the saline removed. The process is repeated and the washed cells are then transfused.

3. Fresh frozen plasma (FFP)
Collect blood in a double bag with ACD or CPD and store at 4°C. Further processing should be done within 24 hours.
In a refrigerated blood bag centrifuge spin at 4000 rpm for 6 min. at 5°C. Transfer 2/3rd of plasma into the satellite bag. Seal the tubing and separate the bags.
Label the plasma bag as FFP with date and store at -30°C. The primary bag is labeled as pack cells and stored as usual at 4°C.

4. Cryoprecipitate
Three bag arrangement is used. Proceed as for FFP. Plasma is frozen with the third bag (second satellite bag) attached to it. For preparation of cryoprecipitate the frozen bag is kept for an hour at 4°C. After an hour the bag is hung upside down in the refrigerator with the satellite bag on the lower shelf. Let the thawed plasma flow into the lower bag. The cryoprecipitate remain, enmeshed in the frozen plasma in the primary bag.
When the primary bag weighs about 30 gm, the tubing between the two bags sealed and the bags separated. Cryoprecipitate are stored at -30°C and the rest of the plasma at 4°C.
Cryoprecipitate from a single unit of blood provides 80 to 100 IU of factor VIII.

5. Platelet rich plasma (PRP)
Make sure that the donor’s platelet count is at least 150,000 per cm. and has not taken aspirin and related substances in the last 5 days.
Use double bag arrangement. Collect blood as usual and keep at 20°C. Do not refrigerate.
Load the centrifuge and let the bags lie undisturbed at 20°C for one hour. Then centrifuge at 900 G for 9 min. Remove 2/3rd volume of plasma to the second bag. This is PRP. It should be stored at 20°C till transfused, which should be done as early as possible, preferably within 24 hours.
6. **Platelet concentrate**

Centrifuge the PRP at 2500 rpm for 20 min. The upper layer of plasma is PPP - platelet poor plasma. Transfer it to the satellite bag, leaving about 50 ml. of plasma with the centrifuged platelets. It should be kept at 20°C with constant but gentle agitation till transfused. This is necessary to prevent aggregation of platelets.

Platelet poor plasma is stored in the refrigerator.

**Whole blood and component transfusion**

Transfusion of blood and its products is of major benefit to the patients in specific situation. The relative safety of the procedure should not blind us to the fact that, inspite of rigid care and preventive measures a small but significant number of untoward reactions occur some of these may carry mortality or severe morbidity. Accordingly transfusion should be given on firm indications. Physician should weigh the advantage of blood transfusion against the possible dangers. As far as possible only that component be transfused that is needed by the patient. (component therapy)

Lack of specific indication is an important contraindication of blood transfusion

**Whole blood and components**

1. Fresh whole blood - (upto three days after drawing) For transfusing in children, transfusion of platelets labile coagulation factors, white cells and exchange transfusions.
2. Whole blood - for cases is acute blood loss e.g. trauma, post partum haemorrhage, hematemesisis, malena etc.
3. Pack cells (erythrocyte suspension) correction of anemia
4. Fresh frozen plasma - coagulation factor deficiency
5. Stored plasma - shock, hypoproteinemia, stable coagulation factor. It can be given as a single unit or pooled.
6. Platelet rich plasma (PRP) or platelet concentrate - for cases of thrombocytopenia due to any cause.
7. Leucocyte suspension - agranulocytosis.
8. Factor 8,9, or 10 concentrate for specific factor deficiencies.
9. Fibrinogen concentrate or cryoprecipitates for cases of hypofibrinogenemia and factor 8.

**Note**: donor for platelets should not have taken aspirin compounds for at least a week before donation

**Indications of blood transfusion**
1. **Anemia**: It is can be due to a variety of causes. Depending on the severity and underlying status of patient, symptoms due to hypoxia may result. In cases where the anaemic is asymptomatic and marrow regenerative activity in good, blood transfusion should be avoided. For most of the cases of anaemia, pack cells are to be preferred, following guide lines are given.
   a. Acute hemorrhagic anemia - whole blood
   b. HDN - whole blood exchange transfusion
   c. Correction of anemia before surgery - whole blood or pack cells
   d. All anemias refractory to treatment - Pack cells
   e. Any severe anemia as a life saving measure - Pack cells
2. **Leukemia**
   a. Acute - whole blood, platelet concentrate
   b. Chronic - whole blood.
3. **Agranulocytosis leucocyte suspension**
4. **Haemorrhagic disease**
   a. Thrombocytopenia - suspension of fresh whole blood
   b. Hemophilia - Factor VIII concentrate or Fresh frozen Plasma.
   c. Hypofibrinogenemia - Cryoprecipitate
   d. Factor IX deficiency - Factor IX concentrate
   e. Factor X deficiency - Factor X Concentrate
   f. Factor V deficiency - Fresh frozen plasma.
   g. Other factor deficiencies eg. VII, XI, XII, XIII - Plasma
5. **Therapy for Shock** - Whole blood or plasma.
6. **Hypoproteinemia** - Plasma or Albumin.
TRANSFUSION TRANSMITTED DISEASE.

Specific objective

- To provide basic information regarding the various types of transfusion transmitted diseases.
- To provide basic information about the side effects of transfusion transmitted diseases and methods to detect them at the earliest.

Mode of teaching

Lecture method, overhead projector and practical demonstration.

The blood which is transfused may sometimes cause disease to the recipient, because of presence of some microorganism in the donor's blood if not tested properly before transfusion. Therefore stress is made especially on providing "SAFE BLOOD" to the recipient.

The main diseases transmitted through blood are hepatitis, syphilis, malaria and AIDS and infrequently brucellosis, toxoplasmosis and some other viral infections like CMV, Polio, herpes and Epstein Barr Virus [EBV].

Hepatitis B and Hepatitis C

Hepatitis B virus is a DNA containing virus of Hepadna viridae family. It represents a spherical double layered Dane particle that has an outer surface envelope of protein, lipid and carbohydrate enclosing a slightly hexagonal core. The genome of HBV is a partially double stranded circular DNA molecule.

It contain a nucleocapsed "core" protein HbC Ag- Hepatitis B core antigen which replicates in the nuclei of infected hepatocytes and possess a distinct antigenic specificity. The virion core antigen contains DNA polymerase activity and double standard DNA molecule.

The major structural protein of Lipoprotein viral coat is HBsAg. the infected hepatocytes are capable of synthesizing and secreting massive quantities of non infective surface protein HBs Ag which is detected in the blood banks by various methods like.
2. Enzyme immuno assay.
3. Radio immune assay

**Hepatitis C virus**

It is a small enveloped single stranded RNA virus of family flaviviridae with a genome that codes for a single poly protein. This HCV virus gives rise to anti HCV (IgG) antibody which can be detected serologically.

**Syphilis**

The blood and its components may transmit syphilis and the incubation period is 1-4.5 months averaging 9-10 weeks. This can also be detected serologically by V.D.R.L test for syphilis, which is a flocculation test.

**Cytomegalovirus (CMV)**

CMV is transmitted by transfusion of blood or components containing white cells in patients with impaired immunity e.g. premature infants and bone marrow transplant recipients.

The main features are fever and splenomegaly with mononucleosis in 3-5 weeks after transfusion of fresh blood in large quantity.

Because of generally mild disease and high prevalence of positive donors screening of all donors is not feasible. However, the blood banks should have CMV negative blood donor on panel.

**Malaria**

The plasmodium causes malaria in man, they infect red blood cells and thus transfusion of cellular component may transmit the disease. In non endemic area it is therefore important to exclude donor who have returned from endemic area. People who have visited an endemic area are deferred for at least 6 months.
**Acquired immuno deficiency syndrome**

AIDS is a complex disease in which the individuals defense mechanism is broken down and thus the person is prone to infection and or malignances.

The etiological agent responsible for AIDS is a typical retrovirus with an outer glycoprotein coat or envelop and an internal protein core containing single stranded RNA and the enzyme reverse transcriptase.

The retrovirus contain three main structural genes the gag gene which codes for the core protein, pol (polymerase) gene which codes for the polymerase enzyme complex (reversal transcriptase) and envelop (envelop) gene which codes for the envelop glycoproteins.

**Enzyme linked immunosorbent Assay (ELISA)**

Total protein material from purified detergent disrupted HIV virions, peptide for recombinant protein is attached to a solid phase [plastic beds or microtitre well].

1. Competitive ELISA

   In competitive ELISA core (p24) and envelop (gp 41) HIV antigens prepared by recombinant DNA techniques are used and they are coated to the micro wells of microplates called solid phase.

**Principle** - The test serum of plasma specimens and control sera are incubated in the wells with human anti-HIV chemically conjugated to the enzyme like horse raddish peroxidase (conjugate) competition for binding to the coated HIV antigen occurs between anti HIV in the test sample or control
sera and in conjugate. A test sample containing anti HIV will block the binding of the conjugate to occur.

After thorough aspiration and washing of the wells to remove the excess sample and conjugate, the anti HIV in test sample or conjugate remain attached to the solid phase. Then a substrate material which changes colour in the presence of enzyme is added to all wells and incubated in the dark. After incubation the enzyme reaction in terminated with blocking agent like sulphuric acid. An intense colour signifies a non reactive, while lack of colour signifies a reactive specimen. The results are read photometrically.

2. Indirect ELISA - It is a solid phase enzyme immunoassay utilizing polystyrene wells of microtitre plate or beads coated with proteins representing HIV core and envelope antigens. Human test serum or plasma and control are incubated with solid phase coated with HIV antigens. Antibodies reactive to HIV if present in the test sample will attach to the solid phase.

After incubation, unbound material are aspirated and the solid phase is washed with washing fluid. Anti HIV will remain attached to the solid phase. These anti HIV are detected by the use of anti human immunoglobulin antibody to which an enzyme has been attached called conjugate. The conjugate binds to human immunoglobulin (i.e. anti HIV) on incubation.

After incubation, unbound material are aspirated and the solid phase is washed with washing fluid. Then a substrate, which changes colour in the presence of enzyme in added to all wells and incubated in the dark. After incubation the enzyme reaction is stopped with blocking agent like sulphuric acid. The intensity of the colour in relation to the cut off value determines whether specimen is reactive or not.

All HIV positive samples are confirmed by western blot method.
1 Viral antigen coated well.

2 Add dilute sample.
3 Incubate
4 Wash off unbound material.
5 Add enzyme labelled anti-human immunoglobulin antibody (conjugate)
6 Incubate
7 Wash off unbound material
8 Add substrate.

9 Incubate in dark.
10 Stop reaction and read.

POSITIVE REACTION

NEGATIVE REACTION

SAMPLES

COLOUR

NO COLOUR

KEY:

Antigen
HIV specific antibody
Enzyme labelled antibody
Substrate

INDIRECT ELISA
References

1. Dr. R.N. Makroo - Compendium of transfusion medicine. 1999.