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STUDY OF DISTRIBUTION OF BLOOD GROUPS AND HAZARDS OF TRANSFUSION REACTIONS

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Abstract— The work was done at Blood Bank where we collected the blood samples of both donor and recipient. For checking out the compatibility of the blood samples with the donor and recipient we done the cross matching and predict the blood group of unknown sample by using forward and reverse technique based on cell and sera grouping through which we obtained the grading of agglutination chart which tell the amount of antigen and antibody present in the blood. These blood samples are tested for pre transfusion and qualitative detection of infectious agent generally viral test include HCV, HBsAg, HIV-1 & HIV-2, VDRL. We plot the graph between infected and healthy person cases which was admitted at hospital. After the successful testing of blood, the healthy blood are used to store in blood bank refrigerator and we extracted whole blood components like FFP, PRBC, Platelets and cryoprecipitate by using centrifugation technique at different temperature, RPM, and time interval which is used in many surgical operation and in emergency.

Keywords— HCV (hepatitis C), HBsAg (hepatitis B surface antigen), HIV (Human Immunodeficiency Virus), VDRL (venereal Disease Research Laboratory), FFP (fresh frozen plasma) and PRBC (packed Red blood cells).

I. INTRODUCTION

ABO and Rhesus (Rh) blood group antigens are hereditary characters and are useful in population genetic studies, in resolving medico-legal issues and more importantly in compatibility test in blood transfusion practice. We made an attempt to retrospectively analyze the records on the blood donors, transfusion recipients, cross matching and HIV, HCV, VDRL and HBV rapid screening test. Blood transfusion is an essential part of modern health care system and if blood with correct group is transfused it can save precious life. Blood grouping plays an essential part of blood transfusion service. ABO and Rh grouping” describes the methodology used for Grouping, sub-grouping, Rh phenotyping of blood for determination of human blood group before transfusion. To strengthen the Blood transfusion services by correct grouping of red blood cells and plasma which can save precious lives and improve health. The purpose of this to assist Blood Transfusion Services - Blood banks, hospitals to strengthen

ABO and Rh blood grouping methodologies which are routinely used in the blood banks.

The recommended methods are provided to help the user to have consistent and reliable products in conformity with specific standards and help to improve the quality of Blood transfusion Services. To ensure the continued safety of the nation's blood supply, it is essential that blood transfusion Services implement effective methodologies for blood Grouping. Before blood transfusion, a cross match is a prerequisite so as to avoid untoward reactions of mismatched transfusion. The donor-recipient compatibility is tested by matching recipient's serum and donor red cells. The importance of blood grouping are in immunology, genetics and anthropology, paternity disputes, as a part of routine health check up and routine licensing for driving, job, etc. The most important role of Rh and ABO blood grouping in HDN (Hemolytic Disease of Newborn). Blood transfusion is an essential part of modern health care system and if blood with correct group is transfused it can save precious life. Blood grouping plays an essential part of Blood transfusion service.

II. REVIEW OF LITERATURE

A. OBJECTIVE OF STUDY

In this study, the main content is:-

- i. Study of distribution of blood groups in Indian population based on cases admitted in SN Hospital and Medical College.
- ii. Transfusion reactions and its hazards.
- iii. By collecting samples of patients we firstly check out the crossmatch of the blood with their compatible donor by using forward and reverse techniques by which we easily and accurately blood group of unknown person can be determined, then we perform different test on the blood of that blood group which check out with different infections like HCV, HIV, HBsAg, and VDRL which help to detect any infection in the blood which may be future problem during blood transfusion of the person.
- iv. After doing all these procedure blood is ready to store in blood bank and can also be used for other purposes by obtaining different components of blood from it by using different techniques.



B. STUDY OF DISTRIBUTION OF BLOOD GROUPS IN INDIAN POPULATION

The distribution of the ABO groups but also on that of the blood group systems elaborated afterwards. It begins with a survey of the genetics and the anthropological significance of the different blood group systems: ABO, Rhesus, MNSs, Lewis, P, Lutheran, Kell, Duffy, Kidd. Detailed geographical surveys follow: Northern and Central Europe, Africa south of the Sahara, the Mediterranean area, Asia, Indonesia and Australasia, America.

The distribution of the four ABO blood types, A, B, AB, and O, varies in populations throughout the world. It is determined by the frequency of the three alleles of the ABO gene in different populations. Blood type O is the most common worldwide, followed by group A. Group B is less common, and group AB is the least common.

A blood group system contains antigens controlled by a single gene. There are 33 blood group systems, including the ABO, Rh, and Kell blood groups which contain antigens that can provoke the most severe transfusion reactions.

Each blood group antigen is assigned a six-digit number by the ISBT. The first three digits represent the blood group (e.g., ABO is 001, Rh is 004), and the last three identify the antigen in the order it was discovered. For example, for ABO, the A antigen was the first to be discovered and has the number 001.001 whereas the B antigen was next and is designated 001.002.

Table-1 Distribution of Blood group in population

	ABO BLOOD TYPE	PERCENT OF GENERAL POPULATION	CHANCE OF FINDING A COMPATIBLE DONOR
1.	O ⁺	38.5%	1 out of 2-50%
2.	O ⁻	6.5%	1 out of 15-7%
3.	A ⁺	34.3%	4 out of 5-80%
4.	A ⁻	5.7%	1 out of 8-13%
5.	B ⁺	8.6%	3 out of 5-60%
6.	B ⁻	1.7%	1 out of 12-9%
7.	AB ⁺	4.3%	100%
8.	AB ⁻	0.7%	1 out of 7-14%

C. ABO Blood Groups and Their Genetic Inheritance

The most well-known and medically important blood types are in the ABO group. They were discovered in 1900 and 1901 at the University of Vienna by Karl Landsteiner in the process of trying to learn why blood transfusions sometimes cause death and at other times save a patient. In 1930, he belatedly received the Nobel Prize for his discovery of blood types.

The cells that make up the body's tissues and organs are covered with surface markers, or antigens. Blood group antigens are either sugars or proteins, and they are attached to

various components in the red blood cell membrane. The ABO blood groups are sugars (glycan or carbohydrate).

ABO Blood type antigens are not only found on the surface of red cells. They are also normally secreted by some people in their body fluids, including saliva, tears, and urine. Whether someone is able to secrete them is genetically controlled. Police agencies now routinely use this so-called **secretor system** data to identify potential victims and criminals when blood samples are not available.

The A and B genes found on chromosome #9. We inherit one gene (allele) from our father and one from our mother. The two co-dominant alleles are A or B. Anytime an individual inherits an A or B gene it will be expressed.

The O gene signifies lack of A or B antigens. It is not expressed unless this gene is inherited from both parents (OO). Therefore the O gene is recessive.

Below is the example of two individuals who are A. One inherited only one A gene along with an O gene and is therefore heterozygous. The other inherited 2 A genes and is homozygous for A.

D. Rh Factor and Its Inheritance

The Rh blood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, it has become second in importance only to the ABO blood group in the field of transfusion medicine.

The antigens of the Rh blood group are proteins. A person's DNA holds the information for producing the protein antigens. The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells. To date, 49 Rh antigens are known.

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will

produce anti-D if they encounter the D antigen on transfused RBCs (causing HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be.

E. BLOOD TRANSFUSION

Blood transfusion is generally the process of receiving blood products into one's circulation intravenously. Transfusions are used for various medical conditions to replace lost components of the blood. In modern medical practice commonly uses only components of the blood, such as RBC, WBC, Plasma, Clotting factor and Platelets. Before a blood transfusion is given, there are many steps taken to ensure



quality of the blood products, compatibility, and safety to the recipient.

Blood donation

Blood transfusions typically use sources of blood: one's own (autologous transfusion), or someone else's (allogeneic or homologous transfusion). Blood is most commonly donated as whole blood intravenously and collecting it with an anticoagulant.

Processing and Testing

Donated blood is usually subjected to processing after it is collected, to make it suitable for use in specific patient populations. Collected blood is then separated into blood components

by centrifugation: red blood cells, plasma, platelets, albumin protein, clotting factor concentrates, cryoprecipitate, fibrinogen concentrate, and immunoglobulin (antibodies). Red cells, plasma and platelets can also be donated individually via a more complex process called apheresis.

All donated blood is tested for infections. The current protocol tests donated blood for HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C, Syphilis (*Treponema pallidum*), Chagas disease (*Trypanosoma cruzi*), and West Nile Virus. In addition, platelet products are also tested for bacterial infections due to its higher inclination for contamination due to storage at room temperature. All donated blood is also tested for ABO and Rh groups, along with the presence of any red blood cell antibodies.

Blood Type and Cross Match

To avoid a transfusion reaction, donated blood must be compatible with the blood of the patient who is receiving the transfusion. More specifically, the donated RBCs must lack the same ABO and Rh D antigens that the patient's RBCs lack. For example, a patient with blood group A can receive blood from a donor with blood group A (which lacks the B antigen) or blood group O (which lacks all ABO blood group antigens). However, they cannot receive blood from a donor with blood group B or AB (which both have the B antigen).

Infection Related To Blood Transfusion

i. HEPATITIS C

The test for antibodies to HCV was proved to be highly valuable in the diagnosis and study of the infection, especially in the early diagnosis of HCV after transfusion. The diagnosis of hepatitis C can be easily made by finding elevated serum ALT levels and presence of anti-HCV in serum/plasma. Recently recombinant DNA techniques have been used to encode the genome of HCV. The genome encodes for three structural proteins (capsid protein, envelope glycoprotein E1 & E2) and several nonstructural proteins (NS2, NS3, NS4 & NS5). Post transfusion hepatitis may be clinically overt, although the majority of cases are subclinical; a variable number of cases may become chronic. Hepatitis B and C are

the most frequently implicated agents. Hepatitis A is rarely seen. The primary diagnosis often occurs because of unexplained elevations in liver enzymes and occasionally hyperbilirubinemia. Definitive diagnosis depends on specific serologic tests (which correlate with time since infection for hepatitis B).

ii. HUMAN IMMUNODEFICIENCY VIRUS (HIV), TYPES 1 & 2

Definitive diagnosis of transfusion-transmitted HIV depends on seroconversion usually occurring within 4 to 12 weeks after transfusion, in the absence of any other risk factors. Molecular techniques can demonstrate effective identity between donor and recipient viral strains. In transfusion-transmitted HIV, the diagnosis is usually made due to a "look back" to recipients of prior donations. Otherwise, onset of symptoms, such as unexplained opportunistic infections, low CD4 counts, or characteristic tumors that mark development of the acquired immune deficiency syndrome (AIDS), may be the first indication of HIV infection. HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent.

As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens. Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple DOT(s) against a white background.

The Human Immunodeficiency Virus (HIV), subsequently named HIV-1 was isolated. Shortly afterwards in 1985 another retrovirus subsequently named HIV-2 was isolated in Africa. These two viruses belong to the retrovirus group and are slow viruses. The structure, gene organization and serological behavior of HIV-1 & HIV-2 and their complete nucleotide sequence has been determined. This knowledge has laid a foundation for the development of a new assay based on Recombinant DNA technology leading to the differential detection of antibodies to HIV-1 & HIV-2 (if present) in Human Serum or Plasma. Research has shown that antibodies produced against envelope gene are found in infected people.

iii. HEPATITIS B

Viral hepatitis is a systemic disease primarily involving the liver, and in most cases is caused by one of three viruses: Hepatitis A (HAV), Hepatitis B (HBV) or Hepatitis C (HCV). The antigen found in the envelope of HBV is designated Hepatitis B Surface antigen (HBsAg) and its presence in serum or plasma indicates active HBV infection.

HBsAg Rapid Test is a simple, one-step test that detects the presence of HBsAg. HBsAg Rapid Test is a lateral flow immunoassay. If HBsAg is present in the sample, it forms a complex with the colloidal gold anti-HBsAg conjugate that is dried onto the test strip. The liquid migrates through the nitrocellulose membrane, and if colloidal gold – antibody – HBsAg is present this binds to a second anti-HBsAg antibody



immobilized on the membrane, forming a visible red line. The test strip contains an internal control line in the control region © that should always show up as a red line regardless of the test line result.

iv. VDRL (VENEREAL DISEASE RESEARCH LABORATORY)

Venereal disease research laboratory (VDRL) test is a nontreponemal test, used for screening of syphilis due to its simplicity, sensitivity and low cost. Prozone phenomenon and biological false positive (BFP) reaction are two shortcomings of this test. Quantitative estimation of VDRL is essential in treatment evaluation. There are three basic methods used in screening for syphilis. These include direct observation of the spirochete by dark field microscopy, and nontreponemal and treponemal serologic antibody studies. More sensitive nontreponemal tests such as the rapid plasma reagin (RPR) and the VDRL are used for initial screening, whereas specific treponemal tests such as the fluorescent treponemal antibody absorption (FTA-ABS) are used to confirm the diagnosis.

v. Other Hazards

The safeties of pharmaceutical products are overseen by pharmacovigilance, the safety of blood and blood products are overseen by Haemovigilance. This is defined by the World Health Organization (WHO) as a system "...to identify and prevent occurrence or recurrence of transfusion related unwanted events, to increase the safety, efficacy and efficiency of blood transfusion, covering all activities of the transfusion chain from donor to recipient." The system should include monitoring, identification, reporting, investigation and analysis of adverse events near-misses and reactions related to transfusion and manufacturing. In the UK this data is collected by a charity called SHOT (Serious Hazards Of Transfusion). Transfusion-associated volume overload is a common complication simply due to the fact that blood products have a certain amount of volume. This is especially the case in recipients with underlying cardiac or kidney disease. Red cell transfusions can lead to volume overload when they must be repeated due to insufficient efficacy (see above). Plasma transfusion is especially prone to causing volume overload due to its hypertonicity.

III. EXPERIMENT AND RESULT

BLOOD GROUPING TECHNIQUES

Three manual methods can be used when performing blood grouping:

- Glass slide or white porcelain tile
 - Glass test tube
 - Microwell plate or microplate
- Newer techniques - Column technique (sephadex gel) & Solid phase tests.

In this report we performed only glass tube method for ABO Blood grouping which are as follow:-

Tube method

Test tubes either of glass or plastic may be used. The tube technique is more sensitive than

Slide technique for ABO grouping. Tube technique is further classified on the basis of grouping which is called forward grouping (cell grouping) and reverse grouping (serum grouping).

Reagents required:-

- CPDA (Citrate Phosphate Dextrose Adenine) anti coagulated Blood samples.
- Working standardized Monoclonal antisera (Anti-A, Anti-B, Anti-A B)
- Anti – A1 (Lectin) & Anti - H (Lectin)
- Reagent cells (A cells, B cells and O cells), 3% in Normal Saline.
- Test red cells samples
- Normal Saline (0.9%)

PROCESSING OF BLOOD SAMPLES

Separation of RBC & Plasma

Centrifuge at 1000 rpm for 1 min at R.T using clean pipette tip, aspirate plasma gently without disturbing settled cells and transfer to a labeled clean test tube for **reverse grouping**.

Preparation of cell Suspension

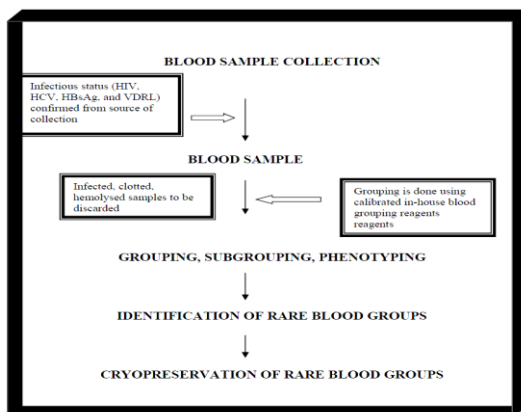
- Label the tubes as per S.No. of samples then add 1ml whole blood in respective S.No. of tubes and Normal saline (N.S) 8 ml, mix well.
- Centrifuge at 2500 rpm for 3 min take the supernatant & discard.
- Wash 3 times as above till supernatant is clear. Prepare 3% red cells suspension in normal saline.
- Set 9 tubes for each test sample as follows;
- 3 tubes labelled - A, B, A B. (forward/cell grouping)
- 2 tubes labelled - H & A1
- 3 tubes labelled a, b and c (reverse/serum grouping)
- 1 Auto control tube, Add 100ul test serum and 50ul test cells suspension of same sample and label it.

I Forward grouping (cell grouping)

- ✓ Set up 3 rows of clean test tubes and label them. Adding 2vol of anti-A & anti-B in the tubes and labelled.
- ✓ After this add one vol of 2-5% suspension of test red cells in each tube and mix the content of each tube by gentle shaking and leave at room temperature 20-24°C for 5mins.
- ✓ Now centrifuge the tube at 1000rpm for 2mins.
- ✓ Observe the supernatant fluid for hemolysis against well lighted background.
- ✓ Check for the agglutination against well lighted background.
- ✓ Record the result immediately.

II Reverse grouping (serum grouping)

- ✓ Take the labeled test tubes a, b, c, and add 100µl each of the test serum in each tube.
- ✓ Add 50µl each of reagent A cells, B cells and O cells in the tubes respectively.
- ✓ Mix the contents of all the tubes and by shaking the tube rack carefully and centrifuge at 1000 rpm for 1 minute.
- ✓ Dislodge cell button by gently shaking the tubes and read against well-lit background.
- ✓ Grade and record agglutination reactions.



SAMPLE DOCUMENTATION

1. Test for the Qualitative detection of antibodies to HEPATITIS C virus in Human Serum or plasma.

Requirements:- HCV Tri Dot Device, Buffer solution containing BSA and Sodium Azide, Protein- A Conjugate in liquid form containing Sodium Azide and sample dropper.

➤ PROTOCOL:-

- ✓ At the center of the device add 3 drops of buffer solution.
- ✓ Then add 1 drop of patient's sample by using sample dropper i.e. 50µl of serum and hold the dropper vertically downward carefully.
- ✓ Wait for 3minutes and add 5 drops of buffer solution in the device again.
- ✓ After adding buffer solution wait for 3 to 5mins and add 2 drops of protein –A conjugate.
- ✓ Again wait for 5 to 10minutes and then add 5 drops of liquid conjugate directly from the conjugate vial.
- ✓ After wait for at least 2 -3mins and again add 5 drop of buffer solution in it.
- ✓ Read result immediately and discard the device immediately considering it to be potentially infectious.



2. Test for the Qualitative and differential Detection of Antibodies to **HIV-1 & HIV-2** in Human Serum/Plasma (Separate Dots for HIV-1, HIV-2 & Control).

Requirements:- HCV Tri Dot Device, Buffer solution containing BSA and Sodium Azide, Protein- A Conjugate in liquid form containing Sodium Azide and sample dropper.

➤ PROTOCOL

- ✓ Firstly add 3 drops of Buffer Solution to the centre of the device and wait for 2mins.
- ✓ Then take out the patient's serum and add 1 drop of patient sample using sample dropper and hold the dropper vertically and the wait for about 2minutes.
- ✓ After adding patient's serum then add 5 drops of buffer solution in it and wait till the 5mins approximately.
- ✓ Then add 2 drops of Liquid Conjugate directly from the conjugate vial. Wait up to 5-10mins.
- ✓ Finally add 5 drops of buffer Solution and observe carefully after 2mins and read results.



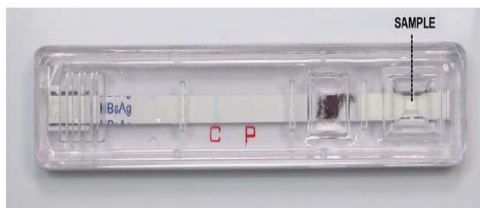
3. Test for the qualitative immunoassay for *in vitro* detection of Hepatitis B Surface Antigen (**HBsAg**) in serum or plasma.

Requirements:- Test device (cassette), Instruction manual, Disposable dropper or micropipette.

➤ PROTOCOL

- ✓ Bring the complete kit and sample to be tested to room temperature prior to testing.
- ✓ Once the device pouch is opened, it must be used within one hour.
- ✓ Bring the required no. of hepacard device foil pouches and specimen to room temp prior testing.
- ✓ Now add 4 drops (~ 150 µL) of serum or plasma into the sample window of the device.
- ✓ The sample will rehydrate and mix with the red colloidal gold conjugate, which flow into the membrane.
- ✓ Allow reaction to occur during the next 20minutes.
- ✓ After 10-15 minutes all the pink color from the conjugate will clear from the membrane except for test and controls lines which form.

- ✓ Discard the hepacard immediately after reading result at 20minutes.
- ✓ The result of the test can then be read.



4. Test for the qualitative immunoassay for *in vitro* detection of Venereal Disease Research Laboratory (VDRL) test in serum or plasma.

Requirements: - Serum, VDRL-Buffered Saline, pH 6.0 $\pm$ 0.1 (1.0% NaCl), Acetone Alcohol, 95% ethanol, Paraffin, Non disposable calibrated needles without bevel, pipetting device with disposable tip delivering 50 μ l, Disposable latex gloves, Glass syringe, 2 ml or 5 ml.

➤ PROTOCOL

- ✓ Bring the complete kit and sample to be tested to room temperature prior to testing.
- ✓ Take 70 μ l of human serum or plasma and 2 drops into the sample by using the sample dropper.
- ✓ Then add 1 drop VDRL buffer solution to the device.
- ✓ Allow the reaction occur during the next 20minutes.
- ✓ Read result after 20minutes.
- ✓ Discard the VDRL card immediately after reading result at 20minutes.



RATIONAL USE OF BLOOD

a) Packed Red Blood Cells

To replace the loss of red blood cells due to trauma or surgery or to treat to severe anemia etc. indications as a substitute of whole blood, severe hemorrhage in surgery, abortion, child birth complications etc. Storage period is 35 days in Blood bank refrigerator at 20°C-24°C.

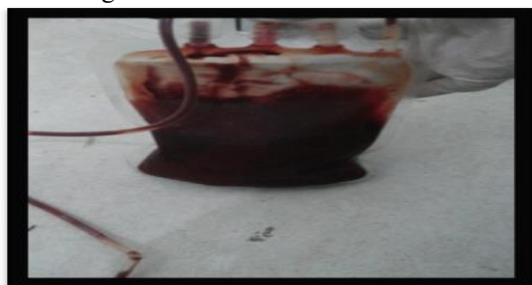


Figure 1

b) Fresh frozen plasma

To correct multiple coagulation factor deficiency as in liver disease, burn, massive bleeding etc. saline Adenine Glucose mannitol solution used as additives which is 80ml while remaining is plasma. Storage period is 1 year in deep freezer at ($<30^{\circ}\text{C}$).



Figure 2

c) Concentrate of platelets

To treat or prevent bleeding due to low platelets counts, thrombocytopenia etc. indications is in bone marrow deficiency, cancer, and dengue. Storage period is 5days at 20°C-24°C under continuous agitation.

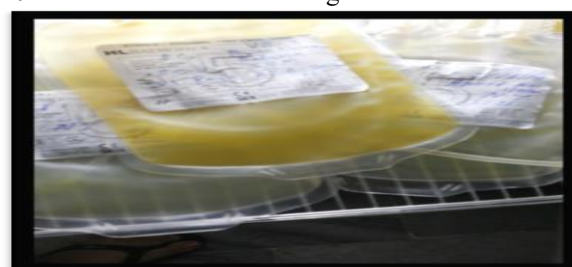


Figure 3

d) Cryoprecipitate

To treat individual coagulation factor deficiencies e.g. fibrinogen factor VIII etc. Storage period is 1year in deep freezer at $<30^{\circ}\text{C}$.

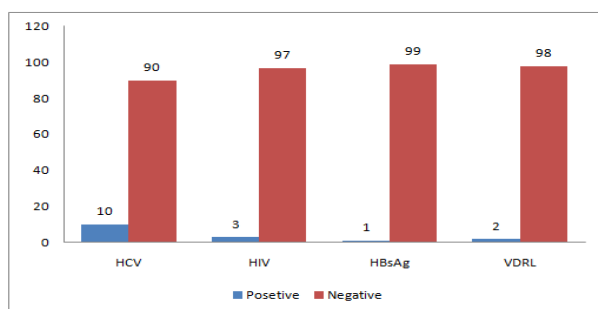


Figure 4

The rational use of blood can be obtained by the process called apheresis. Packed red blood cells, fresh frozen plasma, concentrate of platelets and cryoprecipitate are extracted from blood by using many techniques and using centrifugation in different rpm.



IV. CONCLUSION



We take about 100 samples of patient in which:-

HCV negative patient are 90 which are healthy this means there serum or plasma is not infected with this virus while 10 patients are HCV positive which may be containing HCV antigen in their plasma.

Same as in HIV test the 97 patient are negative and only 3 patients are suffering from this disease which may be due to contamination during blood transfusion, illiteracy or using contaminated sterile.

In hepatitis B (HBsAg) test only one patient is having infected antibodies in their serum or plasma and 99 patients are healthy and free from this virus.

In a healthy person, the VDRL test is negative. This means that no antibodies to the organism that causes *Treponema pallidum*/bacteria. As antigen used in nontreponemal test is component of all mammalian cell membranes, the damage to host tissue by infection, immunization, pregnancy, age-related changes, or autoimmune diseases can result into false-positive nontreponemal test results. Even if the test is positive, one cannot certainly give an opinion in regards to the infective status of the patient with syphilis. The sensitivity of nontreponemal and treponemal tests for syphilis increases with duration of infection, and ranges from approximately 75% in the primary stage to virtually 100% in the secondary stage.

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