

**21**

SCREENING FOR BLOOD TRANSFUSION TRANSMITTED DISEASES

21.1 INTRODUCTION

The microbial agents of importance to blood transfusion services are those that are transmissible by blood transfusion and can cause morbidity and mortality in recipients. Screening of all blood donations should be mandatory for infections namely HIV-1, HIV-2, HBSAG, HCV, Syphilis and Malaria.



OBJECTIVES

After reading this lesson, you will be able to

- explain about the importance of screening blood donations
- describe the infectious agents causing infections
- explain the laboratory diagnosis of transfusion transmitted infections
- perform the laboratory diagnosis of transfusion transmitted infections

21.2 SCREENING OF BLOOD DONATIONS

Screening of all blood donations should be mandatory for the following infections using following markers

- HIV-1 and HIV-2: screening for either a combination of HIV antigen-antibody or HIV antibodies.
- Hepatitis B: screening for hepatitis B surface antigen (HBsAg).

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- Hepatitis C: screening for either a combination of HCV antigen antibody or HCV antibodies.
- Syphilis (*Treponema pallidum*): screening for specific treponemal antibodies.
- Screening of donations for other infections, such as those causing malaria, should be based on local epidemiological evidence

21.3 INFECTIOUS AGENTS

There are four main groups of micro-organisms known to cause infections namely viruses, bacteria, protozoa and fungi

Only first three groups of microbes – viruses, bacteria and protozoa – have been reported to be transmitted by blood transfusion. Individuals with fungal infections are usually too sick to be accepted as blood donors. Viruses are most commonly transmitted by transfusion.

21.4 TRANSMISSION OF INFECTIOUS AGENTS BY BLOOD TRANSFUSION

In order to be transmitted by blood transfusion, an infectious agent must be present in the donated blood. Each blood transfusion service or blood bank or laboratory should, therefore, screen for evidence of the microbes that are known to cause infections with this route of transmission.

21.4.1 Human Immunodeficiency Virus (HIV)

HIV causes AIDS. This syndrome was recognized in 1981, well before the discovery of the causative virus.

A. Laboratory Diagnosis of HIV Infection

Shortly after exposure, the core protein, p24, has been found in some individuals. Within few weeks antibodies to both envelope (gp41) and core (p24) proteins appear in almost all infected individuals. During the early phase of infection a non-specific acute “viral illness” may occur.

Once antibodies appear, they increase in titer even though the host is asymptomatic. During this phase of infection viral cultures of isolated lymphocytes demonstrate the presence of virus. As infection progress, changes in the ratio of T-lymphocytes with specific surface markers, CD4 (helper) to CD8 (suppressor) cell, are observed. The ratio of CD4 to CD8 in healthy and immune competent individual is about 2:1. In acquired immune deficiency syndrome (AIDS), the

virus destroys CD4 cells and their number in the body decline and there is decrease in CD4:CD8 ratio.

HIV positive persons with fewer than 200CD4+ T cells per μl are considered as having AIDS in the absence of symptoms and / or opportunistic infection.

The presence of anti-HIV in an asymptomatic individual means that the individual has been exposed to the virus. It is accepted that, in almost all cases, the virus will be present in the individual. Seroconversion is sequential samples means that the infection has been recent.

Antibodies are present as early as 14 days after infection, while others indicate that they may not be present until 28 days or more after infection. The antibodies produced are directed against both core and envelope proteins. The most important antibodies are specifically anti-p24 (core) and anti-gp41 (envelope). Although antibodies to other proteins are produced, the presence of these two antibodies has been found to provide the best confirmation to infection. It has also been found to be the best means of monitoring the progress of infection.

Following infection and prior to the production of antibodies, there is 'window period' of varying length during which the infection establishes. During this period when no antibodies are detected, viral antigen (p24, gp41) could be detected. The length of time that antigen can be detected is very short, often no more than 1-2 weeks.

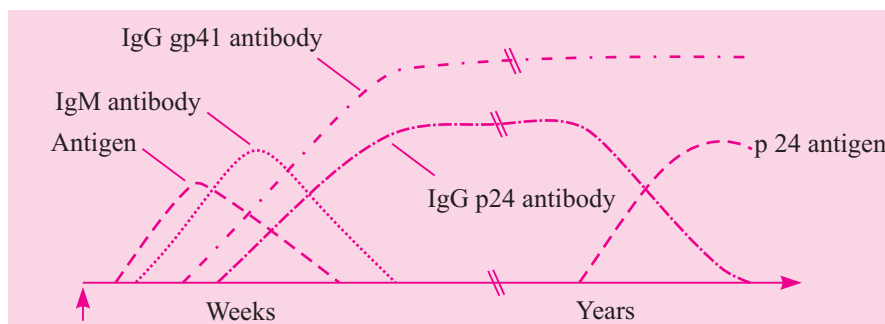


Fig. 21.1: Serological events following HIV infection

B. Testing for HIV Viral Markers

The detection of anti-HIV is the most suitable approach for identifying HIV-infected blood donations. The three main kinds of screening assays to detect anti-HIV available are:

- (i) Enzyme Linked ImmunoSorbent Assays (ELISA/EIA)
- (ii) Particle agglutination assays
- (iii) Specialized rapid assays



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While selecting assays for testing anti-HIV following features may be taken into account:

- High specificity
- High sensitivity
- Simplicity of test
- Incubation time
- Cost

(i) Enzyme Linked ImmunoSorbent Assay (ELISA)

It is the most commonly used assay and is based on the use of immobilized viral antigen which captures anti-HIV antibodies present in the test sample.

Manufacturers often use the terms first generation, second generation and third generation assay.

- First generation assays are purified or unpurified native virus or virus infected cells lysates prepared from cell structure.
- Second generation assays use recombinant antigen produced by cloning fragments of viral nucleic acid into yeast, growing large amount of the engineered yeast in bulk culture and purifying the viral proteins produced.
- Third generation assays are synthetic viral polypeptides artificially produced by chemical synthesis.

Assays are based on the same principles but differ in the way the viral antigen is immobilized:

- On the sides of wells of a polystyrene micro plate
- On the small polystyrene beads. This method needs specialized equipment.

There are three types of ELISA

(a) Antiglobulin type ELISA:

Viral antibody present in test sample is bound to immobilized viral antigen and is detected by enzyme labeled anti-human antibody.

(b) Competitive ELISA:

It is widely used assay, in which antibody present in test sample competes with enzyme linked specific antibody for binding sites on immobilized antigen.

(c) Sandwich ELISA:

This is highly specific type of ELISA in which viral antibody in test sample is bound to immobilized antigen and then detected by free enzyme-labeled viral antigen.

(a) Antiglobulin Type ELISA method (or EIA)

It is solid phase enzyme immunoassay utilizing polystyrene wells of microplates or beads coated with HIV specific proteins representing HIV core and envelope antigens.

1. Serum or diluted serum is added to the well coated with HIV specific proteins (p24 & gp 41). Positive and negative controls are added to a number of wells on each plate run.
2. They are incubated for the defined period of time and at the correct temperature.
3. During the incubation, any specific antibody present in the test serum binds to the viral antigen.
4. At the end of incubation, the wells are washed at least three times with washing fluid to remove unbound serum. (Manual washing is done using multi-channel washer to fill and then empty the wells with wash fluid or by mechanical washing using an automated plate washer).
5. After final wash, the wash fluid is removed. It is very important that wells are as drier as possible. The plate can be turned upside down and gently tapped dry on some absorbent tissue if the wells are still wet.
6. Conjugate solution is added to all wells and they are incubated at the defined period of time and at correct temperature.

Conjugate solution contains anti-human globulin antibody which has been chemically linked to an enzyme usually horse radish peroxidase or alkaline phosphate. Conjugate binds to only human antibodies that are bound to the antigen immobilized on the wells. Conjugate does not bind in those wells that did not contain anti-HIV bound to antigen.

7. At the end of incubation, the wells are again washed three times to remove excess, unbound conjugate and are prepared for the next stage of the assay as described earlier in step 4 & 5.
8. Substrate solution is added immediately to all wells and incubated in dark for the defined period of time at correct temperature.

When the substrate solution is added, color develops in the wells containing bound conjugate due to the activation of substrate by enzyme. Wells having no bound conjugate do not change the color of the substrate. Thus reactive wells having anti-HIV positive sera are colored, and the wells



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containing no anti-HIV sera are colorless. The controls show appropriate color changes.

9. At the end of incubation, diluted acid (1 N H₂SO₄) solution is added to all wells to stop reaction. The acid inactivates the enzyme and fixes the color. The intensity of color change is directly proportional to the antibody concentrate present in the samples/controls.

The color change can be read visually.

10. The optical densities (OD values) of the solutions in the microwells are measured by ELISA reader at the specific wave length after determining the cut-off value and the results are determined.

(b) Competitive ELISA Method

The principles of the competitive Elisa are the same as that of antiglobulin assay but it differs slightly in the way in which anti-HIV is detected.

- The conjugate and the test sera are added at the same time in wells and incubated together.
- The conjugate is enzyme-labeled anti-HIV antibody in competitive ELISA, rather, than labeled non-specific antibody as in antiglobulin-type ELISA.
- The conjugate anti-HIV competes with anti-HIV in test sera for the antigen binding site.

A test sample containing anti-HIV will block the binding of conjugate to antigen while sample not containing anti-HIV will allow binding of the conjugate to antigen.

Method

1. Undiluted sera and conjugate are added in wells at the same time. Positive and negative controls are also put.
2. The wells are incubated for the defined period and at correct temperature. During this period anti-HIV present in the test serum competes with the conjugated anti-HIV for the binding sites on the viral antigen.
3. At end of the incubation period, the wells are washed with washing fluid to remove excess sera and conjugate and prepared for the next step as described in the antiglobulin type assay.
4. Substrate solution is immediately added to all wells and incubated in dark for the defined period and at correct temperature.
5. At the end of the incubation period, dilute acid (1 N H₂SO₄) solution is added to all wells to stop reaction.

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- An intense color signifies a non-reactive sample, while lack of color signifies a reactive specimen.
- The optical densities (OD values) of the solutions in the microwells are read by the ELISA reader after determining the cut-off value and the results are determined.

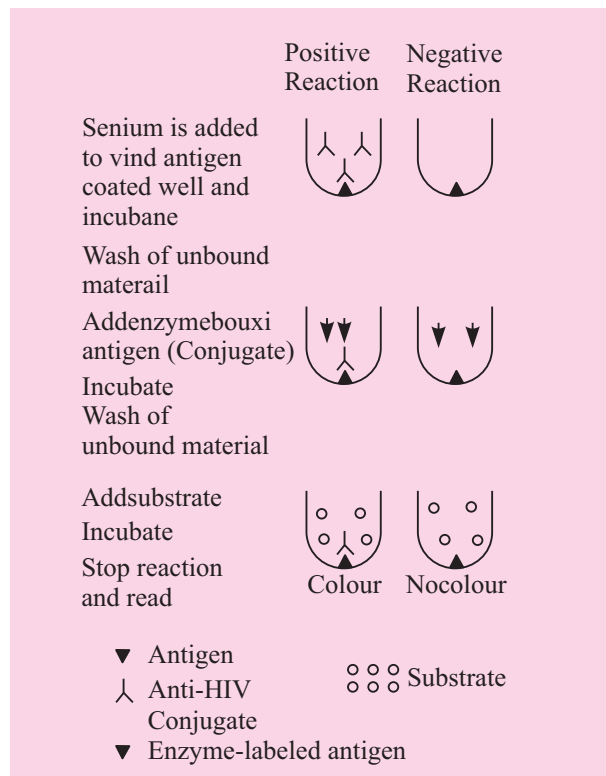


Fig. 21.2: Sandwich Elisa Method

(c) Sandwich ELISA Method

The basic principle of the sandwich ELISA is again the same as that of antiglobulin-type ELISA, but it differs in the way in which the anti-HIV is detected.

- Antigen, usually synthetic peptides are attached to the surface of wells in microplates.
- The conjugate is enzyme-labeled synthetic antigen in sandwich ELISA, rather than enzyme-linked anti-human immunoglobulin in the antiglobulin-type assay.
- During the incubation period, the conjugated antigen binds anti-HIV antibody bound to the antigen immobilized on the microwells.
- A Sandwich is built of antigen-antibody-antigen.

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- At the end of incubation, diluted acid (1 N H_2SO_4) solution is added to all wells to stop reaction.
- An intense color signifies a reactive sample having HIV, while lack of color signifies a non – reactive specimen having no HIV.
- The optical densities (OD values) of the solution in the microwells are read by the ELISA reader after determining the cut-off value, and the results are determined.

Method

1. Sera or diluted sera are added to antigen bound wells. The positive and negative controls are also put. The wells are incubated for the defined period of time and at the correct temperature. During this period anti-HIV present in the sera bind to the antigen.
2. At the end of incubation period. The wells are washed with washing solution to remove the excess sera and the wells are prepared for the next step as described earlier.
3. The conjugate is added to the wells. The wells are incubated for the defined period of time and at the correct temperature. During this period, the conjugate binds to antibody bound to the immobilized antigen. A sandwich is built up of antigen-antibody-antigen.
4. At the end of incubation period. The wells are washed with washing fluid to remove unbound conjugate and the wells are prepared for the next step, as described earlier.
5. Substrate solution is immediately added to all wells and they are incubated at the defined period of time and at correct temperature.
6. At the end of incubation period, dilute acid (1 N H_2SO_4) solution is added to all wells to stop reaction.
7. Color develops in the wells having sera containing anti-HIV and no color will develop in the wells having sera with no anti-HIV.
8. The optical densities (OD values) of the solutions are read by the ELISA reader after calculating cut-off value, and the results are recorded.

Precautions in ELISA

- Hemolysed, lipemic or contaminated sample sera should not be used.
- Correct volumes of test samples, conjugate and substrate are added.
- Instructions given by the manufacturers of kits should be strictly followed.



(ii) Particle Agglutination Assays

Particle agglutination assay detects the presence of anti-HIV by the agglutination of particles coated with HIV antigens. Particles are made of gelatin or latex. The assay is performed in microplates.

Method

1. HIV antigen is immobilized on particles made out of gelatin or latex.
2. Test samples and controls are diluted in microwells with test diluent which is provided with assays.

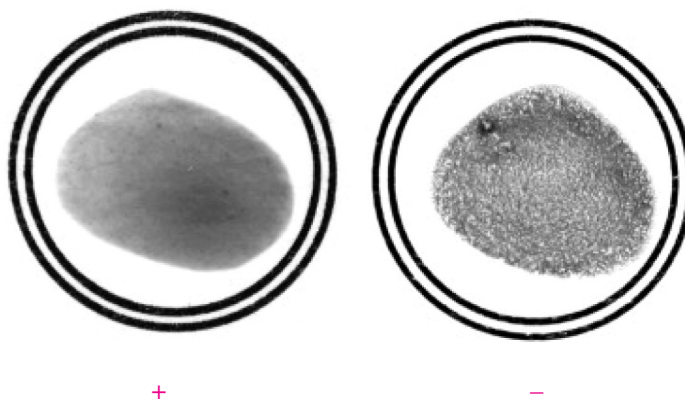


Fig. 21.3: Particle agglutination assays

3. The HIV coated particles are added to dilute samples and controls. Then they are incubated, usually at room temperature (20-24^o C) for the defined period. During incubation, the particles are agglutinated by anti-HIV present in the serum.
4. At the end of the incubation period, the results of tests can be read with naked eyes. If gelatin particles are used, they appear in bluish color. If latex particles are used, they appear white in color which can be seen against a black background.
5. A reactive result appears as an event mat of agglutinated particles across the bottom of wells. A non-reactive result appears as a button or ring of non-agglutinated particles, that settle in the center of well.

Advantages of particle agglutination assay

- Expensive equipment is not needed.
- Do not have different stages of reactions.
- Do not need washing equipment.
- Results can be read visually

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Disadvantages of particle agglutination assay:

- Subjective error in weak reaction
- Test serum having non-specific agglutinins, may agglutinate both sensitized and nonsensitized particles.

(iii) Specialized Rapid Spot Test

It detects anti-HIV. It is rapid and simple and based on the ELISA/EIA technique. The HIV antigen, usually recombinant or synthetic peptide, is immobilized on either porous or semi-porous membrane usually set in a well, in a plastic cassette absorbent pad. Most of the specialized rapid assays are in the form of a kit having everything required for the test.

Method

1. The test sample and buffer solution (provided with the kit) are put on the porous membrane and allowed to soak in. Pre-dilution of the test sample may be required. This is achieved by the addition of drops of diluents and sample in a suitable vial. The diluted sample is directed put on the porous membrane.
2. It is incubated for 10-15 minutes. During this period the sample passes through the membrane and if it contains anti-HIV antibodies, it will bind to the HIV antigen on the membrane.
3. The membrane is rinsed with the buffer solution to remove unbound antibodies.
4. Then conjugate is added. The composition of the conjugate varies between assays use an enzyme conjugate anti-human immunoglobulin as in antiglobulin-type ELISA. When such conjugate is used, a further wash step and addition of chromogen is required to visualize the results. Some assays use proteins A labeled with colloidal gold as the conjugate. It will bind to anti-HIV present and gives a red/purple color.
5. The final result is read visually and compared with the expected results described by the manufacturer.
6. Although control is not required, it is good practice to set up a negative control and a weak positive control.

C. HIV Antigen Detection Assays

These are sensitive and specific tests targeting viral antigens or nucleic acids and their availability has led to proposals for using these assays in screening of donated blood to detect HIV infected blood earlier than with current antibody assays.

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The detection of HIV antigen has very limited value in blood transfusion service; however, it may be useful in the following conditions:

- Detection of HIV infection during window period before seroconversion.
- Confirmation of HIV infection in infants born to HIV infected mothers.
- May help to resolve the indeterminate Western Blot test results.
- To monitor HIV infected patients on antiviral therapy.

Methods for HIV antigen testing.

- ELISA for testing HIV p24 antigen
- Polymerase Chain Reaction (PCR)
- Viral isolation

Screening for HIV Antigen (ELISA)

Screening for HIV antigen (usually p 24) is performed using sandwich ELISA. The difference between HIV-antigen and anti-HIV antibody screening is that HIV-antigen test uses a sandwich of antibody-antigen-antibody, unlike HIV antibody screening which comprises a sandwich of antigen-antibody-antigen.

- Antibody (usually mono-clonal) is bound to the surface microwells.
- Test serum and positive and negative controls are added to the microwells and incubated. At the end of incubation period, the excess serum is washed off.
- Conjugate is added and incubated. The conjugate is an enzyme-labeled specific antibody (usually monoclonal).
- During the incubation, the conjugate binds HIV-antigen bound to the anti-HIV immobilized on the microwell. A sandwich is formed of antibody-antigen-antibody.
- The excess conjugate is washed away and substrate (chromogen) is added in the same way as in the antiglobulin assay and incubated.
- At the end of the incubation period, diluted acid (H_2SO_4) is added to all wells to stop the reaction.
- An intense color signifies a reactive sample having HIV, while lack of color signifies a non – reactive specimen having no HIV.
- The optical densities (OD values) of the solution in the microwells are read by the ELISA reader after determining the cut-off value, and the results are determined (Fig. 21.4).

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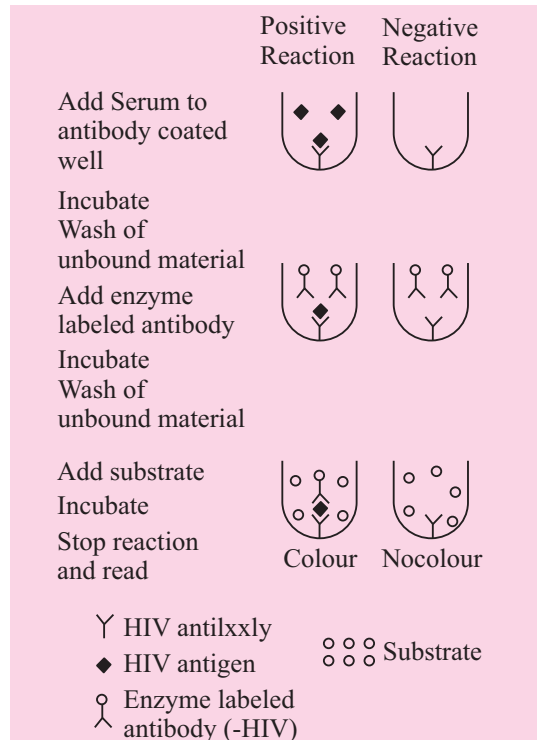


Fig. 21.4: HIV Antigen ELISA

21.4.2 Transfusion Associated Hepatitis

At least four viruses have been associated post-transfusion hepatitis:

1. Hepatitis A virus (HAV)
2. Hepatitis B virus (HBV)
3. Hepatitis C virus (HCV)
4. Hepatitis D virus (HDV)

Several other viruses causing hepatitis have been reported. Hepatitis E virus has been reported to cause epidemic hepatitis associated with contaminated water. The majority of transfusion associated hepatitis is due to HBV or HCV.

Serological Findings

The incubation period of HBV infection is about 30 to 150 days during which the patient has no sign and symptom but the virus may be detected.

- When an individual is infected by HBV several of antigens and antibodies can be detected by serological tests. Usually the first marker of HBV to appear is HBsAg. It remains detectable from a few a days to several months. This marker is also found in some (5% - 10%) infected persons who become chronic carriers of HBV.



- Anti-HBs becomes detectable after HBsAg disappear which indicates recovery from acute illness. Sometimes appearance of anti-HBs is delayed for weeks and months after HBsAg becomes undetectable. During this period, called 'core antibody window', anti-HBc may be the only detectable marker of recent HBV infection.

Figure 21.5 typical serologic course of acute hepatitis B virus infection with recovery

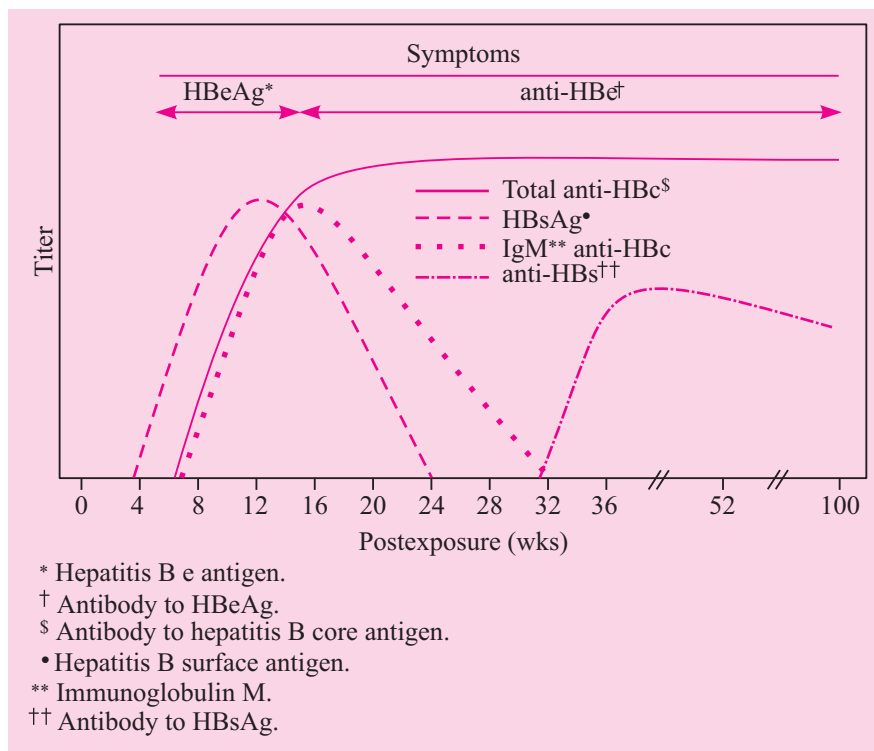


Fig. 21.5: Markers in HBV infection

- Shortly after infection and before clinical signs and symptoms or biochemical changes in liver functions occur, two other markers – HbeAg and anti-HBc are detectable in the serum of infected person.
- Initially the anti-HBc is IgM, however as the infection progress IgG anti-HBc appears and the later persists in persons who recover from the infection.
- HbeAg usually disappears when the patient enters the convalescent phase.
- In others with chronic infection (persistence of HBsAg for longer than 6 months), HbeAg is cleared, and anti-HBe is found.
- In persons who do not develop immunity to HBV, HBsAg, HBeAg, and anti-HBc can be present.

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- Individuals, who recover from infection, will have anti-HBs and/or anti-HBc in their sera.

Serological markers of hepatitis B infection and their diagnostic significance.

Marker	Significance
HBsAg	Active infection, acute or chronic
Anti-HBs	Clinical recovery, infection resolved, immunity develop
Anti HBC (IgM)	Early acute infection
Anti-HBc (IgG)	Active or past infection (carrier state)
HBeAg	Acute or serious chronic infection
Anti-HBe	Resolution of acute infection, may signal late sequelae

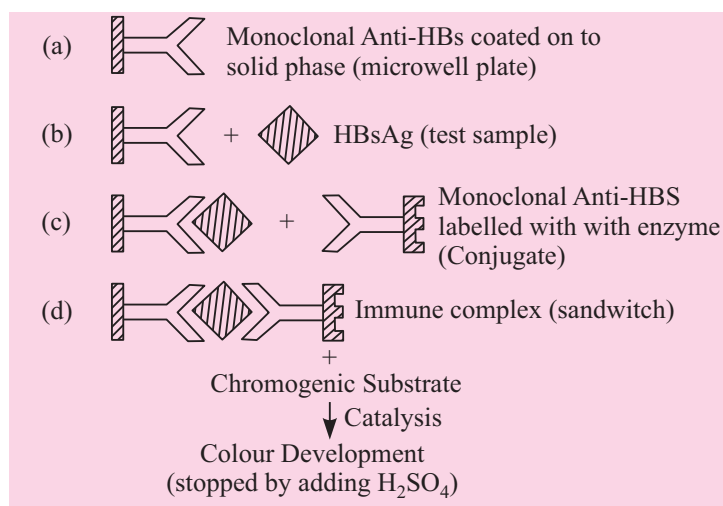


Fig. 21.6: Diagrammatic sequence for detection of HBsAg by ELISA

Enzyme Immunoassay (ELISA/EIA)

It is based on a one step 'sandwich' principle. It involves the use of solid support (wells of microplate or breads) coated with unlabeled anti-HBs antibody. Number of commercial kits is available and the recommended procedure by the manufacturers should be followed. The general steps of the technique of the test in microplate are given below:

See figure 21.6.

1. Put appropriate volume of the test sample and equal volume of positive and negative controls in the wells of microplate coated with anti HBs.

2. Incubate for the correct period of time at defined temperature.
3. At the end of incubation period, the wells are washed to remove excess of serum and dry the wells as much as possible before the next stage of assay.
4. Then add appropriate volume of conjugate (enzyme-linked anti-HBs antibody) to each well. Enzyme label is usually horseradish peroxidase.
5. Incubate for the correct period of time at defined temperature.
6. After incubation, the wells are washed and are prepared for the next step.
7. Add appropriate volume of chromogen substrate into wells.
8. Incubate the plate in dark for correct time, development of colour suggests the presence of HBsAG, and no or low color suggests absence of HBsAG.
9. The results can be read visually or by ELISA reader.

21.4.3 Syphilis

Etiological agent for syphilis is *Treponema pallidum*. Blood and its components may transmit syphilis. The incubation period for transfusion transmitted syphilis is 1 to 4 months, the blood recipient exhibiting the signs and symptoms of secondary syphilis.

Transfusion-transmitted syphilis is not a major hazard of modern transfusion therapy, its chief reasons are:

- The spirochetes do not survive in citrated blood stored 4°C for 72 hours.
- Now the blood is mostly collected from the voluntary donors and sexually promiscuous persons are excluded from blood donation.
- Most patients who need transfusion of blood or its components receive antibiotics therapy because of their clinical condition.
- Widespread use of penicillin and other antibiotics to treat syphilis and its carrier might account for the rarity of infected cases.

Role of serological tests for syphilis in prevention of posttransfusion syphilis:

Spirochetemia usually is common in the early stages during the invasion of lymph nodes. The screening tests used for blood donors often are negative in early syphilis when spirochetes could be transmitted by blood transfusion. Only 25% of patients with primary syphilis have a reactive serological test for syphilis (STS), and the rest do not become positive until the 4th week after the onset of primary syphilis. By the time the person develops a positive STS, the spirochetemia has typically cleared.

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In addition, a high proportion of healthy people have biological false positive reactions even they do not have circulating spirochetes. It may be due to viral infections, immunization, lupus, erythematosus and dysproteinemias.

The prevention of spirochete transmission is not well accomplished by STS test. Anyhow it is considered safe to test blood donation for syphilis due to the following reasons:

- There is possibility of transmitting syphilis.
- Demand of fresher blood for exchange transfusion, and platelets.
- Screening for spirochetes helps to exclude donors who are in high risk group for HIV and HBV infection.
- Cost for STS is low.

Tests

The serological tests are of two types:

- Non-specific tests
- Specific test

Non-specific tests

The most commonly used test in blood donor screening are non-specific tests because they are simple, rapid and economical, they are:

- Venereal Disease Research Laboratory (VDRL) test
- Rapid plasma regain (RPR) test

Specific tests

These tests are mainly confirmatory tests and have lengthy procedures. They are not suitable for routine screening of donor's blood. Tests are:

- Treponema pallidum hemagglutination test (TPHA)
- Fluorescent treponemal antibody absorption test (FTA-ABS)
- Treponema pallidum immobilization test (TPI)
- Enzyme Immuno Assay

21.4.4 Malaria

Malaria is caused by intra-erythrocytic protozoan parasites, Plasodium vivax, P. falciparum, P. ovale or P. malariae. The usual mode of transmission is via the bite of anopheles mosquito.



Screening tests for the malaria

1. Microscopic examination of thick and thin blood smears.
It is difficult to find parasites in blood film in short time especially if the density of parasites is less than 100 per microlitre of blood. However it is best practical method for testing malarial parasites
2. Tests for antibody to malarial parasites are:
 - Indirect fluorescent antibody test (IFA)
 - ELISA
3. Immuno-diagnostic testing, including EIA methodology
4. Nucleic acid probe methodology, including polymerase chain reaction (PCR)
5. Rapid card test – malaria pLDH test
6. Clean the area to be lanced with an alcohol swab.
7. Squeeze the end of the fingertip and pierce with a sterile lancet provided.
8. Wipe away the first drop of blood with sterile gauze or cotton.
9. Take a sample pipette provided, and while gently squeezing the tube, immerse the open end in the blood drop and then gently release the pressure to draw blood into the sample pipette up to the black line.
10. Add 5µl of whole blood into Sample Well (sample well)
11. Add two drops (60µl) of assay buffer into buffer well.
12. Read the test result in 20 min.
13. Negative reaction
The presence of only one band within the result window indicates a negative result.
14. *P. vivax* or other plasmodium sp. Positive reaction
The presence of two color bands indicates a positive result for *P. vivax* or other plasmodium sp. The pLDH present in the sample reacts with the pan anti-pLDH conjugate and move through the test strip where the pLDH is captured by pan specific anti-pLDH.
15. Invalid
The test is invalid if the line in C area does not appear. If this occurs, the test should be repeated using a new strip.

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None of the above methods except microscopic examination of blood smear is practical and possible.

Since there are no appropriate methods for screening malarial parasites in blood donation, it has been suggested that chemoprophylaxis therapy for malaria should be given to all recipients of blood in highly endemic areas.



INTEXT QUESTIONS 21.1

1. The ratio of CD4 to CD8 in healthy and immune competent individual is
2. In Acquired Immune Deficiency Syndrome (AIDS), the virus destroys cells and the CD4:CD8 ratio is
3. HIV positive patients with number of CD4 cells per μl are considered as having AIDS
4. The three main kinds of screening assays to detect anti-HIV are, &
5. The most commonly used assay is
6. Types of ELISA are, &
7. The most commonly used ELISA assay is
8. The highly specific type of ELISA is
9. Methods for HIV antigen testing
10. Example of Non-specific test for screening of syphilis is
11. Example of Confirmatory test used for screening of syphilis is



WHAT HAVE YOU LEARNT

- The microbial agents of importance to blood transfusion services are those that are transmissible by blood transfusion and can cause morbidity and mortality in recipients.
- Screening of all blood donations should be mandatory for infections namely HIV-1, HIV-2, HBSAG, HCV, Syphilis and Malaria.
- There are four main groups of micro-organisms known to cause infections namely viruses, bacteria, protozoa and fungi

Screening for Blood Transfusion Transmitted Diseases

- Only first three groups of microbes – viruses, bacteria and protozoa – have been reported to be transmitted by blood transfusion. Viruses are most commonly transmitted by transfusion.
- The ratio of CD4 to CD8 in healthy and immune competent individual is about 2:1.
- In Acquired Immune Deficiency Syndrome (AIDS), the virus destroys CD4 cells and their number in the body decline and there is decrease in CD4:CD8 ratio.
- HIV positive persons with less than 200CD4+ T cells per μl are considered as having AIDS in the absence of symptoms and / or opportunistic infection
- The presence of anti-HIV in an asymptomatic individual means that the individual has been exposed to the virus. It is accepted that, in almost all cases, the virus will be present in the individual.
- Seroconversion is sequential samples means that the infection has been recent.

The three main kinds of screening assays to detect anti-HIV available are:

- d. Enzyme Linked ImmunoSorbent Assays (ELISA/EIA)
 - e. Particle agglutination assays
 - f. Specialized rapid assays
- Enzyme Linked ImmunoSorbent Assay (ELISA) is the most commonly used assay and is based on the use of immobilized viral antigen which captures anti-HIV antibodies present in the test sample.
 - There are three types of ELISA namely Antiglobulin type ELISA, Competitive ELISA, Sandwich ELISA
 - Precautions in ELISA
 - Hemolysed, lipemic or contaminated sample sera should not be used.
 - Correct volumes of test samples, conjugate and substrate are added.
 - Instructions given by the manufacturers of kits should be strictly followed.
 - Particle agglutination assay detects the presence of anti-HIV by the agglutination of particles coated with HIV antigens.
 - It detects anti-HIV. It is rapid and simple and based on the ELISA/ EIA technique.

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Screening for Blood Transfusion Transmitted Diseases

- The detection of HIV antigen has very limited value in blood transfusion service; however, it may be useful in the following conditions:
 - Detection of HIV infection during window period before seroconversion.
 - Confirmation of HIV infection in infants born to HIV infected mothers.
 - May help to resolve the indeterminate Western Blot test results.
 - To monitor HIV infected patients on antiviral therapy.
- Methods for HIV antigen testing are ELISA for testing HIV p24 antigen, Polymerase Chain Reaction (PCR), Viral isolation
- At least four viruses have been associated post-transfusion hepatitis namely Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV)
- Etiological agent for syphilis is *Treponema pallidum*. Blood and its components may transmit syphilis.
- The incubation period for transfusion transmitted syphilis is 1 to 4 months, the blood recipient exhibiting the signs and symptoms of secondary syphilis.
- The serological tests are Non-specific tests and Specific test
- Non-specific tests are most commonly used test in blood donor screening are non-specific tests because they are simple, rapid and economical, they are Venereal Disease Research Laboratory (VDRL) test and Rapid plasma regain (RPR) test
- Specific tests are mainly confirmatory tests and have lengthy procedures. They are not suitable for routine screening of donor's blood, they are *Treponema pallidum* hemagglutination test (TPHA), Fluorescent treponemal antibody absorption test (FTA-ABS), *Treponema pallidum* immobilization test (TPI), Enzyme Immuno Assay
- Screening tests for the malaria are Microscopic examination of thick and thin blood smears
- Tests for antibody to malarial parasites are Indirect fluorescent antibody test (IFA) and ELISA
- Immuno-diagnostic testing, including EIA methodology, Nucleic acid probe methodology, including polymerase chain reaction (PCR), Rapid card test – malaria pLDH test



TERMINAL QUESTIONS

1. Write a short note on screening of blood donations
2. What are the screening assays to detect anti-HIV
3. Describe briefly the types of ELISA
4. Write a short note on methods of HIV antigen testing
5. Explain the non specific test of diagnosis of syphilis
6. Explain the specific tests of diagnosis of syphilis
7. Write a short note on screening test for malaria



ANSWERS TO INTEXT QUESTIONS

21.1

1. 2 : 1
2. CD4
3. Reduced
4. < 200 cells
5. Enzyme Linked ImmunoSorbent Assay (ELISA)
6. Antiglobulin type ELISA, Competitive ELISA & Sandwich ELISA
7. Competitive ELISA
8. Sandwich ELISA
9. ELISA for testing HIV p24 antigen, Polymerase Chain Reaction (PCR), Viral isolation
10. Venereal Disease Research Laboratory (VDRL) test
11. Treponema pallidum hemagglutination test (TPHA)

MODULE

Hematology and Blood
Bank Technique



Notes