61 Human Immunodeficiency Virus: AIDS

INTRODUCTION

The emergence and pandemic spread of the acquired immunodeficiency syndrome (AIDS) has posed the greatest challenge to public health in modern times. After the sudden appearance of syphilis in Europe five hundred years ago, rarely has any new disease had as great an impact on medicine, science and society and caused as much panic among the public and governments globally as has AIDS. The full consequences of this phenomenon may not be evident for several years because of the silent spread and slow evolution of this infection.

History

The first indication of this new syndrome came in the summer of 1981, with reports from New York and Los Angeles (USA), of a sudden unexplained outbreak of two very rare diseases, Kaposi's sarcoma and *Pneumocystis carinii* (jirovecii) pneumonia in young adults who were homosexuals or addicted to injected narcotics. This condition was given the name **acquired immune deficiency syndrome (AIDS).**

In **1983, Luc Montagnier** and colleagues from the Pasteur Institute, Paris, isolated a retrovirus from a West African patient with persistent generalised lymphadenopathy, and called it **lymphadenopathy-associated virus (LAV).** It produced lytic infection in fresh peripheral blood lymphocytes. In **1984, Robert Gallo** and colleagues from the National Institutes of Health, USA, reported the isolation of a retrovirus from AIDS patients and called it **human T cell lymphotropic virus III** or **HTLV III**. Retroviruses HLTV I and II had already been described in association with human T cell leukemia. International Committee on Virus Nomenclature in 1986 decided on the generic name **human immunodeficiency virus (HIV)** for these viruses.

In 1985, serological tests (ELISA) became available for the detection of anti-HIV antibodies. Serological screening of high-risk groups, blood donors and others revealed a very large and expanding reservoir of HIV in patients and carriers in different parts of the world.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV, the causative agent of AIDS, belongs to the **lentivirus** subgroup of the family **Retroviridae**. Besides HIV, related animal immunodeficiency viruses are also assigned to this group

Members of the Lentivirus group causing immunodeficiency

I. In primates
1. Human immunodeficiency viruses (HIV) types 1, 2
2. Simian immunodeficiency viruses (SIV) causing Simian AIDS (SAIDS):
   a) isolated from sooty mangabeys (SIV-SM) and from rhesus macaque (SIV-MAC) closely related to HIV type 2
   b) isolated from chimpanzee (cpz)—closely related to HIV type 1

II. In non-primates
1. Feline T lymphotropic virus (FTLV) causing feline AIDS (FAIDS)

Structure

Envelope: HIV is a spherical, enveloped virus, about 90–120 nm in size (Fig. 61.1). The nucleocapsid has

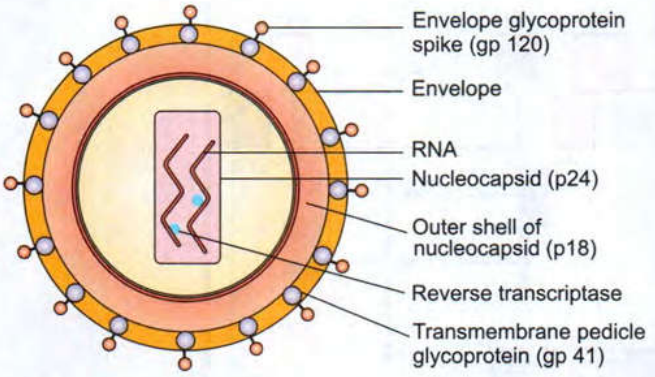

Fig. 61.1 Structure of HIV (diagrammatic representation)

an outer icosahedral shell and an inner cone-shaped core, enclosing the ribonucleoproteins.

Genome: The genome is composed of two identical single-stranded, positive-sense RNA copies, with the reverse transcriptase enzyme, (a characteristic feature of retroviruses). When the virus infects a cell, the viral RNA is transcribed by the enzyme, first into single-stranded DNA and then to double-stranded DNA (provirus) which is integrated into the host cell chromosome. The provirus can remain latent for long periods, influencing host cell function. In response to viral promoters, the provirus initiates viral replication by directing the synthesis of viral RNA and other components.

Lipoprotein envelope: When the naked virus buds out through the host cell surface during viral replication, it acquires a lipoprotein envelope, which consists of lipid derived from the host cell membrane and glycoproteins coded by the virus. The major virus-coded envelope proteins are the projecting knob-like spikes on the surface and the anchoring transmembrane pedicles. The spikes constitute the main surface component of the virus, which binds to the CD4 receptors (along with co-receptors CXCR4 and CCR5) on susceptible host cells. Transmembrane pedicles cause cell fusion.

Viral genes and antigens

The HIV genome contains the three structural genes (*gag*, *pol* and *env*) as well as other non-structural and regulatory genes specific to the virus (Fig. 61.2). The products of these genes, both structural and non-structural, act as antigens. Detection of these antigens and their antibodies is useful for diagnosis and prognosis of HIV infection.

Major antigens of HIV

A. Envelope antigens
1. Spike antigen—gp120 (Principal envelope antigen)
2. Transmembrane pedicle protein—gp 41

B. Shell antigen
1. Nucleocapsid protein—p18

C. Core antigens
1. Principal core antigen—p24
2. Other core antigens—p15, p55

D. Polymerase antigens—p31, p51, p66

Genes coding for structural proteins:

- The *gag* gene determines the core and shell of the virus. It is expressed as a precursor protein, p55. This precursor protein is cleaved into three proteins, p15, p18 and p24, which make up the viral core and shell. The major core antigen is p24 which can be detected in serum during the early stages of HIV infection before antibodies appear. Decline of free anti-p24 antibody and reappearance of p24 antigen in circulation indicates exacerbation of the illness.
- The *env* gene determines the synthesis of the envelope glycoprotein gp160, which is cleaved into the two envelope components: gp120, which forms the surface spikes, and gp41, which is the transmembrane anchoring protein. Antibodies to gp120 are present in circulation till the terminal stage of the infection.
- The *pol* gene codes for polymerase reverse transcriptase and other viral enzymes, such as protease and endonuclease. It is expressed as a precursor protein, which is cleaved into proteins p31, p51 and p66.They do not have much diagnostic or prognostic significance.

Non-structural and regulatory genes
- *tat* **(trans activating gene)** enhances the expression of all viral genes
- *nef* **(negative factor gene)** down-regulates viral replication
- *rev* **(regulator of virus gene)** enhances the expression of structural proteins
- *vif* **(viral infectivity factor gene)** influences the infectivity of viral particles
- *vpu* **(only in HIV-1) and vpx** (only in HIV-2) enhance the maturation and release of progeny virus from cells **(detection of the type-specific sequences *vpu* and *vpx* is useful in distinguishing between infection by HIV-1 and 2)**
- *vpr* stimulates the promoter region of the virus
- **LTR (long terminal repeat) sequences**, one at either end, contain the sequences that give the promoter, enhancer and integration signals.

Antigenic variation and diversity of HIV

HIV is a highly mutable virus, unlike HTLV. It exhibits frequent antigenic variation as well as differences in

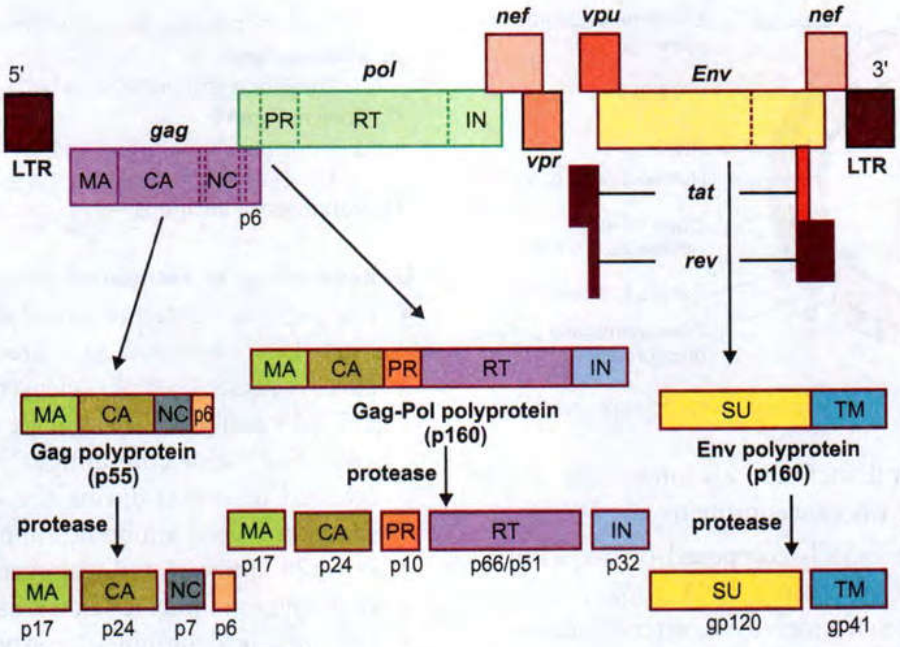

Fig. 61.2 HIV genome—diagrammatic representation

other features such as nucleotide sequences, cell tropism, growth characteristics and cytopathology. Antigenic variation exists in isolates of HIV from different places or persons and also between sequential isolates from the same person. This variability of HIV is believed to be due to the error-prone nature of reverse transcription.

HIV 1 and HIV 2: Based on molecular and antigenic differences, two types of HIV have been recognised. The original isolates of HIV and the related strains prevalent all over the world belong to HIV type 1. HIV strains, first isolated from West Africa in 1986, which react with HIV type 1 antiserum very weakly or not at all have been termed HIV type 2. HIV 2 has only 40 per cent genetic identity with HIV 1. It is more closely related to simian immunodeficiency virus than to HIV 1. It is much less virulent than HIV 1. It is largely confined to West Africa with a few reports from other areas, including western and southern India.

HIV 1 subtypes

HIV 1 strains have been classified into at least ten subtypes based on sequence analyses of their *gag* and *env* genes. These subtypes are designated A to J.

- All the subtypes constitute Group M (for 'major'), which cause most of the HIV 1 infections worldwide.
- A few HIV 1 strains isolated from West Africa (Cameroon, Gabon) do not fall within Group M and have been designated Group O (for 'outlier').
- Some later isolates of HIV 1 from Cameroon, distinct from the M and O groups have been called Group N (for new).

HIV 1 subtypes show geographical distribution, though this is often blurred by viral trafficking. All known HIV virus groups and subtypes are present in Cameroon, West Africa. Subtype A is the most prevalent, worldwide, while B is the most common in the Americas and Europe. The common subtypes in Africa are A, C and D, while in Asia the common subtypes are E, C and B. Type E is presently considered a recombination of A and E called the AE type (also known as CRFs or circulating recombinant forms). **In India and China, subtype C is the most prevalent.**

Antigenic differences between HIV strains may be important in serodiagnosis. Infection by HIV 1 or 2 may not be identified unless the corresponding type is represented in the test antigen.

Transmissibility of subtypes: The subtypes seem to vary in frequency of transmissibility by different routes. The subtypes common in Asia and Africa (C and E) are more readily transmitted by heterosexual contact than the American strains (subtype B) which are preferentially spread through blood—by injection and homosexual contact.

Growth characteristics: Differences in growth characteristics are sometimes observed between HIV isolates from asymptomatic carriers (grow slowly) and from AIDS patients (grow faster). Variations may account for differences in the clinical course of HIV-infected persons.

Resistance

HIV is thermolabile, being inactivated in 10 minutes at 60°C and in seconds at 100°C. At room temperature (20–25°C) in dried blood, it may survive for up to seven days. At autopsy, HIV has been isolated from various tissues up to 16 days after death. It withstands lyophilisation. The virus in lyophilised blood products can be inactivated by heating at 68°C for 72 hours and in liquid plasma at 60°C for 10 hours.

HIV is inactivated in 10 minutes by treatment with 50% ethanol, 35% isopropanol, 0.5% lysol, 0.5% paraformaldehyde, 0.3% hydrogen peroxide, 1% nonidet p40 or 10% household bleach. It is also inactivated at the extremes of pH (pH 1 and 13). Bleaching powder and household bleach are effective for surface decontamination. The standard recommendation is a hypochlorite solution at a concentration of 0.5% available chlorine (5 g/l; 5000 ppm). For the treatment of contaminated medical instruments, a 2% solution of glutaraldehyde is useful.

Pathogenicity

Infection is acquired when the virus enters the blood or tissues of a person and comes into contact with a suitable host cell, principally the CD4 lymphocyte.

Cell receptors for virus attachment: The receptor for the virus is any cell bearing **CD4 antigen,** primarily the CD4+ (helper/inducer) T lymphocyte. About 5–10 per cent of B lymphocytes and 10–20 per cent of monocytes and macrophages, including alveolar macrophages in the lungs and Langerhans cells in the dermis are susceptible. Glial cells and microglia in the central nervous system are also susceptible. **Follicular dendritic cells from tonsils can be infected by HIV without the involvement of CD4.**

Specific binding of the virus to the CD4 receptor is by the envelope glycoprotein gp120. Cell fusion is essential for infection to take place. This is brought about by transmembrane gp41. Binding to the CD4 receptor requires the participation of a co-receptor molecule, which has been identified as CXCR 4 for T cell-tropic HIV strains and CCR 5 for macrophage-tropic strains.

Replication: After fusion of the virus with the host cell membrane, the HIV genome is uncoated and internalised into the cell. Viral reverse transcriptase mediates the transcription of its RNA into double-stranded DNA, which is integrated into the genome of the infected cell through the action of the viral enzyme integrase, causing a latent infection. The long and variable incubation period of HIV infection is because of the latency. In an infected individual, HIV can be isolated from the blood, lymphocytes, cell-free plasma, semen, cervical secretions, saliva, tears, urine and breast milk.

The **primary pathogenic mechanism** in HIV infection is the damage to the CD4+ T lymphocyte. The T4 cells decrease in number with reversal of T4:T8 (helper:suppressor) cell ratio. Infected T4 cells do not release normal amounts of interleukin-2, gamma interferon and other lymphokines, suppressing cell-mediated immune response.

Though the main damage is to cellular immunity, humoral mechanisms are also affected. Helper T cell activity is essential for optimal B cell function. AIDS patients are unable to respond to new antigens. An important feature in HIV infection is the polyclonal activation of B lymphocytes leading to hypergammaglobulinemia of all classes of immunoglobulins, particularly IgG and IgA. In infants and children, IgM levels are also elevated. The hypergammaglobulinemia may also be responsible for allergic reactions due to immune complexes (type 3 hypersensitivity).

Monocyte–macrophage function is also affected, apparently due to lack of secretion of activating factors by the T4 lymphocytes. As a result, chemotaxis, antigen presentation and intracellular killing by monocytes/macrophages are diminished. The activity of NK cells and cytotoxic T lymphocytes is also affected.

The principal immunological abnormalities seen in HIV infection are listed in the box below.

Immunological abnormalities in HIV infection

I. Features that characterise AIDS
1. Lymphopenia
2. Selective T cell deficiency—Reduction in number of T4 (CD4) cells, inversion of T4:T8 ratio
3. Decreased delayed hypersensitivity on skin testing
4. Hypergammaglobulinemia—predominantly IgG and IgA; IgM also in children
5. Polyclonal activation of B cells and increased spontaneous secretion of Ig

II. Other consistently observed features:
1. Decreased in vitro lymphocyte proliferative response to mitogens and antigens
2. Decreased cytotoxic response by T cells and NK cells
3. Decreased antibody response to new antigens
4. Altered monocyte/macrophage function
5. Elevated levels of immune complexes in serum

Clinical manifestations in HIV infections are due not primarily to viral cytopathology but secondary to the failure of immune response. This renders the patient susceptible to opportunistic infections and malignancies. Dementia and other degenerative neurological lesions are due to the action of the virus on central nervous system (CNS) cells.

ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

Clinical Case: A 35-year-old commercial sex worker presented to the medical OPD with history of weight loss, decreased appetite and cough with expectoration for a duration of one month. Total WBC count was low, sputum AFB was positive and graded 2+; HIV serology was reactive with three test strategy of National Aids Control Organisation (NACO). CD4 count was 350 cells/μl. The patient was referred to the ART centre for antiretroviral and antitubercular chemotherapy. She was advised to return for a follow-up after two weeks and to use a condom during sexual contact.

Clinical features of HIV infection

AIDS is the last stage in the wide spectrum of clinical features in HIV infection. The Centers for Disease Control and Prevention, USA, have classified the clinical course of HIV infection under various groups (see box below).

The natural course of HIV infection passes through the following stages:

Group I—Acute HIV infection: Within 3–6 weeks of infection with HIV, about 50 per cent of persons experience low-grade fever, malaise, headache, lymphadenopathy, sometimes with rash and arthropathy resembling glandular fever. Rarely, there may be acute encephalopathy. Spontaneous resolution occurs within weeks.

- Seroconversion illness: Tests for HIV antibodies are usually negative at the onset of the illness but become positive during its course, though in many of those infected there may not be any apparent clinical illness.
- Acute retroviral syndrome: Patients with this syndrome may have fever, fatigue, rash, pharyngitis or other symptoms. HIV antigenemia (p24 antigen) can be demonstrated at the beginning of this phase.

Group II—asymptomatic or latent infection: All persons infected with HIV, whether or not they experience seroconversion illness, pass through a phase of symptomless infection (clinical latency) which may last up to several years. They are positive for HIV antibody and are infectious.

The infection progresses in course of time through various stages, CD4 lymphocytopenia, minor opportunistic infections, persistent generalised lymphadenopathy (PGL), AIDS-related complex (ARC), ultimately terminating in full-blown AIDS. The median time between primary HIV infection and the development of AIDS has been stated as approximately 10 years.

About 5–10 per cent of the infected appear to escape clinical AIDS for 15 years or more. They have been termed **'long-term survivors'** or **'long-term non-progressors'**. The mechanisms for such prolonged survival are not clear, though many viral and host determinants may be responsible.

This period of clinical latency, however, does not mean latency as virus multiplication goes on throughout.

Classification system for HIV infection (Centers for Disease Control and Prevention, USA)

Group I		Acute HIV syndrome
Group II		Asymptomatic infection
Group III		Persistent generalised lymphadenopathy
Group IV		Other diseases
	Subgroup A	Constitutional disease—AIDS-related complex (ARC)
	Subgroup B	Neurologic diseases
	Subgroup C	Secondary infectious diseases
	Subgroup C1	Specified infectious diseases listed in the CDC surveillance definition for AIDS, such as *P.carinii* pneumonia, cryptosporidiosis, toxoplasmosis, generalised strongyloidiasis, cryptococcosis CMV or herpes infections
	Category C2	Other specified secondary diseases, such as oral hairy leukoplakia, salmonella bacteremia, nocardiosis, tuberculosis, thrush
	Subgroup D	Secondary cancers, such as Kaposi's sarcoma, lymphomas
	Subgroup E	Other conditions

The virus load in the plasma is of prognostic value. Viral killing of cells goes on throughout the illness. The steady state of virus (**virus set point**) in a patient varies with individuals. High set point correlates with rapid disease progression.

The host mounts an immune response against the virus, both humoral and cellular, which can only limit the virus load, but not clear it completely. A chronic persistent infection with varying degrees of viral multiplication is the result. The CD4+ T cell count decreases steadily, from over 1000 per microlitre to about 500 or less in the stage of acute infection. When the count falls to < 200, clinical AIDS usually sets in. Hence, case definition by CDC includes all HIV-infected cases with CD4+ T cell counts of 200 or less, irrespective of their clinical condition (*Case*).

Group III—persistent generalised lymphadenopathy (PGL): This has been defined as the presence of enlarged lymph nodes, in two or more non-contiguous extrainguinal sites, that persist for at least three months. This is in the absence of any current illness or medication that may cause lymphadenopathy. The cases may progress to ARC or AIDS.

Group IV—AIDS-related complex (ARC): The typical constitutional symptoms are fatigue, unexplained fever, persistent diarrhea and marked weight loss ('diarrhea and dwindling') of more than 10 per cent of body weight. The common opportunistic infections are oral and esophageal candidosis, herpes zoster, hairy cell leucoplakia, salmonellosis or tuberculosis. Generalised lymphadenopathy and splenomegaly are usually present. ARC patients are usually severely ill and many of them progress to AIDS in a few months.

AIDS: This is the **end-stage disease**, representing the irreversible breakdown of immune defence mechanisms, leaving the patient open to progressive opportunistic infections and malignancies (see box below).

The clinical severity of AIDS varies with the type of infection or malignancy present. In early AIDS, many patients are ill only during episodes of infection, which may respond to treatment. Between episodes, they may be relatively well and able to resume normal life. Patients with Kaposi's sarcoma are less ill than those with other malignancies. The illness progresses inexorably and death ensues in months or years.

- **Respiratory symptoms:** The commonest presentation is **dry cough, dyspnea and fever**. In developing countries including India, the most important pathogen is *M.tuberculosis,* with increasing incidence of multidrug-resistant strains. A double epidemic of HIV and drug-resistant tuberculosis is the current challenge in developing countries. Pneumonia may be viral (CMV) or fungal (cryptosporidium, Cryptococcus or histoplasma).
- **Gastrointestinal system:** Oral thrush, herpetic stomatitis, gingivitis, hairy leukoplakia or Kaposi's sarcoma are common oral manifestations. Dysphagia may be due to esophageal candidosis. Cryptosporidium, salmonellae, mycobacteria, isospora, CMV or adenoviruses frequently cause intestinal infections. Disseminated strongyloidosis may also occur.

'Gay bowel syndrome' is chronic colitis commonly seen in male homosexuals. Amoeba, giardia and a host of diarrheagenic bacteria have been reported to be responsible for this condition.

- **Central nervous system:** The typical CNS opportunistic infections are toxoplasmosis and cryptococcosis. Infections are also seen with CMV, herpes simplex, papovaviruses, mycobacteria, aspergillus and candida. Lymphomas of the central nervous system are common.
- **Malignancies:** Kaposi's sarcoma, Hodgkin's lymphoma and other non-Hodgkin's lymphomas are associated with AIDS.

Major opportunistic infections and malignancies commonly associated with untreated AIDS patients

Parasitic	1. Toxoplasmosis 2. Cryptosporidiosis 3. Isosporiasis 4. Generalised strongyloidiasis
Mycotic	1. *Pneumocystis jirovecii* 2. Candidosis 3. Cryptococcosis 4. Aspergillosis 5. Histoplasmosis
Bacterial	1. Mycobacterial infections—tuberculosis and non-tuberculous infections 2. Salmonellosis 3. Campylobacter infection 4. Nocardia and actinomycetes 5. Legionellosis
Viral	1. CMV 2. Herpes simplex
Malignancies	1. Kaposi sarcoma 2. Lymphomas—Hodgkin and non-Hodgkin types

- **Cutaneous:** Herpes lesions, candidosis, xeroderma, seborrheic dermatitis, prurigo, folliculitis, impetigo and molluscum contagiosum are the common cutaneous lesions besides Kaposi's sarcoma.

Dementia: HIV may cause direct cytopathogenic damage in the central nervous system. It can cross the blood–brain barrier and cause encephalopathy leading to loss of higher functions, progressing to dementia.

Pediatric AIDS: About a third to half the number of babies born to infected mothers are infected with HIV. Virus transmission to the fetus may occur as early as the first trimester, but infection is more common perinatally. Many of the infected children may not survive the first year of life. Children may also acquire the infection from blood transfusion or blood products.

Differences between adult and pediatric AIDS:

- Children develop humoral immunodeficiency early, leading to recurrent bacterial infections.
- They fail to thrive.
- Chronic diarrhea is more common.
- Lymphadenopathy is more pronounced.
- Tuberculosis and opportunistic bacterial infections are common manifestations in pediatric AIDS.
- Lymphocytic interstital pneumonia is seen mostly in children.
- Kaposi's sarcoma, toxoplasmosis and cryptococcosis are less common than in adults.

Laboratory Confirmation of HIV AIDS

Laboratory tests are done for

- Diagnosis of clinically suspected cases
- Monitoring of treatment
- Screening of blood
- Antenatal screening of mothers
- Screening high-risk groups

Methods: Tests depend on the stage of the disease. Principally, three methods can be employed

- Viral isolation
- Detection of antibody to various antigens of the virus
- Detection of viral DNA, RNA or antigens

Policy of National Aids Control Organisation (NACO), Government of India

To bring about a reliable set of standardised tests in India, the **National AIDS Control Organisation (NACO),** Government of India, is credited for implementing a strategy for testing different categories of suspected individuals exposed to HIV. To ensure quality and uniformity in reporting the incidence and prevalence of the disease, a set of guidelines have been provided for conducting serological tests and their interpretation. It has been made mandatory for all testing laboratories to follow these guidelines. A **pre-** and **post-test counselling** must be conducted to educate the patient. And no test can be carried out without the prior consent of the patient.

Specific tests for detection of HIV infection:

Antigen detection: Following a single massive infection, as by blood transfusion, the viral antigens may be detectable in blood after about two weeks. The major core antigen, p24, is the earliest virus marker to appear in blood, hence, is tested for early diagnosis. IgM antibodies appear in about 4–6 weeks, to be followed by IgG antibodies (Fig. 61.3).

Seroconversion: This refers to the appearance of IgM antibody in the patient's serum, following the initial period of p24 antigenemia and viremia. Later, free p24 antigen disappears from circulation and remains absent during the long asymptomatic phase, to reappear only when severe clinical disease sets in.

p24 capture assay

Antibody-bound p24 antigen may be demonstrable after dissociation. The p24 antigen capture assay (ELISA) which uses anti-p24 antibody as the solid phase can be used for this. The test is positive in about 30 per cent of HIV-infected persons. With prior dissociation of the antigen–antibody complex, the positive rate increases to about 50 per cent. The test is most useful in persons recently exposed to risk of infection, in whom the antibody test is negative in the first few weeks of infection and in the terminal phase of illness.

Virus isolation: Once infected with HIV, a person remains infected for life. The virus is present in circulation in body fluids, within lymphocytes or is cell-free. Virus titres parallel p24 titres.

The virus isolation is done in containment laboratories. HIV is isolated from infected persons from the peripheral lymphocytes, by **co-cultivation.** The patient's lymphocytes are cultivated with uninfected healthy lymphocytes, in the presence of interleukin-2. Viral replication can be detected by the demonstration of reverse transcriptase activity as well as antigens, in the culture supernatant. However, viral isolation is not routinely done for diagnosis. The test is positive only in a proportion of persons infected with HIV.

Detection of viral nucleic acids: Amplification of viral DNA and RNA are the most sensitive and specific tests. They can be detected by DNA PCR, RNA PCR (RT-PCR) and bDNA assay.

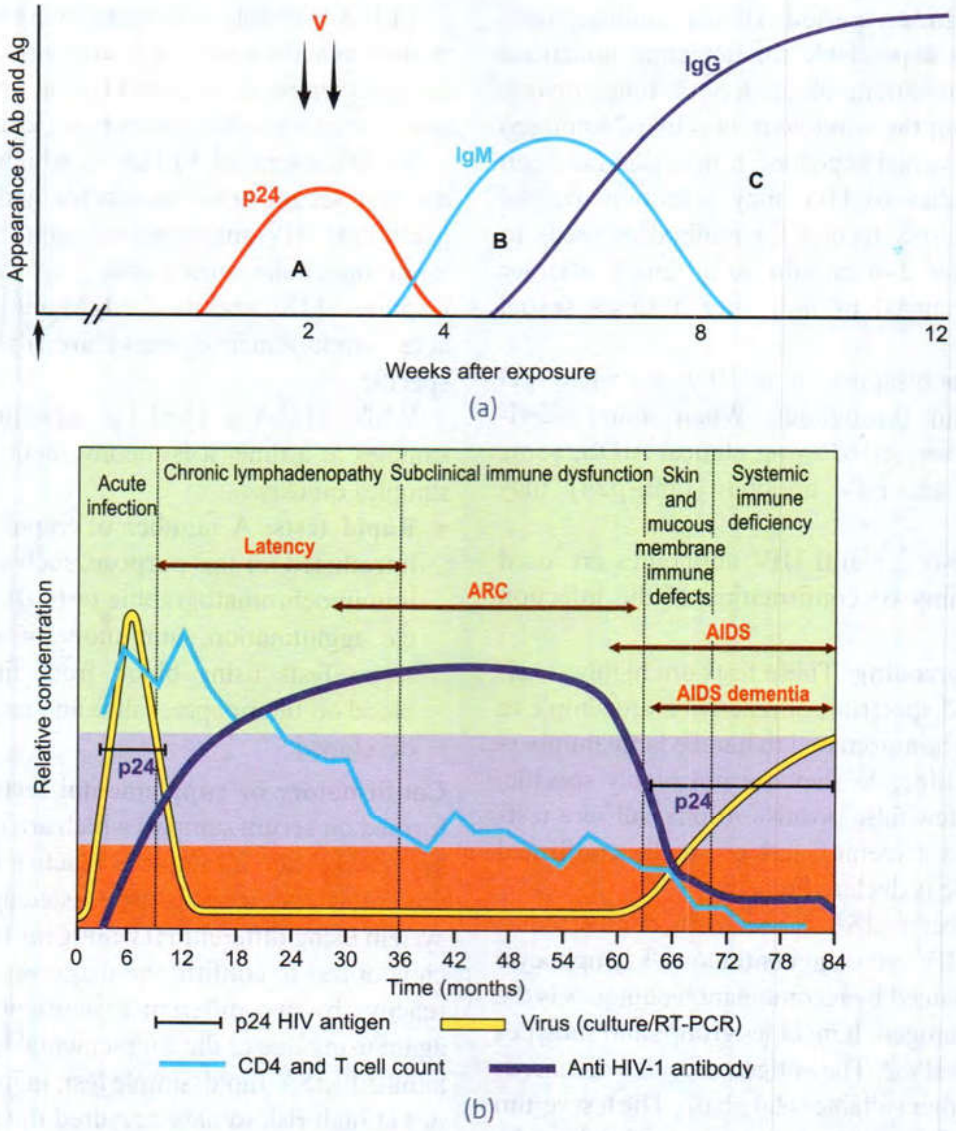

Fig. 61.3 (a) Sequence of appearance of p24 antigen and antibodies after a massive HIV infection; (b) Illustration of the usual time-course of immune response, viremia, and disease resulting from untreated HIV-1 infection.

- **DNA PCR**: Peripheral lymphocytes from the subject are lysed and the proviral DNA is amplified using primer pairs from relatively constant regions of the HIV genome (from the gag and LTR regions). The amplified DNA is detected by probes based on nucleic acid hybridisation. The test is highly sensitive and specific when done with proper controls and can detect HIV proviral DNA at a frequency of one copy per 10,000 cells.
- **RT-PCR**: This method uses an enzymatic method to amplify HIV RNA. This is a useful test to detect the disease progression and monitor response to therapy.
- **bDNA assay**: Sequential oligonucleotide hybridisation steps are used to amplify the viral RNA.

Dried blood spots on filter paper may be used as an alternate to plasma. This is adopted to transport specimens to molecular testing facilities from remote places in developing countries.

The PCR tests are complex and costly and are indicated for confirming, monitoring and as tools for early infant diagnosis.

Antibody detection: Demonstration of antibodies is the simplest and most widely employed technique for the diagnosis of HIV infection. However, it may take 2–8 weeks to months for antibodies to appear after infection. During this period, the individual may be highly infectious. **This seronegative infective stage is**

known as the window period. Hence, antibody testing is not totally dependable for detecting infectious persons, e.g., from among blood donors. Infection can be detected during the window period by p24 antigen assay. Following sexual exposure, if infection has been acquired, antibodies to HIV may take two months to appear. Therefore, testing for antibodies needs to be done only after 2–6 months to ascertain whether infection has occurred or not, after a single sexual exposure.

IgM antibodies disappear in 8–10 weeks while IgG antibodies remain throughout. When immunodeficiency becomes severe following clinical AIDS, some components of anti-HIV antibody (anti-p24) may disappear.

Serological tests for anti-HIV antibodies are used for either screening or confirmation of the infection (Table 61.1).

Tests used for screening: These tests are highly sensitive, have a broad spectrum of reactivity, are simple to perform and can be automated to handle large numbers of samples at a time. As they are not highly specific, they may give a few false positive results. All sera testing positive on a screening test are to be confirmed before the sample is declared reactive.

- **ELISA**: Indirect ELISA is the method most commonly used. HIV grown in continuous T lymphocyte cell line or obtained by recombinant techniques is the source of the antigen. It includes groups and subtypes of HIV 1 and HIV 2. The antigen is coated on microtitre wells or other suitable solid phase. The test serum is added, and if the antibody is present, it binds to the antigen. After washing the excess unbound antibodies, antihuman immunoglobulin linked to a suitable enzyme is added, followed by a colour-forming substrate. If the test serum contains anti-HIV antibody, a photometrically detectable colour is formed, which can be read by the ELISA reader.

ELISA is simple and relatively inexpensive but false positive reactions are not uncommon, particularly with sera containing rheumatoid factor, anti-lymphocyte or other autoantibodies and in hepatic disease.

Modifications of ELISA in which the antibody in the test serum either competes with enzyme-conjugated anti-HIV antibody or is captured by antihuman immunoglobulin onto a solid phase are more specific. Capture ELISA specific for IgM antibody is also available. Immunometric assays are highly sensitive and specific.

While ELISA is ideal for screening several serum samples at a time, it is inconvenient for testing single samples quickly.

- **Rapid tests**: A number of 'rapid tests' have been introduced for this purpose, such as cassette ELISA, immunochromatographic tests (ICT), coated particle agglutination, immunoperoxidase or dip-stick tests. Tests using blood from finger-prick, dried blood on filter paper, saliva and urine have also been developed.

Confirmatory or supplemental tests: These are performed on serum samples which are reactive in screening tests. When a sample is reactive by any one of the screening tests, it needs to be tested again by a different system using different HIV antigens or a different principle of test to confirm the diagnosis. If a specimen is reactive by two different systems, it has to be tested again using one of the supplemental tests which may be a third ELISA/rapid/simple test, in individuals who are not at high risk to have acquired the infection.

The confirmatory tests may also be needed to resolve discordant results of two or more rapid or ELISA tests.

Western blot test: This is the most commonly used confirmatory test (Fig. 61.4).

Procedure: HIV proteins, separated according to their electrophoretic mobility (and molecular weight)

Table 61.1 *Serological markers detected during various phases of HIV infection*

State of infection	*Antigens/Antibodies*	*Anti-HIV IgM*	*Anti-HIV IgG*	*Western blot pattern*
Early infection	+ (P24 antigen)	–	–	–
Acute (seroconversion)	+ → –	– → +	– → +	+
Partial illness	gp120, gp41, gp160 antibodies gp120	+ → –	+	+
Carriers and asymptomatic individuals	P24 ag – gp120, gp41, gp160 antibodies +	–	+	+
PGL	P24 antibodies → –	–	+	+
AIDS	P24, P17 and P55 antibodies → decline	–	+	+

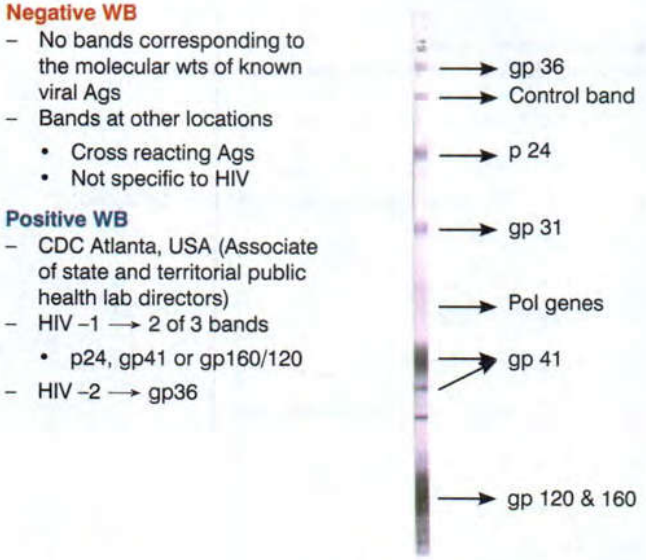

Fig. 61.4 Western blot test for HIV antibody

by polyacrylamide gel electrophoresis, are blotted onto strips of nitrocellulose paper. Patient's sera are reacted with these proteins on the strips followed by an enzyme-conjugated antihuman globulin. A suitable substrate is then added, which produces a distinct colour band where the specific antibody has reacted with the blotted viral protein.

Results: The position of the band on the strip indicates the antigen with which the antibody has reacted. In a positive serum, multiple proteins' bands are seen, Mainly, p24 (*gag* gene, core protein), p31 (*pol* gene, reverse transcriptase) and gp41, gp120 or gp160 (*env* gene, surface antigens) bands are noted. A positive reaction with proteins representing three genes is conclusive. The test may be considered positive if it also shows bands against at least two of the following gene products: p24, gp41, gp120/160. However, interpretation becomes difficult when bands other than those specified above appear. This may happen in early infection or may be non-specific. Western blot is a useful confirmatory test but the interpretation remains subjective and demands considerable experience. In indeterminate cases, the Western blot may be repeated after a specified period of time. It may be necessary to do a p24 assay in indeterminate results.

Apart from diagnosing HIV infection, the laboratory needs to identify the opportunistic infections that are a feature of AIDS. Routine microbiological methods would suffice for this. Serological diagnosis markers of infection may not be reliable in AIDS as antibody formation may be affected by the immune deficiency.

Line immunoassays (LIAs): These are based on the application of recombinant and synthetic peptide antigens on a plastic support strip in a manner similar to the immunoblot assay. LIAs are second- or third-generation assays and have the potential to be used as supplemental tests. Combination assays and differentiating infection by HIV 1 and HIV 2 can also be done in LIA.

HIV screening or testing is required for the following reasons:
- Epidemiological surveillance using unlinked anonymous HIV testing
- Transfusion and transplant safety
- Diagnosis of HIV infection in symptomatic and asymptomatic individuals
- Prevention of parent-to-child transmission
- For post-exposure prophylaxis (PEP)
- Research

Strategies for HIV testing

In India, the National Aids Control Organisation (NACO) of the Ministry of Health, Government of India, has laid out guidelines and strategies for screening, testing and monitoring HIV infected/suspected individuals.

- ELISA/Rapid tests/Supplemental tests (E/R/S) used in strategies I, II and III
- Supplemental test in cases of indeterminate/discordant result of E/R/S

NACO strategies for testing different categories of samples:

Strategy I: This strategy is used to screen blood/blood products organ, tissues, sperms, etc. The sample is subjected once to E/R for HIV (Fig. 61.5).

NACO recommends the use of ELISA kits with a sensitivity of ≥99.5 percent and specificity of ≥98 percent and rapid kits with a sensitivity of ≥99.5 percent and specificity of ≥98 percent.

Strategy II: This strategy is used for surveillance (2A) and for diagnosis (2B), if some AIDS indicator disease is present (Figs 61.6 and 61.7) . If a serum sample is positive in the first ELISA, it is subjected to a second ELISA which utilises a system different from the first one. By second ELISA, if the test is positive, then it is reported as positive. If it is negative (by the second test) the result is considered negative.

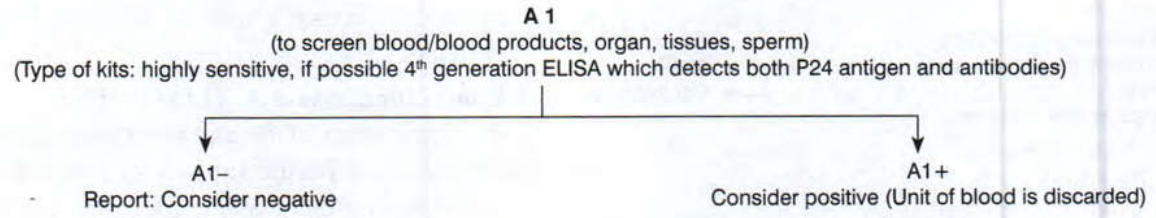

Fig. 61.5 Interpretation of tests A1– and A1+

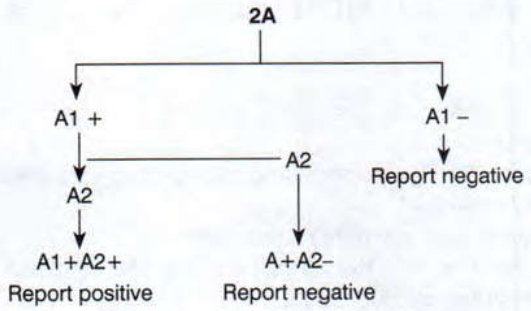

Fig. 61.6 2A (for surveillance- anonymous unlinked)

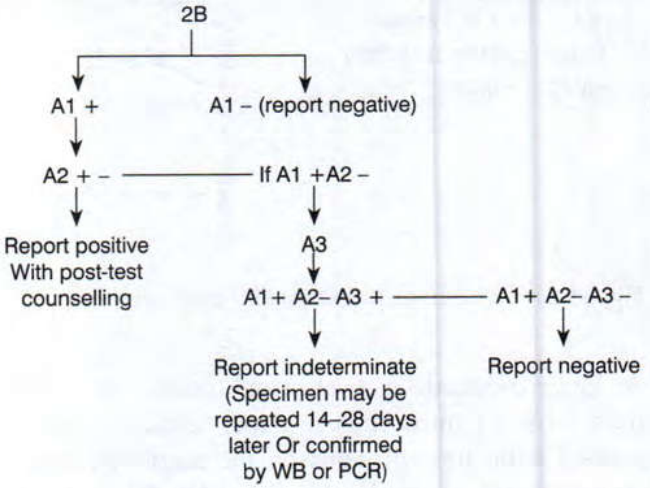

Fig. 61.7 2B (for diagnosis of symptomatic or high-risk individuals)

Strategy 2B: This strategy is used to determine the HIV status of clinically symptomatic suspected AIDS cases in which blood/serum/plasma is tested with highly sensitive screening and confirmatory tests based on different principles and/or antigens as compared to the first test.

Strategy III: This is used in asymptomatic individuals. It is similar to strategy II, with the added third positive ELISA test being required for a sample to be reported HIV-reactive. In this, the first ELISA is the one with the highest sensitivity and the second and third ELISAs have the highest specificity (Fig. 61.8).

Applications of serological tests

Serological tests for HIV infection are used in the following situations:

Screening: Screening is done of populations or selected target groups for epidemiological purposes. Screening of entire populations is neither feasible nor practicable. However, screening of a target population is useful. It is mandatory for all donors of blood, blood products, semen, cells, tissues and organs to be screened. Screening for the p24 antigen can detect those in the window period also. Hence, tests to detect both P24 antigen and HIV antibodies are recommended in blood banks. HIV-positive individuals must not donate blood tissue or organs. As the infection can be transmitted from mother to baby before, during or after birth, antenatal screening of all antenatal women has been made mandatory.

Seroepidemiology: Antibody surveys have been most useful in identifying the geographical extent of HIV infection and in other epidemiological studies such as spread of the infection from identified sources.

Diagnosis: Serology is almost always positive in persons with clinical features of AIDS. It may, however, be negative in acute illness and sometimes in the very late cases where the immune system is non-reactive. Routine serology may also be negative when the infection is with a different AIDS virus. For example, HIV 2 infections are likely to be missed if antibody testing is done with the HIV 1 antigen alone. Test antigens should be updated when new virus types or subtypes are identified, and should be able to detect antibody against all prevalent types of HIV.

Although HIV-1 and HIV-2 are related, there are important structural differences between them. Accurate diagnosis and differentiation of HIV-1 and HIV-2 is crucial for treatment, as HIV-2 is intrinsically resistant to NNRTI, the pillar of national first-line ART regimen.

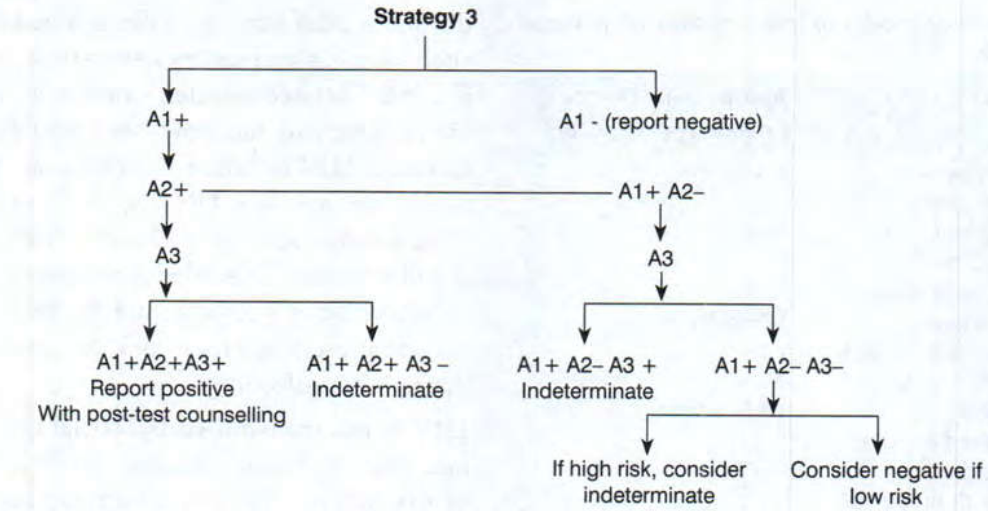

Fig. 61.8 Strategy 3 (for diagnosis of asymptomatic individuals)

Antibody testing may also help to check whether infection has taken place following an exposure, such as sexual contact, blood transfusion or needle-stick injury. Serology after two months and, if negative, after six months, would be sufficient. If serology is negative six months after exposure, infection is unlikely to have occurred.

Prognosis: In a person infected with HIV, loss of detectable anti-p24 antibody indicates clinical deterioration. This is also associated with HIV antigenemia and increased virus titre in circulation.

Non-specific or immunological tests: The following parameters indicate immunodeficiency in HIV infection:

- Total leucocyte and lymphocyte count to demonstrate leucopenia (count usually below 2000/mm^3)
- T cell subset assays. Absolute CD4+ T cell count is usually less than 200/mm^3. T4:T8 cell ratio is reversed
- Thrombocytopenia (low platelet count)
- Raised IgG and IgA levels
- Diminished CMI as indicated by skin tests
- Lymph node biopsy shows abnormalities

Laboratory monitoring of HIV infection: Some laboratory tests are important in monitoring the course of HIV infection. CD4+ T cell count which reflects the current immunological competence of the patient is most often used to monitor the course. A count below 500 indicates disease progression and the need for antiretroviral therapy. Counts below 200 denote risk of serious infection.

It is necessary to monitor measurement of HIV RNA during the course of treatment. This is usually done by two methods, RT-PCR and bDNA assay.

Beta-2-microglobulin and neopterin are two substances that have a predictive value on the progression of HIV disease as they rise with advancing disease.

Epidemiology and prevention

Progenitor of HIV 1 entered the human population from chimpanzees of the subspecies *Pan troglodytes troglodytes* living in equatorial West Africa (Cameroon, Gabon, equatorial Guinea). HIVs are believed to have been present in monkeys for over 100,000 years. Simian Immunodeficiency virus may have taken root in humans by converting to HIV through mutation or recombination.

HIV 1 M, O, N types may represent independent transmissions from chimpanzees to humans. The source of HIV 2 has been established as SIV from the Sooty Mangabey monkey *Cercocebus atys.*

Transmission: The virus has spread globally, with geographically different prevalence rates. HIV is spread only by three modes (Table 61.2):

- Sexual contact with infected persons (heterosexual or homosexual)
- By blood and blood products
- Infected mother to babies (intrapartum, perinatal, postnatal)

There is no evidence of HIV transmission by other means including casual contact or through insects.

- HIV is primarily a **sexually transmitted infection**, initially predominant in male homosexuals. In the affluent countries, homosexual and bisexual men are infected

Table 61.2 *Common modes of transmission of HIV and their relative risk*

	Types of exposure	Approximate chance of infection per exposure
I	Sexual intercourse: anal,vaginal, oral	0.1–1.0%
II	Blood and blood products, Factor VII, etc., blood transfusion	>90%
III	Tissue and organ donation: semen, cornea, bone marrow, kidney, etc.	50–90%
IV	Injections and injuries: shared needles by drug addicts Injections with unsterile syringes and needles Needle-stick and other injuries in health staff Surgical wounds	0.5–1.0%
V	Mother to baby: Transplacental At birth After birth Breast milk	30%

far more often than heterosexuals. Hence, it is found predominantly in men and only occasionally in women. However, the situation in Africa and Asia shows men and women are equally affected. Transmission in the developing countries is almost always heterosexual and can take place in both partners.

The best method of checking sexual transmission and other high-risk activities for infection is through counselling and health education.

- The second mode of transmission is **through blood and blood products**. Screening of blood donors is now mandatory, which must include p24 antigen screening.
- **Contaminated needles** can transmit the infection. This is particularly relevant in drug addicts who share syringes and needles. Higher incidence has been detected in northeastern states of India besides some other parts of the country. The use of **unsterile syringes and needles** by health workers makes iatrogenic infection likely. The use of disposable syringes, needles and other equipment has reduced the incidence.

The risk of **needle-stick injury** is present for health-care personnel, though the chances of infection are much less than with the hepatitis B virus. Transmission of infection from **mother to child** can take place before, during or after birth. As infection occurs in about half such infants, a mandatory testing in the antenatal period is carried out and infected women are informed. Early infant diagnosis has now been introduced by NACO to detect HIV infection in newborns. This is done by testing for pro-viral DNA by PCR on blood collected from the newborn by heel prick. This is absorbed on dry filter paper. This dried blood spot is sent to a referral laboratory for testing, and the baby is treated. HIV may be present in breast milk and may be transmitted through breastfeeding.

HIV is not transmitted by: social and domestic contact, shaking hands, hugging, putting cheeks together or dry kissing. There has been no confirmed case of transmission through saliva, though the virus may be present in the saliva of infected persons. A salivary protein called secretory leucocyte protease inhibitor has anti-HIV activity. There is no evidence of mosquitoes, bed bugs or other blood-sucking insects transmitting the virus.

HIV infection was detected rather late in India, the first cases having been found in female sex workers in Madras (Chennai) in 1986 and the first AIDS patient the same year in Bombay (Mumbai). Since then, the rate of infection has been increasing among high-risk group in certain states and target populations. With several intervention programmes of the NACO, it is hoped to control the infection.

Prophylaxis

Prevention of AIDS depends on general measures such as health education, identification of sources and decrease in high-risk behaviour. No specific vaccine is available. The high mutability, diverse antigenic types and subtypes, long latency and persistence as provirus in infected cells pose several problems in the development of vaccines.

Vaccine research

Several possible strategies have been explored for vaccine production. These include **immunisation** of experimental animals like chimpanzees and monkeys with:

- Modified whole virus
- Subunits, based on envelope glycoproteins expressed in animal cells, bacteria, viruses—or as synthetic epitopes on adjuvant carriers
- Target cell protection by anti-CD4 antibody or genetically engineered CD4. A number of candidate vaccines are being tested in clinical trials in humans
- Post-exposure prophylaxis

This refers to the comprehensive management given to minimise the risk of infection following potential exposure to bloodborne pathogens, such as HIV.

'**Exposure**' for risk of developing bloodborne infections is defined as:

- Percutaneous injury (needle-stick injury)
- Contact with mucous membrane of eye or mouth
- Contact with non-intact skin
- Contact with intact skin when the duration of contact is prolonged with blood or other potentially infectious body fluids

It is expressed as exposure code (EC).

Although the risk of HIV transmission by these routes is less than 1%, PEP is recommended within 72 hours of exposure, depending on the exposure category of HCP and the HIV status code of the patient (Figs 61.9 and 61.10).

Management of AIDS

Approaches to the treatment of AIDS include:

- The treatment and prophylaxis of infections and tumours
- General management
- Immunorestorative measures
- Specific anti-HIV agents

Prompt diagnosis, counselling and appropriate treatment of opportunistic infections and tumours in the early stage of AIDS can be very useful and the patient may be able to resume normal life in between episodes of illness. General management of the patient requires the understanding and cooperation of the health staff in the hospital and of relatives at home. Fears about imaginary risks have to be allayed and reassurance given that the patient can be kept at home or treated in the hospital without danger to contacts, if proper precautions are taken.

Antiretroviral treatment: Highly Active Anti-Retroviral Treatment (HAART) is the mainstay of treatment. It leads to complete suppression of plasma viremia, opportunistic infection rates are decreased and the quality of life for people living with HIV and AIDS (PLHA) is improved. Adherence to treatment can delay the development of drug resistance and the need for second-line treatment. Specific treatment with antiretroviral drugs is the mainstay in the management of HIV infection. A number of effective drugs have become available in

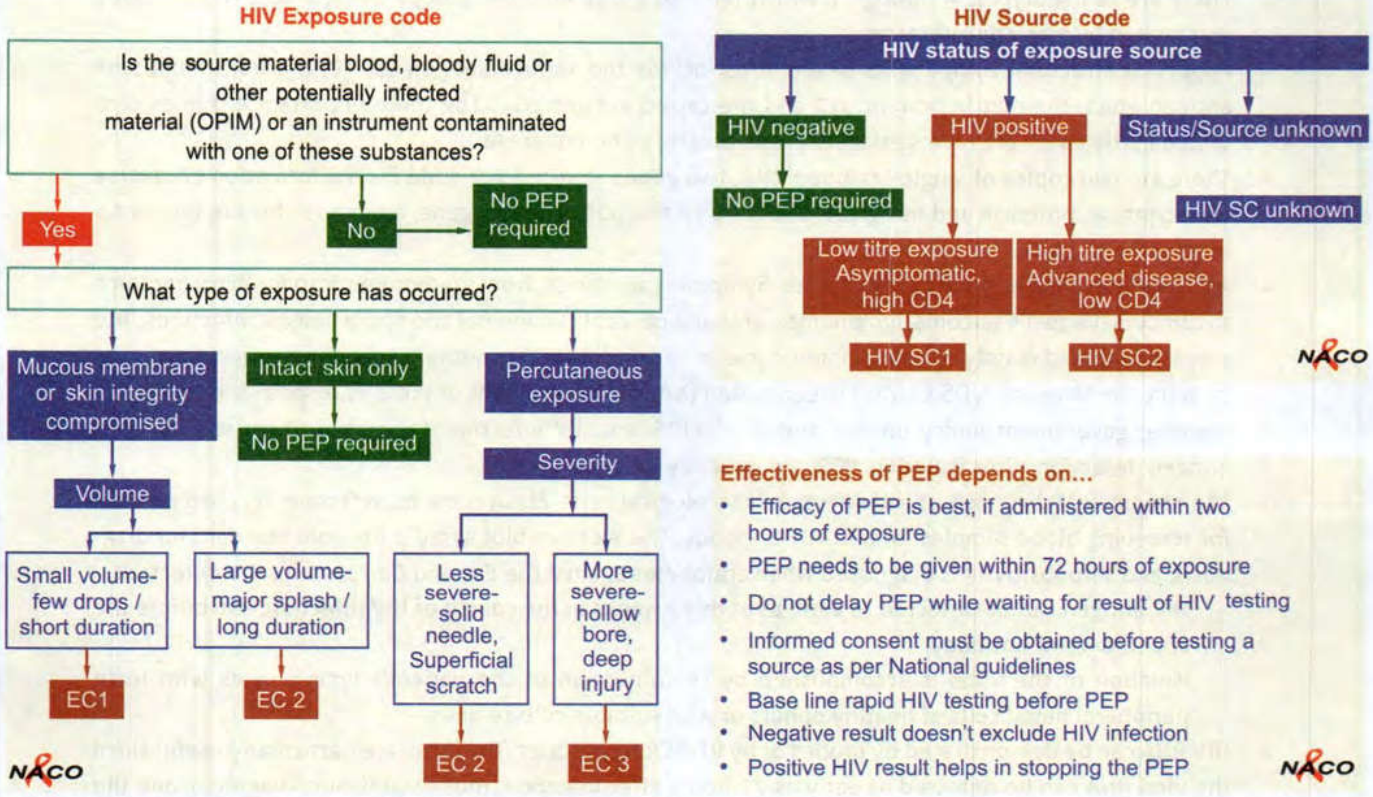

Fig. 61.9 Guidelines of National AIDS Control Organisation (NACO)

Determining PEP recommendation

EC	HIV SC	PEP recommendation
1	1	May not be warranted
1	2	Consider basic regimen (Two drugs)
2	1	Recommend basic regimen (Two drugs)
2	2	Recommend expanded regimen (Three drugs)
3	1 or 2	Recommend expanded regimen (Three drugs)
2/3	Unknown	Consider basic regimen if HIV prevalence is high in the given population

NACO

Fig. 61.10 Recommendations for post-exposure prophylaxis (PEP)

recent years. These include nucleoside analogues like zidovudine (azidothmidine, AZT), didanosine, zalcitabine, lamivudine and protease inhibitors like saquinavir, ritonavir, indinavir, which have been used as monotherapy or in various combinations. Adverse reactions and high cost restrict their wide use in resource-poor countries.

The National AIDS Control Organisation (NACO) provides free anti-retroviral treatment through several ART centres across India.

Steps at immunorestorative therapy such as administration of interleukin-2, thymic factors, leucocyte transfusion and bone marrow transplantation have not been very helpful.

RECAP

- Human immunodeficiency virus types 1 and 2 (HIV 1 and HIV 2) are the causative agents of acquired immunodeficiency syndrome (AIDS) worldwide. The HIV viruses are about 90–120 nm in size, spherical in shape, icosahedral in symmetry and enveloped.
- There are ten subtypes, A through J, which fall into group M, other groups being O and N. It is easily inactivated by many disinfectants.
- Important structural components of the virus include the surface antigen gp120, the transmembrane antigen gp41, the matrix protein p17 and the capsid antigen p24. The three important enzymes contained in the virion are reverse transcriptase, protease and integrase.
- There are two copies of single-stranded RNA. Two genes, *gag* and *pol*, code for the formation of reverse transcriptase, protease and integrase, and for p17 and p25. Another gene, *env*, codes for the formation of gp120 and gp41.
- AIDS is a late manifestation of HIV disease. Symptoms are varied, from asymptomatic to flu-like symptoms to tumours (Kaposi's sarcoma, lymphomas, anal and cervical carcinoma) and opportunistic infections. The disease is spread mainly by sexual intercourse, blood and from the mother to the fetus or newborn.
- In India, the National AIDS Control Organisation (NACO), Government of India, is responsible for implementing government policy on the control of AIDS and HIV infection. Laboratory diagnosis includes antigen detection, virus isolation, PCR and antibody detection.
- The mainstay of diagnosis of HIV infection is serological tests: ELISA is the most frequently used method for screening blood samples for anti-HIV antibody. The Western blot assay is the gold standard for diagnosis, and seropositivity is diagnosed when antibodies against the *Env* and *Gag* proteins are detected.
  - HIV antigen can be detected as early as at three weeks in the course of HIV infection, and before the appearance of antibody.
  - Isolation of the virus is accomplished by co-cultivation of the patient's lymphocytes with fresh peripheral blood cells of healthy donors or with suitable culture lines.
- HIV RNA can be demonstrated by probes or by RT-PCR techniques. The latter are particularly useful since the viral RNA can be detected as early as 72 hours after infection, thus establishing diagnosis, and the response to therapy can be assessed.