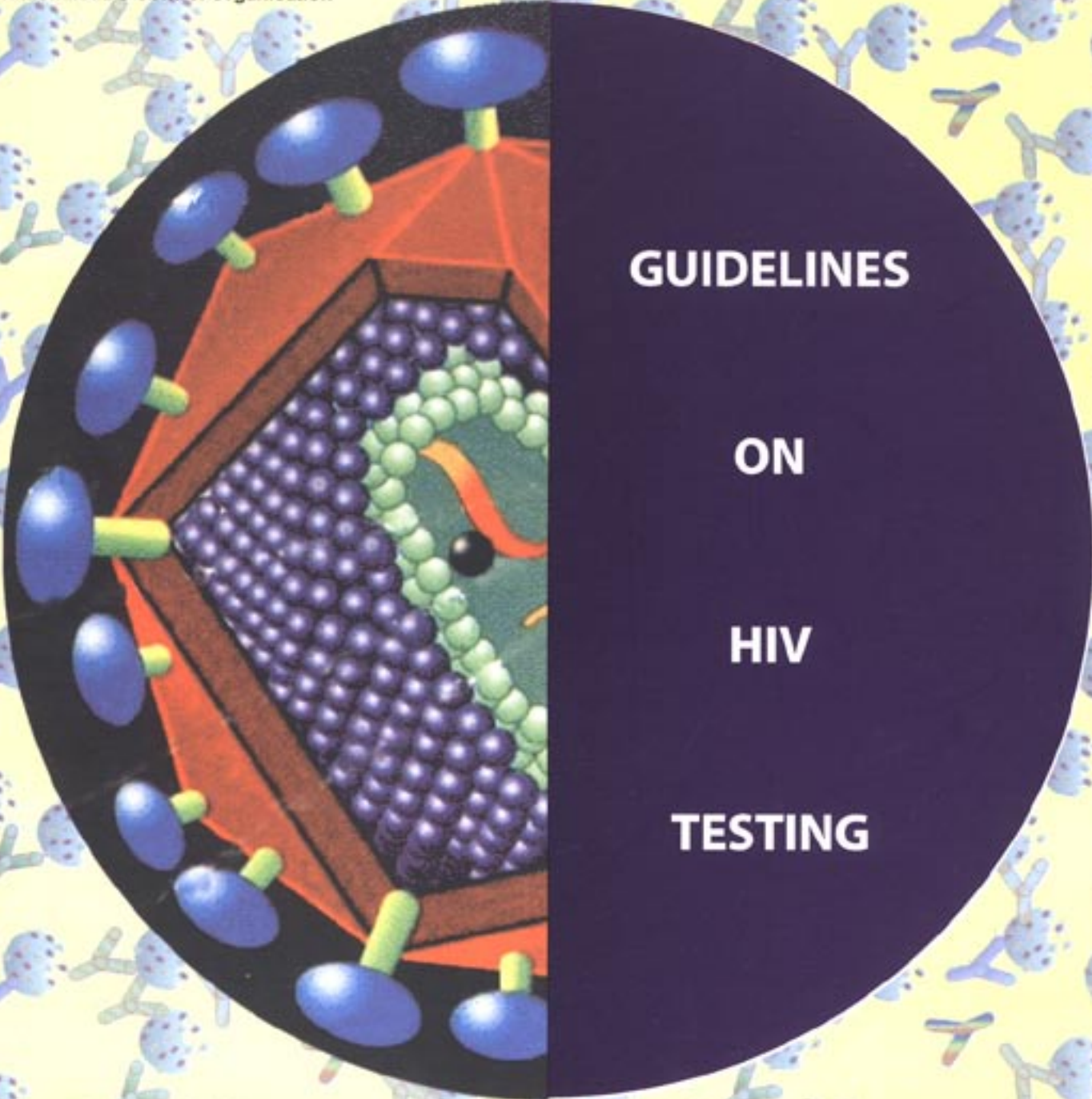




Ministry of Health and Family Welfare  
National AIDS Control Organisation



**GUIDELINES**

**ON**

**HIV**

**TESTING**

**March 2007**



# **GUIDELINES FOR HIV TESTING**

**March 2007**



National AIDS Control Organisation  
Ministry of Health and Family Welfare

Foreword

Message

Acknowledgements

## Chapter

1.	Basic Epidemiology and Natural history of HIV/AIDS	1 - 6
2.	Virology	7 - 12
3.	Immunopathology	13 - 18
4.	Biosafety in laboratories	19 - 33
5.	Collection and transport of specimens for HIV testing	34 - 37
6.	Detection of HIV infection	38-53
7.	HIV testing at counselling and testing sites (ICTCc and PPTCTCs) using rapid tests	54-58
8.	Procedure of testing at ICTCs and PPTCTs	59-62
9.	Selection of HIV test kits	63-70
10.	Direct evidence of HIV infection.	71-74
11.	National strategies and algorithms for HIV testing	75-85
12.	Quality Assurance Program	86-90
13.	Laboratory tests for monitoring, staging and progression of HIV infection	91-96
14.	Transfusion Transmitted Infections other than HIV	97-112
15.	Counselling and HIV/AIDS testing	113-115
16.	Legal and ethical issues of HIV/AIDS	116-119
17.	Infrastructure for HIV testing laboratories at different levels	120-126
18.	Occupational exposure and Post exposure prophylaxis	127-132
19.	ELISA tests which have been evaluated by WHO collaborating centre on AIDS in the Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium	133 - 136
20.	List of references for further study	137
21.	Laboratory registers	138-156

## List of Contributors

157



## Message

HIV/AIDS are a major concern of health care professionals all over the world. Laboratory diagnosis is the only method of defining/establishing HIV status of an individual. India has well developed laboratory approaches for : ensuring blood safety and donation (tissue, organs) safety ; surveillance of high risk groups ; sentinel surveillance ; diagnosis and research.

Most of HIV antibody screening and supplemental (some Labs. only) testing is performed at the local level (ICTCs, PPTCTCs, Surveillance Centres under NACO), while centralised (referral) labs. provide reference diagnostic services for problematic sera and perform sophisticated technique like W.B., PCR and viral load assay, etc.

Regardless of the level of the laboratory involved in HIV testing, the training and expertise of staff members, the type of reagents and infrastructural facilities available are major determinants of the quality of test results produced. Laboratory personnel, one and all, must strive for total quality assurance programme, seeing to it that every step of the testing process over which they have control is monitored and verified for accuracy and precision. Within the laboratory, the personnel must feel confident that they are protected from injury so that they can do their best work safely, without fear and distraction.

This manual provides information that goes beyond practical aspects of laboratory analysis of HIV, including the biology of the HIV virus, the pathobiology of HIV infection in man, the epidemiology of HIV infection in India and quality assessment of test systems and laboratories. The manual therefore provides a comprehensive document not only for use at the bench but also for teaching and training of HIV



**(K. Sujatha Rao)**

Addl Secy. and Director General,  
National AIDS Control Organisation

## Acknowledgements

HIV infection is spreading rapidly in India. The infection can only be detected by laboratory tests, as there is a long asymptomatic period when the individual is infectious and can spread disease but has no specific symptoms or signs of disease. The role of the laboratory is very important and it is essential that the highest standards are not only maintained in each laboratory but are also regularly monitored.

There are a number of diagnostic procedures and various types of commercial diagnostic kits available. More over the results of the laboratory tests can be influenced by many factors. It is essential that each laboratory has a Standard Operating Procedure (SOP) which details each step of the test including bio-safety precautions. The laboratories are also expected to follow the national guidelines circulated by NACO on ethical issues related to HIV testing.

The HIV Testing Manual has been prepared as a practical guide for laboratory personnel. The Manual is comprehensive and covers relevant topics of interest. The chapters have been drafted and reviewed by national experts during the two meetings. The suggestions and inputs provided by various experts have been incorporated in the final draft.

Four new chapters have been added. Some chapters have been merged for better understanding. All the chapters have been updated.

It is hoped that this manual will go a long way in improving the HIV testing services across the country.

**The contributors**

# 1. BASIC EPIDEMIOLOGY AND NATURAL HISTORY OF HIV/AIDS

## The Epidemic : Global

Acquired Immunodeficiency Syndrome or AIDS was first reported in the world from a group of homosexual men in North America in 1981. A couple of years later, the Human immunodeficiency virus or HIV was discovered as the causative organism leading to AIDS. Since then, HIV/AIDS has been reported from all parts of the globe with Sub-Saharan Africa and parts of Asia as the worst affected areas. According to UNAIDS, an estimated 40.3 million people globally are living with HIV at the end of 2005. These include 4.9 million persons newly infected in the year 2005. A majority of the infections occur in young adults between ages 15 to 24 years. HIV/AIDS is one of the major causes of death worldwide and has killed 3.1 million people world wide in 2005 alone. It is the leading cause of death among adults in sub-Saharan Africa and the fourth biggest killer worldwide. Of the total 3.1 million AIDS deaths that occurred worldwide in 2001, 2.2 million deaths were in sub-Saharan Africa.

## Adults and children estimated to be living with HIV as of end of 2005



**Fig 1 : Total: 40.3 (36.7 – 45.3) million**

## The Epidemic: National

Since the first detection of HIV infection in commercial sex workers (C.S.W) in Chennai in 1986, the infection has now spread to all parts of the country. There are an estimated 5.21 million HIV infected persons at the end of 2004. Although the magnitude of disease has been found to be varied in various parts of the country, the states of Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka, Nagaland and Manipur are the hard hit high prevalence

states. Heterosexual transmission has been found to be the commonest route of transmission, accounting for 86% of the total reported cases. Sentinel surveillance data also suggest that HIV has begun to spread in several rural areas. The spread is as diverse as the culture and social practices in different regions of India. The epidemic varies from state to state with heterosexual transmission predominating in the southern states and transmission due to injecting drug use concentrated in the northeastern states. Even within the states, there are large variations of HIV prevalence among the districts. Only some districts of each high prevalence state are high prevalence districts. This most probably is due to practice of high risk behaviours in these districts.

India remains a low prevalence country with overall HIV prevalence of 0.9%, however, it masks various sub epidemics in various foci in the country as indicated by the sentinel surveillance data. In absolute numbers, India continues to stand second next to South Africa in the number of existing HIV infections. Sentinel surveillance data also suggest that HIV has begun to spread in several rural areas.

### Factors affecting the epidemic

Agent factors :

- i) Agent : HIV, the virus which causes AIDS is a RNA virus belonging to the family Retroviridae. These viruses have a unique enzyme, reverse transcriptase, which copies the viral ribonucleic acid (RNA) into deoxyribonucleic acid (DNA), which eventually integrates into host cell chromosome. Hence, HIV persists within the cells and cannot be eradicated from host cells with any of the currently available anti-retroviral drugs. There are two types of HIV; HIV-1 and HIV-2. Both have many subtypes.
- ii) Reservoir of infection: People harboring HIV in their body are the reservoir of infection. They may be asymptomatic or full blown AIDS cases. In developing countries the average interval between infection and development of AIDS is about 9.8 years.
- iii) Source of infection: Contaminated blood and other body fluids (e.g.; semen, vaginal fluids) are the prime source of infection. Breast milk is another source of infection for transmission from infected mother to child. Saliva and tears are not considered to be epidemiologically important sources of infection though the virus is present in these fluids.

Host Factors

- i) Age and sex : Young people are disproportionately affected by HIV/AIDS due to the risk behaviour practiced by this segment of population. Around half of the new HIV infections are in people aged 15-24 years, the range in which most people start their sexual activity. Globally, 50% of new HIV infections are in women. Women are more vulnerable due to the large area of exposure and presence of inflammation.
- ii) Risk Behaviour : HIV prevalence in certain populations is relatively higher. These include IDUs, CSWs, multiply transfused individuals, truck drivers and migrant workers.
- a) Presence of sexually transmitted infections (STIs): There is strong evidence that men and women with genital ulcer disease or urethral discharge are at increased risk of acquiring and transmitting HIV



through unsafe sexual contact with an HIV infected individual. If there is data suggesting high prevalence of STD in a population, this would be an influencing factor for increased risk for acquiring HIV infection.

- b) Frequency of exposure: The probability that a person will become infected with HIV sexually is, in general, proportional to the frequency of unprotected sex acts and number of high risk partners with whom the person has had sexual contact in recent years.
- c) Mixing pattern of population : The way (risk) behaviours are mixed among groups of people can be very unevenly distributed. For example, injecting drug users might only share injecting equipment within their own group but have sexual partners that are both within and outside their identified group.

### 3.3 Social and economic factors :

- i) Low literacy : May limit access to written, risk reduction information. Lack of awareness may lead to practice of high risk behaviours and acquisition of HIV.
- ii) Urbanization : For economic reasons many people may move to the larger cities, where they may indulge in high risk behaviours such as commercial sex and injecting drug use, etc.
- iii) Imprisonment: This may restrict men's access to women and encourage men to have sex with men. The risk of acquiring HIV increases with this type of sexual practice.
- iv) High mobility: Certain target populations (migrants, CSWs) are highly mobile, stay away from family practice risk behaviour which increases the geographic spread of HIV transmission.
- v) Migration and separation from families: Industries such as fishing, trucking and mining may force people to travel to another country or region of the country to find work. The resulting separation from families and situations may drive them to prostitution and casual sex exposing them to the risk of HIV.
- vi) Substance use: Drug and/or alcohol use may impair judgment and limit the ability to practice safer sex.

### Mode of HIV transmission

Epidemiological studies throughout the world have shown four modes of HIV transmission. Unsafe sexual contact, transfusion of contaminated blood, use of contaminated syringes and from infected mother to the newborn child are routes of transmission of HIV.

- i) Sexual intercourse : Whether heterosexual or homosexual, is the major route of transmission. HIV can be transmitted through any individual act of unprotected sexual intercourse, that is any penetrative sexual act in which a condom is not used where one partner is infected with HIV. The risk of becoming infected through an act of unprotected sexual intercourse depends on four main factors:

The likelihood that the sex partner is infected: The probability that a person has become infected with HIV is in general proportionate to the number (frequency) of unprotected sex acts and the number of high risk partners with whom the person has had sexual contact in recent years.

+

The type of sex act: All unprotected acts of sexual penetration (anal, vaginal, oral) carry a risk of HIV transmission because they bring sexual secretions directly into contact with exposed mucous membrane. Injury to the mucous membrane of the rectum, the vagina or the mouth may help the virus to enter into the blood stream. "Receptive" partners are thus at a greater risk than "Insertive" partners in acts of intercourse. However, HIV can be transmitted even through unbroken mucous membrane.

The amount of virus present in the blood or sexual secretions (semen, vaginal or cervical secretions) of the infected partner: Individuals with HIV infection become more infectious as they progress to HIV related diseases and AIDS. There is also an early period of high infectiousness around the time of seroconversion.

The presence of other sexually transmitted diseases and/or genital lesions in either partner : It is important to be aware that HIV can be transmitted sexually even when neither partner has any of the other sexually transmitted diseases. However, there is strong evidence that men and women with genital ulcer disease or urethral discharge are at 5-9 times increased risk of acquiring and transmitting HIV.

- ii) HIV infected blood, blood products, transplanted organs or tissues and the use of improperly sterilized needles and syringes that have been in contact with infected blood can transmit HIV.
- iii) HIV infected woman can transmit HIV to her foetus or infant before, during, or after birth : A pregnant women with HIV infection has an approximately 20-40% chance of passing the virus to her foetus or newborn baby. There is evidence that infection can occur as early as the first 12-15 weeks of gestation. 50% of perinatal infections are in utero or during the birth process. It is estimated that a large number of perinatal infections occur through breast feeding.

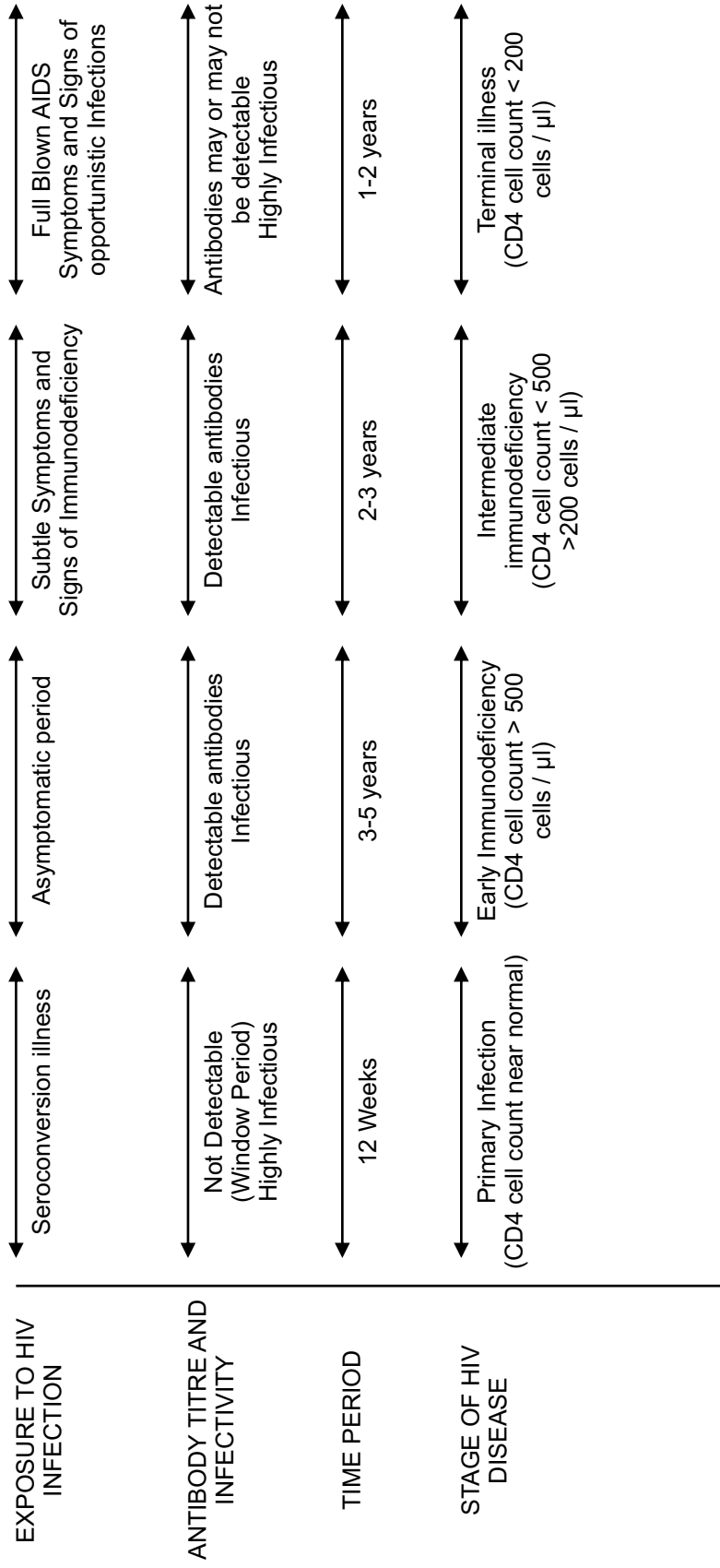
## Natural history of HIV/AIDS

People infected with HIV are both infected and infectious for life, even when they look and feel healthy, they can transmit the virus to others. The signs and symptoms of infection with HIV are varied and complex. Four stages of HIV infection are described below:

- i) Primary infection: Infection with HIV results in rapid proliferation of the virus in blood and lymph nodes. The infected person may experience a seroconversion illness, which usually resolves within weeks. The CD4 cell count declines rapidly before virus is controlled by the immune system, whereupon the count returns to near normal.
- ii) Early immune deficiency (CD4 cell count > 500/μl) : During this phase the immune system has controlled the virus, which is largely restricted to lymphoid tissue. In this phase, damage inflicted by the virus is limited to the regenerative capacity of the immune system and people with HIV are usually without symptoms.

- +
- iii) Intermediate immune deficiency (CD4 cell count 200-500/ $\mu$ l) : Viral replication is very high and CD4 cell turnover is rapid. Subtle signs and symptoms indicating compromise of immune system begin to appear.
  - iv) Advance immune deficiency (CD4 cell count <200/ $\mu$ l) : The virus which proliferates throughout the body overcomes the immune system. Major opportunistic infections and malignancies become increasingly common and require increasing medical intervention.

Fig. 2 : Natural History of HIV Infection in adults



## 2. VIROLOGY

### Introduction

It is very important to understand the causative agent of AIDS (Acquired Immunodeficiency Syndrome), the agent which has caused a pandemic and a disease which has enormous social, economic and behavioural impact on individuals, families, communities and the whole world. AIDS has shattered the global economy with no successful treatment and vaccine in sight.

### History and origin

AIDS was officially recognised for the first time in June, 1981 at the Centers for Disease Control, U.S.A. in previously healthy homosexual men dying with *Pneumocystis jirovecii* pneumonia and candidiasis. Since then, AIDS has been reported from all the continents. The virus causing AIDS was independently identified by a team of French scientists led by Dr. Luc Montagnier of Pasteur Institute and American scientists led by Dr. Robert C. Gallo of National Cancer Institute in 1983-1984. The virus has been called by different names LAV i.e. Lymphadenopathy Associated Virus by the French and HTLV III i.e. Human T Lymphocytotropic Virus type III by the Americans. The International Committee on Nomenclature of Viruses named it the "Human Immunodeficiency Virus" (HIV) and to date two types, HIV-1 and HIV-2 are identified.

Classification : Human immunodeficiency viruses (HIV) belongs to the Family Retroviridae and subfamily Lentivirinae. Two types are recognised HIV-1 and HIV-2. Both differ in geographical distribution, biological and molecular characteristics and extent of transmissibility. These viruses store their genetic information as ribonucleic acid (RNA). RNA must be converted to DNA by a special enzyme reverse transcriptase. HIV-1 has 3 groups, HIV-1 major group (HIV1-M), outlier (HIV1-O) and HIV1-N group. The strains of HIV-1 isolated from people in U.S.A. and Europe are genetically diverse from strains isolated in Africa and Asia. HIV-1 major group can be further classified into subtypes or clades designated A through K excluding I. Such subtypes have envelope gene sequences that vary by 20% or more between subtypes. The subtypes differ in geographical distribution, biological characteristics and major mode of transmission etc. HIV-1 subtypes O and N are more distant to all other HIV-1 subtypes but less so compared to HIV-2. So these are classified under HIV-1 only and have limited distribution in West Africa. HIV-2 has also been reported from other countries and this also comprises of heterogenous group of viruses. HIV-2 has been divided into 5 subtypes (A-E). India predominantly has HIV-1M subtype C. Subtypes A and B are less frequent. However western developed countries have HIV - 1M subtype B as predominant subtype. Subtype C is usually acquired by heterosexual contact and subtype B by homosexual contact

### Structure

HIV is 120 nm icosahedral, enveloped, RNA virus. HIV comprises of an outer envelope consisting of a lipid bilayer with uniformly arranged 72 spikes or knobs of gp 120/gp140 and gp 41/gp36 in HIV 1 and HIV 2, respectively. Glycoprotein (gp) 120/140 protrudes out on the surface of the virus and gp 41/36 is embedded in the lipid matrix. Inside is the protein core surrounding two copies of RNA. Core also contains viral enzymes reverse transcriptase, integrase and protease, all essential for viral replication and maturation. Proteins p7 and p9 are bound to the RNA and are believed to be involved in regulation of gene expression.

## Genetic structure

The genetic structure of virus contains both highly conserved and highly variable regions. The high variability of the virus accounts for drug resistance and evasion from immune response. This also poses problems for development of a successful vaccine. In an infected individual, quasispecies of a particular viral subtype may be found on account of constant variability. Also variability has resulted in a no of recombinant strains circulating in the population (e.g. AE< AG<AGI< and AB, etc.)

HIV has structural and regulatory genes coding for structural and regulatory products, respectively. Structural genes direct the synthesis of physical components of the virus and are also responsible for viral size, shape, structural integrity and its compartmentalization in host cell. The regulatory genes direct synthesis of proteins that effect the synthesis of level of viral components and viral replication. Structural genes are gag, pol, env and others shown in the figure are the regulatory genes. HIV also has some accessory genes like vpu, vpr and vif, etc.

## Replication

Glycoprotein 120/140 of HIV binds to a receptor/receptors on HIV permissive host cell. Predominant receptor is the CD4 molecule present on T lymphocytes and macrophages, though others such as galactosyl ceramide (gal C) have also been proposed. Receptors are molecules (proteins and or glycoproteins) present on the surface of host cells which facilitate the attachment and entry of viruses in to the cell. Entry of virus into the host cell requires certain cellular coreceptors/factors e.g. CCR-5, CXCR-4, CCR-2 and CCR3 etc designated collectively as cell infectivity factor (CIF). CIF may be a coreceptor or enzyme helping in virus interaction with host cell. Most convincing candidate is the chemokine receptor related protein, fusin (CXCR-4). Once the gp41/36 of the virus fuses with the host cell membrane the capsid is uncoated and a ribonucleoprotein complex capable of reverse transcription is formed. During the process of reverse transcription cDNA is formed under the effect of viral enzyme, the reverse transcriptase. Reverse transcription is inefficient in quiescent cells suggesting the involvement of host components in the process. The nucleoprotein complex formed after transcription comprises of linear double stranded DNA, the gag matrix (MA) protein, the accessory vpr protein and the viral integrase (IN). This is called preintegration complex and is transported into the host cell nucleus. IN mediates a complex series of enzymatic steps and integration occurs at cellular loci with open chromatin structure. Integration probably is an essential step for viral replication. The integrated virus is called provirus. The virus may not be expressed in many cells and is considered latent. Virus expression can be stimulated by many viral, cellular and exogenous factors. Other, co-existent viral infections e.g. cytomegalovirus, Herpes virus, etc. can make the non permissive cells permissive. Maturation of virus also takes place after virus assembly and budding.

### HIV Replication - summary

- gp 120/140 binds to host cell receptors
- Reverse transcription
- Proviral DNA synthesis
- Integration with host cell DNA
- Viral proteins synthesis

- Virus assembly and budding
- Maturation of core-proteins

### Heterogeneity/genetic diversity

Studies conducted on different isolates of HIV revealed that the HIV is highly heterogeneous in a variety of biological, serological and molecular features . These include

- Level of virus production
- Replication kinetics in vivo and in vitro
- Cellular tropism (types of cells infected)
- Cytopathicity
- Syncytium forming ability
- Latency and inducibility
- Sensitivity to neutralizing/enhancing antibodies
- Genetic structure

There are two main types HIV-1 and HIV-2. HIV-2 which was isolated in Portugal from patients with AIDS who had come from West Africa showed sequence difference of more than 55% from earlier isolated strains of HIV, so was designated a different type status. HIV-2 sequences are closer to SIV than HIV-1. HIV-1 and to a lesser extent HIV-2 are undergoing mutation during each replicative cycle. Quasispecies are being thrown constantly into circulation of the infected individual. HIV-1 strains from different geographical areas are different genetically (>20% sequence differences). Three groups major, outlier (O) and N have been identified for HIV-1 type. Major group has been classified into different subtypes/clades A through K excluding I on the basis of genetic, geographical, molecular and biological differences as above. Each subtype again comprises of genetically heterogeneous strains on account of high rate of variability of the virus even in the same host. Heterogeneity of virus has implications for development of a successful vaccine and a therapeutic agent.

### Human cells / cell lines and tissues susceptible to HIV

HIV practically multiplies in all cells but the extent of replication varies in different cells :

#### Haematopoietic system

T lymphocytes, B lymphocytes, Macrophages, NK cells, Megakaryocytes, Dendritic cells, Promyelocytes, Stem cells, Thymic epithelium, Follicular dendritic cells.

#### Brain

Capillary endothelial cells, Astrocytes, Macrophages, Microglia, Oligodendrocytes, Choroid plexus, Ganglia, Neuroblastoma cells, Glioma cell lines and Neurons.

#### Skin

Fibroblasts and Langerhans cells.

#### Bowel

Columnar and goblet cells, Enterochromaffin cells and Colon carcinoma cells.

#### Others

Myocardium, Renal tubular cells, Synovial membrane, Hepatic sinusoid epithelium, Hepatic carcinoma cells, Kupffer cells, Pulmonary fibroblasts, Foetal adrenal cells, Adrenal carcinoma cells, Retina, Cervix epithelium (?), Prostate, Testes, Osteosarcoma cells, Rhabdomyosarcoma cells, Foetal chorionic villi, Placental trophoblast cells.

### Mechanism of cell death

- Increase in cell permeability due to budding of virus. Virus punches holes and kills the cell.

- Increase in cell permeability due to toxic effects of virus replication.
- Syncytia formation - involving uninfected cells.
- Apoptotic cell death of activated T cells.
- Autoimmune phenomenon involving CD4 molecule.
- ADCC i.e. antibody dependent cell cytotoxicity.

## Susceptibility of HIV

Fortunately HIV is a very fragile virus. It is susceptible to heat, a temperature of 56°C for 30 minutes or boiling for a few seconds kills the virus. Most of the chemical germicides used in hospital/laboratories and health care settings kill HIV at much lower concentrations. Thus 0.5% to 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, acetone, ether, beta propiolactone (1:400 dilution) and sodium hydroxide (40 m Mol/litre) inactivate the virus.

Sterilization and disinfection - summary of methods which kill all microorganisms including HIV .

### Sterilization

- Autoclaving at 121°C, 15 lbs pressure for 20 minutes.
- Dry heat 170°C for 1 hr.
- Boiling for 20-30 minutes

### Chemical disinfection

- Sodium hypochlorite : 5gm/litre. (0.5 to 1% ordinarily, 5-10% for high organic matter content e.g. discarding tissues etc.)
- Calcium hypochlorite : 1.4 gm/litre.
- Chloramine : 20gm/litre  
(Available chlorine 0.1%)
- Ethanol : 70%
- Formalin : 3-4%
- Glutaraldehyde : 2% for 30 minutes
- Polyvidone iodine (PVI)

## Transmission

Risk factors for HIV infection include multiple homosexual or heterosexual partners; contaminated blood transfusion; injections with contaminated needles and syringes and infected mother to foetus/infant, (before, during or shortly after birth). The efficiency of transmission of HIV is determined by the amount of virus in a body fluid and the extent of contact. High concentrations of free infectious virus and virus infected cells have been reported in blood, genital fluids

and cerebrospinal fluid. Breast milk and saliva yield varying numbers, whereas, other body fluids have a low viral content. High levels of virus are always associated with symptoms and advanced disease.

Saliva in adults contains some nonspecific inhibitory substances like fibronectins and glycoproteins which could prevent cell to cell transfer of virus. Thus, saliva is not a likely vehicle of transmission. Urine, sweat, milk, broncho-alveolar lavage fluid, amniotic fluid, synovial fluid, faeces and tears have been reported to yield zero or a few HIV particles. Hence, these vehicles also do not appear to be important in virus transmission.

Breast milk at the time of primary infection in a feeding mother has a high content of virus and may transmit the



infection to the baby. Cerebrospinal fluid (CSF), on the other hand, also has a high content of virus particularly in individuals with neurological disease, but, CSF is not a natural source of virus transmission.

**Table 1. Efficiency of different routes of HIV transmission and their contribution to total number of cases reported in India.**

Exposure Route	Percent Efficiency (World over)	Percentage of total (World over)	( India)
Blood transfusion	90-95	5	2.5
Perinatal	20-40	10	
Sexual intercourse	0.1 to 1	75	86
Vaginal	0.05-0.1	(60)	85 (heterosexual)
Anal	0.065-0.5	(15)	0.548(homosexual)
Oral	0.005-0.1	case reports only	
Injecting drugs use	0.5-1.0	10	7.3
Needle stick exposure	< 0.5	0.1	0
Others			10.92

(Percentage of total in India from NACO website)

The most efficient vehicle of HIV transmission is blood (Table-1). However, the risk of infection via blood transfusion is now extremely low due to strict HIV screening of donated blood. The most common route of transmission is unprotected, penetrative sexual contact. Different forms of sexual practices carry a variable risk gradient of acquiring HIV. Cell associated rather than free virus is responsible for disease transmission. Anal intercourse carries a high risk of transmission because of bowel mucosa which acts as a portal of entry for virus, and also because of a greater chance of injury to the mucosa. Risk to insertive partner is through infection of lymphocytes and macrophages in the foreskin or along the urethral canal. In females HIV transmission occurs when infected cells in the semen gain entry into the female genital tract, and infect the resident lymphocytes, macrophages and probably the uterine epithelial cells.

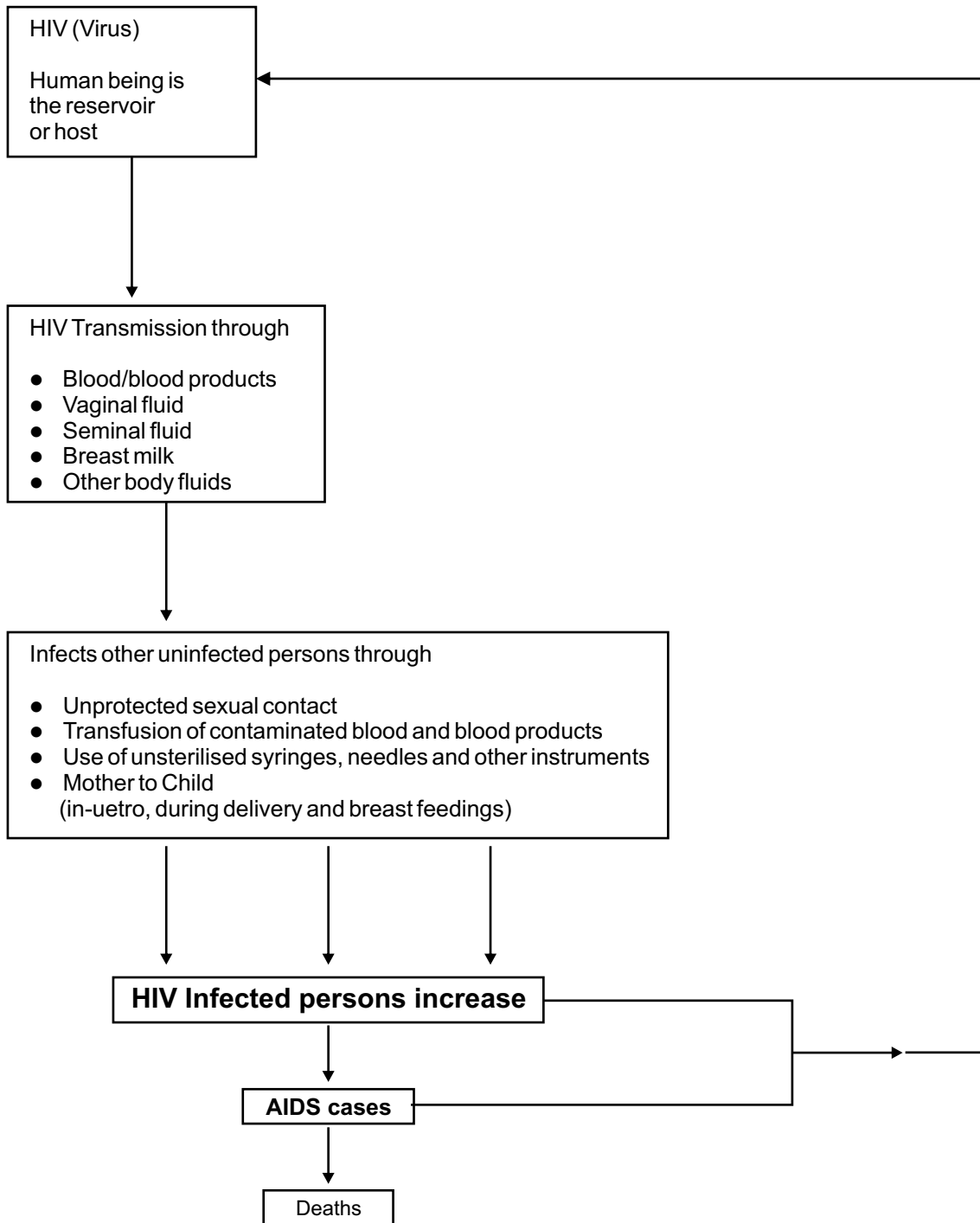
The transmission from infected mother to child appears to occur in 20-40% children born to HIV positive mothers. The source of virus in the newborn is controversial. HIV infection can occur via amniotic fluid, genital secretions, maternal blood and through the breast milk. Transmission to the baby can occur in-utero, and during or after delivery.

Transmission of HIV infection to Health Care Workers (HCW) is extremely uncommon. Pooled data from 20 prospective studies suggests that risk associated with needle injury from HIV infected blood is approximately 0.3 percent. Further, the risk associated with mucocutaneous contact is too low to be reliably estimated. The risk from mucosal or non-intact skin is also minimal.

Fig. 1 depicts the different routes of transmission of HIV.

So far, there has been no report of HIV transmission through a casual social contact, by the enteric or respiratory route, and through an insect e.g. mosquito bite. Prospective studies offer a conclusive evidence that family members and close household contacts of HIV infected individuals are not at risk of acquiring HIV infection through casual human contact (shaking hands, kissing and by sharing of utensils, toilet, linen, bed, etc.) or by providing routine nursing care.

Fig. 1 Transmission cycle of HIV



Transmission of HIV through casual contact, sharing utensils, lavatories and through insect bites has not been documented so far

### 3. IMMUNOPATHOLOGY

#### Introduction

To understand the effects of HIV infection a reminder of body defence is a must. T Lymphocytes and B Lymphocytes together defend the body against all kinds of assaults the body is exposed to, at all times. Both T and B cells migrate from bone marrow, but T cells mature in thymus, where they develop special functions. Both kinds of lymphocytes when activated by antigen, multiply and change. B cells develop into plasma cells, produce specific antibodies which trap and kill microorganisms (bacteria mostly). This is “humoral immunity”. T lymphocytes are responsible for cell-mediated immunity, a very important defence against fungi, protozoa, mycobacteria and viruses. T Lymphocytes include helper T cells (CD4) and cytotoxic/killer T cells (CD8). They release soluble factors lymphokines (cytokines), can regulate humoral suppressor cells and can become memory cells. So, CD4 cell population is central in defending the body. It is the key cell and this is the cell that HIV infects and destroys progressively. Infection with HIV irrespective of type (HIV-1 or HIV-2) and route of infection leads to protracted disease and depletion of CD4 cells in most cases resulting in AIDS. The rate of progression of disease depends upon viral characteristics on one hand and host factors on the other hand and may take from 1 year to more than 15-20 years.

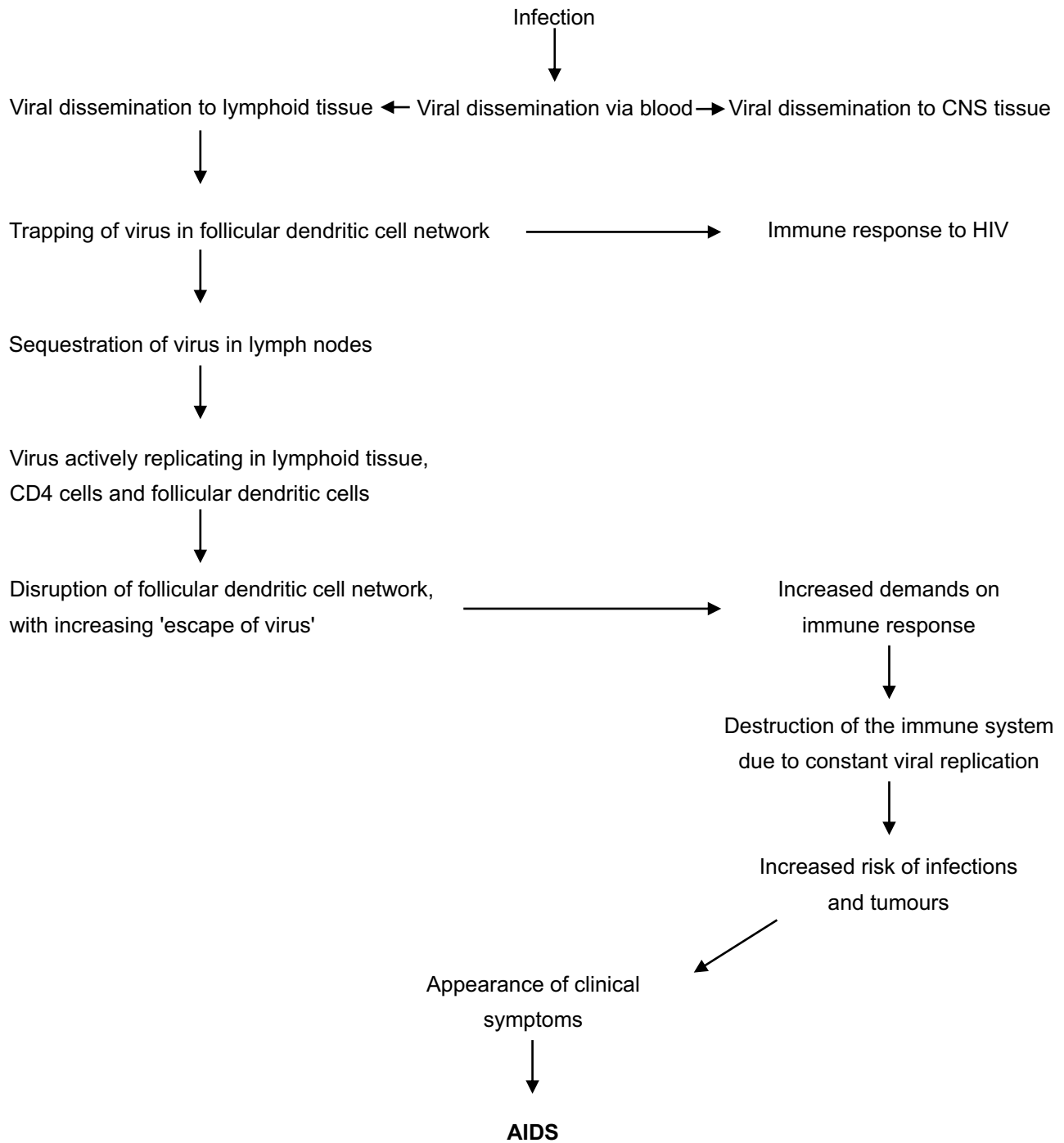
#### Viral entry

Cell free or cell associated HIV enters the body during high risk practices through any route via blood, semen and vaginal secretions from an infected person. HIV infection is facilitated by presence of ulcerative and to a lesser extent nonulcerative sexually transmitted infections. HIV immediately targets on to cells displaying complementary receptors (CD4, CCR-5 and CXCR-4/fusin) which may be CD4 cells, resident macrophages or Langerhans cells depending upon the site of exposure. Virus gp120/140 fits on the receptor like a lock and key system. Viral replication starts immediately after entry into the cell and dissemination occurs through circulatory and lymphoid systems.

#### Primary HIV Infection

During this stage HIV and HIV infected cells reach the lymph nodes and the other lymphoid tissues, where active immune response to viral antigens occurs and at the same time intense replication of virus occurs in activated T lymphocytes. This is a paradox because lymphocytes are activated on account of infection and HIV replicates better in these activated cells. The peak in number of virus expressing cells and spread of virus throughout the lymphoid tissue precedes the increase in plasma viraemia i.e. the virus in the blood. The virus spills over from lymph nodes. These phenomena occur during the first 2-3 weeks after infection and there is intense virus spreading during this period so this is called the stage of virus dissemination. Clinically it coincides with “flu like illness” also known as acute HIV disease. There is significant fall in CD4 cells and viral levels may be high. The next stage is that of down regulation of viraemia. This coincides with robust, intense immune response by the host. Both effective cell mediated immune response carried out by HIV specific cytotoxic T lymphocytes (CTL) and humoral response carried out by complement fixing and neutralizing HIV specific antibodies comes into play. The period from the entry of HIV in the host and the appearance of detectable levels of HIV specific antibodies is called “window period”. During this period individual is infected, is infectious to others but is seronegative i.e. HIV tests for detecting antibodies are negative. Window period ranges from 3 weeks to 3 months on an average, can be longer sometimes. Virologic tests like PCR

Fig. 1 Viral pathogenesis and immune response



and p24 antigen detection may be positive during this period. Both HIV specific antibodies and CTL kill the virus infected cells. As a result the viraemia drops and CD4 cells bounce back to levels slightly lower than the previous normal level. Most of the virus trapping and killing occurs in Follicular Dendritic Cells (FDC) of the lymph nodes and lymphoid tissue. This may be one reason of generalised lymphadenopathy seen in HIV disease. Appearance of neutralizing HIV specific antibodies heralds the transition from acute to chronic stage of HIV disease. Although the immune response succeeds in downregulating the viraemia, HIV is never completely eliminated and progression to chronic phase of HIV disease occurs in most cases. What determines the progression of the HIV disease is the quality of T cell response. This may be genetically determined.

Fig. 1 depicts the course of events following viral entry in the body

**Primary infection summary**

- Dissemination of HIV to lymph nodes and other lymphoid organs
  - Viraemia
  - Window period
  - Acute HIV disease
  - CTL response
  - Antibody response
- Acute glandular fever like syndrome  
 Acute meningoencephalopathy (less common)
- 

**Clinically latent period/chronic illness**

This stage is marked by disappearance of symptoms of acute viral disease, down regulation of viraemia, CD4 cell count becomes almost normal and the neutralizing and complement (C1) fixing virus specific antibodies appear in the blood. All virological parameters in the peripheral blood (viral RNA copies/viral load, virus expressing mononuclear cells, etc.) are very low. However, active and continuous virus replication goes on in the lymph nodes and lymphoid organs which express virus 1-3 logs higher than the peripheral blood. As long as the CD4 counts are higher than 500 cells/µl, the immune response mounted by the lymphoid tissues is effective, there is follicular hyperplasia of germinal centres indicating immune activation of lymph nodes. The important paradox to note is that cellular activation seen in lymph nodes is critical for viral replication i.e. virus replication is better in activated CD4 cells. There is gradual reduction in number of CD4 cells and increase in virus load during the long asymptomatic stage. Increase in virus load in peripheral blood indicates failure of and progressive deterioration of effective immune response

Humoral immunity is intact during the asymptomatic stage that is specific antibodies are produced against different viral proteins, but the antibodies are not protective, are not able to interfere with cell to cell transmission and infectivity of virus on account of constant variation of virus. This period on average lasts for 8-10 years. Progressive impairment of HIV specific and nonspecific cell mediated and humoral immune responses heralds the onset of AIDS. The CD4 cell counts range between >200 to 500 cells/µl in peripheral blood.

**AIDS**

Advanced stage of HIV infection is characterised by increase in all virological parameters (virus load, p24 antigen etc.) in both peripheral blood and lymph nodes. Lymphoid tissue is totally destroyed and replaced by fibrous tissue. Virus

trapping by whatever lymphoid tissue remains is minimal or nil. There is profound immune suppression and opportunistic infections may prove fatal at this stage. The CD4 count is usually less than 200 cells/ $\mu$ l and progressively falls.

### Points to Ponder

Infection with HIV is lifelong .

- Severe immunodeficiency develops in infected persons within 10-12 years on average.
- Consequent to sufficient immune damage, susceptibility to opportunistic infections and cancers increases.
- Cancers and opportunistic infections act as surrogate clinical indicators of AIDS.
- Survival after diagnosis of AIDS is short, 1-2 years on an average in absence of antiretroviral treatment.

### Mechanism of CD4 cell depletion and dysfunction

CD4 cells are the main targets of HIV and progressive destruction of these cells is characteristic of all stages of HIV disease. CD4 cells serve as surrogate markers to monitor the progression of HIV infection. These cells can be destroyed by two mechanisms :

- i) Direct damage by the virus.
- ii) Immune mechanisms triggered during the course of HIV infection.

HIV can kill cells singly or after giant cell and syncytia formation. Single cell killing occurs due to accumulation of unintegrated viral DNA and inhibition of cellular protein synthesis. Syncytium formation is induced by virulent strains of HIV in a multistep mechanism. CD4 cells expressing viral antigens on the surface attract CD4 uninfected cells and the membranes of these fuse producing giant cells and syncytia. One such HIV infected cell can eliminate hundreds of uninfected cells by syncytium formation. Glycoprotein 120 and other intracellular adhesion molecules bring about the cellular adhesion and subsequent damage.

The non virologic mechanisms which can damage/destroy CD4 cells include autoimmune mechanisms, anergy, superantigens, apoptosis (programmed cell death) and virus specific immune responses. A number of hypothesis and complex immune mechanisms have been postulated for CD4 cell depletion involving one or more of above mentioned pathways.

### The course of progression HIV infection

Three dominant patterns of HIV disease progression have been described. These are based on the kinetics of immunologic and virologic events described above.

- i) 80%-90% of HIV infected are "typical progressors" with a median survival time of 10 years, approximately.
- ii) 5% to 10% of HIV infected individuals are "rapid progressors" with a median survival time of 3-4 years approximately.
- iii) About 5% of HIV infected individuals do not experience disease progression for an extended period of time and are called "long term non progressors" (LTNPs).

## Typical progressors

The typical course of HIV infection includes three phases: primary infection (seroconversion), clinical latency and clinically apparent disease.

Primary phase may be totally inapparent or may be associated with acute flu like or mononucleosis like syndrome in 50% to 70% individuals. This occurs within 3-6 weeks of infection and may last for 9-12 weeks. There is high level of virus in the blood. The course of HIV disease is as described above. Progression of HIV infection to AIDS on average occurs in 8-10 years approximately.

## Rapid Progressors

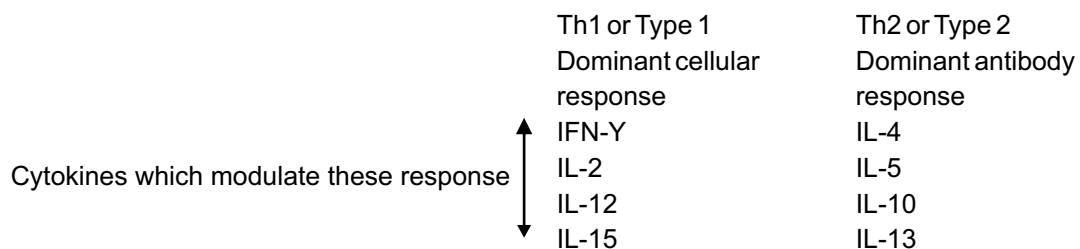
In about 5% to 10% HIV infected rapid progression to AIDS occurs within 2-3 years after seroconversion. Immune response is defective in these individuals. Levels of neutralizing and C1 fixing HIV specific antibodies are low and CD8 cell mediated suppression of HIV replication is 'impaired'. As a result progression to AIDS is rapid in these individuals.

## Long term non-progressors (LTNPs)

A small percentage (5%) of HIV infected individuals do not experience clinical progression of HIV and have stable CD4 cell counts over long period without any therapy. CD4 counts stay at around  $>500$  cells/ $\mu$ l., cell mediated and humoral immune responses are comparatively strong in these individuals. Absolute number of CD8 cells is also persistently high in these persons. In addition the cytotoxic T lymphocytes retain their cytotoxic activity against HIV. The titer of neutralizing antibodies against HIV is also higher in these individuals. The virus specific parameters like virus load, virus replication in peripheral blood and lymph nodes are four fold to 20-fold lower in long term nonprogressors. Also the virus infecting these persons may be of low pathogenicity. Some host genetic factors may also be responsible for these LTNPs.

## Type 1 and Type 2 responses in HIV infection and exposure

The loss of helper cell function in asymptomatic individuals is seen even before the fall in number of CD4 cells. This is evident from in-vivo and in-vitro studies. Progression of HIV infection from asymptomatic to symptomatic stage and then AIDS involves shift of Th1 type responses (cell mediated immune responses) to Th2 responses (humoral immune responses).



Cytokine induced immune dysregulation is a central component in progression to AIDS . This shift in type of immune response and consequent progression to AIDS may be on account of the fact that HIV infects Type-1 cells easily compared to Type-2 cells. The shift in immune response is associated with shift in cytokine profile also (from Type-1 to Type-2) and progression to AIDS. This change in cytokine profile results in inability of host macrophages to get rid of opportunistic pathogens and this results in opportunistic infections and neoplasms which ultimately prove fatal.



## 4. BIOSAFETY IN LABORATORIES

### Introduction

Since the advent of Acquired Immuno-deficiency Syndrome (AIDS) epidemic, extraordinary efforts all over the world have been made to prevent laboratory acquired infections with Human Immuno-deficiency Virus ( HIV).The recent statistics published by CDC relative to transmission risks for HIV and Hepatitis B virus ( HBV) are that the risk for acquiring HIV following needle stick prick (per-cutaneous exposure) from a source patient with HIV is very low (0.25 - 0.3%). This is because the virus generally circulates at a much lower concentration (10-100 infectious doses /mL) and because it is not able to survive as well in the environment outside the body. In contrast, acquiring HBV infection following needle stick prick from a HBV carrier ranges from 9 - 30% as HBV is found in very high titres (>10,000,000 infectious doses /mL) in the blood of many acutely and chronically infected individuals and can survive for long periods in dried blood at room temperature. The chance of acquiring Hepatitis C virus (HCV) is 3 - 10%. The risk of transmission of HIV through muco-cutaneous exposure is 0.05%. In different studies carried out in the U.S., there were 56 documented sero-conversions in the context of occupational exposure up to June 2000. Most of these were as a result of per-cutaneous exposure, some were on account of muco-cutaneous exposures, very few had history of both and some had unknown type of exposure. Globally, it is reported that there 98 confirmed and 194 possible cases of HCP infected occupationally. The risk of occupational HIV transmission depends on :

Prevalence of infected individuals in the patient population

- The frequency of exposure to contaminated medical instruments/blood and body fluids
- The relative infectivity and concentration of the virus
- Volume of blood involved in the exposure
- Depth of needle stick injury
- Type of needle causing Injury solid/ hollow bore needle
- Source patient asymptomatic/symptomatic with AIDS
- Exposure with device visibly contaminated with blood
- Whether PEP was taken within the recommended period

In India, there is no published data available on HIV transmission due to needle stick injury / muco-cutaneous exposure in health care setting. There is just one published report of transmission of HIV to a HCW following occupational exposure with a stillite visibly contaminated with blood from source patients vein. The lack of proper infrastructure, low levels of awareness about safety precautions and a certain degree of complacency amongst laboratory staff are some of the concerns for the laboratory managers.

### Importance of biosafety practices

There is no vaccine for prevention of HIV/ AIDS, treatment is expensive and is lifelong. Hence, prevention of infection is the cornerstone of control of this epidemic. In the health care settings also, prevention is considered most important. The irrational fear of accidental infection from HIV/AIDS patients invariably influences the behaviour of the health care workers (specially those who routinely handle blood and body fluids) towards their patients and their specimens. Therefore, on the one hand, it is extremely important that the laboratory workers should be aware of the risks involved in their day to day work and apply effective infection control practices to reduce the possibility of transmission of these

(blood borne) fatal infections to a minimum. On the other hand, they should not be prejudiced and should not show discrimination towards patients of HIV/AIDS. Table 1 shows the different high risk procedures for the health care workers (H.C. P.) and the various modes of acquiring infection.

**Table 1 Modes of exposure to blood borne pathogens in the laboratory**

<b>Lab. Procedure</b>	<b>H.C.P. at Risk</b>	<b>Source/Modes of transmission</b>
Collection of blood/body fluids	Laboratory technician/Nursing staff	Needle stick injury, Broken specimen container, Blood contamination of hand with skin lesion/breach.
Transfer of specimen	Laboratory technician and transport worker	Contaminated exterior of the container/requisition slip.
Processing of specimen	Laboratory personnel	Puncture of skin or contamination of skin/mucous membrane from - Contaminated work surface - Spill/splash of specimen - Broken specimen container - Faulty techniques
Cleaning/washing	Support staff	Puncture/contamination of skin from - Contaminated glassware - Sharps - Contaminated work surface
Disposal of waste	Laboratory personnel support staff	Contact with infectious waste specially sharps
Transport of specimen to distant laboratory	Transport/Postal staff	Broken/leaking container

Bio safety in a HIV testing laboratory may be discussed under the following headings:

- Precautions in relation to blood and body fluids Standard Work Precautions
- Effective use of sterilisation and disinfection
- Safe disposal of hospital waste

**Standard blood and body fluid precautions for laboratory workers**

Although the risk of transmission of infection due to blood borne pathogens in the laboratory cannot be eliminated altogether because chances of accidents are there, application of universal precautions minimise these to a large extent. Standard work precautions refer to precautions consistently used for all patients all the time while providing care services, regardless of their blood borne infection status. Under standard precautions, all patients and blood and body fluids of all patients are considered potentially infectious for HIV, HBV, HCV and other blood borne pathogens.

Similarly, all instruments and other equipments that have come into contact with blood are assumed to be potentially contaminated with blood borne pathogens and must be properly handled, cleaned and sterilized/ disinfected or safely disposed of. Standard precautions are intended to prevent accidental parenteral, mucous membrane and nonintact skin exposures of health care workers to the above blood borne pathogens in patients. Routine and /or mandatory testing of patients for HIV antibody or HBsAg is not an effective strategy for controlling the transmission of these infections in health care settings.

Body fluids to which standard precautions apply

- Blood is the single most important source of HIV, HBV and other blood borne pathogens in the occupational setting
- Other potentially infectious body fluids (especially those containing visible blood)
  - Semen & vaginal secretions
  - Cerebrospinal fluid
  - Synovial fluid
  - Pleural fluid
  - Peritoneal fluid
  - Pericardial fluid
  - Amniotic fluid

The risk of HIV transmission is extremely low or negligible with other body fluids/secretions/excretions unless these contain visible blood. These include:

- Faeces
- Nasal secretions
- Sputum
- Sweat
- Tears
- Urine
- Vomitus

Standard precautions include:

- i) Barrier protection
- ii) Hand washing
- iii) Safe techniques
- iv) Safe handling of sharp items
- v) Safe handling of specimen (blood, etc.)
- vi) Safe handling of spill of blood/body fluid
- vii) Use of disposable/sterile items
- viii) Safe techniques including mechanical pipetting device
- ix) Immunisation with Hepatitis B vaccine

### **Barrier protection**

Protective barriers reduce the risk of exposure of the laboratory worker's skin or mucous membrane to potentially infective materials including blood and the body fluids.

### **Gloves**

Can reduce the incidence of contamination of hands but cannot prevent penetrating injuries by needles and other sharp instruments.

#### **Gloves should be:**

- Worn while collecting/handling blood specimens, blood soiled items or whenever there is a possibility of exposure to blood or body fluids containing blood.
- Worn while disposing laboratory waste
- Well fitting, disposable, made of vinyl and must be changed if visibly contaminated with blood/breached
- Heavy duty general purpose rubber gloves for washing infected glassware/sharps. These utility gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, discoloured or if they have puncture, tears, etc.
- Removed before handling door knobs, telephones, pens, performing office work and leaving the laboratory.

### **Laboratory gowns**

- Laboratory gowns or uniforms (preferably wrap-around gowns), front closed should be worn when in the laboratory and should be removed before leaving the laboratory.
- Plastic aprons should be used while cleaning infected reusable articles and during disposing waste.

### **Facial protection**

- Simple and cheap deflector masks and protective glasses may be worn if splashing or spraying of blood/body fluids is expected.

### **Occlusive bandage**

- All skin defects e. g. cuts, scratches or other breaks must be covered with water-proof dressing before patient care.

### **Hand washing**

This is an ideal safety precaution and is one of the most important in preventing HIV transmission in health care settings.

- Hands should be washed thoroughly in running water with soap without missing any area.
- Washing of hands is mandatory:  
Immediately after contamination with blood / body fluids  
After removing gowns / coats and gloves  
Before eating / drinking and leaving the laboratory
- Ideally, liquid soap dispensers should be provided to the laboratories, which should be regularly cleaned and

maintained. If not feasible, soap bars after washing should be left in a dry tray to prevent contamination with some microorganisms which grow in moist conditions

- A moisturising hand cream should be used after every hand wash
- Gloves should not be regarded as substitute for hand washing

### **Safe techniques**

- Biological safety cabinets Class 2 should be used, preferably, for handling materials containing higher concentration of infectious agent than usually present in clinical specimens (e. g. while carrying out HIV culture).
- All procedures and manipulations of potentially infectious material should be performed carefully to minimise the formation of droplets, aerosols, splashes or spills.
- Mouth pipetting should be strictly prohibited. Mechanical pipetting devices should be used for pipetting of all liquids in the laboratory.
- Centrifugation should be done in tubes with safety caps.

### **Safe handling of sharps**

- Extreme care should be used to avoid auto-inoculation.
- All chipped or cracked glassware should be discarded in appropriate containers.
- Broken glass should be picked up with a brush and pan. Hands must never be used.
- The disposable needles should never be manipulated, bent, broken, recapped or removed from syringes.
- The used sharps should never be passed directly from one person to another.
- Always one should dispose of his/her own sharps by self.
- Used needles should be discarded in puncture-proof rigid containers (plastic or cardboard boxes) after disinfection in 0.5-1% freshly prepared sodium hypochlorite solution (common bleach) and never in other waste containers. If a needle shredder/destroyer is available, only the needles or the needles along with syringe nozzle may be shredded depending upon the type of the shredder
- Sharp disposable containers should be located close to the point of use/care.
- Sharp disposal containers should be sent for disposal when three-fourth full.

### **Safe handling of specimens ( blood, etc.)**

- Specimens, especially blood and body fluids should be collected in pre-sterilised screw-capped plastic

- containers properly sealed to prevent spillage or leakage.
- Autoclaved / pre-sterilised disposable syringes and needles for venipuncture or lancets / cutting needles for finger prick should be used.
  - Cuts in hands should be properly covered with waterproof occlusive bandages.
  - Disposable gloves should be worn while collecting blood / body fluids and proper asepsis should be maintained.
  - If a sample container shows evidence of breakage (in case not collected in the above container), leakage or soiling, it should be transferred with a gloved hand into a second sterile container. Any pertinent information should be rewritten from the old to the new container.
  - If the requisition slip is contaminated with blood, it should be rejected. In case of emergency, the contaminated slip may be handled using gloves.
  - Hands should be thoroughly washed with soap and water particularly after handling specimens.
  - Blood and other specimen containers should be labelled with a special warning sign "Biohazard Precaution". If the outside of the container is visibly contaminated with blood it should be cleaned with disinfectant. All blood specimens should be placed in small leak-proof impervious plastic tubes for transportation to the laboratory.

#### **Safe Handling of blood/body fluid spills**

In case of a spill of blood / body fluid in the laboratory, wear gloves and cover the area with adsorbent material and then flood with a disinfectant solution. e. g. freshly prepared 10% Sodium hypochlorite solution and leave for 30 minutes. Wearing gloves, lift the material and place in discards bucket and thoroughly mop the area with soap and water. All contaminated materials should be disposed of as infectious waste.

#### **Hepatitis B vaccine**

Appropriate use of HBV vaccine plays a key role in prevention of transmission of HBV from patient to health care worker. Therefore it is important that all the HCW's including the laboratory workers should be immunized as recommended by WHO.

#### **Laundry and linen**

Although soiled linen has been identified as a source of large numbers of certain pathogenic organisms, the risk of actual disease transmission is negligible.

Soiled linen may be handled as little as possible and with minimum agitation to prevent gross microbial contamination of the air and of persons handling the linen.

All soiled linen must be handled with gloved hands and if feasible, decontaminated in 0.5- 1% sodium hypochlorite in the laboratory before sending to the laundry.

Soiled linen after decontamination, should be put in heavy plastic bags which are tied and sent to the laundry. In the laundry, decontamination in bleach is recommended in case not done earlier. This should be followed by washing in hot water (>70°C) with detergent. Usual wash cycles in washing machines kill HIV.

Management of Accidental Exposure to HIV in the Laboratory: Accidental exposure and post-exposure prophylaxis is discussed in a separate Chapter 18.

## General biosafety guidelines for laboratory workers

- Access to laboratory should be limited at the discretion of laboratory- in-charge when experiments are in progress. The door should have a symbol of BIOHAZARD:NO ADMITTANCE. Laboratories should be well ventilated so as to ensure that the personnel do not breathe in contaminated air.
- Eating, drinking, smoking and application of cosmetics are prohibited in the laboratory. Sandals and open style shoes do not afford proper foot protection and are not to be used. Contact lenses especially the soft ones should not be worn.
- Laboratory and work tables should be scrupulously cleaned with liquid detergents and disinfectants. Laboratory work surface should be decontaminated once a day after completion of day's activity and immediately after spill of viable material with disinfectant.
- Biological safety cabinets and other primary containment devices e.g. centrifuge safety caps should be used whenever handling hazardous specimens and when it is likely to produce aerosols or infectious droplets.
- Blood and other specimen containers should be labelled with a warning "biohazard" sign. The outside of the specimen container should be cleaned with sodium hypochlorite solution in case of visible contamination.
- Gloves should be worn while dealing with blood specimens, blood-soiled items, body fluids. excretions, secretions, surface materials and objects exposed to them.
- Gowns / laboratory coats must be worn while working with potentially infective materials and removed before leaving the laboratory.
- Hands should be washed immediately after contact with blood and before leaving the laboratory.
- Mechanical pipetting devices should be used. Mouth pipetting is strictly prohibited. Careful techniques should be followed to minimise the creation of aerosols.
- Accidental wounds from sharp instruments should be avoided. Extreme caution is warranted when handling needles and sharps to avoid auto-inoculation. Needles should be promptly placed in a puncture resistant container immediately after decontamination.
- Paper work should not be done on potentially contaminated surface.
- All potentially contaminated materials and wastes from the laboratory should be disposed after decontamination preferably by autoclaving. A label with a globally accepted biological hazard sign should be applied.

## Effective use of sterilization and disinfection

### Definitions

Cleaning is a process which removes foreign material (e.g. soil, organic material, micro-organisms) from an object.

Disinfection is a process which reduces the number of pathogenic micro-organisms, but not necessarily bacterial

spores from inanimate objects or skin, to a level which is not harmful to health.

High level disinfection is often used for a process which kills Mycobacterium tuberculosis and enteroviruses in addition to other vegetative bacteria, fungi and more sensitive viruses.

Sterilization is a process which destroys all micro-organisms including bacterial spores.

The level of decontamination should be such that there is no risk of infection when using the equipment.

### ***Classification of infection risk from equipment or environment into three categories and suggested levels of decontamination.***

Low risk : Items in contact with normal and intact skin, or the inanimate environment not in contact with the patient (e.g. walls, floors, ceilings, furniture, sinks and drains). Cleaning and drying is usually adequate except when there is spill of blood/ body fluids.

Intermediate risk : Equipment which does not penetrate the skin or enter sterile areas of the body but is in contact with mucous membranes or non-intact skin, or other items contaminated with virulent or transmissible organisms (e.g. respiratory equipment, gastrointestinal endoscopes, vaginal instruments, thermometers). High level disinfection is usually adequate.

High risk : Items penetrating sterile tissues, including body cavities and the vascular system (e.g. surgical instruments, intra-uterine devices, vascular catheters, syringes and needles etc.). Decontamination followed by cleaning and sterilisation is required. High level disinfection may sometimes be appropriate if sterilisation is not possible.

## **Methods**

### ***Cleaning of glassware***

- Thorough cleaning and drying with detergents and water remove most organisms from an object/surface and should be carried out meticulously before sterilization.
- It may be manual or mechanical (ultrasonic cleaners or washers/ disinfectors). For manual method all contaminated items after prior disinfection should be dismantled before cleaning. Cold water is preferred as it will remove most of the protein materials (blood, sputum, etc.). The most simple, cost effective method is to thoroughly brush the item keeping the brush below the surface of the water to prevent the release of aerosols. The brush should be decontaminated after use and dried. The items should be rinsed finally in clean, warm water and dried. Items are then ready for use for sterilisation. Personnel handling contaminated items should wear good quality gloves for personal protection.

### ***Environmental cleaning***

- Floors, surfaces, sinks and drains should be cleaned with warm water and detergent. Routine use of disinfectants is unnecessary.
- If there is spillage of blood, body fluids or sputum, disinfection before cleaning is recommended. In high risk areas or following spillage from a known infected patient, the surface is cleaned using freshly prepared 0.5-1% sodium hypochlorite solution. Gloves should be worn. Release of chlorine gas from disinfection of large spillage can be hazardous to staff. If spillage is immediately removed, general disinfection of the room is not necessary. Thorough cleaning will suffice.



### **Disinfection**

Most of the disinfectants used in health care settings in this country, if used in proper concentration and for suitable period of time are effective against HIV. Either thermal or chemical processes are used for disinfection. Thermal disinfection is preferred, whenever possible. It is generally more reliable, leaves no residue, is more easily controlled and is non-toxic. Organic matter (serum, blood, pus or faecal matter) interferes with the antimicrobial efficiency of either method. The larger the number of microbes present, the longer it takes to disinfect. Boiling (100°C) for 20- 30 minutes (holding time) is a very simple and reliable method for the inactivation of all micro-organisms including hepatitis B virus, HIV and mycobacterium. Carefully done, it is a high-level disinfection procedure. The main use of chemical disinfectants is for heat labile equipments where single use is not cost effective. The properties of common chemical disinfectants are shown in Table-2.

### **Decontamination/disinfection of used needles and syringes**

- The needle is not detached from syringe.
- The disinfectant is aspirated into the syringe
- The needles and syringes are immersed with disinfectant horizontally in a flat tray for 30 minutes
- The disinfectant solution is discharged from the syringe and needle and rinsed with disinfectant by filling and emptying a number of times
- The disposable syringes and needles are disposed as given above
- The reusable syringes and needles are autoclaved/boiled for 30 minutes.
- Chemical disinfection should never be used as a method of sterilization particularly for syringes and needles.

### **Sterilisation**

- Sterilisation is carried out by steam under pressure, dry heat, gas or liquid chemicals.
- The choice of the methods like autoclaving, use of hot air oven etc, depends on a number of factors including type of material of the object, number and types of organisms involved and risk of infection to patients or staff.
- HIV is a fragile virus and is adequately inactivated by standard sterilisation procedures like autoclaving at 15 pounds pressure for 20 minutes at 121°C or hot air oven at 160°- 180°C for 1 hour (holding time).
- Pressure cooker (household pressure cooker or WHO/UNICEF modification) achieves 15 p.s.i. equivalents to 121°C for 20-30 minutes and may be used in small laboratories.
- Any sterilization procedure should be monitored routinely by mechanical, chemical and biological techniques.
- Sterile items should be protected against recontamination.

**Table 2 Disinfectant properties of commonly used disinfectants**

	Anti microbial activity					Other properties				
	Spore	Myco bacteria	Other bacteria	Virus		Stable	Inactivation of organic matter	Corrosive/ damaging	Irritant/ sensitising	
				Enveloped	Non.Env.					
1. Alcohol (70%)**** (1-10 min.)	None	Good	Good	Good	Moderate	Yes in closed container)	Yes (Fixative)***	Slight (Lencement)	No	
2. Chlorine release compds. (0.5-1%) (10-60 min.)*****	Good	Good	Good	Good	Good	No (< 1 day)	Yes	Yes	Yes*****	
3. Glutaraldehyde 2% (5 min - 3 hrs)*****	Good (3 hours)	Good* (20 min.)	Good (5-10 min.)	Good (5-10 min.)	Good (5-10 min.)	Moderate (14-28 days)	No (Fixative****)	No	Yes***	
4. Clear soluble phenolic (1-2%)**	None	Good to moderate	Good	Moderate	Poor	Yes	**No	Slight	Yes	

\* Less active against M.avium intracellulare

E-enveloped

\*\* Potentially toxic, not to be used in neonatal ward

NE-non enveloped

\*\*\* Poor penetration

\*\*\*\* Flammable

\*\*\*\*\* Irritant/sensitizing in high concentration

## Safe disposal of laboratory waste

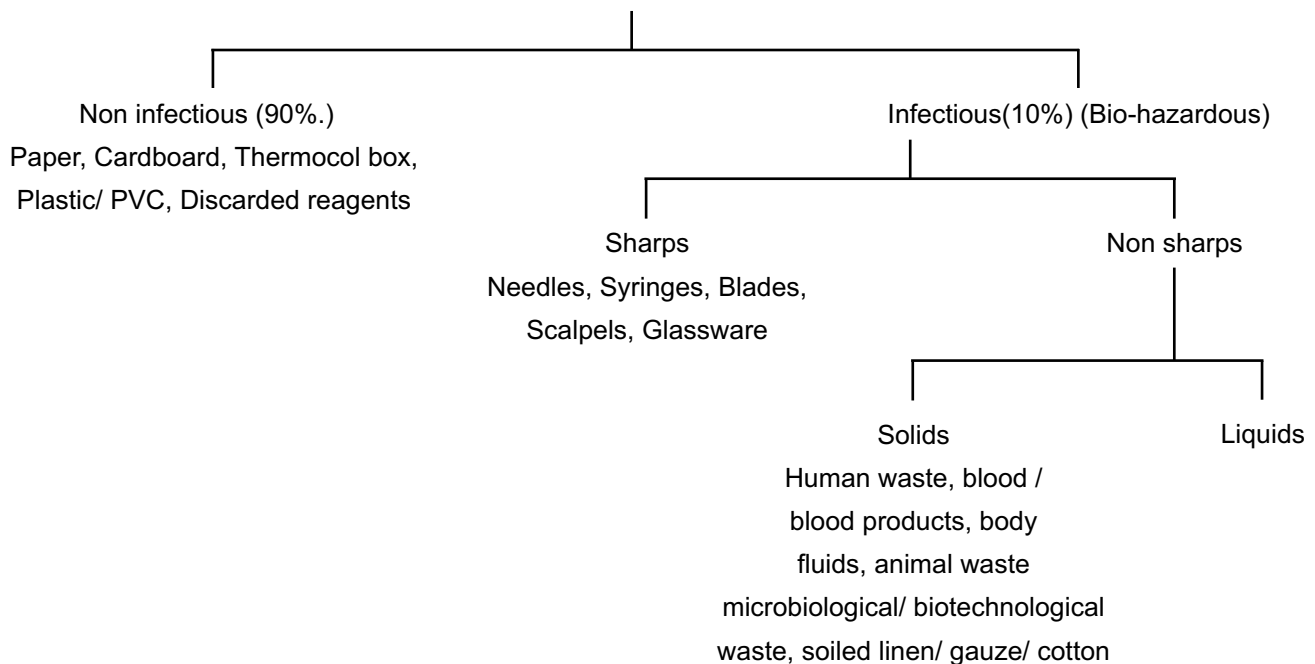
Laboratory waste is a potential hazard to HCP. Infectious waste can transmit numerous diseases in the community and put those who handle waste and live in its proximity, at risk. Besides, the increasing use of disposables in health care is also posing an additional burden on the waste management facility. It is extremely important that the recycling of these items is prevented. Only a small percentage (<10%) of the waste generated in a health care settings is infectious, remaining, approximately 90% is household type non-infectious waste. The most practical approach to the management of biomedical waste is to identify and segregate infectious waste (with a potential for causing infection during handling and disposal), for which some special precautions appear prudent. This will drastically reduce the cost of the disposal methods in health care settings.

### Setting up of a biomedical waste facility

Every hospital, nursing home, veterinary institution, animal-house, blood bank, research institute generating biomedical waste should install an appropriate biomedical waste facility in the premises or should set up a common facility in accordance with the directions given by the appropriate authority. Biomedical waste should not be generated without authorisation. Every hospital should have a waste management programme. Waste survey is an important part of the waste management programme and helps in determining both the type and quantity of waste being generated in the hospital including the laboratory and determine the feasible methods of disposal.

### Classification of laboratory wastes

Table-3 Laboratory waste



## Containing waste at generation point

At the generation point ie. the laboratory, waste is managed in the following way :

- i) Collection
- ii) Segregation and weighing
- iii) Storage

Waste segregation is the key to any waste management scheme. It consists of placing different types of waste in different containers or colour-coded-bags at the site of generation. This helps in reducing the bulk of infectious waste and contains spread of infection to general waste. This practice reduces the total treatment cost, the impact of waste in the community and the risk of infecting workers. Proper segregation should identify waste according to source and type of disposal/ disinfection.

Waste should be segregated into different categories (Table-3) at the site of generation ie. in the laboratory and weighed separately at the time the waste is being disposed.

Solid non infectious waste is collected in black bags and disposed as household waste.

Infectious waste must be separated at the point of generation itself and should be decontaminated prior to it's storage, transport and disposal. Solid infectious waste are disposed as follows:

- Sharps-
  - a) Needles and syringe nozzle- shredded in needle-destroyer (if available); if not available, decontaminated as in b.
  - b) Scalpel blades/ lancets / broken glass-should be put in separate container with bleach, transferred to plastic/ cardboard boxes, sealed to prevent spillage and transported to incinerator.
- Glass ware should be disinfected, cleaned and sterilised.
- Culture plates with viable culture should be autoclaved in plastic autoclavable bags, media are removed and collected in yellow bags and disposed of by incineration/ micro-waving/ hot air oven. The plates can be reused after sterilization.
- Swabs should be chemically disinfected followed by incineration.
- Disposable single use products (syringes, gloves, sharps etc.), as these items are often recycled and have the risk of being reused illegally, these should be disinfected by dipping in freshly prepared 1% Sodium hypochlorite for 30 minutes to 1 hour. Bins which can be used for this purpose are a set of twin bins, one inside the other with the inner one being perforated and easily extractable. This minimizes contact when the contents are being removed.

Disposable items like gloves and syringes, etc. should be shredded cut or mutilated (high temperature in hot air oven) before disposal followed by deep burial or properly accounted, before disposal. Extreme care should be taken while handling the needles.

Liquid waste generated by the laboratory are either pathological or chemical in nature and are disposed of as follows:

- Non-infectious chemical waste should first be neutralised with appropriate reagents and then flushed into conventional sewer system.
- The liquid infectious waste should be treated with a chemical disinfectant for decontamination then neutralized and flushed into the sewer.

### *Types of collection bags*

Solid wastes are collected in leak-resistant single heavy duty bags or double bags may be used. Bags having different colour codes (Table 4) with red labels mentioning date and details of waste are recommended. The bags are tied tightly after they are three-fourths full.

### *Packing, storage and transport*

- All segregated and disinfected waste should be packed in proper containers and colour-coded bags (Table 4) with red labels mentioning details of biomedical waste and biohazard signs . All containers used for storage of such waste should be provided with properly covered lids.
- Such containers should be inaccessible to scavengers and protected against insects, birds, animals and rain.
- There should not be any spillage during handling and transit of such waste.
- The waste sharps, after pre-treatment should be broken before packing in the container.
- The waste should be transported in vehicles authorised for this purpose only.
- No such waste should be stored in the place where it is generated for a period of more than two days.

### *Treatment and disposal*

#### *Disposal methods*

Disposal may be done by following method:

- Municipal corporation disposal system
- Sanitary landfill at designated site
- If incinerator is not available, deep burial in controlled landfill sites is recommended. Decontamination should be carried out before burial
- Incineration (Temp.750°C)  
Incinerator burns /reduces the infectious waste to ashes and therefore is favoured by hospitals. It may be of two types common or individual. There are some disadvantages like pollution /incomplete melting of needles. Hospitals with more than 30 beds or >1000 patients per month should have an incinerator. Plastics cannot be incinerated.

#### *Guidelines for waste disposal*

- All biomedical wastes should be treated and disposed off strictly in accordance with the options mentioned in the table 4.
- Waste which cannot be incinerated, (plastics) should be pre-treated by disinfection and disposed of in an environmentally sound manner.
- Waste should not be dumped, discharged or disposed in any place other than a site identified for the purpose.
- All precautions and personal safety measures should be taken (including provision of protective clothing, masks, gloves, gumboots, goggles, etc. as may be necessary). Hepatitis B vaccine is recommended for affording protection to all personnel engaged in handling biomedical waste, or being exposed to such wastes against infection from handling or exposure.
- All treatment and disposal facilities should be located at a specified area away from the general service area of the hospital, public places and residential areas.
- When the treatment option is burial, the pits should be located at sites away from agricultural land, residential areas, ground-and safe water sources. There should be no leakage from the pits in to surrounding areas.
- All plastics should be disinfected, shredded and disposed of in an environmentally friendly manner. Recycling of disposables eg. syringes, needles, gloves, transfusion bags etc. should be prevented.
- All liquid waste should be disinfected and flushed in the sinks at the point of generation.

- Biomedical waste should not be disposed of on open land and municipal dustbins. Untreated liquid waste should not be let into sewers.

### **Maintenance of records**

Separate records for classification of waste and their regular disposal should be maintained in the laboratory. The waste disposal programme should be supervised and monitored regularly.

### **Reporting of accidents**

In the event of an accident occurring at any location or site where biomedical waste is handled or during transportation, the appropriate authorities must be informed and needful action taken.

### **Training**

Training regarding need of and national guidelines of biosafety practices is extremely important and should be provided at regular intervals for different levels of health care workers. Guidelines for biosafety should be provided which may be modified from time to time according to the requirements.

**Table-4 Container and colour coding for disposal of bio-medical wastes**

Waste category	Waste class	Type of container	Colour coding	Treatment / Disposal option
No. 1	Human anatomical waste, blood & body fluids	Single-use containers/ Plastic holding bags	Red	Incineration / Deep Burial
No. 2	Animal & slaughter house waste	Single-use containers/ Plastic holding bags/ sacs	Orange	Disinfection & Deep Burial
No. 3	Microbiology & bio-technology waste	Single-use containers/ Plastic holding bags	Yellow	Autoclaving / Microwaving & Incineration
No. 4	Waste sharps	Re-usable/ single-use sturdy containers of plastic, glass or metal	Blue	Shredding & Deep Burial
No. 5	Discarded medicines	Re-usable/ sturdy cardboard/ glass/ plastic holding bags	Blue	Shredding & Deep Burial
No. 6	Soiled wastes (linen)	Plastic bags/ sacs	Yellow/ Black	Disinfection and machine cleaning
No. 7	Disposables (other than sharps)	Re-usable/ sturdy containers/ plastic holding bags	Yellow/ Black	Disinfection-Chemical / Autoclaving, Shredding, Burial
No. 8	Liquid wastes	NA	NA	
No. 9	Chemical wastes	Sturdy containers/ plastic holding bags	Yellow/ Black	

## Implementation of biosafety practices in laboratory

Although guidelines regarding standard work precautions and other biosafety practices are available since long, strict implementation is not in practice in healthcare settings in India even in the capital city. With increase in the prevalence of HIV infection, there is a definite need that the HCPs take biosafety practices seriously. For effective compliance, the laboratory managers should ensure adequate supply of personal protective equipment, availability of materials for handwashing, disinfectants and set up an effective waste disposal programme for disposal of biomedical wastes.

## 5. COLLECTION, TRANSPORT AND STORAGE OF SPECIMENS FOR HIV TESTING

### Introduction

Almost all laboratory procedures for HIV testing are performed on patients' blood; serum or plasma; hence the collection of blood is described below.

### Performing venipuncture:

- Gloves should be worn and sterilised /disposable syringes and needles should be used.
- For avoiding soiling, a piece of linen with a layer of dressing pad ( a sheet of absorbent cotton between two layers of gauze piece) or simply a big piece of absorbent cotton may be placed below the forearm before commencing veni-puncture.
- After collecting 3- 5 ml of blood aseptically, it should be carefully transferred from the syringe without squirting into a sterile plastic leak proof specimen container preferably screw capped. The containers should be labelled before commencement of venipuncture. The cap may be tightly screwed after the blood has been transferred to the vial.
- After blood is collected, the tourniquet is removed and the needle is withdrawn. The patient is given a dry sterile cotton swab to press over the site of venipuncture. Elbow may be flexed to keep the cotton swab in place till the blood stops. Any blood spill is carefully wiped with 70% ethanol/10% bleach solution.
- All the swabs and cotton pieces are placed in plastic bags for disposal. If the outside of the vial is visibly contaminated with blood, it should be cleaned with 10% freshly prepared sodium hypochlorite solution.
- The blood is allowed to clot for 30 minutes (not more than 2 hours) at room temperature. The clot may be gently broken if necessary using sterile pasteur pipettes.
- Alternatively vacuum based blood collection systems (vacutainers) can also be used. These vacuum based collections are relatively safe for usage and harbour minimum risk of unwanted exposure to infected blood.

### Separation of sera samples:

- The vial / vacutainer should be centrifuged at 3000 rpm for 10 minutes to separate serum to avoid haemolysis. If no centrifuge is available, the blood with clot may be left overnight in the refrigerator at 4°C. The clot will retract and get separated from serum.



- The specimen vial is un-stoppered, the serum is drawn off by sterile Pasteur pipette and transferred to a sterile plastic screw capped leak proof tube.

### Addition of preservative:

- The usual preservative should not be added since it inactivates conjugates and gives rise to false serological results.
- If necessary, 5 bromo, 5 nitro, 1-3 dioxane in propylene glycol at a final concentration of 0.05% is recommended as preservative.
- Thiomersal at a final concentration of 0.01% is effective only for a few weeks as it loses activity when exposed to light.

### Storage of serum specimens:

- The sera samples are placed in leak proof plastic containers in the refrigerator at 2-8°C, for upto 48 hours for storage. The storage of samples should be as per test requirements.
- The outside of the container is checked for visible contamination with blood which should be cleaned.
- All the specimen vials must be adequately labelled with patient details.
- Then the specimen vials are packed in a second tightly capped unbreakable container surrounded by adequate packing material (see figure 1 ahead).
- For storage for a longer time, specimens must be frozen at -20°C or deep-freezing at -70°C is advised, in labs where deep freezers are available.

### Sample transportation

These instructions are recommended for specimen transportation:

The shipment of infectious agents is regulated by the Transportation of Dangerous Goods Act and the International Air Transport Association (IATA) dangerous goods regulations. HIV infected specimens are classified as infectious class 6.2 substances under the United Nations (UN) no. 2814. The packaging must adhere to UN class 6.2 specifications. Packaging requires a 3 layer system as described below (see Fig. 1 for a diagrammatic representation):

- The specimen tube, in which serum is to be transported, should not have cracks / leakage. It should preferably be made of plastic and be screw capped. The outside of the container should be checked for any visible contamination with blood which should be disinfected.
- Place the tube containing the specimen in a leak-proof container (e.g. a sealed plastic bag with a zip lock or alternatively the bag may be stapled and taped) and pack this container inside a cardboard canister / box containing sufficient material (cotton gauze) to absorb all the blood should the tube break or leak.
- Cap the canister/box tightly.
- Fasten the request slip securely to the outside of this canister. This request slip should have all details i.e. name, age, sex, risk factors, history of previous testing, etc. and should accompany the specimen. The request slip should be placed in a plastic ziplock bag to prevent smudging on account of spillage.

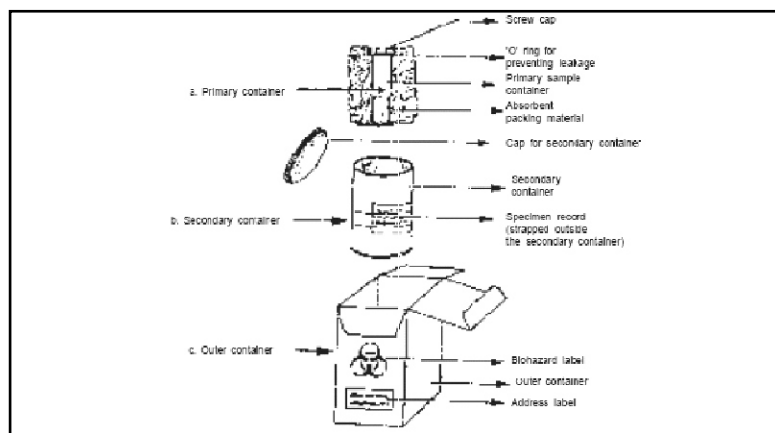
- For mailing, this canister/box should be placed inside another box containing the mailing label and biohazard sign.

The diagram ( fig 1) depicts the method of sample transport for a single/ few ( 2-3) samples that could fit into the secondary container shown in the diagram. The size of the primary sample container will vary with the number of samples being transported. For a larger number of samples ,a tube rack (or some such container ) may be used wherein the samples can be transported in the upright position and at appropriate temperature. The packaging instructions for the transport of a larger number of samples are given below:

- The specimen should be carefully packaged to protect it from breakage and insulated from extreme temperature
- Label appropriately and mention the test/s being requested for that sample. The collection site should make use of a unique identification number as sample identity. Names of the patients should be avoided to prevent confusion on account of duplication of names as well as to maintain confidentiality.
- Secure the vacutainer cap carefully and seal it further with sticking tape ( placed so that it covers the lower part of the cap and some part of the tube stem.
- During packaging, the tubes containing specimens should be placed in a tube rack and packed inside a cool box (plastic or thermocol) with cool/ refrigerated / frozen gel packs ( as appropriate to keep the sample at the recommended temperature for the test ) placed below and on the sides of the tube rack. Place some cotton or other packaging material between the tubes to ensure that they do not move or rattle while in transit.. Cool box required for transportation could be a plastic bread box or a vaccine carrier. Seal/secure the lid of the cool box .
- This cool box should then be placed in a secure transport bag for purposes of shipping to the testing facility. The request slips should be placed in a plastic zip lock bag and fastened securely to the outside of the cool box with a rubber band and sticking tape.
- A biohazard label should be pasted on the visible outer surface of the package containing the samples. The package must be marked with arrows indicating the 'up' and 'down' side of the package
- Samples should be transported to the receiving laboratory by commercial courier or be hand delivered by a trained delivery person.
- The collection site must have prior knowledge of the designated testing days of the laboratory to which the samples are being sent.
- No transport should be done during weekends and holidays or non-testing days of the testing laboratory unless prior arrangement has been made with the receiving laboratory.

Note: Use overnight carriers with an established record of consistent overnight delivery to ensure arrival of specimen within the specified time.

**Figure 1 Packaging of specimen for transport to the laboratory.**



## Safe Handling and disposal of sharps:

- Extreme care should be used to avoid auto-inoculation.
- All chipped or cracked glassware should be discarded in appropriate containers.
- Broken glass should be picked up with a brush and pan. Bare hands must never be used.
- The disposable needles should never be manipulated, bent, broken, recapped or removed from syringes.
- The used sharps should never be passed directly from one person to another.
- One should always dispose of his/her own sharps.
- Used needles should be discarded in puncture-proof rigid containers (plastic or cardboard boxes) after disinfection in 0.5-1% freshly prepared sodium hypochlorite solution (common bleach) and never in other waste containers. If a needle shredder/destroyer is available, only the needles or the needles along with syringe nozzle may be shredded depending upon the type of the shredder
- Sharp disposable containers should be located close to the point of use.
- Sharp disposal containers should be sent for disposal when three-fourth full.
- In case suitable means of disposal of syringe / needles are not available, these disposable syringes should be heated in dry ovens and be allowed to mutilate to prevent recycling of plastic syringes. Needles can be incinerated.



## 6. DETECTION OF HIV INFECTION

### Introduction

HIV/AIDS is not like other infectious diseases. It is far more complex because HIV infection cannot be diagnosed clinically in asymptomatic individuals, is life long, outcome is invariably fatal and no cure or vaccine is available so far. Since, commonly HIV/AIDS is acquired through sexual contact, individuals known to be HIV infected are stigmatized and discriminated against. A number of moral, ethical, legal and psychosocial issues are related with a positive HIV status. So, anyone attempting to assess the HIV status of an individual must be conversant with these issues, strategies/algorithms of HIV testing, protocols of testing, rationale of using test kits, correct method of informing the client, counselling, importance of confidentiality, technical and other pitfalls and quality assurance to name some. Confidentiality of a positive test result is of utmost importance. Counselling should be undertaken to motivate the individual to tell the spouse/family and induce behaviour change. In only 50-93% of cases primary HIV infection is symptomatic with a variety of symptoms ranging from influenza-like or mononucleosis-like illness to more severe neurological symptoms which can persist from a few days to as long as two months.

Acute stage is followed by a long asymptomatic phase.

Important point to understand is that laboratory diagnosis is the only method of determining the HIV infection status of an individual during the acute and the long asymptomatic period.

### Purpose of HIV testing

- Information is useful for prophylaxis, medical management and treatment of HIV and related illnesses.
- To assure blood safety and donation safety.
- To assess the efficacy of targeted intervention in a defined cohort.
- To monitor trends of epidemic (sentinel surveillance etc.).
- Identification of asymptomatic individuals (practising high risk behaviour).
- To plan personal and family's future if the result is positive.
- To motivate for behaviour modification through counselling amongst those who test negative and who practise high risk behaviours.
- To induce behaviour change and prevent transmission by counselling in those who test positive.
- To diagnose clinically suspected cases.
- For peace of mind of individuals practising high risk behaviour.

### Kinetics of humoral immune response

An understanding of the sequence of events that follow the entry of virus into the body will help to understand the optimal usage of various HIV tests during different stages of HIV disease.

Viral entry into the body leads to a transient period of plasma viraemia (usually high level) and p24 antigenaemia. However, the levels of these components come down with concomitant immune response. Humoral response is evidenced by formation of antibodies of different classes(IgM, IgA, IgG) against different structural

proteins (gag: p15, p17, p24, p55; env: gp 41, gp 120, gp 160; and pol: p31, p51 and p66), regulatory proteins (nef, rev, tat) and accessory proteins (vif and vpr and vpr). All structural components are strongly immunogenic and induce formation of antibodies, whereas, immunogenicity of regulatory and accessory proteins is variable.

The antibodies appear in blood within 2-8 wks after infection but usually become detectable after 3 wks to 12 wks with the assays available presently. This period following the entry of HIV into the body and the appearance of detectable levels of antibodies with the available test kits is called the “window period”. During this period the individual is infected, infectious and non-reactive with the antibody detection tests. The antibodies to gag protein (p24 and p55) are first to appear usually, though antibodies to env proteins and pol proteins may also be produced simultaneously. As infection progresses to AIDS, antibody to p24 usually declines as p24 antigen levels rise concomitant with progression of disease to AIDS. However, antibodies to env proteins persist throughout the infection.

Anti-HIV antibodies may be IgA, IgM and IgG. The IgA and IgM responses are inconsistent. IgG response is consistent and better understood. IgM response appears earlier than IgG but the sensitivity of assays available to detect this class of immunoglobulins is low and also IgM is detectable for a short period. Detection of IgM is valuable for identifying early seroconversion particularly following needle stick injury (2-11 days) and infection in newborn. IgA is the predominant immunoglobulin in seromucous secretions (saliva, colostrum, genitourinary secretions, etc.). Since IgA does not cross placenta and therefore detection of anti-HIV IgA by HIV-IgA assay in a newborn is diagnostic of congenital HIV infection. However, Enzyme Linked Immunosorbent Assay (ELISA) and Western Blot (WB) to detect IgA and IgM need further evaluation.

### Informed consent after pretest counselling

HIV testing for the purpose of identification of an individual must always be undertaken after pre test counselling and informed consent. Testing without informed, written and explicit consent has proven to be counter productive and has driven the HIV positive individuals underground. This makes institution of prevention and intervention measures more difficult. Pre test counselling also empowers the individual to face the HIV test result. (Refer to chapter on counselling for HIV testing).

### Confidentiality

The confidentiality of the test result (both negative as well as positive) should be strictly maintained in most cases. This is to respect the privacy and rights of the individuals and to protect them from discrimination, victimization and stigmatization. The test result, name of the individual, etc. must never be discussed loosely. The test report must be placed in a sealed envelope and submitted to the clinician who requisitioned the test. The envelope should be marked “confidential”. The records in the laboratory must also be kept secure to prevent access by unauthorised persons. The results are never communicated via telephones/fax/email etc.

### Detection of HIV specific antibodies

Detection of anti-HIV antibodies is the mainstay of testing for HIV and diagnosis of HIV.

Tests to detect specific HIV antibodies can be classified into :

- Screening tests (ELISA/EIA and Rapid)
- Supplemental tests (ELISA/EIA and Rapid and Western Blot)

Screening tests are performed to screen units of donated blood and blood products and for surveillance. These include a myriad of ELISAs which usually take 2-3 hrs to yield results. Rapid screening tests give results within minutes and include visual assays like dot-blot tests, particles (gelatin, RBC, latex, microbeads) agglutination, HIV spot and comb test and fluorometric microparticle technologies. Simple screening tests are also based on ELISA principle but take about half an hour or so.

Supplemental tests are performed on serum sample reactive in screening test for the purpose of diagnosis of the individual. When a serum specimen is reactive by any one of the screening tests it has to be tested again by a different system using different HIV antigens or different principle of test to confirm the diagnosis. If a specimen is reactive in 2 different systems it has to be tested again using one of the supplemental tests which may be a third ELISA/Rapid test or a Western Blot test (WB) as the case may be.

A healthy individual reactive in three different systems of testing is confirmed to be having HIV infection. The other supplemental tests like W.B. /IF are used to resolve discordant results of ELISA and research, as far as India is concerned as these tests are expensive, time consuming and need expertise.

Whichever commercial kit is selected, it should be ensured that it detects antibodies against both HIV-1, HIV-2 and their subtypes.

Presence of HIV antibodies indicates the individual is infected with HIV and can transmit infection to others through unsafe risk behaviour.

### Specimens to be collected for detection of anti-HIV antibodies

Most common specimen collected is blood. HIV test kits are available for detecting HIV antibodies in various kinds of specimens like blood, plasma, serum, saliva and urine. Detection of HIV antibodies in specimens like saliva and urine has not been evaluated and standardised in India.

Antibody detection:

- Blood/serum/plasma
- Saliva      ↑      not as good
- Urine        ↓      as blood

### HIV antibody screening assays

The list of the screening assays for HIV testing is given below

- ELISA (2-3 hours)
- Rapid tests (minutes)
  - Dot blot assays (immunoconcentration, vertical flow of reagents)
  - Particle agglutination
  - HIV spot and comb tests
  - Immunochromatography (lateral flow of reagents)
  - Dipstick and comb assays (based on ELISA technology)

ELISA is a commonly performed screening test. Screening assays must detect all positive sera i.e. should be highly sensitive even if some false positive results do occur. However, results of a screening test should never be used as the final interpretation of HIV status, and individual is never identified on the basis of one screening assay as technical errors can occur. The serum reactive in screening assay is subjected to confirmatory tests (as per policy and strategy of testing) to be classified as reactive only if it is reactive in repeated assays.

Other screening tests were also introduced subsequently. These include latex, red cell and gelatin particle agglutination, comb tests, line tests and dot- blot assays. These tests are easy to perform, are rapid, do not require sophisticated equipment, technical expertise and are mostly cost effective. Some of them, particularly comb tests, line tests and dot- blot assays, are also discriminatory for HIV 1 and HIV 2 antibodies.

## ELISA as the screening assay

ELISA is the most commonly performed screening test at blood banks and tertiary care sites testing a large number of specimens a day. It is easy to perform, adaptable to large number of samples, is sensitive and specific and cost effective. There is a wide variety of ELISA assays available commercially and so the appropriate test choice can be made taking into consideration the available resource, storage facility, technical expertise available, infrastructure available, objective of testing, prevalence of infection and performance characteristics of test kits etc.

### *Different types of HIV kits (based on type of HIV antigen) available commercially*

First generation of ELISA developed were very sensitive but not specific because whole viral lysates were used as antigen. These lysates usually contained small amounts of host cell components which gave rise to false positive reactions. The ELISA technologies were improved and 2nd and 3rd and fourth generation kits were developed using recombinant and synthetic peptides as antigens. So, ELISA assays available in the market may be:

- First generation kits use antigens derived from detergent disruption of viruses grown in human lymphocytes.
- Second generation kits use artificially derived recombinant antigens expressed from bacteria or fungi.
- Third generation kits use chemically synthesized oligopeptides of about 15-40 amino acids (synthetic peptides).
- Fourth generation kits use a combination of recombinant and synthetic peptides and can detect both HIV antigen (p24) and antibodies concurrently.

### *Principles of ELISA*

On the basis of the principle of the test ELISA can be divided into:

- Indirect
- Competitive
- Sandwich and
- Capture assays.

All ELISAs consist of either HIV antigen or antibody (depending upon the principle) attached on a solid phase (matrix or support) and, incorporate a conjugate and substrate detection system. Viral antigens may be whole virus lysates, recombinant or synthetic peptides. The matrix can be “wells” or “strips” of a microplate, plastic beads or nitrocellulose paper. Conjugates are most often antibodies (IgG, sometimes IgM and IgA also) coupled to enzymes (alkaline

phosphatase or horseradish peroxidase), fluorochromes or other reagents that will subsequently bring about a reaction that can be visualised. In case of enzyme conjugates the signal generated is a colour reaction and in case of fluorochrome it is fluorescence. The substrates used are 4-nitrophenylphosphate for alkaline phosphatase and o-phnyxylencdiamine dihydrochloride (OPD) and TMB for horseradish peroxidase, which produce colour on being acted upon by the respective enzymes and the colour can be either detected visually or measured on a ELISA Reader as OD values.

All kit controls as specified in the package insert of the kit must be included with each test run to validate the test. Internal controls also must be included with each run to ensure quality results. (Preparation of internal controls for practising quality assurance is detailed in the “Manual on Quality Standards for HIV Testing Laboratories”).

### **Indirect ELISA**

HIV antigens are attached covalently to the solid phase support allowing HIV antibodies present in the specimen to bind, and these bound antibodies are subsequently detected by enzyme labelled anti-human immunoglobulin and specific substrate system. If the test specimen contained antibodies colour reaction will take place.

Indirect ELISA is the most commonly used system.

### **General procedure**

The instructions given in the package insert provided in the kit are read carefully and followed to the letter. All controls as specified must be included with each test run to validate the test result.

- Dilute specimen appropriately, add to the solid phase and incubate for a specified time and at specified temperature.
- Solid phase is washed to remove unbound antibodies
- Appropriately diluted enzyme conjugate is added and incubated as specified
- Solid phase is washed to remove excess conjugate
- Substrate made appropriately is added
- Colour change produced is measured after specified time using an ELISA Reader at wavelength specified
- The result is calculated as detailed in the package insert from the various OD values obtained.

The indirect ELISA produces a colour change directly proportional to the concentration of specific antibodies in the specimen.

### **Competitive ELISA**

In this assay the HIV-antibodies present in the specimen compete with the enzyme conjugated antibodies in the reagent for binding to the antigen on the solid phase. If the test specimen contains HIV antibodies, these will compete with the labelled antibodies in the reagent for binding to antigen. So that less or not labelled antibodies can attach to the solid phase. Hence, faint or no colour is produced on addition of substrate if specimen contained HIV antibodies.



Unlike indirect ELISA, here reduction and or absence of colour indicate the presence of HIV antibodies in the test specimen. Development of strong colour means specimen is non reactive for HIV antibodies (Fig. 3).

Procedure:

Follow the instructions given in the package insert.

- A dilution of test specimen as per the kit insert and the appropriate amount of enzyme labelled HIV antibody conjugate are mixed and added simultaneously to the solid phase antigen support. The same is incubated for specified time at specified temperature.
- Plate is washed and substrate is added.
- Colour change developed is measured by ELISA reader and expressed as OD value.

Competitive ELISA takes less time than indirect ELISA and usually no predilution of test specimen is required.

### **Sandwich ELISA**

This is a modification of indirect ELISA to improve sensitivity and specificity of the test. Antigen bound to the solid phase binds antibody in the test specimen in first step. Since antibody molecules are bivalent they are still able to bind to another molecule. The next step is addition of similar enzyme labelled HIV antigen i.e. same antigen as on solid phase. This will attach to the antibody molecule which is already bound to the solid phase antigen with one arm. Thus forms a sandwich of antigen + antibody + enzyme labelled antigen complex. The next step is addition of specific substrate which results in development of colour which is measured by ELISA reader. One big advantage of sandwich ELISA is that all classes of HIV-antibodies can be detected.

The sandwich ELISA that is done to detect p24 antigen is described in Chapter on “Direct Evidences of HIV infection”.

Procedure

Same as indirect ELISA only difference being that in this case enzyme labelled antigen is added in place of enzyme labelled anti-human immunoglobulins.

### **Antigen and antibody capture ELISA**

Antigen capture ELISA can be based on principle of indirect or competitive ELISA, only difference being in the initial step of attaching antigen to the solid phase in case of indirect ELISA.

A monoclonal antibody directed against an HIV antigen is bound to the solid support. Next step is addition of HIV antigen supplied as reagent. This antigen is captured by the monoclonal antibody bound to the solid phase. Test specimen appropriately diluted is added next. HIV antibodies if present in the specimen bind to HIV antigen on solid support. Remaining principle is same as indirect ELISA.

Only advantage of antigen capture ELISA is that it is more specific than indirect assay.

Antibody capture assays were developed to test specimens with low concentration of HIV antibodies (e.g. urine and saliva) or to detect specific class of antibodies (e.g. IgG, IgM or IgA). In this test an anti-human

immunoglobulin (anti-IgG, IgM or IgA) is attached to solid support. The patient specimen is added. The concentrated immunoglobulin in patient's specimen binds to the anti-globulins on solid phase. Next labelled antigen is added which binds to HIV antibodies of the patient bound to solid support. Next, the substrate is added and the OD value is read on the ELISA Reader.

### **Interpretation of results**

Each test run must be validated according to the validation criteria given in the package insert of the kit and the known value of the quality control sample.

Cut-off-value:

- Each kit manufacturer has devised a method of calculation that produces a cut off value, to classify a test sample as positive or negative.
- This cut-off value can be based on an average of the negative controls multiplied by a factor or is based on a relationship of positive controls, to optimize sensitivity and specificity of assay.

### **Grey zone identification**

- All infected individuals start with low levels of antibody and gradually the level of HIV antibody increases as the infection progresses. Therefore, some laboratories testing high risk, high incidence populations institute "grey zone" identification where specimens which are just (10%) below the cutoff (i.e., cut-off multiplied by 0.9) are classified as "grey zone reactive", which are retested and subjected to confirmatory testing. This method may identify seroconverting patients early in the course of disease.

### **Repeat testing**

- Reactive and "grey zone" reactive specimens are normally repeated in duplicate in ELISA and consensus results (2 or 3 test results) are accepted.

### **Performance variables/troubleshooting**

ELISA tests are generally easy to perform but require careful adherence to procedures; any deviation in incubation times and / or temperature and pipetted volume can dramatically change test results.

Specimen problems - things to avoid :

- Haemolysis
- Repeated freezing and thawing
- Gross lipaemia
- Bacterial contamination
- Heat inactivation - may cause falsely reactive ELISA

Operator/technical error :

- Improper timing of incubations can give
  - low or high absorbance readings
- Incubation temperature
  - Low temperature - low absorbance readings
  - High temperature - high absorbance readings
- If conjugate precipitates, low reading; if heat inactivates enzymes, low reading.

Equipment problems :

- Incubator

Poor air circulation (hot spots, cold spots)  
Stacking plates (>2); improper heating

- Water Bath :  
Contaminated water, splashes into plates or tubes
- Improperly maintained plate washer  
Improper washing, high O.D. background, left over conjugate on plates creates colour change.
- Improperly calibrated/maintained microplate reader

Operator technique : Variation within or between plates

- Improper washing
  - (a) Incomplete removal of wash solutions - low absorbance readings.
  - (b) Inadequate washing - high absorbance readings non-specific reactions.
- Inaccurate/incorrect pipetting - absorbance readings, high or low.
- Failure to add sample controls, or reagents to correct wells.
- Timing of reagent additions, especially stop solution in relation to substrate.
- Reagents reconstituted or diluted in wrong diluent or wrong volumes.

### **False positive and false negative ELISA results**

There are some conditions other than HIV infection which may give a reactive HIV results i.e. false positive result. Also, sometimes the result may be negative even in HIV infected i.e. false negative result. Some of the common conditions giving erroneous results are listed below.

False positive result:

- Auto-immune diseases
- Multiple pregnancies
- Multiple transfusions
- Antibody to Class II HLA Ag (HLA-DR4)
- Hyper gammaglobulinemia
- Antipolystyrene antibodies
- Chronic alcoholics
- Patients with hepatitis
- Hepatitis B immunisation
- Technical error etc.
- Others

False negative result:

- Infected but not yet seroconverted, window period
- Late stage disease (immune collapse)
- Technical error

### **Rapid assays**

A number of rapid assays based on principle of agglutination, immunoconcentration (vertical flow), immunochromatography (lateral flow) and ELISA based have been developed for ease of performance and quick results. These assays generally require less than 30 minutes to perform and do not require special equipments.

These tests have been validated in India. The results of the blinded multicenteric study showed the rapid tests to be as sensitive and specific as the ELISA in the field. Consequently the tests were sanctioned for use at ICTCs, PPTCTCs and emergency situations as the screening as well as supplementary/confirmatory tests following the national strategies/algorithms of testing.

### **Agglutination assays**

Agglutination assays incorporate a variety of antigen coated carriers like red cells, latex particles, gelatin particles and microbeads. These particles are used to support or carry the antigen. HIV antigens are attached to the carrier particles by non specific attachment.

Agglutination assays have good sensitivity, do not require sophisticated equipment, are easy to perform, require no wash procedures and are cost effective. However, specificity is somewhat compromised and prozone reaction may be seen. To overcome the prozone reaction, diluted specimen is used to perform the test. During the agglutination reaction HIV antibody combines with HIV antigen on the carrier particles and since all antibodies are multivalent, a sort of lattice network is formed which can be visualised macroscopically or microscopically as per the directions of the manufacturer.

#### **Procedure**

The instructions given in the package insert should be strictly followed.

- A dilution of test specimen is mixed with HIV antigen containing carrier particles.
- If test specimen contains HIV antibodies, a lattice network will form between the antigen carrying particles and antibodies and is visualized as formation of clumps.
- Agglutination is seen visually except when carrier particles are microbeads.
- Weak reactions read subjectively must be confirmed by ELISA.

### **Dot blot assays / Comb tests**

These assays are rapid, easy to perform, can usually discriminate between HIV-1 and HIV-2 and do not require sophisticated equipment. The results are read by development of colour. Only drawback of the test is the high cost. Sensitivity and specificity of these assays compares with ELISA.

The assays utilize recombinant or synthetic peptides spotted on to nitrocellulose paper/micro particles. The antigen containing matrix is housed in a plastic device (cassette) containing adsorbent pads underneath to collect reactants or made as a comb and the antigens are spotted onto the tooth of the comb card. Each assay contains an immunoglobulin/procedure capture control to validate the result. These assays are very good for single test application i.e. in emergency, autopsy room, ICTCs, PPTCTCs and peripheral blood banks. They have not been automated so far.

#### **Procedure (comb and dot blot tests) :**

The instructions given in the package insert should be followed.

- Dilution of test specimen is added which filters through the membrane/microbeads by action of gravity. During the process HIV antibodies in the test specimen bind to HIV antigens on the membrane.
- Wash solution is added to remove unbound antibodies.
- The appropriately diluted conjugate is added (antihuman - immunoglobulin conjugated with enzyme as in

indirect ELISA or protein A conjugated with colloidal gold particles. Protein A conjugate can bind to human IgGs directly).

- Rinse with wash solution to remove excess of reactants.
- Substrate appropriately diluted is added which forms a precipitate at sites on membrane which contain antigen antibody complexes.
- Colour development in the control and test dots is read visually.

Fig.1 and 2 show diagrammatic representation of comb and dot blot assays

Intrepretation of result is done as per the instructions in the package insert. However, reactive results have to be confirmed by ELISA.

Immunocomb test is similar to blot assay based on principle of indirect ELISA. Here also the instructions given in the package insert with the kit are followed to perform the test.

Immunochromatography (lateral flow of reagents)

These assays are rapid, easy to perform, can usually discriminate between HIV-1 and HIV-2 and do not require sophisticated equipment. The results are read by development of colour. Only drawback of the test is the high cost. Sensitivity and specificity of these assays compares with ELISA. These are lateral flow one step tests. With this technique we can detect antibodies as well as antigens. These tests are usually temperature stable. For HIV testing the rapid tests are used to detect the HIV antibodies. Examples of kits based on this technology are Determine, Unigold and Hemastrip.

### HIV antibody detection in other fluids

The standard specimens for detection of HIV antibodies are serum/plasma / blood. However, HIV antibodies can be detected in other fluids also. HIV antibodies can be detected in oral fluids (saliva/oral mucosal transudates). The levels are less than 1% of the levels in serum, but can be detected by sensitive ELISA technology. The isotype of antibody detected is secretory IgA, so appropriate ELISA should be used. The tests have shown high sensitivity and specificity as per the reports from U.S., Tanzania, Thailand, Brazil and other countries and Orasure is licensed by F.D.A.. These tests may find better use once the issues concerning confidentiality, counselling and follow up have been resolved. HIV antibodies can also be detected in urine using appropriate ELISA kits. These tests have also been validated. However, guidelines for using these tests as well as strategies for follow up, etc. need to be developed and tests need to be validated in India.

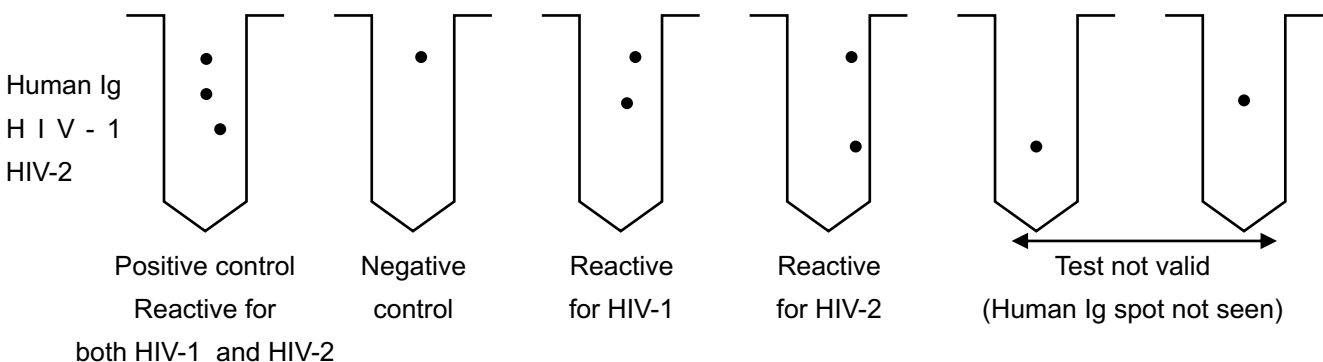
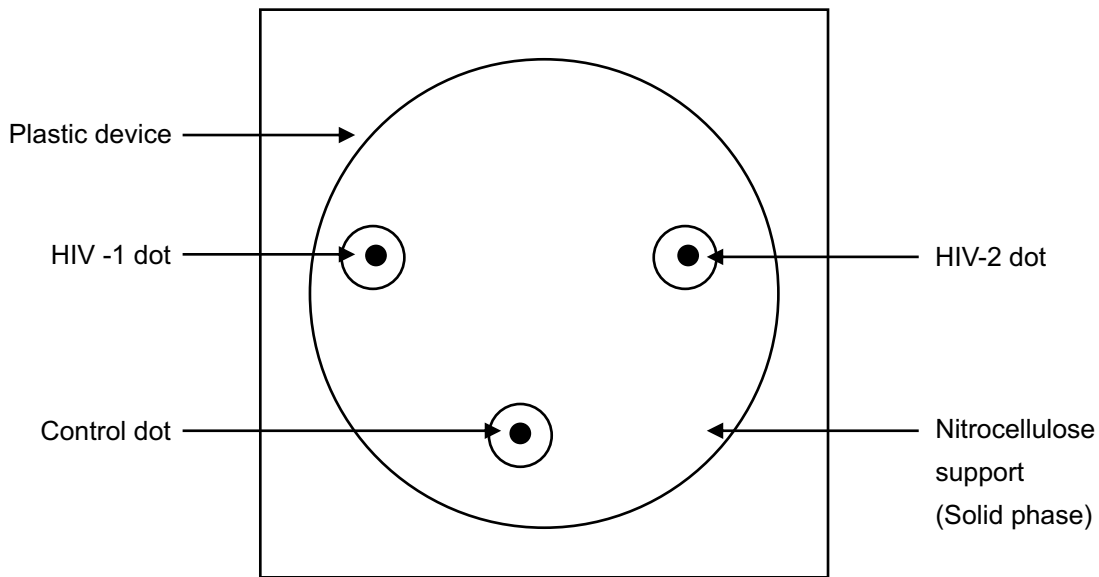


Fig. 1 Diagrammatic representation comb test (Tooth of comb card shown)



**Fig. 2 HIV-1/HIV-2 dot-blot assay to detect and discriminate HIV infection**

### Choice of the screening assay

Before choosing a particular protocol/kit/strategy/algorithm for HIV testing one has to be clear about the following parameters before testing is undertaken.

- Objectives of testing
- Sensitivity
- Specificity
- Prevalence of HIV infection in the population.
- Cost effectiveness.
- Appropriateness to the strategy and national guidelines of testing and according to the infrastructure facilities available.

### Objectives of HIV testing

- i) Blood and blood products safety. This is achieved by mandatory testing of all donated blood units and blood products.
- ii) Screening of donors of sperms, organs and tissues.
- iii) Diagnosis of HIV infection in clinically suspected cases.
- iv) Voluntary counselling and testing
- v) Epidemiological surveillance using unlinked anonymous HIV testing. Here the result of test cannot be linked with the identity of the person.
- vi) Research and surveys

### Sensitivity of HIV test

It is the accuracy with which a test can confirm the presence of an infection. Tests with high sensitivity show few false negatives and are meant to be used to screen blood prior to transfusion and ensure blood safety.

$$\text{Sensitivity} = \frac{\text{True positive (TP)}}{\text{TP} + \text{False negative (FN)}} \times 100$$

### Specificity of HIV test

It is the accuracy with which a test can confirm the absence of an infection. Tests with high specificity show few false positives and are to be preferred for the diagnosis of HIV infection in an individual.

$$\text{Specificity} = \frac{\text{TN (True negative)}}{\text{TN} + \text{FP (False positive)}} \times 100$$

### Prevalence of HIV and its implication for test results

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HIV infection in the population from which the person comes. The higher the prevalence, greater is the probability that a person testing positive is truly infected i.e. greater the positive predictive value of the test (PPV).

The likelihood that a person showing a negative result is truly uninfected the negative predictive value (NPV) decreases as the prevalence of HIV infection among the general population increases. The details for calculation of PPV and NPV are given in “Manual on Quality Standards for HIV Testing”

### Strategies/algorithms of HIV testing in India

Because of the enormous risk involved in transmission of HIV through blood, safety of blood and blood products is of paramount importance. Since the PPV is low in populations with low HIV prevalence, WHO/GOI have evolved strategies/algorithms to detect HIV infection in different population groups and to fulfill different objectives as given in chapter on “National HIV testing strategies/algorithms”. The various strategies, so designated, involve the use of categories of tests in various permutations and combinations.

1. ELISA/Rapid tests (E/R) used in strategies/algorithms I, II, IIA, IIB & III
2. Supplemental test are E/R with different antigens and or different principle of test. Western Blot and Line Immunoassay are used only in problem cases e.g. in cases of indeterminate/ discordant result of E/R.

**Strategy/algorithm I :** Serum is subjected to one E/R for HIV. If negative, the serum is considered free of HIV and if positive, the sample is taken as HIV infected for all practical purposes. This strategy/algorithm is used for ensuring donation safety (blood/blood products organ, tissues, sperms etc.). The unit of blood testing reactive (positive) is discarded. Donor is informed only if written consent for knowing the result was taken. Donor is given the provisional positive report and is referred to ICTC/VCTC for counselling and confirmation of test result.

### Strategy/algorithm II A

A serum sample is considered negative for HIV if the first ELISA report is so, but if reactive, it is subjected to a second HIV which utilizes a system different from the first one. It is reported reactive only if the second test confirms the positive report of the first. This strategy is used for surveillance.

### Strategy/algorithm II B

This algorithm is used for diagnosis of individuals who are symptomatic and have AIDS defining illnesses. Two to three different test kits are required to practice this strategy. A symptomatic individual testing positive with two HIV tests done serially is diagnosed as HIV infected. However, a symptomatic individual testing positive with one test and negative with the second test is subjected to a third tie breaker HIV tests & if positive the individual is given the report as indeterminate; if negative with the third tie breaker test, the individual is given a HIV negative report. However, in cases where 2 tests are negative and one is positive & risk behaviour is positive or in cases where result is indeterminate, then repeat testing and follow-up is undertaken after 14-28 days.

**Strategy/algorithm III :** It is similar to strategy IIB, with the added confirmation of a third consecutive reactive test being required for a sample to be reported HIV positive. The test to be utilized as the first test should be the one with the highest sensitivity and the second and third tests should be the tests with the highest specificity. The first, second and the third tests used either have different antigens or different principles of test.

Strategy IIB & III are to be used for diagnosis of HIV infection. E/R 2 and E/R 3 ought to be tests with the highest PPV possible to eliminate any chances of false positive results. Strategy III is used to diagnose HIV infection in asymptomatic individuals indulging in high risk behaviour.

### Supplemental (confirmatory) assays

Supplemental/confirmatory test are undertaken to confirm the HIV infection status of an individual who is either symptomatic or asymptomatic and has history of high risk behaviour.

Supplemental assays are used for the purpose of diagnosis of HIV infection and identification of the individual i.e. the individual is informed about the test results in the proper way (with counselling and confidentiality). This is because the screening assays are highly sensitive and are bound to give biologic false positive reactions whereas supplemental assays are sensitive as well as specific and are performed to rule out false positive reactions. Supplemental E/R using different antigen and/or principle of test are performed to fulfil the diagnostic objective of strategy/algorithm IIB and III of HIV testing policy.

However, supplemental tests do not always give conclusive results in which case either Western Blot or other virologic tests (PCR, p24 antigen detection etc.) have to be resorted to or follow up testing after 14-28 days is practised.

Various supplemental assays available commercially are listed below

- E/R with different antigen system (recombinant or synthetic peptides) or with different principle of test which makes the test more specific
- Western blot (WB)
- Immunoblot (IB)
- Line immunoassay



- Indirect fluorescent antibody test (IFA)
- Radioimmunoprecipitation test (RIPA)

As per the National HIV testing policy of India, supplemental tests are used only for strategy II and strategy III of HIV testing. For the purpose, E/R with higher specificity can be used as supplemental tests, provided these assays incorporate different antigen system and/or different principle of test. Only in case of unequivocal/discordant result WB is to be used. This is to make the HIV testing cost effective as well as easy to perform. The other supplemental tests i.e. IFA, RIPA are not used in India. So, only the supplemental assays used in India are being described.

#### **E/R tests as supplemental assay**

Supplemental E/R are recommended for specimens reactive in the screening assay for strategy II and III of HIV testing policy. If the screening assay contained virus lysate as antigen the second and third assays should have recombinant or synthetic peptides as antigens. Use of recombinant and synthetic peptides makes the assay more specific while retaining the sensitivity. Alternatively the second and third assays can also be based on different principle of ELISA e.g. if the screening assay was indirect ELISA the supplemental assays should be based on principle of competitive ELISA/Sandwich ELISA.

#### **Western blot/Immunoblot/ Line immunoassay**

These are most widely accepted supplemental assays, are highly specific but are expensive, labour intensive, need expertise to interpret and may also give unequivocal/indeterminate results. The specificity of these tests is based on two factors: separation of antigens and their concentration.

IB and WB use viral antigens from whole virus lysates electrophoretically transferred to a membrane support. So these blots may contain contaminating cellular components.

Recombinant or synthetic HIV antigens mechanically applied onto the support membrane are used in Line Immunoassays (LIA). These do not contain contaminating cellular components and are highly specific.

Indeterminate results can be seen by any of the above assays.

WB/EIA HIV kits are commercially available.

#### **Procedure**

The instructions given in the package insert must be diligently followed both for performance of test as well as interpretation of result.

#### **General Procedure:**

- HIV Ag test strips are placed in individual troughs of plastic tray.
- A dilution of the patient serum/plasma or control is added to individual test troughs and incubated on a rocking platform.
- At the end of incubation, the troughs are aspirated and the test strips are washed to remove unbound antibodies.
- An enzyme conjugate antihuman immunoglobulin is added to bind to human antibodies bound to HIV antigens on the test strips.
- The troughs are aspirated and strips are washed to remove unbound conjugated immunoglobulins.

- A substrate is added and colour development occurs where antigen/antibody complexes are localized on the strips.
- The colour development is stopped; the strips are washed, dried and interpreted.

### **Reading and interpretation**

The presence or absence of bands of the major viral proteins is scored for each strip. Unusual or atypical band locations are also noted.

### **Interpretative criteria**

Results are interpreted either as per WHO criteria i.e. presence of at least two envelope bands (gp 120, gp160, gp41 etc.) or as per the manufacturer's criteria for positive, indeterminate and negative.

In case of indeterminate result, retesting of the individual is done after two weeks, 1 month, 3 months and sometimes upto 1 year. During this time either the test becomes negative or positive or may stay indeterminate. Decision about the result has to be reached after correlation with history of high risk behaviour and clinical parameters.

## **Laboratory diagnosis of HIV infection in newborn (congenital HIV infection)**

Transplacental transmission of HIV can occur from infected pregnant mother to the foetus as early as 8 weeks of gestation or may be even earlier. It is estimated that > 80% of AIDS cases in infants < 1 year old are due to perinatal transmission of HIV-1. Diagnosis of HIV infection in infants born to seropositive mothers is difficult because maternal antibody (IgG) to HIV-1 crosses the placenta and can persist for up to 18 months making the distinction between maternal and neonatal IgG difficult. The tests which can be undertaken to diagnose HIV infection in neonates before 18 months of age are detailed below.

### **Detection of IgA and/or IgM anti-HIV antibodies**

This class of antibodies do not cross placenta. The IgA class of HIV antibody assay using Western Blot technique in infected children at 3 months of age has a sensitivity of 97.6% and specificity of 99.7% as reported in a study. IgM class of antibodies are produced by infected infants by six months of age. Production of IgM is erratic, false positive results are obtained due to rheumatoid factor and polyvalent nature of IgM which leads to nonspecific binding.

### **Estimation of p24 antigen (core antigen)**

The immune-complex dissociation assays which involve pretreatment of serum/plasma to liberate p24 antigen complexed with p24 antibody prior to performance of ELISA are sometimes used to identify HIV -infected infants. However, the test is not sensitive.

### **Polymerase chain reaction (PCR)**

The technique specifically amplifies viral DNA sequences of interest. Theoretically it is possible to identify one infected cell in the specimen as also latent HIV infections. Various reports indicate the specificity of PCR to be invariably >95% regardless of age of testing while sensitivity ranges from 15% in neonates (within 48 hrs. after birth) to more than 95% in infants over 1 month of age. This test is recommended to detect infection in children born to HIV infected women as per the algorithm of testing.

### ***In-vitro antibody production assay***

The method is tedious and involves in-vitro culture of antibody producing B lymphocytes from peripheral blood. The HIV antibodies are detected in culture supernate from truly infected infants. It is not used any more on account of the availability of better diagnostic tests.

### ***In-vitro isolation of virus from blood or tissues***

The method is time consuming, expensive and not sensitive. This is due to the lower number of available cells cultured and paucity of infected cells in the sample that are present. Additionally, it may take upto 6 weeks or longer to obtain a result. Very few laboratories in India are equipped to culture HIV. The test has no place for diagnosis of HIV in infants.

### ***Indirect indicators of HIV infection***

These include various nonspecific markers like hypergammaglobulinemia, absolute lymphopenia, hematologic abnormalities, low CD4 cell counts and clinical markers like severe failure to thrive or wasting syndrome, recurrent severe bacterial infections, opportunistic infections, HIV associated malignant disease, encephalopathy, lymphocytic interstitial pneumonia, etc. which point towards acquired immunodeficiency syndrome. However, these parameters are employed to supplement the conventional serodiagnosis of HIV infection. The tests available for diagnosis of vertical transmission of HIV from infected mother to the neonate err on the side of high specificity with a lower sensitivity.

### **Laboratory diagnosis during window period**

HIV infection during window period can be detected by demonstrating the presence of virus and virus components. PCR and detection of p24 antigen may be helpful. However, in case of accidental occupational exposure the virologic test if undertaken must be substantiated by serological test for a positive diagnosis.

## 7. HIV TESTING AT COUNSELLING AND TESTING SITES ( ICTCs AND PPTCTCs) USING RAPID TESTS

### Introduction

Rapid tests are in vitro qualitative tests for the detection of antibodies to Human Immunodeficiency Viruses (HIV) types 1 and 2 in human serum, plasma, whole blood saliva and urine. Currently HIV testing in India is performed on serum/whole blood (fingerprick), and plasma. This is because the HIV testing on urine and saliva samples has not been evaluated and validated in India. In recent times, a large number and wide range of rapid tests of high quality have become available and are being currently used world wide in laboratories under the following conditions:

- Facilities to perform ELISA test are absent,
- In emergency cases
- Remote blood banks where the collection volume is low and the facilities for ELISA are not there( ELISA is the test of choice for HIV testing at blood banks) .
- Point of care settings like VCTCs, PPTCTCs, and also truck driver intervention healthcare sites, etc.

### Types and technologies of rapid tests

Different types of rapid tests are available.

The various technologies on which rapid tests are based include:

- Immunoconcentration ( flow through) ( dot blot assays)
- Immunochromatography ( lateral flow assays)
- Particle agglutination (latex, gelati, RBCs, etc)
- Immunocomb (Dip stick/ comb tests) ( mostly ELISA based)

Immuno concentration:

This technology is available in the form of dot blot assays. These are multi step tests, very rapid to perform and results are obtained within a few minutes. The antigens (recombinant / synthetic peptides) are passively blotted on the nitro cellulose membrane matrix which is bound to the solid support and contains the adsorbent pads to collect the serum and reagents after their addition. Inbuilt control (internal control) in the form of a spot or line to indicate the validity of the test is available with each test. Semi-quantitative results are possible with the reader equipment.

Agglutination:

Agglutination assays are easy to perform and require no wash procedures. The antigens are adsorbed passively on the carriers (red blood cells, latex particles, gelatin particles and or micro beads). The antibody present in the serum sample reacts with the antigen adsorbed carriers, resulting in clumping of agglutinated particles. However, if in the reaction mixture the concentration of antigen and antibody is not optimum, phenomenon of prozone reaction (antibody excess) occurs which may lead to a false negative reaction. To overcome this initial dilution of the test sample is to be done as recommended by the manufacturer. Reactions are read visually. The control (uncoated or un sensitized particles) is run to detect non specific agglutination. If agglutination is detected in both the control and the test samples the assay needs to be repeated. Examples of these tests are Capillus and Serodia.

## Capillus agglutination test



### Immunocomb assays:

The immunocomb is a rapid test intended for the qualitative and differential detection of antibodies to HIV-1 and HIV-2 in human serum or plasma. The immunocomb is an indirect solid phase enzyme immunoassay (EIA). The solid phase is a comb with 12 projections. Each tooth has 3 spots:

- Upper spot:     Goat antibodies to human immunoglobulin (control to validate the test)
- Middle spot:   HIV-1 synthetic peptides
- Lower spot:     HIV-2 synthetic peptides

The developing plate has 6 rows (12 wells each) with each row containing a reagent solution ready for use at different steps in the assay. Immunoglobulin present in the testing samples is captured by the anti human immunoglobulin antibody on the upper spot (internal control). Unbound components are washed away. IgG from the sample is captured on the teeth and reacts with antihuman IgG antibody labeled with alkaline phosphatase which react with chromogenic components and the results are seen as gray blue spots on the surface of the teeth of the comb

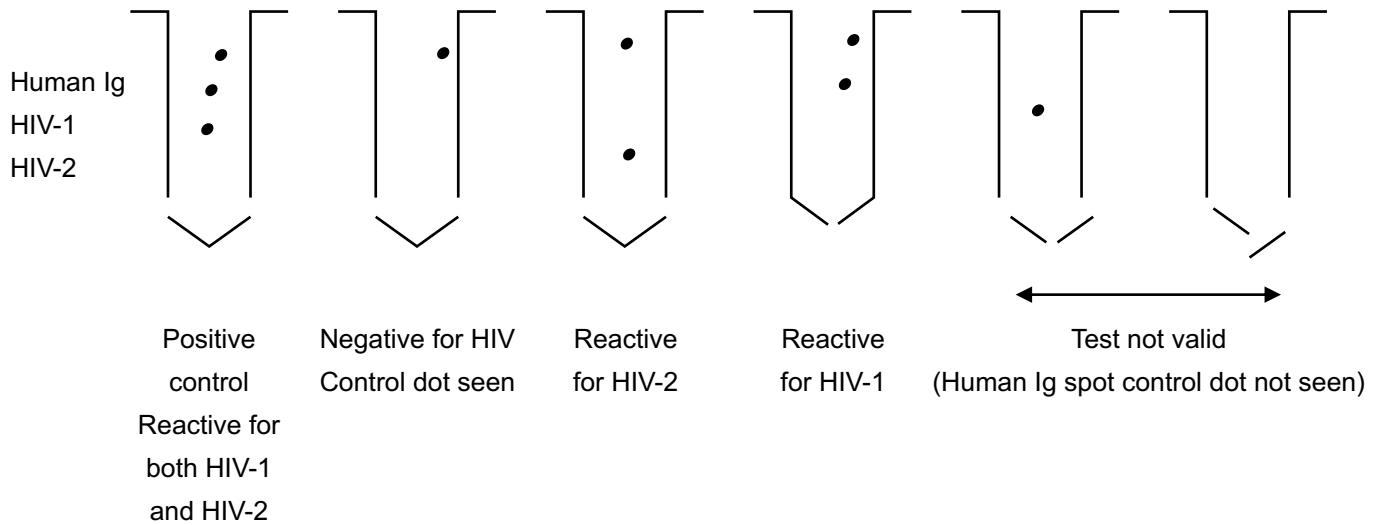
### Interpretation of controls:

- |                            |                    |
|----------------------------|--------------------|
| Appearance of upper spot   | - Negative control |
| Appearance of all 3 spots  | - Positive control |
| Upper spot does not appear | - Invalid result   |
- Interpretation of results:
- |                                     |                                |
|-------------------------------------|--------------------------------|
| Appearance of upper spot            | - Non reactive sample          |
| Appearance of upper and middle spot | - Reactive for HIV-1           |
| Appearance of upper and lower spot  | - Reactive for HIV-2           |
| Appearance of all 3 spots           | - Reactive for HIV-1 and HIV-2 |

### Post-assay activities:

- After the assay, discard the test devices in the box containing 1% sodium hypochlorite
  - Discard the used spreadsheets into the bio hazard bags
- Swab the workbench and all equipments after use with 1% sodium hypochlorite followed by 70% alcohol

## Diagrammatic representation immunocomb test

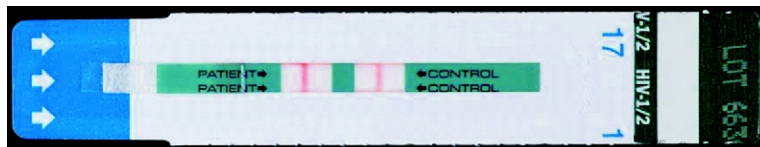


### Immunochromatography:

These are lateral flow one step tests. With this technique we can detect antibodies as well as antigens. These tests are usually temperature stable. For HIV testing the rapid tests are used to detect the HIV antibodies. Examples of kits based on this technology are Determine, Unigold and Hemastrip.

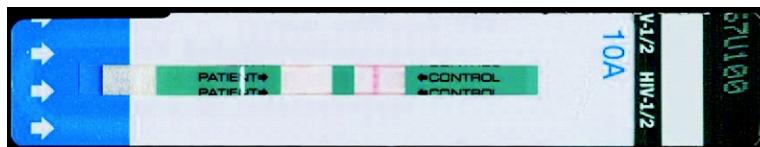
### Reactive

2 lines of any intensity appear in both the control and patient areas.



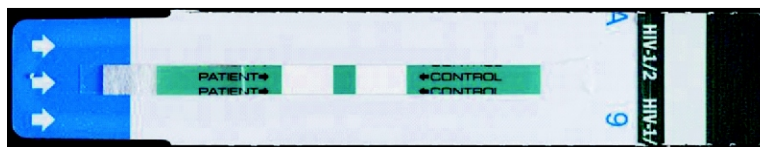
### Non-reactive

1 line appears in the control area and no line in the patient area.



### Invalid

No line appears in the control area. Do not report invalid results. Repeat test with a new test device even if a line appears in the patient area.



Possible outcomes of these rapid tests (Immunoconcentration, Immunofiltration, Dipsticks and Immunocombs) :

- Reactive or positive : Appearance of test band and control band
- Non reactive or negative : Appearance of control band only
- Invalid : Absence of control band. Test has failed. Repeat the Test with a new device

### Advantages:

- No equipment is required to perform the test. Micropipettes may be needed to dilute the sample for some assays eg. agglutination assays
- Limited infrastructure is needed. Some of the rapid tests can be stored at room temperature. These tests have wide temperature range stability.

- Rapid assays can be used in remote peripheral labs and for circumstances where same day results are required e.g VCTCs and PPTCTs
- The rapid assays are versatile as far as sample stability is concerned. These can be used on whole blood, serum, plasma, saliva or urine. Different kits are available for use on these different samples
- Most of the rapid tests comprise of one to five step procedures
- Reading is subjective and visual with naked eye examination. However now rapid test readers are available for some tests, allowing us to perform semiquantitative analysis. Rapid immunoassay results can now be read objectively using CCD chips (as in digital cameras).
- Rapid tests are now more robust
- Management of waste generated by performing rapid tests is easier to manage as compared to that generated by performing ELISA
- Rapid tests work out more cost effective overall particularly in set ups like that in VCT and PPTCTCs as they are more robust, there is lesser cost of the requisite lab infrastructure and lesser cost for sample collection

#### Disadvantages

- More expensive because of single test packaging and performance.
- Test performance may vary by the product.
- Refrigeration required for some of the products.
- Each test card cannot be quality controlled with an external quality control sample.
- There may be issues of sensitivity, specificity, negative predictive value and positive predictive value in relation to the reference gold standard. These should be carefully considered while procuring the product.

#### Accuracy of rapid tests

Many rapid tests available in the market are as accurate as the best classical assays, e.g., ELISA. Some of the rapid tests may be less accurate. This happens when manufacturers do not follow total quality management and good manufacturing practices to cut the costs. In order to ensure quality of rapid tests these must be validated vis-à-vis the classical assays in the specific locations before use. This was done by NACO before the rapid test kits for use at VCTC's were recommended i.e., the available rapid test kits were evaluated in a blinded multicentric study vis-à-vis available ELISA's by NICD on behalf of NACO and the rapid test kits were recommended for use at VCTC's and later at PPTCTC on the basis of evaluation results obtained. HIV rapid tests were found to perform as reliably as ELISA's in the field.

#### Strategies for rapid test use

Rapid tests are used following the algorithm based on serial testing following the national strategies of testing appropriate to the objective of testing. The rapid test selected for screening should be 100% sensitive. Highly specific (> 98.5%) rapid tests should be selected for supplementary and confirmatory testing. The testing algorithms are given in the chapter on National strategies/algorithms for HIV testing

#### Uses of rapid tests

These tests can be performed on patients at point of care areas like peripheral health care centers, pharmacies, emergency vehicles, out patient clinics, VCTC's, blood banks, PPTCT centers, hospital wards, homes, in the field and hospital wards.

## Quality assurance practices

Most of the rapid test kits have inbuilt Ig spot/line which monitors the quality of kit as well as the quality of performance of the test. The Ig spot/line always shows up as positive provided the kit reagents are functioning optimally and the test has been performed as per the manufacturers instructions. However these are the kit controls. In order to ensure the validity of the test result, and quality performance of test, a known positive external control must be tested along with the test samples using the same kit (same lot number and expiry, etc.) on day of performance of the assay. This will ensure quality of the kit as well as the procedure.



## 8. PROCEDURE OF TESTING AT ICTC AND PPTCT

### Introduction

Integrated Counselling & Testing Centre (ICTC) is an extremely important component of HIV / AIDS care, prevention and control program. ICTC serves as an entry point for counseling, testing, antiretroviral treatment, treatment for opportunistic infections, psychosocial support and referral to the appropriate services.

HIV counseling and testing are two extremely important functions of a ICTC .The ambience, the infrastructure of the ICTC should be conducive for the client to confide in the counselor and undergo testing if required for risk behaviour change and prevention of transmission of HIV.

### Integrated counseling and testing center (ICTC)

A model VCTC set-up should be established under the following guiding principles

#### Location

- Should be easy to locate .e.g. through signboards , and use of symbols for non- literate clients
- The name should be a non-stigmatising one, easily understandable and should preferably be in local language
- In a hospital setting, it should be located near the general OPD in the vicinity of the STI or Obstetrics and Gynecology department.
- The counseling room, blood collection site and Medical Officer should be located in close proximity to one another.
- The Microbiology / Pathology department/s where blood testing is to be done should be located in close proximity.

#### Waiting area

- Should be in close proximity to the counseling and blood collection site
- Should be well lit and adequately ventilated
- Should have sufficient sitting space for the clients
- Should have accessibility of the clients to
  - books ,posters, IEC materials
  - information about ICTC services
  - Provision of clean drinking water
  - Health dispenser including condoms
- Client suggestion box
- Waste basket
- TV/Video in local and simple understandable language
- Toys for children accompanying their parents to the ICTC

#### Counseling rooms

- There should be two separate rooms for counseling (one for the male counselor and the other for the female counselor)
- They should be in close proximity to the waiting area and the blood collection site
- The rooms should preferably be sound proof to provide proper client counseling environment where privacy of the clients can be maintained

- Availability of counseling material
  - Counseling guidelines
  - Visual aids: flip charts and posters
  - Condoms
  - Client register
  - Appointment diary
  - Pre and post test counseling forms
  - Informed Consent
  - Referral directory / Referral cards
  - Model of a penis for condom usage demonstration

## ICTC staff and qualifications

### Core staff

Depending on resource availability and size of the ICTC, the core staff should include:

1. ICTC In charge / ICTC manager (Ideally Microbiologist)
2. Two trained counselors (one male and one female)  
(per counselor 8-10 sessions per day)
3. One trained laboratory technician

### Optional

- Counselor supervisor
- Peer counselors (PLHAs, community workers)
- Dietician
- Data entry clerk
- Receptionist
- Support and cleaning staff

### Qualifications of HIV / AIDS counselor

He/She should hold a post graduate degree in Psychology /Social work (M.A. Psychology or M.A. Social work)  
Qualified PLHAs with the required degree and qualifications should be given preference.

### Qualifications of laboratory technician

Laboratory technicians trained from institute approved by State /UT Governments in medical laboratory technology should be appointed.

### Qualification of ICTC Medical Officer

The ICTC Medical Officer should be trained in principles of HIV /AIDS case management, in diagnosis and treatment of opportunistic infections and should understand the concept of HIV /AIDS counseling.

### Procedure of counseling and testing

- The client on reaching the VCT is registered, a Personal Identification No./Digit (PID) is given to the client.
- Client is given the pretest counseling during counseling the client is informed about HIV; how it causes disease; how it is transmitted; how it is not transmitted; the window period; possible need for testing; false

negative and false positive test; the risk behaviors; the treatment, drugs and services available and the networks available for psychosocial and other problems. This information has to be updated and is conveyed to the client in a language understood by the client.

- On the basis of the knowledge gained, the client makes an informed decision to either under go HIV testing or not to under go testing.
- If the client agrees to undergo the testing, a written informed consent is taken.
- Next the blood sample is collected as per the blood collection guidelines and the client is asked either to wait, if there is time to do the testing the same day or is given the paper with his/her PID No. to come and collect the report on a specified date (usually by third day).
- The rapid HIV testing is performed as per the guidelines, results are interpreted and report is prepared as negative, positive or indeterminate. Necessary entries are made in the relevant registers.
- The client comes to collect the report. The report is given with the post-test counseling. In case the report is negative the counseling is aimed at stressing the need for change of risk behavior. Practice of safe sex and use of safe needles is stressed. Use of condom is promoted. This is very important for prevention of transmission of HIV. In case the test result is positive the client is provided psychosocial support; is informed about availability of treatment and other services. This is done to help the client to cope with the positive result and empower the client through hope of treatment. The importance of safe behaviour (safe sex, safe needles) is stressed again to prevent secondary transmission of HIV. In case the result is indeterminate the client is called for repeat testing after 14-28 days. In case the result continues to be indeterminate, the client is referred to the NRL for further testing.
- The client is referred to the A R T center, and in case of female if pregnant is referred to PPTCT for further management.
- All the records are maintained at the ICTC as per the guidelines confidentiality of test result is maintained.
- The client is counseled to get the spouse/ children get tested for HIV.
- The client is provided the referral as per the requirements.

### Prevention of parent to child transmission centers (PPTCT)

Mother (Parent) to child transmission of HIV is a major problem worldwide. Most of the children (90%) who are HIV positive were born to HIV-infected mother. HIV-1 may be transmitted from an infected mother to the baby during pregnancy i.e. in-utero, during delivery and during breast feeding. The transmission rates through this route range from 20 to 40 %. Transmission of HIV-1 from mother to child depends upon various viral, host and obstetric factors. There are a number of interventions which reduce the parent to child transmission of HIV-1. These include early identification of the mother who is HIV-1 positive through counseling and testing, administration of antiretroviral drugs to reduce the viral load in the mother, appropriate obstetric and pre natal care of mother, counselling about

breast feeding and administration of antiretroviral drugs to the HIV- exposed newborn.

Prevention of parent to child transmission (PPTCT) of HIV-1 is a very important component of NACP II and NACP III which is aimed to minimize transmission of HIV through this route.

### *The floor plan of space required for PPTCT*

The area required is one room less than the ICTC otherwise the movement of the client is some what similar. The PPTCT should be located near/ in the Gynae. and Obst. departments so that the pregnant women attending Antenatal clinics can access the PPTCT service comfortably.

### *The procedure of enrollment and functioning of PPTCT.*

- Group counselling of pregnant women is done at the ANC, in a language which the women understand. The content of counseling is similar to that at ICTC. The additional information given is on the interventions available for positive pregnant ladies to prevent PTCT.
- Group education about breastfeeding and other relevant facts is also given
- Women are offered the HIV test.
- Those women who consent for HIV testing are given one to one counselling. The content of counselling is same as at ICTC. However, the women are also explained about the drugs available to prevent PTCT, the possibility of a child being born who may be HIV positive, the facilities available for management of such a child and the role of breastfeeding in transmission of HIV to the baby and how to prevent it.
- A written informed consent is taken from women who wish to undergo the testing. Patient ID is given and the necessary records are maintained as per guidelines.
- Blood sample is collected.
- Rapid HIV testing is performed as per the guidelines.
- The women is given the PID and is asked to collect the report at specified time (usually the same day or within 3 days).
- HIV positive women are again given the post test counselling and enrolled for the drug intervention program as per National PPTCT protocol.
- The HIV negative women are counselled about the safe sexual practices (condom use) and safe syringes for primary prevention of HIV.

## 9. SELECTION OF HIV TESTING KITS

### Introduction

Selection of the appropriate HIV test kits is an essential prerequisite of an accuracy and reproducibility of test results and good quality management. A large number of assays are available commercially each offering essential as well as attractive performance characteristics namely sensitivity, specificity, efficiency and predictive values. However, the performance of the test will depend upon the conditions in the laboratory, technical expertise, population being tested, etc.

New and improved kits are also being developed continuously and these also need evaluation in the same way as the established kits. Evaluation of the performance characteristics of the HIV test kits (preferably WHO pre qualified) by the designated Statutory agency should be done and is mandatory prior to the test kit being available for diagnostic testing.

Situations under which these kits may have to be evaluated can be as follows:

- Kits under import; evaluation with local serum panel is very important.
- New kits developed/manufactured indigenously; to assess efficacy.
- To resolve controversies regarding discordant results or deterioration of components and reagents. This may be due to break in cold chain during transit and/or storage of in-use kits

In India, Drugs Controller General of India directs the manufacturer to submit the representative sample of kits for evaluation, using a locally made and well characterized serum panel by the Institutes identified for this purpose. The National Institute of Biologicals in NOIDA UP is the statutory body that has been assigned the responsibility of evaluating and approving these kits prior to their distribution in the Government and Private sector. Each lot of kits has also to undergo evaluation by the National Reference Laboratories after obtaining NIB approval and prior to the lot being released for use in government testing labs. The evaluations must be performed under identical conditions

The parameters to be evaluated to assess the performance characteristics of the kits include:

- Sensitivity
- Specificity
- Efficiency
- Positive predictive value
- Negative predictive value

The parameter's values should be within those recommended by the technical expert committee, G.O.I. on this subject. The final approval of the test kit is given by DCGI on the basis of the evaluation reports submitted by the identified evaluation centres

**Sensitivity :** Sensitivity of a test is defined as its ability to detect truly infected individuals as also its ability to detect very small amounts of analyte. Sensitivity can be calculated by the following formula:

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}} \times 100$$

**Specificity:** This is the ability of an assay to correctly identify all the uninfected individuals i.e. there should be no false positives. The specificity can be calculated by the following formula:

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}} \times 100$$

**Efficiency:** It is the overall ability of a test to correctly identify all positives as positive and all negatives as negative. Efficiency is determined as below:

$$\text{Efficiency} = \frac{\text{True positives} + \text{True negatives}}{\text{True positives} + \text{False negatives} + \text{True negatives} + \text{False positive}} \times 100$$

**Predictive values:** This is the measure of value of a test in relation to the prevalence of the disease in the population. The value of a test in addition to the parameters described above depends on the population being tested.

**Positive predictive value (PPV)** is the ability of the test to identify actually infected individuals among all persons giving a positive result with the kit being evaluated.

$$\text{PPV} = \frac{\text{True positives}}{\text{True positives} + \text{false positives}} \times 100$$

**Negative predictive value (NPV)** is the ability of the test to correctly identify the really non infected individuals from among all the persons giving negative result.

$$\text{NPV} = \frac{\text{True negatives}}{\text{True negatives} + \text{false negatives}} \times 100$$

If the prevalence is high the PPV of a test will be high i.e. an individual testing positive will mean real infection. Whereas, in low prevalence area the chance that an individual testing positive is really infected is lower.

## RECOMMENDATIONS FOR SELECTION OF TEST KITS

The testing centre should procure and have available at all times HIV test kits based on three different principles and /or antigen systems to enable them to perform HIV testing as per NACOs HIV testing strategies at ICTCs and PPTCTs and one highly sensitive (100%) to screen blood at blood banks to ensure blood safety.

HIV (ELISA) test kits general specifications

1. Should be solid phase microplate ELISA using HIV I & II recombinant and/or synthetic peptide antigens.
2. The assay should detect HIV I and II antibodies.
3. The assay should detect antibodies to all sub-types of HIV I.
4. The assay should be able to detect antibodies of HIV I and II during early seroconversion period.
5. The assay should have reactive and non-reactive controls with each kit.
6. The kit should have a shelf life of minimal 12 months at the port of discharge of consignees end which ever is applicable.
7. Adequate literature detailing the components, methodologies, validity criteria, performance characteristics, storage conditions, manufacturing and expiry dates should be provided with each kit.
8. The assay should have sensitivity level at 99.8% and above and specificity level at 98% and above.
9. The manufacturer/ authorized agent should ensure maintenance of cold chain during storage and transport at 2°C–8°C.

HIV test kits (sandwich ELISA)

1. Should be solid phase microplate sandwich ELISA using HIV I & II recombinant and/or synthetic peptide antigens

The selection criteria 2-9 are identical to those applicable as above for HIV (ELISA) Test Kits General Specifications

HIV (ELISA) capture/competitive test kits

1. Solid phase microplate capture ELISA in which solid phase is coated with anti-immunoglobulin (anti IgG, IgM, IgA).
2. If assay is competitive ELISA and the solid phase should be coated with recombinant and/or synthetic peptide antigen.

The selection criteria 3-9 are identical to those applicable as above for “General Considerations”

HIV Rapid test kits-general specifications

1. Should be solid phase/particle coated with synthetic/recombinant HIV I & HIV-II antigens.
2. The assay should detect antibodies to HIV I & HIV II antibodies by Enzyme Immuno Assay / Agglutination/Immunochromatography/any other principle.
3. The product should be able to detect antibodies to HIV I and II during early sero-conversion period.
4. The product should have positive and negative controls.
5. The kit should have a shelf life of minimal 12 (twelve) months at the port of discharge of consignees end which ever is applicable.
6. Adequate literature dealing the components methodologies validity criteria and performance characteristics of the product should be provided with each kit.
7. Should have sensitivity level at 99.8% and above and specificity level at 98% and above.
8. The supplier/local agent should have facility for storage of kits at 2°C–8°C
9. The total procedure time shall not be more than 30 minutes.
10. Provision shall be made for conducting single test at a time.

11. The tests should be easy to perform and the kits should preferably be stable at room temperature (22-25°C)

#### HIV Rapid test kits- principle of Enzyme Immuno Assay

1. Should be a Solid phase/particle coated with synthetic/recombinant HIV I & HIV II antigens.
2. The assay should detect HIV I and II antibodies by principle of Enzyme Immuno Assay.

The selection criteria 3-11 are identical to those applicable as above for "HIV Rapid test kits (General Considerations)

#### HIV Rapid test kits -principle of agglutination

1. Should be solid phase/particle coated with synthetic/recombinant HIV I & HIV II antigens.
2. The assay should detect HIV I and II antibodies by Agglutination.

The selection criteria 3-11 are identical to those applicable as above for "HIV Rapid test kits (General Considerations)

#### HIV Rapid test kits - any other principle excluding Enzyme Immuno Assay and Agglutination

1. Should be solid phase/particle coated with synthetic/recombinant HIV I & HIV-II antigens.
2. The assay should detect antibodies to HIV I & HIV II antibodies by any other principle excluding Enzyme Immuno Assay and Agglutination.

The selection criteria 3-11 are identical to those applicable as above for "HIV Rapid test kits-general considerations



**DETAILS OF SOME OF THE COMMERCIALY AVAILABLE HIV RAPID TEST KITS**

Name of kit	Antigen	Principle	Manufacturer	Sensitivity	Specificity	Capacity to differentiate between HIV 1&2	Inbuilt control
Immunofiltration*							
HIV Tridot	Recombinant proteins	Immunofiltration	Biotech Inc, Himachal Pradesh India	100%	100%	Yes	Yes
HIV Spot	Recombinant	Membrane filtration	Gene Lab. Diagnostics Ltd. 85, Science Park Division No. 04/01 Singapore Science Park	100%	99.5%	Yes	Yes
Immunoassay							
Immunocomb II HIV 1 & 2 BiSpot	Synthetic peptides	Indirect EIA	PBS Orgenics, France	100%	99.4%	Yes	Yes
HIV EIA Comb	Recombinant proteins	Dot Immunoassay	J. Mitra Co.Ltd., India	100%	99.9%	Yes	Yes
Comb AIDS RS	Recombinant & Synthetic peptides	Dot Immunoassay	Span Diagnostics Ltd. G.I.D.C. Sachin 394230 Surat, India	100%	98.7%	No	No
Agglutination							
Capillus HIV1&2	Recombinant proteins	Latex agglutination	Trinity biotech. USA	99.6%	100%	No	No
NEVA HIV	Recombinant molecules having RBC binding sites	Particle agglutination	Cadila pharmaceuticals. "Cadila corporate campus" Sarkhej Dholke Road, Bhat Ahmedabad 382210 Gujarat	99%	95%	No	No
Lateral Flow/ Immuno chromatography							
SD Bioline HIV ½ 3.0	Recombinant	Immuno Chromatography	SD Standard Diagnostics Inc. 575/34 Pajang/Dang JanGanKu, Suwon/Sikyong Do. Korea	100%	99.8%	Yes	Yes

Retrocheck HIV	Recombinant	Lateral flow Immuno Chromatography	Qualpro Diagnostics, India	100%	99.8%	No	Yes
Precise	Recombinant	Immuno Chromatography	Ranbaxy Lab. Ltd. Diagnostic Division A-3, Okhla, Industrial Area Phase 1, New Delhi-110020	100%	99.6%		Yes

\* Different manufacturers use different raw materials as matrix for adsorbing the HIV peptides. The immunofiltration involves the vertical flow of the reagents. As the reagents pass through the matrix the reactants get adsorbed and concentrated (immunoconcentration) on the viral peptides on the matrix in case of HIV positive sample to produce a colored spot /line

ELISA TESTS

Name of the kit	Antigen	Principle	Manufacturer	Sensitivity	Specificity
Ani Labsystems (Elisa based)	Synthetic peptide	Indirect Solid Phase (EIA)	Ani Lab. systems Ltd. OY Museekatu 13B Fin-00100 Helsinki, Finland	100%	99.5%
Micro lisa HIV	Recombinant Protein	Indirect Elisa	J. Mitra & Co Ltd. A-180, Okhla Area Ph. 1 New Delhi-20	100%	99.5%
Eliscan HIV	Synthetic peptide	Indirect Solid phase	Ranbaxy Lab. Ltd. Diagnostic Division A-3, Okhla Industrial Area Phase 1, New Delhi-110020	100%	99.5%
HIV ASE 1+2	Recombinant	Direct sandwich method	General Biological Corporation Innovation 1st Road Science Based Industrial Park HSINCHU Taiwan, Roc	100% (done at NICD)	99.6% (done at NICD)

Combinations as per principle

- I. Immunofiltration + Immunoassay + Agglutination
- II. Immunofiltration + Immunoassay + Lateral Flow/ Immuno chromatography
- III. Immunofiltration + Agglutination + Lateral Flow/ Immuno chromatography
- IV. Immunoassay + Agglutination+ Lateral Flow/ Immuno chromatography

Examples

- I. Tridot/Pareekshak/ HIV Spot + Immunocomb HIV 1& 2Bispot/HIV EIA Comb/CombAIDS RS + Capillus/NEVA
- II. Tridot/Pareekshak/ HIV Spot + Immunocomb HIV 1& 2Bispot/HIV EIA Comb/CombAIDS RS + SD Bioline HIV 1/2 3.0/ Retrocheck HIV/ Precise
- III. Tridot/Pareekshak/ HIV Spot + Capillus/NEVA+ SD Bioline HIV 1/2 3.0/ Retrocheck HIV/ Precise
- IV. Immunocomb HIV 1& 2Bispot/HIV EIA Comb/CombAIDS RS + Capillus/NEVA + SD Bioline HIV 1/2 3.0/ Retrocheck HIV/ Precise

Care has to be taken that atleast two kits selected should be able to differentiate between HIV 1 & 2

For example: Do not select Pareekshak + Comb AIDS RS + Capillus as none of these kits can differentiate between HIV 1 & 2, Instead Tridot + HIV EIA Comb + Capillus can be selected

Combinations as per antigen

All the kits have Recombinant Antigen except + Immunocomb HIV 1& 2 Bispot which has synthetic antigen and CombAIDS RS which has a combination of Recombinant & Synthetic peptides. To avoid confusion it will be better to use kits with different principles rather than different antigens. But in dire situations a combination of two immunoassays can be used along with one more test of other principle.

## 10. DIRECT EVIDENCE OF HIV INFECTION

### Introduction

The diagnosis of infectious diseases can also be made by the direct demonstration of the presence of infecting organisms in the clinical specimens collected from the patient. While isolation of the infecting organism is possible in many infectious diseases, the diagnosis may also be made by detection in the clinical specimens of nuclear material or antigens specific for the infecting organisms.

HIV infection is diagnosed by the presence of anti-HIV antibodies in the blood. Serological assays provide a sensitive procedure to screen blood for the presence of antibodies to HIV. Samples found to be positive for HIV antibodies by screening E/R tests can be further tested by two different E/R tests or by the Western Blot test for confirmation. However, there are situations where the serology is negative although there is definite evidence of exposure to HIV. Direct detection of HIV would be needed as seen in the following settings:

- To determine HIV status during window period
- In health care workers following accidental exposure to contaminated blood etc.
- Detection of HIV infection in babies born to HIV positive mothers
- Indeterminate Western Blot results
- Discordant results on antibody testing

HIV infection cannot be detected / diagnosed in infants (<18 months) born to HIV-infected women on the basis of serological tests. This is because maternal antibodies, acquired through passive transplacental transfer can persist up to 15-18 months in the infant. The HIV antibodies can also be transmitted through the breast milk of infected mothers. A positive serological test in these infants may merely be a reflection of the maternal antibodies. Virologic tests (DNA-PCR) are recommended in such cases and are important diagnostic indicators of HIV infection.

The diagnosis in these situations may be made by:

- Detection of HIV-specific DNA by Polymerase Chain Reaction (PCR)
- Detection of p24 antigen
- Isolation of HIV by viral culture.

### Polymerase Chain Reaction (PCR) for HIV :

The laboratory procedure used to test for the genetic material of HIV is called the Polymerase Chain Reaction (PCR) test. PCR helps to find a specific gene sequence amongst an abundance of other DNA. PCR testing in HIV may be performed for:

- Diagnosis as in settings enlisted above
- Research like:
  - Subtyping of HIV virus
  - For cloning genes for use in generating reagents for diagnostic purpose or for preparing vaccines
  - For generating chimeric viruses to serve as candidates for vaccine development.
  - For studying functional changes induced by site directed mutagenesis

## Method and Principle of Polymerase Chain Reaction

Specific oligonucleotides are chemically synthesised (called primers) which are complementary to DNA flanking the sequence of interest. Genomic DNA is arranged in the form of a double stranded helix held together by hydrogen bonds. On heating, the double stranded DNA gets denatured to single stranded DNA. On cooling, the DNA solution, the primers bind to the single strands of DNA. One primer is designed to anneal to the sense strand and the other primer to the anti-sense strand, with their 3' ends pointing towards each other.

Primers are mixed with a buffered solution of dNTPs, magnesium, thermostable polymerase enzyme and the template DNA. The mixture is then covered with a layer of mineral oil to prevent evaporation and placed in a programmable heating block (thermal cycler). The double stranded DNA template is then denatured by heating to a temperature above its melting point. The temperature is then lowered sufficiently for hybridization between primers and template to occur. The temperature is however, kept high enough to prevent mismatch hybridisation of the primers to similar sequences elsewhere in the genome. High concentration of the primers is maintained to ensure that this reaction is favoured over re-annealing of the complementary template DNA strands.

The temperature is then raised towards the optimum for the thermostable polymerase enzyme (72 deg C), which has attached itself to the end of the primer-template duplex. Synthesis proceeds from the 3' end of each primer until the reaction is stopped by heating to the melting point for the second time. The product of this reaction is of indefinite length and is known as long PCR product. This completes the first cycle of PCR.

The second PCR cycle commences with the melting step, followed by primer annealing. Here, the primers anneal not only to the original DNA but also to the newly synthesised strands from the first cycle of PCR. The second cycle repeats the first cycle with respect to the original DNA, but synthesis on the new strands can proceed only as far as the end of the molecule which corresponds to the 5' end of the opposite primer.

After the third cycle of PCR, synthesis directed by the PCR products of the first two cycles will be bound by both ends of the primer sequences and this PCR product will accumulate exponentially with subsequent cycles of amplification. The major product of PCR will thus be of defined length, consisting of the sequence between the primers and includes the primer sequences themselves.

In the HIV PCR reaction, often a nested PCR reaction is used. Here, an aliquot of the PCR product can be re-amplified with a second set of primers which are located at 3' or internal to the original set of primers used for the first round of PCR amplification.

Different commercial assays based on PCR technology use variations of the basic PCR reaction. The kit manufacturer instructions for all steps of test performance & quality control are to be followed.

### *Precautions to be taken for PCR reaction :*

Because of the sensitivity of the PCR test, it is important that care is taken to avoid cross contamination of samples or carry over of amplified products that can result in false positive results. For all amplification techniques, greatest attention is directed towards the prevention of contamination, because locating its source around the laboratory is time-consuming and tedious. Once a contamination has occurred, testing has to cease, until the source is identified.

Without exception, test results must be rejected, even if only one of the accompanying contamination controls reveals contaminating fragments.

Setting up a molecular diagnostics laboratory has its own unique requirements, and those should be carefully considered when evaluating what all is needed to start and operate this kind of a lab successfully. The PCR laboratory needs to be designed as per the standard recommendations to ensure unidirectional workflow. The problems associated with the avoidance of contamination in PCR necessitate a decisive and strictly- adhered-to laboratory organization, including room and space and environmental planning. Ideally, a molecular diagnostics laboratory should be divided into four separate work areas, each having dedicated special equipment for:

- Reagent storage and set-up,
- Sample/Template preparation,
- Reaction mix assembly and amplification, and
- Product analysis.

When arranged in this fashion, these activities can be classified into

- Preamplification activities (Reagent storage and sample preparation)
- Postamplification activities (Amplification and product analysis)

These events as mentioned earlier must be separated in time and space

In the Preamplification lab there should be a slight positive pressure as compared to the air in the connecting hallway. The Postamplification lab in contrast should be at a slightly reduced pressure to pull in air from outside and thereby prevent escape of amplicons from the analyzed samples.

In addition to ensuring strict workflow measures as described above, the following precautions also need to be taken:

- All blood samples must be handled carefully to avoid possibility of transmission of HIV.
- Lab coats and gloves should be used while handling the samples and PCR products.
- Biosafety laminar flow hoods should be used to prevent cross contamination as well as for biosafety precaution.
- Use of barrier tips or positive displacement pipettes for dispensing of samples.
- Reagents to be stored as small aliquots.
- Preparation of master mixes to minimize handling of reagents.
- Equipment, gowns, gloves should not be moved from one work area to another in the PCR laboratory.

The two methods normally used for virus isolation are direct method or co-culture method. In the direct method, PBMCs from the patient are cultured in vitro in presence of PHA. In the co-culture method PBMCs from heterologous HIV uninfected donor are stimulated with PHA, and after 48-72 hours the stimulated cells are cultured along with the PBMCs from the patient.

## Detection of p24 antigen

The p24 antigen test identifies actual HIV viral particles in blood (p24 is a protein specific for HIV). However, the p24 antigen test is generally only positive from about one week to 3 - 4 weeks after infection with HIV. The p24 protein cannot be detected until about a week after infection with HIV, because it generally takes that long for the virus to become established and multiply to sufficient numbers that they can be detected. The p24 antigen then becomes undetectable again after sufficient antibodies to p24 have been produced, because they bind to the p24 protein and

eliminate it from the blood. Once antibodies are produced, the p24 antigen test may thus be negative even in people who are infected with HIV. Of course, at that point the regular HIV antibody test will then be positive. Recently antigen dissociation tests have been developed and shown to be more sensitive.

The p24 antigen in the serum of infants is bound to maternal HIV antibody. This test is relatively insensitive for the detection of HIV infection in infants. Less than 20% have detectable p24 antigen at 1-6 months of age. p24 antigen can be detected in serum by ELISA in 30% patients during the window period, 10% of asymptomatic patients and 50-60% of AIDS patients.

Until the availability of tests for HIV RNA load, it was the main assay available for monitoring the progress of HIV infection. However, the drawbacks of this test are that the antigen is poorly quantifiable, may not be detectable in many individuals and does not change very well with anti-retroviral therapy. It is therefore not particularly considered a useful prognostic marker. The ultrasensitive p24 antigen assays developed recently need evaluation and validation vis-à-vis conventional viral load assays.

### Method and principle:

ELISA p24 antigen capture assay kits are available for detection of p24 antigen. If acid hydrolysis is used to disrupt antigen-antibody complexes in serum, the sensitivity of p24 antigen detection can be increased, and this assay may be a tool for early diagnosis (during the window period). Therefore, most kits presently used for diagnosis include immune complex dissociation (ICD) at acidic pH, to increase sensitivity. Quantitation for monitoring p24 is possible by testing serial dilutions of the plasma/serum.

On the whole, p24 antigen detection assay is neither a good diagnostic nor a good monitoring test for HIV infection on account of low sensitivity. However, a positive p24 test in case of discordant serological test results in an infant born to HIV infected mother and during the window period supports HIV infection.

### Culture and isolation of HIV

HIV may be isolated from the blood and other body fluids but this is predominantly a research tool and the facilities for viral culture are only available in a few select reference laboratories. Isolation of HIV is a specialized procedure requiring at least P2+ containment facility and high degree of expertise. HIV isolation has therefore remained mostly as a tool for research. HIV is isolated from the peripheral blood mononuclear cells (PBMC) or plasma and other body fluids. The activated CD4+ cells are susceptible for HIV infection. Hence autologous or heterologous PBMCs activated with mitogen phytohaemagglutinin (PHA) are cultured with the infectious material. The cultures are maintained at 37°C in 5% CO<sub>2</sub> atmosphere for up to 28 days. The cultures are fed with freshly activated PBMCs at regular intervals. The presence of virus in the culture supernatant is detected either by demonstration of the presence of p24 antigen or enzyme reverse transcriptase. The infected cells may also demonstrate syncytia in culture. Virus infected cells may also be detected by HIV-specific immunofluorescent assay.



## 11. NATIONAL STRATEGIES AND ALGORITHMS FOR HIV TESTING

### Introduction

Two distinct human immunodeficiency viruses, HIV-1 and HIV-2 are the aetiologic agents of AIDS. Phylogenetically, HIV-1 is divided into Group M [10 subtypes A-K excluding I] and Group O [9 subtypes) Group N (new virus) and HIV-2 into 6 subtypes (A to F). In Thailand, India and sub-Saharan Africa, ~ 90% of HIV-1 infections are acquired through heterosexual transmission in contrast to 10% in the U.S. and Western Europe. Subtypes A, C and D predominate in Africa, subtypes E and B are commonly found in Thailand and C is the main subtype in India; whereas, subtype B predominates in the U.S. and Western Europe.

Laboratory diagnosis is the only method for determining HIV status of an infected individual. A number of tests and diagnostic kits are available to assess the HIV status of individuals. Serological tests are most commonly performed.

The choice of test protocol depends upon:-

- Objectives of HIV testing
- Sensitivity and specificity of the test used
- The prevalence of HIV infection among the population
- Resources available
- Appropriateness to the strategy of testing
- Infrastructural facilities available

Objective of HIV testing

- Blood and blood products safety. This is determined by mandatory screening of each unit of donated blood
- Donors of sperms, organs and tissues are tested for HIV to prevent HIV transmission to the recipient
- Diagnosis of HIV infection in clinically suspected individuals
- Prevention of parent to child transmission after counseling and informed consent
- Voluntary testing: high risk groups after counseling and informed consent
- Sentinel surveillance to monitor epidemiological trends
- Research, surveys to identify population groups requiring specific interventions, etc.

### Sensitivity

It is the accuracy with which a test can confirm the presence of an infection in an infected individual. Tests with high sensitivity show few false negatives and are meant to be used to screen blood prior to transfusion and ensure blood safety.

### Specificity

It is the accuracy with which a test can confirm the absence of an infection in an infected individual. Tests with high specificity show few false positives and are to be preferred for the diagnosis of HIV infection in an individual.

### Prevalence of HIV infection

The probability that a test will accurately determine the true infection status of a person being tested varies with the

prevalence of HIV infection in the population from which the person comes. The higher the prevalence, greater is the probability that a person testing positive is truly infected i.e. greater is the positive predictive value (PPV) of the test. The likelihood that a person showing a negative result is truly uninfected the negative predictive value (NPV) decreases as the prevalence of HIV infection among the general population increases.

## Laboratory investigations for HIV infection

HIV infection can be detected in the laboratory either by detection of antibodies to HIV, or by detection of the virus, its antigen and its DNA. Detection of specific antigens, viral nucleic acid, isolation / culture of virus are all confirmatory tests in that the presence of the virus is detected. But they are risky because of the danger of infection to laboratory workers, are very laborious and difficult to perform, require skilled expertise and hence are to be done only in specified laboratories

The indirect predictors of HIV infection (CD4 cell count,  $\beta$ 2 microglobulin, etc.) are monitors of immunity status of patients and are to be done at routine intervals to monitor the progression of disease.

The specimens which can be utilized to detect various markers of HIV infection are given below.

- (i) Antibody detection
  - Blood / serum / plasma3-5 ml. of blood is collected in clean, screw capped plain vial for ELISA and for the supplemental tests. Saliva and urine have been used to detect antibodies to HIV but the assays have not been validated in India.
- (ii) Antigen detection
  - Serum / plasma
  - Cerebrospinal fluid
  - Cell culture supernatant (i.e. the tissue culture fluid)
- (iii) Virus isolation

Virus Isolation can be attempted on HIV infected tissues. It may be isolated from blood (PBMN cells), semen, vaginal / cervical specimen, tissue, CSF and plasma. It is less successful on other body fluids like saliva, urine, breast milk, tears and amniotic fluid. Virus isolation is done for research purposes only and never for diagnosis in India.

## Detection of specific antibodies

This is done by performing initial screening tests, which if positive, are followed up by supplemental tests to confirm the diagnosis.

### Screening tests

The screening assays can be either ELISA & or rapid HIV tests. ELISA is the preferred test at blood banks, NRLs and SRLs where a large number of samples are tested at a time. Rapid tests are preferred at ICTCs and PPTCTCs where the number of samples to be tested is less and where same day/emergency testing is required.

ELISA (Enzyme Linked Immunosorbent Assay) is the most commonly performed test at blood bank and tertiary labs to detect HIV antibodies.

There are various kinds of ELISA based on the principle of test:

- Indirect ELISA
- Competitive ELISA
- Sandwich ELISA
- Immune capture ELISA

ELISA is also classified on the basis of the antigens utilized into:

- 1st generation: Infected cell lysate is used as the antigen.
- 2nd generation: Glycopeptides (recombinant antigens) are used as the antigen.
- 3rd generation: Synthetic peptides are used as the antigen.
- 4th generation: Antigen and antibodies are detected simultaneously. The assays may use a combination of recombinant and synthetic peptides as antigens.

Rapid tests include:

- Dot blot assays (immunoconcentration based)
- Particle agglutination (gelatin, RBC, latex, microbeads)
- Dip stick and comb tests, etc (ELISA technology based).
- Immunochromatography based tests

When a serum sample tests reactive once by a system of ELISA / Rapid (E/R) test, the test is to be repeated immediately by a different system in order to confirm the diagnosis. The sample is then to be taken up for supplemental tests to confirm the diagnosis. Supplemental tests may be E/R/, WB, IFA, RIPA etc.

ELISA takes up to three hours to yield results. It has a major advantage of being economical. Although rapid tests give result within minutes these are far more expensive. Commercial kits are available for ELISA and rapid tests.

Tests which detect antibody to both HIV 1 and 2 and all the subtypes are to be employed

**Supplemental tests**

- Second and third Elisa/Rapid
- Western blot



WB is done in legal cases, problem cases and for research

WB is expensive, time consuming and requires expertise to perform. This is to be done to confirm the diagnosis on samples which given discordant results in E/R.

**Strategies/Algorithms of HIV testing**

Because of the enormous risk involved in transmission of HIV through blood, safety of blood products is of paramount importance. Since the PPV is low in populations with low HIV prevalence, WHO/GOI have evolved strategies to detect HIV infection in different population groups and to fulfil different objectives. The various strategies, so designated, involve the use of categories of tests in various permutations and combinations.

1. ELISA/ Rapid tests:  
Used in strategy I, II & III
2. Supplemental test: E/R and Western Blot(WB)  
WB is used in problem cases where discordant serological results are obtained, if WB is not available than client should be retested all over again after 2-4 weeks.

### Strategy/Algorithm I

Blood/Plasma/Serum is subjected once to a highly sensitive E/R for HIV. If negative, the sample is to be considered free of HIV and if positive, the sample is taken as HIV infected for all practical purposes. This strategy is used for ensuring donation safety (blood, organ, tissues and sperms, etc.). Unit of blood testing positive is destroyed as per guidelines. A donor who gives consent to know the result of HIV test is informed about the result. In case the test is positive donor is referred to ICTC for counselling, testing and confirmation of the test result.

### Strategy/Algorithm II A

Sample is tested as above and in case of positive is tested further. A serum sample is considered negative for HIV if the first ELISA or rapid test reports it so, but if reactive, it is subjected to a second ELISA or rapid test which utilizes a system different from the first one i.e. the principle of test and/or the antigen used is different. It is reported reactive only if the second ELISA/ rapid test also gives the positive report like the first test. In case the second E/R is non reactive, then the result is taken as negative for sentinel surveillance purposes. This type of HIV testing is anonymous and unlinked.

### Strategy/Algorithm II B

This strategy is used to determine HIV status of clinically symptomatic suspected AIDS cases in whom Blood/Serum/Plasma is tested with the highly sensitive screening test. The sample is considered negative if the test gives non-reactive result. In case the test result is reactive the same sample is tested with another test kit (based on different principle of test or having different antigens compared to the first test), if the result is reactive with second test kit also the sample is considered to be positive in a symptomatic AIDS case. So, in cases where the physician indicates that the patient is suffering from clinical AIDS like symptoms, the HIV status of the patient can be confirmed as positive on the basis of two positive test results. In case sample is positive by first test kit and negative by second test kit, the sample is subjected to a tiebreaker third test. If third test is reactive, sample is reported as indeterminate and follow-up testing is undertaken after 2-4 weeks. In case the tie breaker third test is negative, sample is reported as negative. Counselling, informed consent and confidentiality are a must in all these cases.

### Strategy/Algorithm III

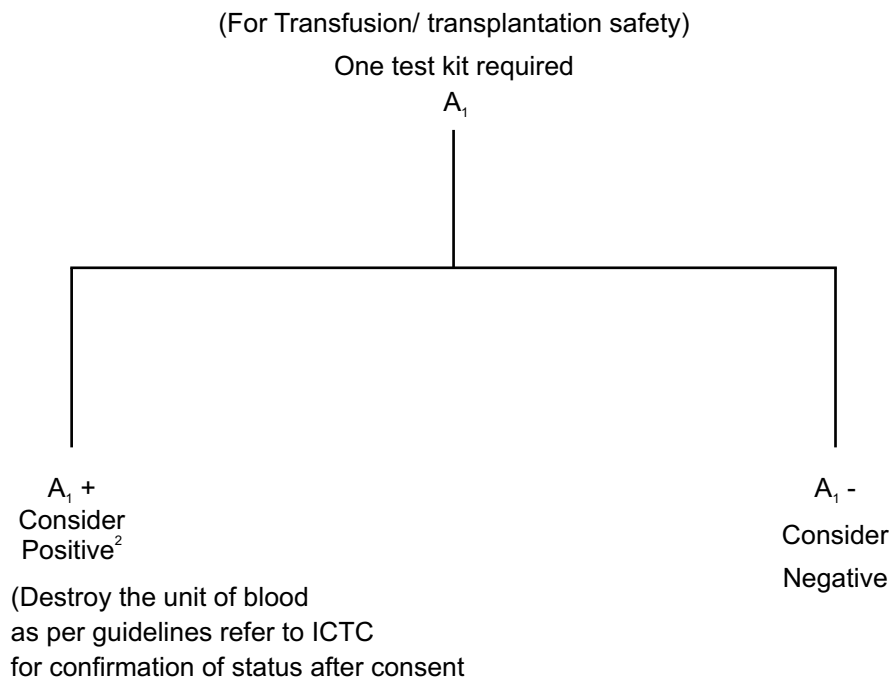
For strategy III the HIV testing done is similar as for strategy IIB, with the added testing by a third test for positive result. Positive confirmation of a third reactive E/R test is required for a sample to be reported HIV positive. If the sample gives reactive result with two E/R and non reactive with the third assay it is reported as "indeterminate" and patient is called back for repeat testing after 2-4 weeks. The test utilized for the first screening test is one with the highest sensitivity (may give high number of false positives) and those used for the second and third tests are those with the highest specificity (to minimize false positive reactions). This strategy is used for diagnosis of HIV infection in asymptomatic individuals at ICTCs and PPTCTCs. Counselling, informed consent and confidentiality are a must in these cases. Three different kits with different antigen system and / or different principle of test are required to follow this strategy.

Strategy IIB & III are to be used for diagnosis of HIV infection. Second and Third test should be tests with the highest Positive Predictive Value (PPV) possible to eliminate any chances of false positive results. If sample gives reactive result with two assays and non reactive with third assay in a healthy asymptomatic individual is reported as “indeterminate” and follow up testing is done after 2-4 weeks. A sample reactive in first assay and non-reactive in second assay is subjected to a third tie breaker assay. In case the third assay also gives non- reactive result, sample is reported as negative for HIV infection. In such cases however, if client history is suggestive of high risk factors/high risk behaviour, he may be asked to come for follow up testing after 2-4 weeks. If sample gives reactive result with third test kit, then the individual is reported as “intermediate” and patient is called back for repeat testing after 2-4 weeks.

Antibodies to HIV-1 are most commonly and reliably detected by ELISA/Rapid and confirmed by ELISA/Rapid tests. Antibody testing by ELISA remains the standard method for screening potential blood donors; simultaneous testing for p24 antigenemia is superfluous because of low sensitivity and expense. Use of improved, third and fourth generation serological assays demonstrates that seroconversion typically occurs 3-12 weeks post-infection, although significant delay can occur in some individuals. p24 antigen positivity and /or PCR detection of proviral DNA can narrow the window period following primary infection.

In diagnosing HIV-1 infection, the specificity of ELISA is >99% when properly performed and the sensitivity is >98%. In low-risk populations, the false-positive rate of combined EIA and WB/IB testing is estimated to be <1 in 100,000. Highly sensitive and specific agglutination and EIA methods for detection of type-specific antibodies to HIV-2 are also available.

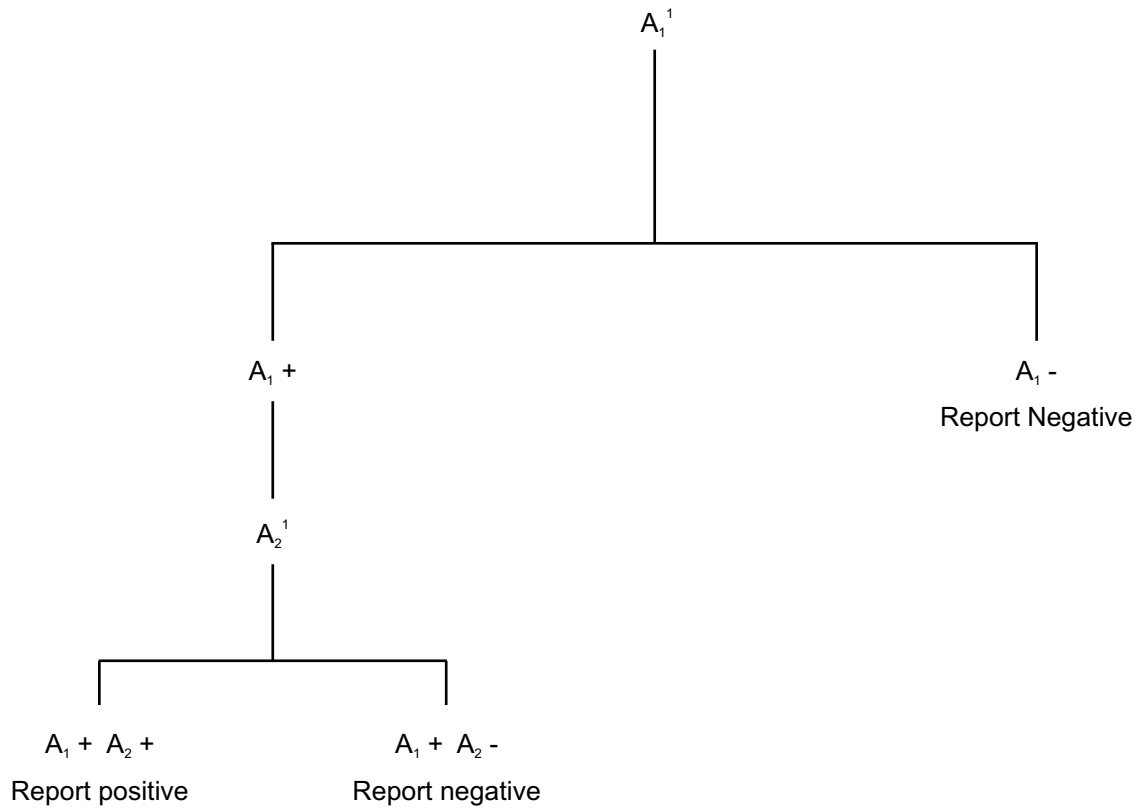
The screening test selected preferably should be fourth generation and the sensitivity of the test should be 100%. The supplemental/confirmatory tests selected should be 99.8% sensitive and at least 98.5% specific. Highly sensitive tests reduce the occurrence of false negative result and fourth generation HIV tests reduce the window period to 10-15



(Explanation of Positive <sup>2</sup> is given in legend after the strategies)

Strategy/Algorithm II A

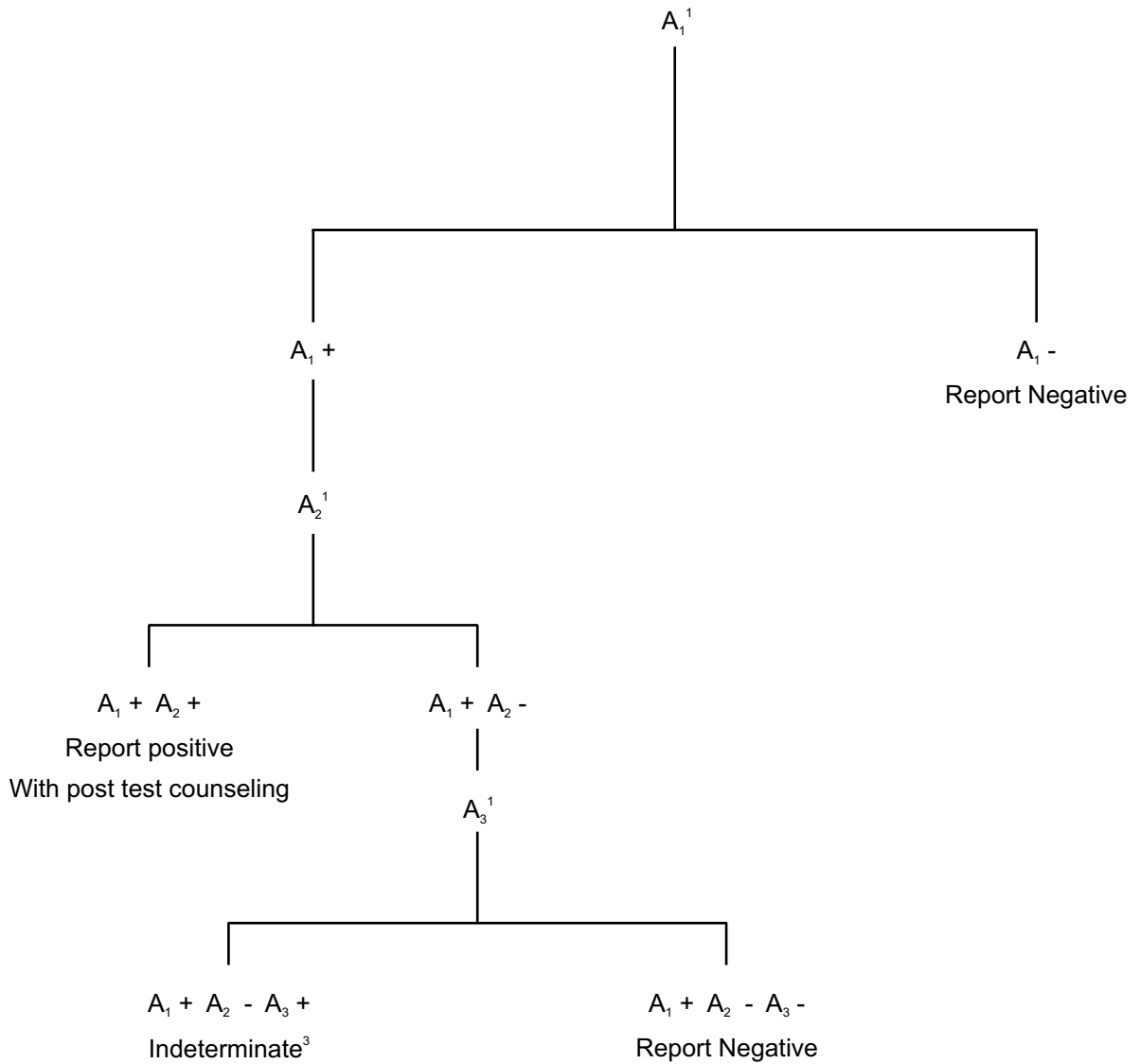
(For surveillance)  
2 Test kits required



Strategy/Algorithm II B

(Diagnosis of an individual with AIDS indicator disease symptoms.)

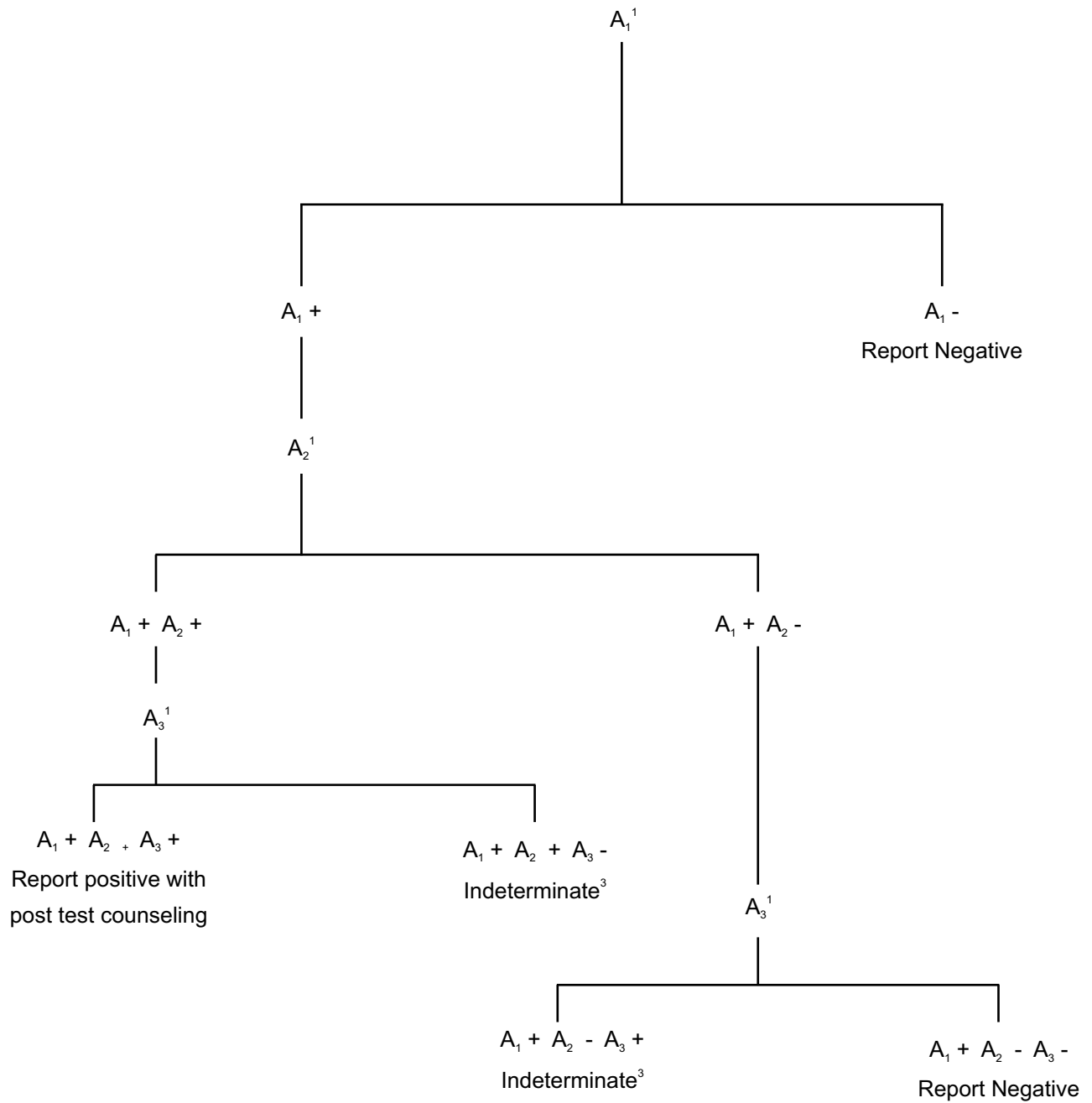
3 Test kits required.



Strategy/Algorithm III

[To detect HIV infection in asymptomatic individuals (VCTCs, PPTCTs)]

3 Test kits required





days thus playing an important part in ensuring blood safety. Highly specific tests reduce the occurrence of false positive result and thus ensuring that a person testing positive as per the strategy IIB/III is really HIV infected.

<sup>1</sup>Assays A1, A2, A3 represent 3 different Assays

<sup>2</sup> Such a result is not adequate for diagnostic purposes: use strategies IIB or III. Whatever the final diagnosis, donations which were initially reactive should not be used for transfusions or transplants. Refer to ICTC after informed consent for confirmation of HIV status

<sup>3</sup>Testing should be repeated on a second sample taken after 14-28 days. In case the serological results continue to be indeterminate, then the sample is to be subjected to a Western blot /PCR if facilities are available or refer to the National Reference Laboratory for further testing.

### **Detection of p24 antigen**

Detection of p24 viral antigen is expensive. The sensitivity of the test is also limited. Though a positive test may confirm HIV infection, a negative test does not rule out HIV infection. It is never used as a screening test. p24 testing may be undertaken in the following situations:

- To detect infection in the newborn (not reliable)
- To help resolve equivocal Western Blot results
- To detect infection during early window phase
- To diagnose CNS disease
- Late stage of disease (immune collapse)
- To monitor response to anti-retroviral therapy ( quantitative assay, needs validation)

EIA for HIV-1 antigen detects primarily uncomplexed p24 antigen, in serum, plasma, CSF or cell culture. It indicates active infection, allows diagnosis before seroconversion, quantitative test can predict prognosis and is useful for monitoring response to therapy. Disadvantages of antigen detection assays include: poor sensitivity (only 69% in patients with AIDS and low in neonates < 1 month old); detection is not possible in patients with high titers of p24 antibody (which complexes with the antigen); and failure to detect HIV-2 antigen.

### **Polymerase chain reaction (PCR)**

PCR is never used as a screening test.

PCR can detect proviral DNA during window period, can differentiate latent HIV infection from active viral transcription and can quantitate the copy number of HIV DNA when used with external standards (e.g. viral load assays). PCR can successfully differentiate between HIV-1 and HIV-2 infections. Proviral DNA can be detected in peripheral blood mononuclear cells before seroconversion. Limitations to the diagnostic use of PCR are rare false-negatives, some of which can be avoided by the use of multiple primer pairs and primers from conserved regions of the genome, and false-positives due to cross-contamination of the PCR reaction mixture.

HIV-1 can be detected by PCR in the CSF of HIV-infected patients independently of disease stage; spread of HIV-1 to the brain represents an early event during infection which occurs in most asymptomatic individuals. PCR is also used to detect HIV infection in neonates born to HIV infected mothers.

### **Virus culture**

Virus culture is another method for identifying HIV infection. Positive culture rates of up 98% are reported in confirmed

seropositive individuals. The culture method is, however, expensive, labour-intensive, can take weeks for complete results and potentially exposes laboratory workers to high concentrations of HIV. Virus culture is used for research (drug sensitivity, vaccine studies, etc.).

### **Viral load assay**

Quantitation of HIV RNA in plasma is useful for determining free viral load, assessing the efficacy of antiviral therapy and predicting progression and clinical outcome. Baseline HIV viral load is predictive of survival at 10 years in patients with nearly identical CD4 counts. Assessment of baseline viral load prior to initiation of therapy is useful in patient management.

### **Surrogate markers**

Prognostic factors for progression to AIDS requiring further study include elevated serum prolactin concentrations, decreased dehydroepiandrosterone concentrations, the presence of antibodies of HIV-1 virion infectivity factor protein, and elevated serum IgE concentrations. The role of cytokines and cytokine receptors and their potential prognostic value in HIV infection also require further investigation in addition to the other well known prognostic factors. Currently CD4 cells enumeration is used to assess the immune status and to assess the response to ART in India. The other indirect predictors of HIV infection such as increased,  $\beta^2$  microglobulin, increased IL 2 receptors, are not used any more to assess the immune status of the infected individual.

### **Unlinked anonymous testing**

Such type of screening or testing is not directed to the individual, but has as its objective, the public health surveillance of HIV infection. It is a method for measuring HIV prevalence in a selected population with the minimum of participation bias. Unlinked anonymous screening offers a distinct advantage over mandatory or voluntary testing. Unlinked anonymous testing involves use of blood already collected for other purposes; therefore, the effect of selection bias will remain though minimal, and will depend upon time, location and other details of blood collection.

### **Voluntary confidential counselling and testing**

Testing is often done for diagnostic purposes. Here it is important that the issues related to confidentiality receive great attention. Since this method is based on voluntary HIV testing or testing for diagnosis of HIV/AIDS cases, it is imperative to respect the individual's need to maintain confidentiality. By maintaining confidentiality, it will not only instill faith in the individual about the health care system in the community but also encourage more and more people practicing risk behavior to come forward for an HIV test. This testing is done after counseling and written informed consent of the client.

### **Mandatory testing**

When testing is done without the consent of the patient and data could be linked to identify the person it is called "mandatory testing". Mandatory testing is recommended only for screening donors of semen, organs or tissues in order to prevent transmission of HIV to the recipient of the biological products.

### **Choice of HIV tests**

The choice of tests is also based on the different objectives of HIV testing. The tests that are adopted are the ELISA or Rapid clubbed together as 'E/R'. One E/R denotes test done on one single antigen preparation; two is when all positive samples on first antigen test are repeated on a second antigen preparation and three is when this test is

repeated on the same sample for a third time using a different antigen system. For transfusion safety purposes one E/R is used; for surveillance, two E/R are used, for diagnosis of full blown AIDS cases two/three E/R are used and for asymptomatic individuals three E/R are used. The test selected for screening units of blood at the blood banks should be third or fourth generation ELISA which is 100% sensitive. This will help to minimise false negative results. The test selected for diagnosis should be >99.8% sensitive and highly specific. This is to minimise the false positive result.

### **General principles of HIV testing**

Testing policy in general should consider the following points:

- It should be part of the overall comprehensive preventive programme.
- Testing should be technically sound and appropriate.
- Test procedure must be appropriate to the field situation.
- Testing procedure must be cost effective.
- Laboratory procedures must be monitored for ensuring quality.

### **HIV testing in health care settings**

The fear and apprehension that exists among health care workers in managing HIV infected individuals and AIDS patients is largely due to the minimal risk that exists of HIV transmission due to a needle stick or other sharp injury. Thus the demand for mandatory HIV testing of patients admitted in hospitals or undergoing surgery, etc is not rational. This demand is neither rational nor appropriate. A mandatory HIV test is no substitute for Standard Work Precautions that need to be adopted for every patient in a hospital or any other health care setting. On the other hand testing without explicit consent of the patient has been proven to be counterproductive in the long run. In the control of the HIV epidemic such testing can drive the target people underground and make it more difficult for launching interventions.

The national testing policy reiterates the following:

- No individual should be made to undergo a mandatory testing for HIV.
- No mandatory HIV testing should be imposed as a precondition for employment or for providing health care services and facilities.
- Any HIV testing must be accompanied by a pretest and post test counseling services, informed consent. Confidentiality of result should be maintained.

## 12. QUALITY ASSURANCE PROGRAMME

### Necessity and importance

The diagnostic tests to detect antibodies to HIV have sensitivity and specificity which are not absolute. In all these tests we have false negative results as well as false positive results which are inherent and cannot be avoided. The percentage of false positive results will increase as the prevalence rate of persons with HIV antibodies in a population increases. These two problems are compounded by the fact that during testing, the laboratories will rarely be able to perform to the level of accuracy that the tests are technically capable of achieving. Thus, the validity of diagnostic test results is dependent to a very large extent on the quality of the technical conditions under which the tests are performed. Meaning thereby, consistent production of reliable results requires a stringent overall assurance programme which would control technical conditions before, during and after each assay.

The Quality Assurance Programme ensures that the final results reported by the laboratory are correct, reliable and accurate as far as possible. This programme oversees reporting results in a timely manner and to the appropriate individual. It also ensures use of the most reliable tests for the diagnosis of HIV infection, using the algorithm/strategy commensurate with objective for which testing is being undertaken. It is dependent on a good Quality Control Programme and its efficacy may be verified by a good Quality Assessment Programme.

### Quality control programme

This programme includes measures which are introduced during each assay to verify the validity and reliability of the test. However, this does not indicate that the results generated are accurate (which, indeed, is the characteristic of the test). It also does not indicate that the results have been reported timely, properly and correctly.

### External quality assessment programme

This is a means to determine the quality of results. It is an external evaluation of a laboratory performance by incorporating proficiency panels as the means to evaluation. For a laboratory to be considered a respected testing facility, it must be a laboratory that can always produce accurate, reliable and reproducible results.

## Guidelines to improve quality of testing

### Condition of the specimens

All specimens must be inspected at the time of receiving and also before testing to ensure that they are suitable. Use of lipaemic, haemolysed and contaminated sera should be avoided. If they have to be used, the reporting officer should mention on the report that the result of the test may not be valid because of the condition of the serum samples. A new specimen should be requested for repeat testing.

All specimens should be properly labeled before acceptance. The label should include following information: a) name of the patient, b) collection date, c) patient's identification number, if applicable. Each specimen must be accompanied with a test request form which should include age and sex of the patient, name of the physician requesting the investigation, risk group of the patient, reason for the investigation in addition to the information given on the label. In case of unlinked anonymous testing, only code number and collection date may be mentioned on the label as well as on the request form.

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If the serum or plasma sample is frozen before testing, it is essential that after thawing at room temperature/37°C, the sample should be inspected for any clot or floating particles. The sample should be clarified before testing. The sample should be well-mixed before testing.

Presently, all the test kits distributed by the NACO, are meant for testing blood, serum or plasma only. Therefore, these kits may not reliably detect presence of antibodies in other body fluids.

### **Quality of kits and equipments**

The test kits must be used within the expiration date stated on the kit to ensure valid results.

Every batch of the kit should be tested and certified for its efficacy (sensitivity and specificity) before distribution.

ELISA reader, washers, incubators, pipettes should be checked regularly for their optimal performance. They need to be calibrated every three months, at least annually.

### **Controls used in the tests**

Each test run requires a set of controls to validate the results. These controls must be treated in the same manner as unknown samples. They are run simultaneously and under the same conditions as the unknown samples. Upon completion of the test, the results of the controls and the samples are examined using the same criteria for interpretation. The assay is valid and the results are reliable when the controls produce acceptable results. The procedure of validation of ELISA and rapid tests is given in details in the “Manual on Quality Standards for HIV Testing Laboratories”.

There are two types of controls which must be included in each run :

#### **Internal controls**

These controls (positive and negative) are included in each HIV-test kit by the manufacturer and are to be included in each test run. These are essential for quality control measures for each run. They are intended for use with the same lot number of the kit in which they have been packed. Internal kit controls are generally adjusted by the manufacturer so that an expected range of values are obtained with each lot of kits. To avoid considerable fluctuations in the OD values due to variable coating of the antigen on the solid surface during manufacturing, the manufacturer, artificially stabilizes kit controls to give the same values as the previous lot.

#### **External controls**

These should be included with each test-run to monitor consistent performance and lot to lot variation which cannot be detected using internal controls for the reasons mentioned above. These controls are made from pooled test kit controls or made from pooled sera from HIV-positive or negative individuals in each laboratory (in-house controls). However, for monitoring the performance of other laboratories, the serum samples for external controls are drawn in sufficient quantities to last for at least 12 months.

The most important external control to include is a borderline reactor. This control would indicate any minor change especially around cut off value because such changes would effect the results of unknown samples with OD values near the cut off. It may take several months to establish ranges for external controls. Nonetheless, this type of

control system can be very effective in helping to identify potential problems and inaccuracies in the laboratory setting.

The preparation and standardization of the quality external controls is given in details in the Manual on Quality Standards for HIV Testing. Reproducibility and quality of internal and external controls must be standardized by intra-run reproducibility and inter-run reproducibility. The control samples (either internal or external) are tested at least three times on the same test-run. This will indicate intra-run reproducibility. This exercise is then carried out on test-runs on 20 consecutive days to determine inter-run reproducibility. By either of these methods variations should not exceed 10%. These serum samples may also be evaluated at the National Reference Laboratories.

### **Interpretation of data**

The test kit inserts carry instructions to establish range of internal controls and to define the outliers. Since the external controls are included in each test run to monitor consistent performance of the test kit, it is necessary to determine the limit of acceptability statistically by calculating arithmetic mean, standard deviation and error, coefficient of variation etc. However, the values for the internal controls, external controls and the cut off can be monitored easily by quality control graphs. This is because the graphic presentation of the control values over time on Levy Jenning chart will make subtle changes in controls more easily discernible.

An accurate method of graphically representing the values of control of each test run is to plot OD/cut-off (CO) ratio on Y-axis. In this method the control values are expressed relative to the cut-off value i. e. "E" ratios. This is important as the OD as well as CO values will change slightly between test-runs. Therefore, the controls should be compared with the respective calculated CO value.

Two types of changes can be observed in the quality control graphs. These are.

### **Shifts**

When control values of six consecutive test runs fall on one side of the mean, it is called shift. This indicates a major change in the test-performance due to i) switching to a new lot of kits, ii) new reagents, iii) changes in incubation temperature, iv) change of pipettes, v) a new technician, etc.

### **Trends**

When control values of six consecutive tests are distributed in one general direction, it is called trend. This is generally due to i) deterioration of reagents, and ii) a routinely used pipette slowly losing its calibration.

### **Calculation of grey-zone reactors**

During routine testing, many samples may have slightly elevated OD values just above CO value which may suggest presence of low antibody activity. Such samples are called grey-zone reactors or borderline reactors. This reactivity may be due to a) early seroconversion; b) very low antibody reactivity present in the serum; c) false positive reactivity. Therefore, it is very essential to repeat any HIV reactive result having OD values greater than or equal to the cut-off value. Any repeatedly positive result is then validated by a more specific supplemental test like Western blot.

Sometimes, due to a technical error a serum with low antibody reactivity may give an OD value just below the cut-off value. Therefore, it is advocated that a repeat HIV test be performed on all the samples which give an OD value of 10% below the cut-off. Although this is very arbitrary, this approach increases the chance of finding some early HIV seroconversion.

### Reporting of results

Unlike any other test report, the HIV-test reports must be handled with care. Since HIV infected individuals may face social stigma, improper reporting may sometimes lead to emotional breakdown of an individual or even suicide. Therefore, before reporting the result of HIV-antibody test it must be borne in mind that:

- a) The results of any screening test (E/R) are only presumptive and should not be reported. These must be validated by a supplemental test.
- b) In blood-banking only donated blood is screened by a single E/R as per recommendations of Govt. of India. Therefore, these results should not be used to identify the individuals. In case the donor has given consent to receive the report, the donor is given the provisional report and is referred to ICTC for counseling and testing for confirmation of result.
- c) In serosurveillance studies the positive results of first E/R are validated by a second E/R, these results are not used to identify the asymptomatic individuals.
- d) For making a diagnosis of HIV-infection, the recommended strategies/algorithms must be followed.
- e) HIV test results should be reported to the physician who has requested the test. All HIV testing, in which an individual is identified, must be preceded and followed by pre-test and post-test counseling, respectively.
- f) All test results must be kept confidential and should never be discussed in public. The test results should never be communicated on telephone.

### Record keeping

- a) A laboratory must maintain a log book for recording of the laboratory specimens. The information contained in the log book should be kept confidential.
- b) A work sheet (see annexure) containing the identification numbers of sera to be tested must be prepared each time before the test run is performed.
- c) Daily records of temperature of water baths, incubators, refrigerators and freezers should be maintained.
- d) Micropipettes should be calibrated, ideally every three months or at least bi- annually.
- e) ELISA readers should be calibrated to ensure accuracy of their readings.
- f) The laboratory should maintain a file where all procedures/package inserts are kept for ready reference.
- g) Numbers of ELISA positive samples being reported by each laboratory from various high risk group, moderate risk group and low risk group should be maintained.
- h) Number of ELISA positive samples which have been found negative by Western blot assay should be maintained.
- i) Number of times an ELISA positive serum sample was rerun before subjecting it to supplemental test should be maintained.

These data will reflect upon the technical problems if any existing in a laboratory.

### Tracking the performance of the kit under field conditions

Each laboratory performing ELISA should maintain the following information to ensure good performance of the kit.

- i) OD values of internal and external positive controls of each test run
- ii) OD values of internal and external negative controls of each test run
- iii) OD values of external borderline positive controls of each test run

- iv) Cut off value of each test run
- v) Name, batch and lot number of the kit used.
- vi) Expiry date of the kit used.
- vii) Date of the test run.

This information will help to keep a track of the performance of the kit under the field conditions.

**Quality control of HIV antibody test kits**

Monitoring of different lots of HIV Kits :

Sometimes a manufacturer produces a reagent-lot that passes quality control requirement in his unit but fails to perform adequately in the field. This could be due to several factors including artificial stabilization of the controls by the manufacturer, substandard storage and shipping conditions, etc. Therefore, it is imperative that these lots of reagents must be identified quickly before distribution to the surveillance centres. This is done by a technique called “Parallel testing” in which performance of new lots of kit with the previous lots via a common control material (external controls and the controls from previous lots) is compared. If all controls produce expected results, the new lot has passed the parallel test and may be used for routine testing.

A quantitative expression of sensitivity of HIV kits in terms of positive delta value (delta +) is better and more reliable than percentage value. This helps in selecting among ELISA kits with equal sensitivity. The higher the delta+value, the higher is the probability that this test will correctly identify antibody positive sera.

For sero-diagnosis and sero-surveillance, an ELISA kit with a high sensitivity and high specificity is needed.

A negative delta value (delta-) is quantitative expression of specificity. The greater the negative delta (delta-) value, the higher is the possibility that this assay will correctly identify the true negative sera.

**Calculation of delta values :**

- a) To calculate the delta values, 50 confirmed positive and 50 confirmed negative sera are tested.
- b) Cut off value is calculated as suggested by the manufacturer.
- c) Mean OD and standard deviation of positive and negative samples is calculated.
- d) Mean OD/CO ratio of positive and negative samples is calculate by the following formula

$$\text{OD/CO ratio} = \frac{\text{Mean OD of the samples}}{\text{Mean CO value}}$$

Delta value is calculated by the following formula :

$$\text{Delta +} = \frac{\text{Mean OD/CO ratio of positive samples (log 10)}}{\text{Standard deviation of positive samples}}$$

$$\text{Delta -} = \frac{\text{Mean OD/CO ratio of negative samples (log 10)}}{\text{Standard deviation of positive samples}}$$



# 13. LABORATORY TESTS FOR MONITORING, STAGING AND PROGRESSION OF HIV INFECTION

## Introduction :

Infection with the human immunodeficiency virus (HIV) may develop to Acquired Immune Deficiency Syndrome (AIDS) at different rates in different individuals, with a spectrum varying from rapid progression to long term non-progression. This variability makes it essential to have tests which can accurately assess the stage of infection in an individual, as well as predict its course and monitor its progression. These laboratory tests are very valuable during the period of clinical latency, and, subsequently, supplement various clinical parameters, which are also extremely important in categorizing the infection / disease stage. Response to anti-retroviral therapy is also monitored using these prognostic tests.

### Laboratory tests :

Increased HIV replication leads to a depletion of CD4 cells, which are its main target. CD4 cell count, a very useful marker, is the most commonly used laboratory test for staging HIV infection and monitoring its progression.

The increased rate of HIV replication which is reflected in an increase in plasma viral RNA load is presently considered one of the most representative and sensitive laboratory test for monitoring progression of HIV infection. However in resource limited settings, CD4 count enumeration remains the mainstay for monitoring the progression of infection in HIV positive individuals.

Markers like antibodies to HIV antigens ( p24, p17, nef,etc.) , soluble markers of immune activation e.g. neopterin, beta 2 microglobulin and some cytokines and their receptors in serum were previously considered relevant for monitoring the course of HIV infection but are no longer recommended for the same .

The laboratory tests currently used for monitoring stage and progression of HIV infection can be classified into:

- Viral markers
  - Plasma HIV RNA load
  - Viral antigenaemia e.g. p24 antigen
- Immunologic markers
  - CD4 cell count

### Viral markers

#### Plasma HIV viral load

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. RNA is the genetic material of HIV that contains the information needed to make more virus. Although total quantification includes cell-free virus, virus in infected cells in all compartments of the body, and integrated provirus, the easiest measurement of viral load is that of cell-free virus in an individual's plasma .

Monitoring Human Immunodeficiency Virus type 1 (HIV-1) viral load has become one of the critical standard of care

for monitoring and managing response to the therapy in HIV-infected patients and progression toward AIDS

Viral load tests measure the amount of HIV-RNA in a small amount of blood .There are different techniques for doing this:

- The PCR (polymerase chain reaction) is the most frequently used test. It uses an enzyme to multiply the HIV in the blood sample. Then a chemical reaction marks the virus. The markers are measured and used to calculate the amount of virus. Test results are reported as copies/ml.
- The bDNA (branched DNA) is a fairly frequently used test. It combines a material that gives off light with the sample. This material binds with the HIV particles. The amount of light is measured and converted to a viral count.
- NASBA (Nucleic acid sequence based amplification assay) is an in vitro nucleic acid amplification test for quantitation of amplified HIV-1 RNA which is measured by means of electrochemiluminescence .

The PCR test results are often different from the bDNA results for the same sample. Because the tests are different, only one kind of test (PCR or bDNA) should be used to measure a person's viral load over time.

Viral loads are usually reported as copies of HIV in one milliliter of blood. The best viral load test result is "undetectable". This does not mean that there is no virus in the blood; it just means that there is not enough to be detected through the test. "Undetectable" depends on the sensitivity of the test used on the blood sample

The viral load test is helpful in several areas:

- The viral load test is valuable for managing therapy, to see if antiviral drugs are controlling the virus.
- PVL helps in monitoring antiretroviral therapy. Suppression of HIV RNA levels followed by rebound levels might signal:
  - suboptimal adherence to the antiretroviral therapy regimen
  - the emergence of drug-resistant HIV variants
  - decreased absorption of antiretroviral drugs
  - altered drug metabolism because of physiologic changes
  - drug interactions
  - vaccinations or concurrent infections.
- For prognosis- viral load can help predict how long someone will stay healthy. The higher the viral load, the faster the progression to AIDS and the terminal complications.
- Viral Load testing can also be used for
  - initial evaluation of newly diagnosed HIV infection
  - surveillance of patients who are not receiving antiretroviral drug therapy. For patients who haven't started taking medications, it helps decide when to start.
  - in pregnant HIV-infected women , the PVL predicts transmission risk.

## ***Viral load options ,methods and principles***

### ***1.Polymerase chain reaction***

- a) Roche Amplicor HIV-1 monitor version 1.5 and Roche Amplicor HIV-1 monitor ultrasensitive (RT-PCR)  
The Roche Amplicor HIV-1 Monitor Version 1.5 and Roche Amplicor HIV-1 Monitor Ultrasensitive Version 1.0 (RT-PCR) are approved by the FDA for the quantitation of HIV-1 RNA in plasma.. HIV-1 RNA is isolated from the plasma; then a complementary strand of DNA (cDNA) is transcribed from the

target RNA using reverse transcriptase. The cDNA is amplified using very specific oligonucleotide primers. Quantification of the RNA is achieved by hybridizing the amplified DNA to specific probes followed by a colorimetric detection assay.

Version 1.5 of the Amplicor HIV-1 Monitor assay was developed to perform equivalently with all group M subtypes of HIV-1. In addition, version 1.5 has a variation in design which allows amplicon capture on magnetic beads rather than on a microwell, and uses a Cobas Amplicor robotic analyzer which facilitates high level assay throughput.

b) **Abbott real time PCR**

The Abbott Real Time™ HIV-1 assay has been developed for use on the Abbott m2000™ system, an automated instrument using real-time PCR technology for the detection and quantification of infectious agents that cause disease. Real-time PCR is a modification of standard PCR, which is often compared to the photocopying of nucleic acid (DNA or RNA). The process involves the alternate heating and cooling of a small sample containing a segment of nucleic acid molecules dozens of times over several hours. Throughout this process, copies of these DNA molecules are "amplified" or exponentially increased so that the nucleic acid can be more readily analyzed. The method is called "real-time PCR" because the amplified DNA can be detected during the PCR process, in real time, rather than at the end of the process. This ensures more accurate and precise quantification of nucleic acid. Abbott has received CE Mark certification for a real-time PCR (polymerase chain reaction) test for monitoring HIV-1 viral load in patients. CE marking allows the test to be sold in the European Union.

## 2. Branched chain DNA (bDNA)

a) **Bayer Versant**

The Versant HIV-1 RNA 3.0 assay is approved by the FDA for quantitation of HIV-1 RNA in plasma. It uses the bDNA technology to measure viral load. The bDNA assay consists of a series of hybridization procedures followed by an enzyme substrate reaction. In this assay, HIV-1 present in the patient's blood is disrupted to release the viral RNA. The RNA is captured by a set of capture probes (bound by solid phase) while a set of target probes hybridizes both the viral RNA and the preamplifier probes. The amplifier probe hybridizes to the pre-amplifier probe forming a branched DNA (bDNA) complex. The bound bDNA is incubated with an enzyme and then with a chemiluminescence substrate.

b) **Chiron Quantiplex**

The Quantiplex HIV-1 RNA assay (Chiron Diagnostics, Norwood, MA) is a branched DNA (bDNA) sandwich method which quantifies plasma HIV-1 RNA by amplifying the signal rather than the target nucleic acid. The bDNA assay does not require viral RNA purification or PCR amplification steps. Instead, virions are concentrated by centrifugation and disrupted by detergent and Proteinase K, releasing viral RNA. This lysate is incubated with two sets of oligonucleotides. The first set captures viral RNA, hybridizing to both conserved regions of the HIV-1 pol gene and to oligonucleotides bound to the microwell. The second set of oligonucleotides provides signal amplification. This set consists of four components: oligonucleotides with homology to both the target RNA and to preamplifier

oligonucleotides; preamplifier oligonucleotides, amplifier oligonucleotides, and oligonucleotide probes bound to alkaline phosphatase (AP). Each of these components binds by hybridization to the next at multiple sites. In this way, the signal is amplified without copying the target RNA. Detection is by chemiluminescence using an AP-specific substrate. The amount of light detected is directly proportional to the amount of bound nucleic acid. The absolute quantity of HIV-1 RNA is determined from an external standard curve run on the same plate.

### 3. Nucleic acid sequence-based amplification (NASBA) assay

#### a) *NucliSens HIV-1 QT assay (bioMérieux)*

The NucliSens HIV-1 QT assay (bioMérieux) is a nucleic acid sequence-based assay and has been approved by the FDA for the quantitation of HIV-1 RNA in plasma. The NucliSens HIV-1 QT assay is based on target amplification using NASBA (nucleic acid sequence based amplification) technology (Organon Teknika, Durham, NC). The NASBA assay selectively and directly amplifies HIV-1 RNA without PCR in a one-step sandwich hybridization procedure using two oligonucleotide primers, three enzymes, nucleoside triphosphates and the appropriate buffers. In this viral load test, the HIV-1 is lysed and HIV-1 RNA is extracted and bound to silica beads. Nucleic acid amplification then occurs using specific primers derived from the gag region of the genome. The amplified RNA is hybridized to capture probes attached to magnetic beads. This cycle is repeated resulting in exponential amplification (1 million to 1 billion-fold) under isothermal conditions. The nucleic acid is detected and quantified by directly measuring electrochemiluminescence which is characterized by very high sensitivity and a broad dynamic range. Quantitation of HIV-1 viral load is accomplished by co-amplification of three internal RNA quantitation standards specific for HIV-1 gag and part of pol.

The isolation technique used in this assay allows diverse sample types (plasma, cerebrospinal fluid, lymph tissue, genital secretions, and cells) to be used as the source of viral nucleic acid; however, the FDA-approved assay, NucliSens HIV-1 QT, has only been validated for use with plasma. Another advantage of this assay is that the purified nucleic acid may be used for other molecular testing, such as sequencing.

#### b) *Primagen Retina TM Rainbow HIV-1*

The Retina Rainbow HIV-1 test is a real time amplification assay based on the NASBA Technology. It is capable of measuring the HIV-1 virus in body fluids like blood, plasma and serum that have been spotted and dried on a dedicated filter paper. Samples spotted on such filter paper are stable at ambient temperature, are not infectious and can be sent to a central testing lab by regular mail. Retina TM Rainbow enables detection of all circulating HIV-1 subtypes including group N and O and combined with the dried blood spot technology can bring HIV-1 virus testing to the resource and infrastructure poor countries.

Dried blood spot viral load protocols have also been developed for the Roche and bioMérieux assays.

Comparative performance characteristics of the FDA approved Versant bDNA, Amplicor HIV-1 Monitor 1.5, and NucliSens HIV-1 QT assays for viral load determinations have been tabulated below.

<b>Comparison of Roche Amplicor, Versant bDNA, and bioMérieux NucliSens</b>			
Properties	Roche Amplicor HIV-1 Monitor 1.5	Versant HIV-1 RNA 3.0	bioMérieux NucliSens HIV-1 QT
Sensitivity (copies/mL)	Standard 1.5 (400) Ultrasensitive 1.5 (50)	Version 3.0 (75)	NucliSens QT (176)
Specificity	Nearly 100%	Nearly 100%	Nearly 100%
Specimen type	Plasma in ACD or EDTA tube	Plasma in EDTA tube	Plasma in ACD, EDTA, or heparin tube
Dynamic range (copies/mL)	Standard 1.5 (400-750,000) Ultrasensitive 1.5 (50-100,000)	Version 3.0 (75-500,000)	NucliSens QT (80-3,470,000)
Specimen volume	Standard 1.5: 0.2 mL Ultrasensitive 1.5: 0.5 mL	1.0 mL 2.0 mL	1.0 mL
Specimen transport	Prepare plasma within 6 hours of collection; store specimens at 20°C or 70°C	Prepare plasma within 4 hours of collection; store specimens at 20°C or 70°C	Prepare plasma within 4 hours of collection; store specimen at 20°C or 70°C
Advantages	Version 1.5: FDA-approved Amplifies subtypes A-F	Better reproducibility (no amplification variation) Amplifies subtypes A-G	FDA-approved Amplifies subtypes A-G
Tests per specimen	One	One	One
Area of HIV genome selected for amplification	Gag	Pol	Gag
Amplification method	Target amplification	Signal amplification	Target amplification
Quantitative internal control	Yes	No	Yes

### **Detection of p24 antigen**

In principle, it is possible to measure the production of the viral protein p24 as an alternative to viral load. The p24 test is detecting a protein that is made inside infected cells and may be released from those cells in various ways, even if they are not producing viral particles. The results of p24 tests may be as consistent as viral load tests; but they are not quite measuring the same thing

## CD4 count

Human immunodeficiency virus (HIV) infects and destroys leukocytes that express the CD4 surface receptors, as a result, depletes its host of CD4 T-lymphocytes. Its decline is the hallmark of HIV infection and the rate of loss in each person is unique. Therefore, obtaining accurate and reliable measures of CD4 lymphocytes is essential to assess and manage persons infected with HIV. Depletion of CD4 T-lymphocytes has been linked to the immunopathogenesis of HIV infection and the progression of the disease. From the early days on CD4 T-lymphocyte assay has been recognized as the hallmark clinical surrogate marker for staging HIV disease progression. A CD4 T-cell count of 200 cells/ $\mu$ l is regarded as an AIDS defining event.

Flow cytometry based methods are currently the standard technology for CD4 T-lymphocyte counting because they are accurate, precise, fast and reproducible. This can be performed either by dual or single platform technology (DPT / SPT). The DPT requires a flow cytometer to generate the percentage of lymphocytes that are CD4-positive, and a hematology analyzer to obtain the total white blood and lymphocyte counts. The DPT is not recommended in this guideline as with this technique there are two sources of instrumental error and the hematology instrument requires blood that is <6 hours old. With SPT, only a flow cytometer is required to provide the results. CD4 T-lymphocytes in HIV infected individuals are monitored for the following reasons:

- to estimate the level of immune competence of an individual
- to stage HIV disease
- to make decisions for initiation of ART.
- monitoring response to anti-retroviral therapy / to estimate rate of progression of HIV disease
- to initiate chemoprophylaxis against opportunistic infections

### Principle:

Flow cytometry is the automated analysis of optical properties of individual particles in a fluidic system. It is a process in which measurement of physical and/or chemical characteristics of cells/particles are made while cells pass through the measuring apparatus in a fluid stream. The current applications of flow cytometers are derived from their ability to define and quantify cell populations.

The three basic components of a flow cytometer (FC) are Fluidics, Optics and Electronics. Fluidics: The flow cytometer requires the cells to be in suspension, flowing in single file through flow cell. Alignment with the laser and the detectors is extremely important if consistent light scatter and fluorescent signals from the same particle are to be obtained.

Optics :A light source needs to be focused on the same point where cells have been focused by the fluidic system. As cells pass in single file through this focus point, light and fluorescence is scattered, absorbed, excited and emitted at different wavelengths. Flow Cytometry measures light scattered, emitted or absorbed by cells, which in turn provide the values of almost all measurable parameters

Electronics: Once the light has been collected from various directions and selected by filters, it reached the detectors where light changes back into photons. Photomultiplier tubes (PMTs) are used to convert these photons into an electronic output. Pulses are converted to numbers through a process called analog-to-digital- conversion. Data acquired can be further manipulated by selecting the cell population of interest by gating

Further details on the test performance and technology options are provided in the "National Guidelines for the enumeration of CD4 lymphocytes with single platform technology for initiation and monitoring of ART in HIV infected".

## 14. TRANSFUSION TRANSMITTED INFECTIONS OTHER THAN HIV

### Introduction :

Transfusion medicine has been constantly evolving through the years with improved technologies that enhance the capability of identifying existing and newly emerging transfusion transmissible infections (TTI). In spite of the efforts made by blood banks, the risk of TTI remains

The transfusion of blood began with the use of whole-blood and continued for many years. However, nowadays whole blood and a variety of blood components and products are available for their use in patients of acute haemorrhage, anemia, thrombocytopenia, neutropenia, abnormal coagulation and immunodeficiency, etc. The term blood-component is used for those blood materials that are separated by means of differential centrifugation followed by expression into satellite/secondary packs attached to the original donor blood pack. Blood products are those blood materials that have been extracted and purified from crude plasma and preserved in stable form. Some of the important blood products and blood components have been summarized in Table 1

Blood and blood products are now recognized as life saving. Unfortunately transfusion of whole blood and these blood products/components is associated with risk of transmission of viral, parasitic and bacterial infections, if blood is not tested properly for the presence of micro-organisms before transfusion. Maximum percentage of transfusion transmitted diseases are viral, followed by parasitic and bacterial infections. The infections transmitted most commonly through blood are Human Immunodeficiency Virus (HIV), Hepatitis, Syphilis and Malaria. Table 2 gives the list of the infectious agents transmitted through blood and blood products.

Apart from the infectious microorganisms that are transmitted through transfusion of infected blood, blood components/products may be contaminated with bacteria through the many stages of preparation, including blood collection, processing, pooling, and eventually transfusion. Bacteria may enter into blood components through several sources: donors' bacteremia, exposure to donor skin bacteria by venipuncture, and contaminated bags and environment in blood banks or hospitals. Transfusion transmitted bacterial reaction has been identified as the most severe infectious complication associated with transfusion. The clinical severity of transfusion transmitted bacterial reactions depends largely on the type and load of bacteria involved as well as the recipient's condition. Table 3 enlists the bacteria that can contaminate the blood products during or after processing.

**Table 1 List of various types of blood components / products**

<b>Cellular components</b>	<b>Blood components</b>	<b>Blood products</b>
<ul style="list-style-type: none"> <li>• Red blood cells</li> <li>• Leucocyte reduced red cells</li> <li>• Washed red blood cells</li> <li>• Frozen/ deglycerolysed red Cells</li> <li>• Platelet concentrate</li> <li>• Granulocyte concentrate</li> </ul>	<ul style="list-style-type: none"> <li>• Single donor plasma</li> <li>• Fresh frozen plasma</li> <li>• Cryoprecipitated antihemophilic factor</li> </ul>	<ul style="list-style-type: none"> <li>• Factor VIII concentrate</li> <li>• Factor IX concentrate</li> <li>• Albumin</li> <li>• Plasma protein fraction</li> <li>• Immune serum globulin</li> <li>• Rh D immunoglobulin</li> <li>• Antithrombin III concentrate</li> <li>• Hyperimmune serum</li> </ul>



**Table 2 List of microorganisms transmitted through blood and blood products**

Viruses	Parasites	Bacteria
<ul style="list-style-type: none"> <li>• HIV-1 and HIV-2</li> <li>• Hepatitis B virus</li> <li>• Delta virus</li> <li>• Hepatitis C virus</li> <li>• Hepatitis G/GB virus C</li> <li>• Cytomegalovirus</li> <li>• Epstein barr virus</li> <li>• HTLV-I</li> <li>• HTLV-II</li> <li>• Human parvovirus B-19</li> <li>• West Nile Virus</li> <li>• Hepatitis A virus</li> <li>• Hepatitis E virus</li> <li>• Transfusion transmitted virus (TTV)</li> <li>• Human Herpes Virus-6</li> <li>• Human Herpes Virus-8</li> <li>• SEN-virus</li> <li>• CJD and vCJD</li> </ul>	<ul style="list-style-type: none"> <li>• Plasmodium spp.</li> <li>• Toxoplasma gondii</li> <li>• Leishmania spp.</li> <li>• Trypanosoma cruzi.</li> <li>• Microfilaria</li> <li>• Babesia microti</li> </ul>	<ul style="list-style-type: none"> <li>• Treponema</li> <li>• Brucella</li> <li>• Ehrlichia</li> <li>• Rickettsiae</li> <li>• Borrelia</li> <li>• Leptospira</li> </ul>

**Table 3 List of Bacteria that can contaminate the blood product during or**

<ul style="list-style-type: none"> <li>• Haemolytic E. Coli</li> <li>• Pseudomonas</li> <li>• Klebsiella</li> <li>• Proteus</li> <li>• Yersinia</li> <li>• Staphylococcus albus</li> <li>• Staphylococcus epidermidis</li> <li>• Enterobacter</li> <li>• Aerobacter</li> <li>• Achromobacter</li> <li>• Alcaligenes</li> <li>• Brucella spp</li> </ul>
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## Microrganisms transmitted through Blood and Blood products

Maximum percentage of transfusion transmitted infections are viral. Transmission of viruses is higher if the blood/components/products are derived from a large pool of plasma taken from many donors.

Transfusion associated hepatitis:

Transfusion associated hepatitis (TAH) develops as a consequence of transfusion of infected blood or blood components. The illness initially often remains sub-clinical but could later result in chronic liver disease and even primary hepatocellular carcinoma.

Viral hepatitis being one of the serious complications of blood transfusion, can be due to different infectious agents like Hepatitis B, C, D, and G viruses (HBV, HCV, HDV, and HGV) besides cytomegalovirus (CMV) and Epstein Barr virus (EBV). Post transfusion hepatitis is mainly due to Hepatitis B virus or Hepatitis C virus. Unfortunately most of the carriers of hepatitis viruses are not even aware of their carrier status.

Transmission of Hepatitis A virus (HAV) and Hepatitis E virus (HEV) through blood transfusion though not firmly established has however been reported among patients with hemophilia in case of HAV and in endemic areas in case of HEV.

Prevention of post transfusion hepatitis

Following points should be kept in mind for prevention of post transfusion hepatitis:

- Promotion of voluntary blood donation.
- Proper donor interview and selection.
- Use of most sensitive and specific test for detection of the causative organisms.
- Good manufacturing practice (GMP) for blood fractionation and incorporation of virucidal technology (Solvent detergent technique) for viral inactivation.

## Viruses

### a) *Hepatitis B virus (HBV):*

Hepatitis B virus is the most important causative agent of TAH. Humans are the only reservoir of Hepatitis B virus (HBV). Hepatitis B virus was discovered in the serum of an Australian aborigine and thus its antigen is also known as Australia antigen.

Hepatitis B is an important vaccine preventable disease. The incubation period of HBV infection is about 50-150 days during which the patient may have no signs and symptoms but the virus may be detected in the blood stream. There is a prodrome of malaise, weakness, loss of appetite, nausea followed by symptoms such as fever, rash, arthralgia and jaundice depending upon the severity of infection. In mild cases jaundice may not occur however, more severe cases can result in serious disease.

In 10 to 20% of clinically diagnosed HBV infections, the infection does not resolve but enters into chronic phase, which may last for just six months and then resolve or persist for life. In some chronically infected persons, no ill effects are seen but the infection may later develop into chronic liver disease which could lead

to chronic active hepatitis, cirrhosis, hepatocellular carcinoma and death. The prevalence of chronic HBV infection is measured by a positive hepatitis B surface antigen (HBsAg) test.

The virus is highly infectious and minute amounts of blood/body fluids as little as 0.01µl can transmit the infection. HBV can be transmitted by any of the following modes:

- Percutaneously through injection drug use or accidental pricks with contaminated needles /cuts with infected sharps
- Through exposure to contaminated blood ( as with transfusion of unscreened blood) or body fluids
- Sexually through heterosexual or male homosexual activities,
- Vertically from mother to infant

HBV infection can be effectively prevented through immunization , which is available for infants and preadolescents. In recent years, with the emergence of HBsAg escape mutants, the investigation of their implications for blood safety has become an important research priority. HBsAg escape mutants may not be detected by the current HBsAg assays used for blood donors, and the routine HBV vaccine may not protect the infection induced by the mutants; therefore, these pathogens pose a potential risk to the safety of blood transfusion.

The virus particle (virion) is a small complex double shelled structure having an external diameter of 42 nm with a nucleocapsid core and lipoprotein coat. It represents the complete HBV. The nucleocapsid core is 27 nm in diameter. It replicates in the nuclei of infected hepatocytes and possesses a distinct antigen called hepatitis core antigen (HBcAg). The major structural protein is the lipoprotein viral coat and is called hepatitis B surface antigen (HBsAg). This protein is synthesized in the cytoplasm of infected hepatocytes in excess quantities than that needed for viral assembly. The excess material in the form of small particles, either 22nm spherical particles or smaller tubular particles of varying lengths are released in the blood stream. Since these smaller particles contain only the major viral protein HBsAg they are considered non-infectious but act as markers of infection. Occasionally complete virus occurs in the blood stream. The virion has an other marker i.e. hepatitis B “e” antigen (HBeAg) which is closely related to core antigen and is considered as a marker of infectivity.

Antibodies to three main antigens i.e. anti- HBsAb, anti- HBcAb and anti- HBeAb appear at different times mainly during the course of disease and may persist for years, some times for life after recovery.

- Anti-HBsAb is usually associated with resistance to reinfection.
- Anti-HBcAb is not protective but is considered as a sensitive indicator of HBV replication. Its presence in asymptomatic blood donor indicates either recent HBV infection or chronic carrier state.
- Anti-HBeAb is seen mostly in healthy carriers of HBsAg.

### **Serological and biochemical markers of HBV infection:**

The development of various serological markers during the acute and chronic HBV infection are shown in table 4 and respectively and the interpretation of various markers is given in table .

- Appearance of HBsAg in patient's serum : It is usually the first detectable serological marker in acute HBV infection and remains detectable from few days to several months depending upon recovery of the patient. In some cases HBsAg persists for years. These are called carriers.

- Appearance of Anti-HBc (HBcAb) : In most cases HBcAb appears in the serum during the acute illness (preicteric phase) and persists for months.
- Appearance of HBV “e” antigen and its antibody: Hepatitis B “e” antigen (HBeAg) appears in the serum almost at the same time as HBsAg but disappears in most cases within a few weeks. Its disappearance is followed by the appearance of anti HBe (HBeAb) in serum which lasts for several months.
- Appearance of anti HBs (HBs Ab): Antibody to HBs Ag (HBsAb) appears after the disappearance of HBsAg and indicates recovery from acute illness. At times the appearance of HBsAb may be delayed for weeks after HBsAg becomes undetectable and during this period called core antibody window, anti HBc-IgM may be the only detectable marker of recent HBV infection.

**Table 4 Interpretation of various HBV markers**

<b>HBsAg</b>	<b>HBeAg</b>	<b>anti-HBc</b>	<b>anti-HBe</b>	<b>anti-HBs</b>	<b>Interpretation</b>
—	—	—	—	—	No evidence of present or past HBV infection.
+	—	—	—	—	Incubation period of HBV.
+	+	+	—	—	Early HBV infection with high infectivity
+	—	+	+	—	Late acute HBV infection or chronic HBV infection with lower infectivity.
—	—	+	+	—	“Window” period in late acute HBV infection.
—	—	+	+	+	Convalescent from HBV infection.
—	—	+	—	+	Late phase of convalescence (anti- HBe has waned).
—	—	—	—	+	Response to HBV vaccine or recent administration of hyperimmune anti- HBs immunoglobulin.

**Prevalence of Hepatitis B infection:** HBV infection occurs virtually in every country of the world. Currently there are 300 million carriers of this virus worldwide. The carrier rate in India is estimated to be around 5%

**Screening tests for Hepatitis-B virus:** Currently the most sensitive and widely used tests for detection of HBsAg are based on the EIA/ELISA principle. Centers having a large blood collection base use ELISA method for screening of blood for presence of HBsAg whereas the smaller units use the rapid card tests for this purpose.

There are numerous commercial kits (ELISA and rapid cards) available for detection of HBsAg and it is recommended that the procedure provided by the manufacturers in their kit is followed.

**Limitations of HBsAg screening:** Even after using the most sensitive screening test for the detection of HBsAg on blood from voluntary donors, HBsAg negative units have been found to transmit the hepatitis B virus infection. The basic aim of blood transfusion services should be to provide zero risk blood for transfusion, however in actual practice, this is not always achievable because of the following reasons:

- Lack of a test which is 100% sensitive probably due to presence of HBsAg mutants.
- Failure to detect HBsAg in some donors in the early incubation period.
- Difficulty in carrying out highly sensitive tests like DNA hybridization and PCR.

#### **b) Hepatitis C virus (HCV)**

HCV is the most common cause of post transfusion non-A non-B hepatitis in the developed world. The virus is an enveloped single stranded RNA virus belonging to the family Flaviviridae.

HCV has shown substantial heterogeneity within several regions of the viral genomes. On the basis of these genomic differences, HCV has been classified into 11 genotypes and each genotype further has several subtypes. This makes development of a vaccine difficult for prevention of Hepatitis C. Currently, no vaccine is available against HCV.

HCV transmission occurs by:

- Needlestick injuries or cuts with infected sharps
- Use of contaminated needles and syringes
- Transfusion of unscreened blood
- Unprotected sexual exposure
- Mother to child- in utero, during parturition and by breast milk
- Organ transplants
- Tattooing
- Transmission by saliva and tears cannot be excluded.

Prevalence of Hepatitis C is higher in under developed countries. The prevalence is estimated to be 1.5% in India

Incubation period of Hepatitis C averages around 6-8 weeks though it may range upto several months. The danger of Hepatitis C is not the acute disease but the persistence of infection. About 75% of the infections are subclinical. However, 85% or more of the acute HCV infections become chronic and may eventually lead to the development of liver cirrhosis and Hepatocellular carcinoma after a few decades.

The detection of antibodies to HCV by ELISA or rapid tests is used for serodiagnosis of HCV infection. Other methods of establishing HCV infection are detection of the viral genome by PCR and by immunofluorescence and in situ hybridization on biopsy material.

### c) Hepatitis D virus

It is also known as Delta hepatitis. It is caused by a defective RNA virus that is unable to produce its own protein coat and thus coats itself with HBV surface antigen. The delta virus is dependent on pre-existing or concomitant HBV infection for propagation. Co-infection causes acute viral hepatitis which often becomes fulminant and is usually fatal, while superinfection causes acute hepatitis progressing to chronic active hepatitis and cirrhosis in 75% of carriers.

Delta hepatitis has parenteral mode of transmission and usually follows HBV infection. Although tests for delta antigen and antibody exist but these need not be used in blood donor screening as all infections with delta virus are positive for HBsAg. So routine testing for HBsAg will eliminate the risk of transmission of Delta virus.

### d) Hepatitis G virus (HGV)

Hepatitis G virus is an enveloped RNA virus and belongs to the Flaviviridae family. The virus genome is similar to that of HCV with which it shares a 25% homology at the nucleotide level. The HGV appears less variable although variants have been described. HGV replicates in peripheral blood cells but its replication in the liver is still questionable. The mode of spread of HGV is almost same as HCV. The HGV can be detected in the serum by PCR. Recently an immuno-assay has been developed to detect antibody to HGV.

The prevalence of HGV in blood donor population by doing antibody tests in the developed countries ranges between 1 to 5% but the detection of antibody generally indicates recovery from and probably the immunity to the HGV agent.

### e) Cytomegalovirus (CMV)

CMV is one of the largest Herpes viruses with double stranded DNA. This infection is wide spread and is transmitted by a number of routes. The infection can also be transmitted by organ transplantation and transfusion of blood/blood products.

After transfusion the virus gets reactivated. The infection can be severe in immunocompromised patients and premature infants. It may be fatal in cases of transplantation of allogenic marrow. Transfusion transmitted CMV can be checked/controlled to a greater extent only by depleting the white cells by filtration or centrifugation. At present the only method to avoid its transmission is by using the CMV negative donors. IgM specific anti-CMV ELISA can be used to screen the plasma products.

### f) Epstein-Barr Virus

Epstein-Barr virus (EBV) infection is common in the general population. It is usually asymptomatic in children, although in adults the infection often results in clinical symptoms such as fever and sore throat. EBV has been associated with the development of Burkitt's lymphoma, nasopharyngeal carcinoma, and B-cell lymphoma in immunosuppressed individuals.

Transmitted primarily through person-to-person contact via saliva, EBV can also be spread through blood transfusion. Leukodepletion may be an effective way to reduce EBV transmission through blood transfusion

### g) Human T-cell lymphotropic viruses

Human T-cell lymphotropic virus type-I (HTLV-1) was the first human retrovirus isolated from a patient with adult T-cell leukaemia/lymphoma (ALT). The disease can persist for a life time but usually does not cause major illness in most people who are infected. In rare instances, HTLV-1 is associated with a chronic degenerative demyelinating neurological disease called tropical spastic paraparesis (TSP) but now often called HTLV associated myelopathy (HAM). HTLV-1 is more common in southwestern Japan and Caribbean islands. The route of transmission for HTLV-1 is by sexual contact (predominantly male to female), by parenteral exposure to blood and mother to child through breast milk.

The second human retrovirus HTLV-II was discovered several years after HTLV-1. It is closely related to HTLV-1 and it is difficult to differentiate between these two viruses in the routine laboratory. Although no firm association between HTLV-II and any disease has been found but the virus was first found in a patient with hairy T-cell leukaemia and subsequently in other T-cell malignancies, viral RNA expression has not been found in malignant cells. High prevalence of HTLV-II has been seen in I.V. drug abusers and their sexual contacts in USA and other countries. The prevalence of HTLV-I and HTLV-II in India is not available.

The viruses are transmitted by transfusion of cellular components of blood but not by cell free components like plasma. Universal screening of blood donors for HTLV-I was introduced in December, 1988 in USA and in July, 1991 in France. In south western Japan where HTLV is, endemic screening of blood markedly reduced the rate of seroconversion in recipients of blood.

Several HTLV screening assays are available like gelatin particle agglutination (P.A.), enzyme immunoassay (EIA) and indirect immunofluorescence (IF). The sensitivity and specificity of ELISA tests ranges from 98.4% to 100%. None of the screening tests available is able to differentiate between anti HTLV-I and anti HTLV-II because of cross reactivity due to 75% homology of amino acid sequences between the two viruses. Confirmatory assays like western blot, RIPA, antibody capture radioimmuno assay and PCR are also available.

### h) Parvovirus :

Parvo virus B-19, a single stranded non enveloped DNA virus was discovered in 1975 in the serum of normal blood donors. It is a common cause of a blood disease known as erythema infectiosum and may be implicated in aplastic anaemias. This clinical entity may be severe in pregnant women, AIDS cases, and haemophiliacs etc. Fortunately this infection is not prevalent in India. Diagnostic methods like haemagglutination, gel test, DNA hybridisation and PCR have been developed to diagnose this infection.

### i) Transfusion transmitted virus (TTV) and SEN virus

TT virus and SEN virus are relatively newly identified viruses. Infection with TTV and SEN may be found in the general population, particularly in recipients who have received multiple transfusions and in injection drug users. Infection with SEN-V and TTV are mostly asymptomatic, and have not been known to be associated with any form of hepatitis or other clinical disease. Blood units are not routinely screened for serologic markers of TTV and SEN virus infections.

### **j) Human Herpes Viruses 6 and 8:**

Human herpesvirus 6 (HHV-6) has been described as the causative agent of the sixth disease. This disease features high and persistent fever and affects primarily children between 3 months and 3 years of age. Recently, HHV-6 has been described as a potential threat to transfusion safety because of its persistent infection and the high prevalence of antibodies to HHV-6 among blood donors.

Human herpesvirus 8 (HHV-8) is a newly discovered virus that has been associated with Kaposi's sarcoma, in particular in immunocompromised individuals such as patients with HIV. Transmission of HHV-8 through blood transfusion has not been established, although the virus has been transmitted through organ/bone marrow transplantation

Currently, blood units are not routinely screened for HHV-6 and HHV-8 serologic markers.

### **k) CJD and vCJD**

Creutzfeldt - Jakob disease is one of the series of transmissible spongiform encephalopathies (TSE) and is thought to be caused by a proteinaceous particle smaller than a virus. This nucleotide infectious particle is called prion. Although transmission of CJD through blood and blood products has been speculated and the possibility cannot be completely ruled out, no such case to date has been reported. It has been speculated that the transmissibility of vCJD through blood may be greater than that of classic CJD.

## **Parasites**

Most common parasites which may be transmitted through blood/blood products are the Malarial parasite and *Toxoplasma gondii*. Sometimes others like *Microfilaria*, and rarely *Leishmania* spp, *Trypanosoma cruzi* and *Babesia microti* etc. may also be transmitted through transfusion of blood/blood products.

### **a) Malarial parasite**

Malaria is a major public health problem throughout the world. The infection is caused by a protozoan parasite of the genus *Plasmodium*. Four species of *Plasmodium* namely *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale* are responsible for human malaria.

Transmission of malaria can occur after transfusion of whole blood, red cells or any blood component contaminated with red cells. Plasma fractions do not transmit the disease. The parasites of all species remain viable for at least one week in stored blood at 2-6°C. However, *Plasmodium falciparum* can remain viable for 2-3 weeks. The incubation period of *Plasmodium vivax* and *Plasmodium falciparum* is one week to one month while that of *Plasmodium malariae* is many months. Post transfusion malaria (PTM) is particularly dangerous in pregnant women and immunodeficient patients.

The parasite can survive in the blood of host for many years followed by relapse or recrudescence. Such cases as blood donors pose potential threat of post transfusion malaria. Transfusion transmitted malaria can be lethal. Quite a good number of deaths have been reported due to transfusion transmitted malaria

To avoid the transfusion transmitted malaria, proper scrutiny and screening of blood donors should be done.



Immunofluorescence method for detection of malaria has been found to be better than the routine microscopy method. Now enzyme based immunodiagnostic assays are available commercially as kits for early diagnosis and screening of blood.

Detection of malaria in carriers

Examination of blood film: The examination of blood film is not suitable for screening large number of blood donations because it is difficult to find parasites in blood film in the short available time especially if density of parasites is less than 100 per microlitre of blood. However, the examination of blood films for parasites within red cells is considered best method for diagnosis in suspected cases.

Test for malaria antibody: Tests for malaria antibody either by indirect fluorescent antibody (IFA) test or ELISA are alright for nonendemic areas but not ideal for malaria endemic areas like India as there is a likely chance that many blood donors will have antibodies and will be rejected.

#### ***b) Toxoplasma gondii***

Toxoplasmosis is caused by an obligate intracellular protozoan parasite known as *Toxoplasma gondii*. The disease is prevalent throughout the world. Generally it is transmitted by ingestion of oocyst / cyst of parasite. The infection can also be transmitted by trophozoites in blood through transfusion. The infections initiated by oocyst or cyst are invariably mild but infection induced by trophozoites of blood stage is more severe and in the acute form. The trophozoite of parasite can survive in blood stored at 4°C upto 50 days. Leukocyte transfusion has also been associated with transmission of Toxoplasmosis in immunocompromised patients. Both these observations suggest that now transfusion associated Toxoplasmosis is posing a serious threat to AIDS patients.

#### ***c) Leishmania donovani***

Leishmaniasis affects approximately 12 million people in tropical and subtropical areas transmitted primarily by the bite of an infected vector -the sandfly. *L. donovani* can also be transmitted through blood transfusion and cause clinical disease in newborns or immunosuppressed recipients. Given the low risk, no recommendations have been made to test antibodies to *L. donovani* in blood donors on a routine basis..

#### ***d) Trypanosoma cruzi***

Chagas' disease is caused by a protozoan parasite - *Trypanosoma cruzi* has become an increasingly important infectious disease in Central and South America. Transfusion transmitted Chagas' disease has been reported in endemic areas for almost 40 years. The risk of *T. cruzi* transmission through transfusion is low and depends on several factors, including the prevalence of asymptomatic parasitemia in blood donors and the immunologic status of recipients. Testing for serologic markers, are neither sensitive nor specific enough to effectively identify asymptomatic blood donors. Leukodepletion may reduce the risk of transfusion transmitted Chagas' disease

#### ***e) Babesia microti***

In general, *B. microti* is transmitted to humans through the bite of deer ticks ; however, it can also be transmitted through blood transfusion to recipients from asymptomatic and parasitemic donors. In immunocompetent individuals,

the acute infection is subclinical, however, severe outcomes may occur in older patients, infants, or immunocompromised individuals. Currently, owing to the low risk of transmission routine screening in blood banks is not recommended.

## Bacteria

Unlike the viruses the direct transmission of bacteria from donor to recipient is less common. *Treponema pallidum* has been found to be associated with transfusion induced syphilis.

### a) *Treponema pallidum*

Syphilis is a disease caused by *Treponema pallidum*. It is primarily a sexually transmitted disease but the organism can gain entrance in to the body through minute lesions on skin or mucous membranes.

Blood transfusion is a potential route of infection, particularly if fresh infected blood is transfused. The incubation period in transfusion induced syphilis is on an average 9-10 weeks and recipient usually presents with secondary eruptions.

The chances of post transfusion Syphilis have been greatly reduced because of the following reasons :

- Exclusion of high risk donors.
- Screening for Syphilis.
- Storage of blood at 2-6°C for 72 hours prior to transfusion. (Spirochaetes are very sensitive to temperature and die at lower temperature)

Serological test : There can be non specific tests and specific tests

Non Specific tests : The non specific tests include :

- i VDRL (Venereal Diseases Research Laboratory) test
- ii RPR (Rapid Plasma Reagin) test.

VDRL : VDRL test is a simple, rapid and economical test for screening syphilitic infection, however the test needs inactivation of the specimens (sample) and the shelf life of the reconstituted antigen is poor.

RPR test : It is a macroscopic non-treponemal flocculation test for screening syphilitic infection. The test is rapid and does not need inactivation of the sample, the antigen used is a modified VDRL antigen (cardiolipin) which contains microparticulate charcoal/carbon to enhance the visual interpretation. The test is supplied in the form of kits by manufacturers and it is advised to follow the instructions provided with the kit.

Specific tests : Specific tests are mainly used for confirmation and are not suitable for routine screening.

- i *Treponema pallidum* haemagglutination test (TPHA).
- ii Fluorescent treponemal antibody test (FTA)
- iii Enzyme immuno assay (EIA).

### **b) *Brucella***

Brucellosis is primarily a zoonosis but may be rarely transmitted from person to person by transfer of tissues including blood and bone marrow.

### **c) *Borrelia***

Lyme disease is an emerging tick-borne zoonosis caused by *Borrelia burgdorferi*. It is not endemic in India. Lyme disease may pose a potential threat to the safety of blood transfusion. Transfusion Transmitted Lyme disease has not yet been reported

Blood donors are not routinely screened for antibodies to *B. burgdorferi*. The most effective strategy to prevent Lyme disease in endemic regions is through vaccination against *B. burgdorferi*

### **d) *Ehrlichia***

The agents are transmitted primarily through bites of infected ticks. In addition, ehrlichiosis, may be transmitted through blood transfusion from an asymptomatic donor to a recipient. However, such a mode of transmission has not been established because of the limited knowledge and information on ehrlichiosis. Currently, there are no effective screening serologic tests available to identify infected blood donors

### **e) *Rickettsiae***

Rocky Mountain Spotted Fever (RMSF) is a severe tick-borne disease caused by *Rickettsia rickettsii*. RMSF is transmitted mainly through the bites of infected ticks. This agent has also been transmitted through blood transfusion. Currently, no specific assays are used for screening and testing of serologic markers of *R. rickettsii* in blood donors.

### **f) *Leptospira***

Leptospirosis is a spirochetal infection caused by the pathogenic leptospire *L. interrogans*. It is endemic in some parts of the world particularly the southern states of Tamilnadu and Kerala, Gujarat, Maharashtra and the Andaman Islands in India. The infection is spread through contact with urine of rats, dogs, cats and cattle. Transfusion transmitted Leptospirosis has been reported from the city of Madras. Routine screening in blood donors for Leptospirosis is not recommended so far. However screening of donor blood by dark field microscopy, and arbitrary primed PCR when available together with temporary deferral would be an ideal policy particularly in endemic areas until DNA based techniques are available for routine use.

## **Bacterial contamination of blood/blood components/blood products:**

Handling and storage of blood, its components and products at room temperature as well as at 4°C may provide sufficient time and opportunity for bacterial contamination.

Transfusion of blood contaminated with bacteria has a typical clinical syndrome of violent chills during the transfusion or shortly after the transfusion. The symptoms may be due to endotoxins. Sometimes transfusion of contaminated blood may cause septic shock syndrome resulting in death of the recipient. Bacteria commonly associated with septic shock are *E. coli*, *Enterobacter* spp., *Pseudomonas*, *Klebsiella* spp. etc. Septicaemia has been reported following the transfusion of plasma and cryoprecipitates etc.

In most of the cases, contamination of blood, its components/products occurs due to the introduction of bacteria at the time of collection, processing, manufacturing or storage. With the improvement in collection and storage methods,

the contamination of blood with bacteria has declined significantly. The blood contaminating organisms are mainly ubiquitous Gram negative bacteria. In blood they grow very fast and unfortunately the contaminated blood may appear normal even with heavy contamination, as these bacteria are not haemolytic. Gram positive bacteria which form a part of skin flora (Staphylococcus and diphtheroids) are less often implicated in transfusion associated diseases.

Apart from blood, which is a good culture medium for micro-organisms the citrate, glucose and anticoagulant preservative solutions are ready made energy source for these bacteria. Fortunately, macrophages and leukocytes present in blood have natural bactericidal capacity.

Bacterial contamination of blood/products/components can be prevented to a certain extent by :

- Use of closed blood collection systems
- Transfusion of blood within 6-8 hrs. of collection and/or removal from refrigeration.
- Preparation of blood components in sterile conditions.
- Proper inspection of blood unit for haemolysis/dicolouration of RBCs.

### Prevention of transfusion associated diseases caused by blood products/components

Blood products/components have been recognised as life saving drugs by WHO. A wide range of blood components and blood products are now available that are being manufactured throughout the world. Till 1980s the major concern of transfusion induced diseases was only hepatitis viruses but the detection of AIDS virus alerted the entire world to combat the menace of transfusion associated infections.

For controlling the transfusion associated transmission of infections quality control/quality assurance ; good laboratory practices (GLP), good manufacturing practices (GMP) including virucidal technologies and biosafety procedures etc. should be implemented strictly.

There are six important steps to ensure quality in transfusion medicine services.

- Immunohematological investigations.
- Microbiological investigations.
- Blood collection
  - Donor recruitment
  - Donor's investigations
- Separation of blood components and preparation of blood products.
- Storage.
- Use of associated equipments.

### General control measures

- Transfusion should be given only when there is a clear medical indication.
- Screening of units of donated blood and products for all the diseases as per the National blood bank policy
- Autologous transfusion should be encouraged.

### Good laboratory practices/good manufacturing practices

Transmission of transfusion associated infections can be effectively controlled by effective implementation of quality control/quality assurance program. To control such transmission following measures should be taken.

- Selection of normal healthy donors.
- Immunodiagnostic kits/reagents for diagnosing the transfusion associated diseases must be validated by quality control authority/regional labs.
- Strict GLP/GMP should be followed.
- All the equipments/instruments used for transfusion must be sterile.
- Transfusion committee must be operational and should have written quality plan for transfusion safety and standard operative procedures (SOPs) for various tests etc.
- Prevention of bacterial contamination during donation, collection, storage and processing of blood.
- Effective viral inactivation of certain plasma products/components.
- Judicious use of blood/blood products/components.
- Regular quality audits.
- Enforcement of corrective measures.
- Biological safety cabinet should be used for handling the biological materials.
- Proper handling and disposal of disposables like needles, syringes and bags should be ensured.
- Effective decontaminants should be used for cleaning/decontamination of work place.
- Blood should be centrifuged in covered containers.
- Equipment should be decontaminated before/after repair/transfer etc.
- Protective clothing (gown, mask etc.) should be used.
- Hands should be washed properly.

## Quality control

### Quality control of equipments and immunodiagnostic kits/methods :

The instruments under use for storage, handling, processing, testing etc. must be properly maintained and monitored to ensure Good Laboratory Practices and Good Manufacturing Practices (GLP/GMP). Biosafety cabinet, automatic cell washers, water bath etc. must be validated with regular instrument maintenance and calibration as per prescribed protocols.

Immunodiagnostic kits/reagents and methods used for screening of the donor must be evaluated for QA/QC for assuring the proper diagnosis of diseases.

**Quality control of personnel :** Quality control of personnel is one of the most important aspects of quality control program as it is central point around which the entire process revolves. Personnel also regulate the entire process of QA/QC.

All personnel should be :

- Trustworthy.
- Properly qualified/trained and able to withstand stressful situations.
- Well acquainted with SOPs.
- Conversant with proficiency testing.
- Trained regularly through continuing medical education programs.
- Given suitable incentive/commendation from time to time.

**Quality control of blood components/quality control of procedures :** Quality Control Programme should be strengthened in GLP/GMP which consist of

- collection of blood

- testing
- separation for components
- preparation of blood products
- viral inactivation
- proper storage
- safe transfusion in patients
- impact assessment.

### **Mandatory screening for transfusion transmitted infections in blood banks in India**

It is mandatory for every blood unit collected at blood banks in India to undergo screening and test negative for the following TTIs prior to being declared fit for transfusion and/or further processing for preparation of blood products and blood components. The results of such testing must be clearly indicated on the label.

- HIV-1 and HIV-2 [ ELISA (rapid cards to be used only in emergency cases or in very small blood bank set ups)]
- Hepatitis B (ELISA)
- Hepatitis C ( ELISA preferably and rapid card only in smaller set ups where ELISA is not available)
- Syphilis (VDRL/RPR)
- Malaria ( Smear examination and history)

Result seeking donors whose blood has tested positive for HIV in the blood bank screening should be referred to the ICTC for further information, counseling and confirmation (by undergoing testing) of their HIV test result.

## 15. COUNSELLING AND HIV/AIDS TESTING

### Definition

Counselling is a process where one person, explicitly and purposefully, gives his/her time, attention and skills to assist a client to explore their situation, identify and act upon solutions within the limitations of their given environment. In simple terms, counselling is a process in which two people meet and have a dialogue to resolve a crisis, solve a problem or make decisions involving highly personal and intimate matters and behaviours.

World Health Organisation (WHO) defines HIV/AIDS counselling as a dialogue between a client and a care provider aimed at enabling the client to cope with stress and to take personal decisions relating to HIV/AIDS. The counselling process includes the evaluation of personal risk of HIV transmission and the facilitation of preventive behaviour.

### *Difference between counselling and health education*

Counselling differs from health education in many ways and they are:

- i Counselling is usually a 'one is to one' process whereas, health education addresses a group of people.
- ii Counselling is useful for not only giving information but also for changing attitudes and motivating behavioural change. Health education is used mostly for information sharing.
- iii Counselling sessions involve personal problem solving. In health education, general issues are discussed.
- iv Counselling is more focussed, specific and goal targeted, whereas health education is much more generalized.
- v Counselling evokes strong emotions in both counsellor and client whereas health education sessions are emotionally neutral in nature.

### *Objectives of HIV/AIDS counseling*

The objectives of HIV/AIDS counselling include :

- i Prevention of HIV infection by changing life styles and behaviours. (Primary prevention).
- ii Providing psychological support to those already infected.
- iii Motivating for change in high risk behaviour ( Secondary prevention).

### *Why is HIV/AIDS counselling necessary*

HIV/AIDS counselling is mandatory for providing voluntary HIV testing services. This is so because diagnosis of HIV in an otherwise healthy individual induces a series of psychological reactions like denial, anger, anxiety, depression, to finally acceptance. So far, there is no successful cure or vaccine available for HIV infection. In order for the individual to be able to accept the infective status, carry on with life, plan future, prevent transmission and continue to

function as a useful member of community, counselling is a must. Counselling induces a positive attitude and high life force in the individual helping him to carry on as before in spite and irrespective of the HIV infection, HIV/AIDS counselling is mandatory (pre and post test) as laid down by WHO/UNAIDS. Government of India is also actively emphasising the necessity of HIV counselling.

### Who requires HIV/AIDS counselling?

HIV/AIDS counselling is recommended for the following groups of people :

- I. Persons already identified as having AIDS or being infected with HIV and their families.
- II. Those being tested for HIV (pre and post test).
- III. Those seeking help because of past or current risk behaviour and planning their future.
- IV. Those not seeking help but practicing high risk behaviour.
- V. Those on ART for adherence, etc.
- VI. Those requiring ART.

### Where can HIV/AIDS counselling be provided?

HIV/AIDS counseling is provided at VCTCs, PPTCTCs and ART centres. HIV/AIDS counselling can also be provided in any other setting including hospital wards, STD clinics, ANC clinics, family planning clinics, blood donation centers, drug deaddiction centers, prisons, primary/secondary health centers and community based programmes.

### WHO can provide HIV/AIDS counselling?

HIV/AIDS counseling is being provided by trained, qualified counselors. HIV/AIDS counselling can be provided by anyone who has a sympathetic ear, can give time to listen, has knowledge of accurate scientific facts about HIV/AIDS and undergoes systematic and periodic training in counselling. In addition to doctors, nurses, psychologists, psychotherapists and social workers, even teachers, community and peer leaders, youth and self help groups can undertake preventive and supportive counselling.

### Pre-requisites of counselling

Some of the pre-requisites of counselling include :

- i Time and availability of counsellor
- ii Acceptance of the activity by counsellor
- iii Easy accessibility to counselling
- iv Aptitude for maintenance of confidentiality
- v Correct knowledge and information about HIV/AIDS and policies etc.
- vi Consistency in counselling

### Contents of preventive HIV counselling

- i Determine whether the behaviour of an individual or group of individuals involves a high risk behaviour which can lead to HIV infection.
- ii Work with individuals to make them understand and acknowledge the risk behaviour.
- iii Define and discuss with them how their life, attitude/values and self image is linked to these behaviours



- iv Help individuals to define their potential for attitude shifts, behaviour modification and change.
- v Work with individuals to introduce and sustain the modified behaviour.

## Types of counseling

- i Pre-test
- ii Post test-positive, negative and indeterminate results.
- iii Follow-up
- iv Adherence

## Counselling

HIV/AIDS is a life long disease which to date, has no cure or vaccine. In addition, lack of a healthy non judgmental and non discriminatory environment has resulted in isolation, victimisation and breach of fundamental rights including denial of basic medical services to people with HIV infection. HIV/AIDS counselling can help people in accessing correct information, assessing risks, making appropriate behavioural changes, leading to protection of self and others. This will help in developing coping mechanisms in people with HIV infection. Counselling can lead to empowerment and raise individual consciousness and can make individual take responsibility for their own behaviour.

## 16. LEGAL AND ETHICAL ISSUES OF HIV/AIDS

### Introduction:

No laws have been enacted on legal and ethical issues in India so far. These issues are under active consideration. However, there are some basic considerations on legal and ethical issues on HIV testing, confidentiality, consent and discrimination, etc. which need to be discussed. Many countries and even the World Health Organisation have put forward certain recommendations on these issues. The issues may be as relevant to India and are discussed below.

HIV/AIDS epidemic has posed an array of legal and ethical challenges. These challenges include limits and significance of confidentiality; obligatory informed consent before testing and initiating treatment; counseling of women to make reproductive decision; burden on infected individuals to protect their sexual partners; obligation of the state to prevent spread of disease; obligation of physicians to care for HIV infected; issues related to insurance, men, women and children, etc.

### Ethics of prevention and care:

HIV is transmitted during sexual intercourse (most common), both homosexual and/or heterosexual, through contaminated needle sharing as in IV drug users, by pregnant mother to the infant and through accidental exposure while providing care to HIV-infected. AIDS is primarily a behaviorally transmitted disease and transmission of HIV can only be interrupted through modifications in behavior e.g. using condom during sexual intercourse, not sharing needles and using therapeutic intervention to reduce mother-to-child transmission of HIV.

Public health system has to develop preventive strategies based on the above biological facts.

The issues which need to be debated to prevent sexual transmission of HIV include the empowerment of women to make sexual relationship decisions in light of their cultural and social status.

The basic principle of ethics dictates that individuals should be treated with respect and their dignity should not be violated. This also applies to their culture. Failure to respect the local cultural norms is regarded as the imposition of will and values of the dominant and powerful on the subordinate and marginal. One example is education of gay men and drug users to modify their behavior (use of condom and sterile needles through needle exchange programme) to protect themselves and others. Conservatives view these strategies as "legitimizing" homosexuality, extramarital sex and encouraging drug use. This opposition from conservatives on the basis of morality limits the state's first ethical responsibility i.e. to protect the vulnerable.

Sex education, empowerment of women to be able to control their sexual lives and provision of condoms challenge the social norms of societies where women are subordinate and subjugated. For control of AIDS the consensus is to use public health strategies which are voluntary emphasising mass education, counseling and respect for privacy.

## Discrimination/right to work

The realization that AIDS was a disease of the socially marginalised (homosexuals) and frequently despised individuals led to discrimination of HIV-infected by employers, landlords, school personnel and even some health care professionals. The central message of the CDC guidelines was: "HIV could not be causally transmitted so there were no public health grounds for exclusion of infected individuals who otherwise were capable of performing their expected functions". In the context of health care setting, standard blood and body fluid precautions would protect the health care workers not only from HIV, but also from the far more infectious Hepatitis B and C, etc. Thus there was no ground for mandatory HIV testing and no possible ethical foundation for discrimination against persons with HIV.

The Centers for Disease Control has estimated the risk of transmission from an HIV infected surgeon during an operation between 1 in 42,000 to 1 in 420,000. Despite these estimates there is no documented transmission from an infected health care worker to a patient.

## Confidentiality and HIV/AIDS:

Confidentiality of physician-patient encounters is a basic medical ethic, even reflected in the Hippocratic Oath.

Ethically, confidentiality is derived from the principles of autonomy (the patient determines who shall know his or her medical history) and fidelity (the fiduciary relationship of the patient and physician requires trust and confidence). Confidentiality allows the physicians to obtain all the information necessary to make a complete diagnosis and motivate the patient to participate in therapy.

Modern codes of ethics recognize this principle:

The patient has the right to confidentiality. The physician should not reveal confidential communications or information, without the consent of the patient, unless provided for by law or the need to protect the welfare of the individual or public interest. Civil and criminal penalties may ensue for unlawful disclosure of HIV positive status.

This is because of the negative connotations of a positive test for HIV (discrimination, psychological and social effects).

However, the principle of confidentiality is never absolute and has always been subject to limits in the interest of society, public welfare and the rights of the other individuals.

Exceptions to confidentiality are appropriate when necessary to protect public health or when necessary to protect individuals, including health care workers, who are endangered by persons infected with HIV. If a physician knows that a seropositive individual is endangering a third party, the physician should within the constraints of the local law, i) attempt to persuade the infected patient to cease endangering the third party; ii) if persuasion fails, notify authorities, and iii) if the authorities take no action, notify the endangered third party.

Some state statutes make exception to confidentiality with regard to the spouse and sexual partners of the patient, other state statutes make no such exceptions. The U.S. presidential commission addressed this issue in 1988. The decision about whether to breach the confidentiality was to remain with the physician and was not to be imposed as a

matter of law. However, confidentiality is central to the control programme. Maintaining confidentiality encourages more and more people at risk to access the testing services and helps to instill faith about the public health system in the community.

### Informed consent for HIV testing:

A physician who performs any invasive procedure on a patient must do so after informed consent, i.e. the patient must have or be given sufficient knowledge about the procedure to make an intelligent decision. The law of informed consent emanates from two sources i) the fiduciary relationship between a patient and a physicians, and ii) protection to the concept of patient autonomy.

Informed consent applies to HIV testing and it is real informed consent and not implied consent.

Full disclosure of the nature of HIV disease, nature of the proposed test, implications of a positive and a negative test result and the consequences of treatment must be made prior to taking consent. The consent must be voluntary and patient must be able to understand and competent to refuse.

Testing should always be accompanied with counseling.

Informed consent for testing and disclosure must be in writing. Because “if it is not documented, it did not happen” literally applies to informed consent for HIV testing.

Specified exceptions to the need for informed consent for HIV testing include HIV testing that is not linked to identity (e.g. sentinel surveillance, research), blood banks and organ procurement. HIV testing in a minor and an incompetent patient can be undertaken with a guardian's consent.

HIV testing should not be undertaken without written informed consent because of the issues of confidentiality, discrimination, victimization and psychological harms and burdens raised by an HIV positive result.

### HIV testing:

As such HIV/AIDS involves issues of privacy, communal health, social and economic discrimination, coercion and liberty. HIV testing outside the context of blood banking evoked a great deal of controversy and debate about issues such as should the individuals at high risk be required to be tested? How and who will counsel those who were to be tested regarding implications of positive test for themselves and others? What about confidentiality? What about the consequence of testing for the right to work, to go to school, to get married, to bear children and to obtain insurance? There emerged a broad consensus for voluntarism. Except for clearly circumscribed circumstances, HIV testing was to be done under conditions of voluntary, informed consent and results were to be protected by stringent confidentiality safe guards. This consensus was supported by professional organizations, activist organizations, public health officials and bioethics.

HIV screening/testing can be undertaken as voluntary testing after counseling for behavior change; for clinical purposes; for seroprevalence studies; for ensuring safety and for research.

There has been and is lot of debate on routine mandatory testing. Mandatory testing is not cost-effective and is rather counterproductive. Voluntary screening after counseling on identified high risk group is more effective and productive for behavior change and case management. Routine mandatory HIV testing should not be undertaken for

the benefit of the health care workers. Rather practice of standard work precautions will be more beneficial in light of the window period and other blood transmitted infections like Hepatitis B and C.

General Medical Council of the United Kingdom has put forward the following guidelines on Ethical aspects of HIV/AIDS.

#### GMC Ethical aspects-on HIV/AIDS

- The doctor/patient relationship is founded on mutual trust and respect.
- The doctors will extend to HIV infected or AIDS patient the same high standard of medical care and support which they would offer to any other patient.
- Doctors who think they may have been infected with HIV should seek appropriate diagnostic testing and counseling and, if found to be infected, have regular medical supervision.
- They should also seek advice regarding the limits to their clinical practice from competent local consultants so as not to put their patients at risk of HIV infection.
- Doctors infected with HIV have the same rights to confidentiality and support as afforded to other patients.
- Explicit informed consent is a must for HIV testing. Only in most exceptional circumstances can testing without explicit consent be justified.
- Confidentiality of result must be maintained. Only under circumstances where the clinicians feels that withholding the test will put either the colleagues or others at risk, the confidentiality can be breached and shared in a limited manner with relevant individuals only.

#### Non-Discrimination, Human Rights and HIV/AIDS (WHO Global Programme on AIDS)

Human rights and freedom involved in the context of HIV/AIDS include:

Principle of Non-Discrimination: right to equal protection of the law; right to life; freedom from inhuman or degrading treatment or punishment; right to privacy; right to liberty and security; right to the highest attainable standard of physical and mental health; right to marry and to found a family; right to work; right to education; right to social security, assistance and welfare; freedom of movement; right to seek and enjoy asylum; right to share in scientific advancement and its benefits and right to participate in public and cultural life.

## 17. INFRASTRUCTURE FOR HIV TESTING LABORATORIES AT DIFFERENT LEVELS

### Introduction

There has been a progressive increase in access to HIV antiretroviral drugs in the country, limitations on laboratory capacity can be an important barrier in the way of reaching country targets. These limitations can be in the form of both laboratory infrastructure as well as staff requirements. In view of this, it is critical to improve the existing standards of lab infrastructure at local, regional and national levels so as to permit uniform availability of HIV testing, OI diagnosis, and CD4 testing, as also improved and wider availability of facilities for hemoglobin and chemistry testing. This should also include the availability of DNA-PCR testing to facilitate infant diagnosis at a national level.

In view of the above, it is important to define the minimum standards in terms of both infrastructure as well as staff requirements for the various levels of laboratories. This will require choosing uniform, simplified and cost-effective methodologies at the various levels (PHC, district and national) as well as establishing ways and means to ensure continued supply of reagents as well as maintenance of testing equipment.

The infrastructure requirements provided below are for both routine investigations (that can be undertaken at the PHC levels), as well as for specialized tests (that may be undertaken at district and national levels). The routine investigations include point of care as well as in lab testing of Hemoglobin (Hb), blood glucose, urine examination, VDRL, pregnancy testing , HIV testing (rapid and ELISA) as well as diagnosis of routine OIs through simple staining procedures.

### Categories of HIV testing laboratories/centres

HIV testing laboratories have been categorised into following major groups by National AIDS Control Organisation, GOI.

Screening laboratories in blood banks : The main function of these laboratories is to ensure donated blood safety. Thus the laboratories in blood banks in addition to other testings e.g. grouping, cross matching must screen the donated units of blood for transfusion transmitted infections (TTI) i.e. HIV, HBV (HBsAg), HCV, syphilis and malaria as per the existing guidelines from GOI.

Sentinel surveillance laboratories and voluntary counselling and testing laboratories : These categories of laboratories are functioning in various medical colleges, hospitals, CHCs, PHCs and institutes. These laboratories carry out voluntary testing (with pre and post test counselling) and testing for surveillance. Being located in medical colleges, hospitals and institutes, the necessary infrastructures for HIV testing in these laboratories are likely to be in place. However, the infrastructure will have to be provided at CHCs and PHCs.

National and state reference laboratories : Presently there are 12 NRLs distributed all over India that are entrusted with the following broad responsibilities:

- Providing referral HIV diagnostic services in the region.
- Evaluation of HIV test kits.
- HIV screening of blood products for human use.

- Carrying out external quality assessment of other laboratories and trouble shooting.
- Providing training in HIV testing, quality assurance and biosafety procedures.
- Providing technical support for formulating guidelines for quality assurance and other HIV testing related issues.
- Voluntary counselling and testing.
- Providing HIV testing services for various groups as directed by NACO, G.O.I. from time to time.
- Providing diagnostic services (including training) for opportunistic infections.
- CD4 cell enumeration
- DNAPCR, Viral load estimation and drug resistance testing

Tables 1 -3 (see below) list the infrastructure and staff requirements for a lab at PHC, district and reference lab levels in reference to HIV testing.

**Table 1** **INFRASTRUCTURE REQUIREMENTS FOR A P.H.C. LAB**

	Quantity	Unit Cost (Rs)	Total cost (Rs)
Infrastructure / civil work			
Room size (shall include phlebotomy)	100 to150 sq feet (includes 50 sq feet for phlebotomy)		
Electrical points	12	36	432
Extension cords	2	50	100
Electrical tubes	4	150	600
Exhaust fan	1	500	500
Fire extinguisher	1	650	650
Stainless steel sinks (18" x 30")	1	2200	2200
Handwashing sinks (16" x 18")		2200	
Water distiller (quartz)	1	10000	10000
Dustbins with foot paddle	3	300	900
Laboratory stools	3	500	1500
Sub total 1			16882
Storage cabinets/ other furniture			
Glassware / chemicals	1	7200	7200
Filing cabinet	1	5500	5500
Desks	1	4000	4000
Chairs	1	2000	2000
Sub total 2			18700
Appliances and general lab equipment			
Airconditioner 1.5 ton	1	25000	25000
Fans	1	1200	2400
Refrigerator (300 L)	2	18000	18000
Desktop computer	1	30000	30000
Printer Laser	1	9000	9000
Water bath (37 degrees) 300x250x175mm	1	5986	5986
Incubator (37 degrees) 14x14x14"			



	Quantity	Unit Cost (Rs)	Total cost (Rs)
Hot air oven 14x14x14"	1	9348	9348
Sample transport boxes	5	250	1250
Desk top Centrifuge + rotor with 16 slots	1	12366	12366
Cyclomixer / vortex	1	2300	2300
VDRL rotator	1	2000	2000
Binocular Microscope	1	15000	15000
UPS - 1 kva (offline)	1	9000	9000
Sub total 3			141650
Phlebotomy /blood collection			
Vacutainer needle holder, case of 500	as per requirement	2000	
Vacutainer needle, 21G, case of 100	as per requirement	500	
Vacutainer tube, KEDTA, 3.5 ml, case of 100	as per requirement	575	
Vacutainer, plain 3.5 ml case of 100	as per requirement	575	
Needle destroyer	1	1500	1500
Bunsen burner/ spirit flame	2	150	300
Torniquet	5	15	75
Medicated spirit (4 liters pack)	as per requirement	400	
Cotton gauge rolls	as per requirement	20	
Butterfly needles 25G, box of 50	as per requirement	145	
Plastic tube racks 31 holes	6	132	792
Plastic tube racks 62 holes	5	500	2500
Sub total 4			5167
Pipetting			
Autopipette 5- 50 ul	1	4560	4560
Autopipette 50-200 ul	1	4560	4560
Autopipette 100-1000ul	1	4560	4560
Plastic disposable pipettes - 5ml, case of 100	2	1500	3000

	Quantity	Unit Cost (Rs)	Total cost (Rs)
Plastic disposable pipettes - 10ml, case of 100	2	1700	3400
Plastic disposable pipettes - 25ml, case of 100	1	1700	1700
Tips (10-100 ul) - pack of 500	as per requirement	240	
Tips (200-1000 ul)- pack of 500	as per requirement	190	
Sub total 5			17220
General labware			
Beakers - 1000ml, polypropylene	5	106	530
Beakers - 500ml , polypropylene	3	50	150
Measuring Cylinders- 500ml , polypropylene	5	152	760
Measuring Cylinders- 1000ml , polypropylene	2	400	800
Glass tubes (small) pack of 100	5	390	1950
Glass tubes (large) pack of 100	5	860	4300
Wash bottles (HDPE) - 250 ml capacity	5	39	195
Staining racks	2	100	200
Filter papers (ordinary)- pack of 500	as per requirement	500	
Electronic timers	2	550	1100
Sticker labels (pack of 100)	as per requirement	100	
Micoslides (pack of 100)	as per requirement	50	
coverslips 18*18mm (pack of 100)	as per requirement	46	
Staining jars	10	13	130
Sub Total 6			10115
Biosafety			
Powderless latex Gloves (small) - pack of 200	as per requirement	1400	
Powderless latex Gloves (medium) pack of 200	as per requirement	1400	

	Quantity	Unit Cost (Rs)	Total cost (Rs)
Powderless latex Gloves (large)- pack of 200	as per requirement	1400	
lab coats (medium)	3	200	600
Lab coats (large)	2	200	400
Autoclave electrical , pressure cooker type -300x 300mm	1	4182	4182
Sub total 7			5182
Biochemistry			
Blood glucose meter (Biosensor technology)	1	2500	2500
Reagent strips for blood glucose (pack of 50 strips)	as per requirement	1100	
Disposable lancets (pack of 100)	3		
Hematology			
Hb meter	1	20000	20000
Hb kits (pack of 100 tests)	as per requirement	600	
Blood rocker	1	3200	3200
Sub total 8			23200
Microbiology & serology			
HIV I & II (rapid) pack of 100 tests (3 types of kits)	as per requirement		
ELISA plate reader with washer	NR		
ZN stain kit for AFB			
Methylene blue (125ml)	as per requirement	44	
Carbol fuchsin (125ml)	as per requirement	50	
Hydrochloric Acid (N/10, Conc. HCl - 500 ml)	as per requirement	92	
VDRL (RPR) - 500 tests/ pack	as per requirement	1200	
Urine pregnancy test - 100 tests/pack	as per requirement	1200	
Urine analysis			
Urine strips (3 parameters - glucose, protein, pH) - pack of 100 strips	as per requirement	250	
Urine Container for routine Samples (pack of 500)	as per requirement	180	
Total set up cost			238116

	Number	Min. Qualifications	Job responsibility
Staff requirements			
Medical Officer cum Lab-incharge	1	MBBS / MD	<ul style="list-style-type: none"> <li>● Overall admin of lab and VCT/ PPTCT,</li> <li>● Signing of reports</li> <li>● Liasoning with ART centre</li> </ul>
<i>Technician</i>	1	Diploma in Medical Lab Technology	<ul style="list-style-type: none"> <li>● Phlebotomy, sample receipt and log-in</li> <li>● Testing of samples</li> <li>● Preparartion of test reports</li> <li>● Maintenance of all records</li> </ul>

The officer-in-charges (OIC) of HIV testing sites (PHC/district) should preferably be laboratory experts. In case the above requirement is not met, the OIC shall be a appropriately trained medical officer.

## 18. OCCUPATIONAL EXPOSURE AND POST-EXPOSURE PROPHYLAXIS

### Introduction

An “exposure” that may place a Health Care Provider (HCP) at risk of bloodborne infection is defined as a percutaneous injury (e.g. needle-stick or cut with a sharp instrument), contact with the mucous membranes of the eye or mouth, contact with non-intact skin (particularly when the exposed skin is chapped, abraded, or afflicted with dermatitis), or contact with intact skin when the duration of contact is prolonged (e.g. several minutes or more) with blood or other potentially infectious body fluids. Body fluids that are potentially infectious include blood, semen, vaginal secretions, cerebrospinal fluid, synovial, pleural, peritoneal, pericardial and amniotic fluids or other body fluids contaminated with visible blood. Exposure to tears, sweat, urine, faeces, saliva of an infected person is normally not considered as an “exposure” unless these secretions contain visible blood. Any direct contact (i.e., contact without barrier protection) to concentrated virus in a research laboratory or production facility requires clinical evaluation.

**Table1 Efficiency of different routes of occupational HIV transmission**

Exposure route	Percent efficiency
Needle stick exposure	0.3%
Mucous membrane splash to eye, oronasal	0.09%

### Practices that influence risk and how to reduce risk

Various epidemiological and laboratory studies have shown that the risk of infection varies with type of exposure, such as:

- Type of needle (hollow bore vs. solid)
- Device visibly contaminated with patient's blood
- Depth of injury
- The amount of blood involved in the exposure
- The amount of virus in patient's blood at the time of exposure.
- Whether PEP was taken within the recommended time (<2 hours, up to 72 hours)

Prevention of exposure to virus remains the mainstay to prevent occupational infection. All health care workers must follow universal precautions at all the times. This means that all the patients must be seen as potentially infectious because it is neither practical nor it is desirable to test all the patients for HIV.

### Prevention of Exposure

- Hand washing
- Barrier use in nursing when indicated: latex or vinyl gloves, gown/apron, protective eyewear or mask

- Immunization against Hepatitis B whenever possible
- Minimisation of invasive interventions, oral vs. injection when possible
- Safe handling of sharps:
  - Careful handling of hollow bore needle is very essential as it may lead to deep injuries.
  - The needles should never be recapped. In situations where recapping is essential, single hand method should be used.
  - Needles should never be bent/broken by hand.
  - Needles should not be left on trolleys and beds and must be disposed of immediately.
  - Never pass used sharps from one person to another directly.
  - Use forceps instead of fingers for guiding sutures.
  - The sharps should be disposed of in a puncture resistant container containing bleach.

## HIV

### Management of Exposure :

Steps to be taken on accidental exposure to blood (or body fluid containing blood) are:

- Wash wound immediately with running water and soap
- Inform the lab /hospital management and document occupational accident
- Consult with nearest ART centre/ resource for Post-exposure prophylaxis, evaluation, and follow-up (as per the National guidelines on PEP)
- Counselling and collection of blood for testing from the exposed HCP with written informed consent must be done.
- Whenever possible confidential counselling and testing of source for Hepatitis, HIV etc must be done. A history should be taken as well to ascertain likely risk of the source. (PEP should be provided to the exposed HCP until report of source is available and confirmed negative).
- Risk of infection and transmission must be evaluated
- Never delay start of therapy due to debate over regimen. Begin with basic 2-drug regimen, and change if warranted, once expert advice is obtained
- Reevaluation of the exposed person should be considered within 72 hours post exposure, especially as additional information about the exposure or source person becomes available. The exposed person is advised to seek medical evaluation for any febrile illness that occurs within 12 weeks of exposure.
- Administer PEP for 4 weeks .PEP should be provided until result of the source's test is available and confirmed negative or until course completed ,if source positive or unknown
- A repeat HIV test of the exposed individual should be performed at 6 weeks, 12 weeks and 6 months post-exposure, regardless of whether or not PEP was taken

***Ideally, prophylaxis should be begun within 2 hours of exposure.***

#### **Donts:**

- Do not panic!
- Do not reflexively place pricked finger into mouth
- Do not squeeze blood from wound, this causes trauma and inflammation, increasing risk of transmission
- Do not use bleach, alcohol, betadine, or iodine, which may be caustic, also causing trauma

### **Dos:**

- Remove gloves, if appropriate
- Wash site thoroughly with running water. Irrigate thoroughly with water or saline if splashes have gone into the eye or mouth.

### **Evaluation of Source**

1. If the HIV status of the source patient is known, and confirmed negative, there is no need for HIV-PEP.
2. If the status of the patient is unknown, and neither the patient nor his/her blood is available for testing, then the choice of whether to use PEP and what regimen will depend on the severity of the wound, and how much is known about the individual's risk history.
3. If the status of the patient is unknown, and the patient is available he is to be counselled and consent obtained for testing.
4. If the patient refuses testing, but a sample of blood is available, it is the right of the exposed person that the sample should be tested, and the source patient may decline to be informed of the result.
5. If the patient has refused counselling and testing, and there is no blood sample available, it is the right of the exposed person to ask that blood be taken for testing, and the source patient may decline to be informed of the result. Under no circumstances should the source patient be charged for the test.
6. If the patient is known to be HIV positive, evaluation of risk is in order. The two key factors to consider are whether the patient is antiretroviral drug naïve, or whether s/he has been exposed to medications; and whether the patient is likely to have a high viral load as determined by testing if available, or by clinical signs and symptoms.
  - Low risk: Asymptomatic, or viral load <1500 copies/ml
  - High risk: Symptomatic with OI, or AIDS, acute seroconversion, high viral load
7. In the case of a high risk exposure from a source patient who has been exposed to or is taking antiretroviral medications, consult an expert to choose PEP regimen, as the risk of drug resistance is high.

### **Seroconversion after occupational exposure**

If transmission of a bloodborne infection occurs after occupational exposure which has been documented, the HCP has a right to receive treatment and care for this illness. It is also the right of all persons infected thusly, to freedom from discrimination regarding their working conditions. Such persons are entitled to have all of their human rights respected, beginning firstly with the right to confidentiality regarding their health care.

**Table 2. HIV Postexposure prophylaxis evaluation**

Exposure	Status of source (see below)			HIV status negative
	HIV + and low risk	HIV + and high risk	HIV status unknown	
Mucous membrane/non-intact skin; small volume (drops)	Consider 2-drug PEP	2-drug PEP	Consider 2-drug PEP	No PEP is required if the source blood is confirmed HIV negative
Mucous membrane/non-intact skin; large volume (major blood splash)	2-drug PEP	3-drug PEP	Consider 2-drug PEP	
Percutaneous exposure: not severe solid needle, superficial	2-drug PEP	3-drug PEP	Consider 2-drug PEP	
Percutaneous exposure: severe large bore hollow needle, deep injury, visible blood in device, needle in patient artery/vein	3-drug PEP	3-drug PEP	Consider 2-drug PEP	

**Table 3. Post-exposure chemoprophylaxis when source patient is drug naïve**

<p><b>Basic regimen:</b></p> <p>2 drugs (NRTIs)</p> <p>(4 weeks therapy)</p>	<p>Zidovudine (AZT/ZDV)–300mg twice/day is used for all types of exposure</p> <p>+ Lamivudine (3TC) – 150 mg twice a day is added to increase the effectiveness of ZDV and to prevent resistance to ZDV</p>
<p><b>Expanded Regimen:</b></p> <p>3 drugs (2 NRTIs + PI)</p> <p>(4 weeks therapy)</p>	<p>Basic Regimen (AZT/ZDV + 3TC)</p> <p>+ Nelfinavir 750 mg three times daily or any other boosted protease inhibitor. (for higher risk categories - consult expert).</p>

Drugs not recommended for PEP:

- ddl and d4t; together
- Nevirapine

In case the source patient is on ART and considered to be having drug resistance (clinically) then the ART



physician should be consulted and appropriate ARVs should be given for PEP.

### **Schedule of necessary measures regarding therapy and follow up in case of PEP**

The person should be provided with pre test counseling and PEP be started as discussed above. Before starting PEP, 3-5 ml of person's reference blood sample is to be taken and sent to the laboratory for testing and storage. It is important that a serum sample is collected from the HCP as soon as possible (zero hour) after exposure for HIV testing, failing which it may be difficult to attribute the acquired infection due to exposure in occupational setting. This may have bearing on the claims of compensation from health authorities. The first sample for HIV testing is collected immediately after exposure and 2nd at 6 weeks, 3rd at 12 weeks and last at 6 months after the exposure. During the follow-up period, especially the first 6-12 weeks when most infected persons are expected to show signs of infection, the recommendations for preventing transmission of HIV are to be followed by the HCP. These include refraining from blood, semen, organ donation and abstaining from sexual intercourse. In case sexual intercourse is undertaken a latex condom must be used correctly and consistently. This reduces the risk of HIV transmission. In addition, women should not breast-feed their infants during the follow-up period after exposure to prevent exposing their infants to HIV in breast milk.

The antiretroviral drugs for PEP are to be given for four weeks. Government of India has already made the resources available with various State AIDS Societies to meet with the expense of PEP for HCWs. The drugs for PEP must be available round the clock.

The report of exposure and PEP has to be sent to Addl. Director (Technical) National AIDS Control Organisation, GOI.

### **Pregnancy and PEP**

If the HCP is pregnant at the time of occupational exposure to HIV, the designated authority/physician must be consulted about the use of anti-viral drugs for post exposure treatment.

### **Facts known about the safety and side effects of these drugs**

Most of the information known about the safety and side effects of these drugs is based on studies of their use in HIV-infected individuals. For these individuals, ZDV and 3TC have usually been tolerated well except for nausea, vomiting, diarrhoea, tiredness, or headache for people taking ZDV.

### **Steps to be undertaken by the infection control officer on receiving information about occupational exposure**

- All the needle stick injuries should be reported to the State AIDS Society giving the details of the type of exposure. and the measures taken to manage the same. The State AIDS Societies should in turn inform NACO about the cases periodically.
- A registry is available with NACO for follow-up of all such cases.
- Infection control officers in all hospital have been directed to ensure that anti retroviral drugs for PEP are available in casualty at all the times.

+

Persons taking PEP should also be advised that for certain symptoms (rash, fever, back or abdominal pain, pain on urination or blood in the urine, or symptoms of hyperglycaemia such as increased thirst and urination) evaluation by a doctor should not be delayed. An exposed person should be advised to use precautions (e.g., avoid blood or tissue donations, breastfeeding, unprotected sexual relations or pregnancy) to prevent secondary transmission, especially during the first 6-12 weeks following exposure.

Each institution should designate a team of persons who have authority to ensure that confidentiality is maintained, and required care given in any case of occupational exposure. At every healthcare facility, there should be 3 persons (ideally the Infection Control Officer, the Nodal Officer, and the casualty Medical Officer), who should be trained to take responsibility to ensure that the following procedure is carried out:

- Evaluate the injury and counsel the HCP for HIV ELISA (and hepatitis testing, HBsAb, if available), ensure proper reporting is completed but that confidentiality is maintained
- Decide regimen counsel HCP about the HIV PEP drugs and potential side effects and precautions to be taken until follow-up tests done
- Interpret results of baseline and follow up tests
- Conduct follow-up visits and testing

In order to ensure that an exposed person has access to prophylactic therapy in a timely manner, it is recommended that PEP ARV medications should be kept available at all times in any location where a doctor is on call 24 hours a day (i.e., casualty, ICU). A minimum of 3 days worth of all 3 drugs in the extended regimen should be included in the HIV exposure-response kit, as well as the reporting/written consent forms, and a rapid test kit to be used to test the source patient. At the PHC level, there should be a 3-days supply of drugs, and PEP is to be begun in consult with the corresponding district hospital.

## 19. TESTS WHICH HAVE BEEN EVALUATED BY WHO COLLABORATING CENTRE ON AIDS IN THE DEPARTMENT OF MICROBIOLOGY, INSTITUTE OF TROPICAL MEDICINE, ANTWERP, BELGIUM

The list of assays given below (ELISA/Rapid/Supplemental) is relevant for use in India as these assays detect antibodies to both HIV-1 as well HIV-2. The list has been prepared from WHO document number WHO/BLS/98.1 with due permission from WHO.

ELISA/EIA For the detection of antibody to HIV-1 and HIV-2

Manufacturer	Sensitivity	Specificity			Reader/ Visual (R/V)	Suitability
			WB+sera	WB-sera		
Detect-HIV TM (Biochem Immunosystems)	100.0 (98.6-100.0)	97.4 (94.0-99.2)	12.65	-2.21	R	LS
BuPont HIV-1/HIV-2 ELISA (DuPont de Nemours)	100.00 (98.7-100.0)	85.6 (79.8-90.2)	9.34	-0.96	R	LS
Anti-HIV-1/HIV-2 EIA <Roche> (F. Hoffman-LeRoche)	100.0 (98.7-100.0)	96.9 (93.498.9)	11.30	-2.37	R	LS
Wellcozyme HIV-1+HIV-2 (Wellcome Diagnostics)	100.0 (98.7-100.0)	96.9 (93.3-98.9)	38.51	-1.99	R	LS
Peptide HIV ELISA (Cal-Tech Diagnostics)	72.6 (69.477.6)	95.4 (91.3-97.9)			V	S
Genelavia Mixt (Sanofi Diagnostics Pasteur)	100.0 (98.6-100.)	98.5 (95.6-99.8)	16.77	-2.10	R	LS
Biotest Anti-HIV-1/-2 Recombinant (Biotest)	100.0 (98.6-100.0)	97.9 (94.9-97.4)	50.47	-3.08	R	LS
Enzymum-Test Anti-HIV-1/+2 (Boehringer Mannheim)	100.0 (98.7-100.0)	100.0 (98.6-100.0)	5.50	-2.48	R	S
Innotest HIV-1/HIV-2 Ab (Innogenetics)	100.0 (98.8-100.0)	97.9 (95.9-99.9)	7.22	-2.30	R	LS
Clonatee HIV (1+2) Ab EIA (Clonatee)	99.6 (98.8-100.0)	95.9 (93.1-98.7)	7.47	-1.68	R	S
Peptide HIV-1 & HIV-2 ELISA Test (Sero-Immuno Diagnostics)	97.6 (95.7-100.0)	98.5 (96.7-100.0)			V	VS
Cobas Core Anti-HIV-1/HIV-2 EIA <Roche> (Hoffmann-La Roche)	100.0 (98.36-100.0)	89.2 (84.6-93.8)	10.8	-1.0	R	LS

Abbott Recombinan HIV-1/HIV-2 3rd Generation (Abbott)	100.0 (98.5-100.0)	100.0 (98.5-100.0)	11.5	-4.3	R	LS
HIV-1 and/or HIV-2 Recombige EIA (Combridge Diagnostics)	100.0 (98.6-100.0)	100.0 (98.6-100.0)	10.4	-50	R	LS
UBI HIV-1/2 EIA 2nd (United Biomedical)	99.5 (98.6-100.0)	92.4 (88.6-96.2)	4.8	-1.5	R	S
HIV1+2 env Peptide EIA (Labsystem OY)	100.0 (98.5-100.0)	76.2 (70.0-82.4)			R	LS
VIDAS HIV-1+2 (Bio Merieux)	100.0 (99.6-100.0)	97.8 (95.6-100.0)			R	S
VIRONOSTIKAHIV Uni-Form II (Organon Taknika)	100.0 (99.6-100.0)	98.8 (97.6-100.0)	7.4	-3.0	R	LS
BIOTEST Anti-HIV-1/2 Recombinant (Biotest AG)	100.0 (99.6-100.0)	99.1 (98.1-100.0)	74.9	-3.3	R	LS
INNOTEST HIV-1/HIV-2 Ab s.p. (Innogenetics n.v.)	100.0 (99.6-100.0)	98.8 (97.6-100.0)	14.0	-3.8	R	LS
UBI HIV 1/2 EIA (United Biomedical Inc.)	90.9 (87.4-94.4)	100.0 (99.7-100.0)	10.8	-3.2	R	LS
HIVisual 1 & 2 (Immuno Diagnostic Inc.)	100.0 (99.6-100.0)	94.9 (92.5-97.3)	1.88	-1.15	R	LS
HIV-1 HIV-2 ELISA (Genelabs Diagnostics)	100.0 (99.6-100.0)	97.3 (95.6-99.0)	72.2	12.7	R	LS
ETI-AB-HIV-1/2K (Sorin Biomedica)	100.0 (99.6-100.0)	98.8 (97.6-100.0)	10.39	-2.15	R	LS
HIV SCREEN (Labsystems OY)	100.0 (99.6-100.0)	99.7 (99.1-100.0)	21.54	-4.11	R	LS
HIV EIA (Labsystems OY)	100.0 (99.6-100.0)	99.4 (98.6-100.0)	14.20	-3.85	R	LS

## Simple / Rapid assays for the detection of antibody to HIV-1 and HIV-2

Manufacturer	Sensitivity	Specificity	Ease of performance	Suitability
Test Pack HIV-1/HIV-2 Ab (Abbott)	100.0 (98.5-100.0)	95.9 (92.0-98.2)	VE	VS
HIV CHECK1+2/HIV SPOT 1+2 (DuPont de Nemours) (Genelabs Diagnostics)	99.3 (97.4-99.9)	100.0 (98.1-100.0)	E	VS
Clonatec rapid HIV-1-HIV-2 Ab (Clonatec)	98.9 (96.8-99.8)	99.5 (97.2-99.8)	E	VS
Recombigen HIV-1/HIV-2 Rapid Test Device (Cambridge Diagnostics)	100.0 (98.7-100.0)	94.5 (91.2-97.8)	E	VS
Serodia - HIV-1/2 (Fujirebio)	100.0 (98.5-100.0)	100.0 (98.5-100.0)	LE	S
SPAN COMBAIDS VISUAL (Span Diagnostics)	96.5 (93.5-99.5)	100.0 (98.3-100.0)	E	VS
CAPILLUS HIV-1/HIV-2 (Cambridge Diagnostics)	100.0 (99.6-100.0)	98.8 (97.6-100.0)	VE	VS
Immunocomb II BiSpot HIV 1&2 (PBS Organics)	100.0 (99.6-100.0)	99.7 (99.1-100.0)	VE	VS
MicroRed HIV-1/HIV-2 Ab Test (Agen Biomedical)	98.5 (97.0-100.0)	95.5 (93.2-97.7)	VE	S
SimpliRed HIV-1/Hiv-2 Ab Test (Agen Biomedical)	99.2 (98.2-100.0)	87.3 (83.7-90.9)	VE	S
HIV (Sav) 1&2 Rapid Sero Test (Diatech Diagnostica Ltd./Healthcare Technologies Ltd.)	97.7 (95.9-99.5)	96.7 (94.8-98.6)	VE	S
ENTEBE HIV Dipstick (Hepatika Laboratories)	100.0 (99.6-100.0)	96.4 (94.4-98.4)	E	S
Dipstick-HIV 1+2 (Immuno-Chemical Laboratories)	100.0 (99.6-100.0)	98.2 (96.8-99.6)	E	VS
SPAN COMBAIDS VISUAL (Span Diagnostics Ltd.)	100.0 (99.6-100.0)	88.0 (84.5-91.5)	E	S
DIA (Dot Immuno Assay) HIV- 1+HIV-2 (Weiner Lab)	99.6 (98.8-100.0)	99.4 (98.6-100.0)	E	VS

## Supplemental assays for the detection of antibody to HIV-1 and HIV-2

Manufacturer	Sensitivity	Specificity	Ease of performance	Suitability
INNO-LIA HIV-1/HIV-2Ab (Innogenetics)	100.0 (98.6-100.0)	100.0 (98.0-100.0)	LE	S
HIV CHECK1+2/HIVSPOT 1+2 (DuPont de Nemours) (Genelabs Diagnostics)	100.0 (99.4-100.0)	66.4 (57.9-74.1)	LE	S
Clonatec rapid HIV-1-HIV-2Ab (Clonatec)	99.3 (96.4+99.9)	100.0 (98.1/100.0)	LE	S

VE = Very easy  
 LE = Less easy  
 LS = Less suitable  
 S = Suitable  
 VS = Very suitable

## 20. LIST OF REFERENCES FOR FURTHER STUDY

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## CHAPTER 21

### LIST OF LABORATORY REGISTERS AND FORMATS

The following should be maintained for keeping a track of various activities in the laboratory:

1. Specimen log-in register
2. Lab test result entry register
3. Daily temperature log (refrigerator, water bath and hot air oven)
4. General equipment maintenance log
5. Microscope maintenance log
6. Autoclave operation log
7. Complaint and corrective action log
8. Format for worksheet
9. Expired kits log
10. New reagent verification log
11. Stock card
12. Monthly assay-reassay format.
13. Consumption record and inventory management.
14. HIV consent form
15. HIV test requisition form
16. HIV test report form
17. Proforma for sending reports to NACO / SACS

Sample formats for the above registers are provided below:





**Specimen log in register**

Patient ID	Patient Name	Age	Sex	Referred by		Time Test received in lab	Required investigations	Sample requirements met?	If not, remarks	Initials of the receiving person
				Clinician (name)	Clinic (name)					



LAB TEST RESULT ENTRY REGISTER

Patient ID	Patient Name	Age	Sex	Name of the investigation	Test result	Test valid / invalid	Time test completed and entered	Whether report generated	Initials of the entering technician

**Daily Temperature log (Water bath, refrigerator and hot air oven)**

Name of the laboratory		Temperature (degree C)																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Month																																	
January																																	
February																																	
March																																	
April																																	
May																																	
June																																	
July																																	
August																																	
September																																	
October																																	
November																																	
December																																	

MICROSCOPE MAINTANANCE LOG

<b>Name of the laboratory</b>																																																							
<b>Daily</b>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31																								
Clean objectives, stage and condenser																																																							
Check fine & coarse adjustment																																																							
Check mechanical stage																																																							
Check light alignment																																																							



**NEW REAGENT VERIFICATION LOG**

Name of the laboratory \_\_\_\_\_

Date	Old reagent				New reagent			Verification		
	Reagent/Kit	Cat No.	Lot No.	Expiry date	Cat No.	Lot No.	Expiry date	Analyte	Value with old kit	Value with new kit

Verified by lab head \_\_\_\_\_



### EXPIRED KITS LOG

Name of the laboratory \_\_\_\_\_

Date	Reagent/Kit	Lot No.	Expiry date	Tick to confirm that kit is discarded after expiry	Technicians's signatures

Verified by lab head \_\_\_\_\_

+  
**Autoclave Operation Log**

Name of the facility & dept			
Equipment		Serial Number of the equipment	

Date	Daily check Inlet PSI Y	Daily check Clean Drain Y	Items being sterilized	Lot # (if needed)	Indicator type used (spore strips)	Time in	Time/ Temp Attained	Time Off	Time Removed	Indicated	Acceptable or not	Initials

**Equipment Maintenance Log**

Health Department		City	
Equipment		Serial Number	

**Scheduled Maintenance Tasks**

Task	Interval	Vendor or Staff Initials

**Scheduled Maintenance Tasks**

Date	Task	Initials



**Compliant and Corrective Action Log**

Name of the Lab / Department :	
Date Reported :	Initiated By :
Source of Communication / Complaint :	
Date of Occurrence :	Time :
Narrative of Event (If necessary) :	
Immediate Corrective Action Taken :	
Does the written procedure cover how to deal with this event? Yes No* Not Applicable "if No - Procedure must be updated within fifteen days from date of event.	
If Yes - Was the written procedure	If No - Why not? Elaborate Below

Followed?					
Yes					
Follow Up Activities Required?	If Yes - Indicate what and date to be completed below.				
Yes					
No					
Signature	<table border="1"> <tr> <td data-bbox="302 1646 737 1711">Who Completed - Signature</td> <td data-bbox="737 1646 922 1711">Date :</td> </tr> <tr> <td data-bbox="302 1073 737 1136"></td> <td data-bbox="737 1073 922 1136">Date :</td> </tr> </table>	Who Completed - Signature	Date :		Date :
Who Completed - Signature	Date :				
	Date :				

## Stock Card

Regularly reported record of orders, receipts and issues of tests

Lab Site : \_\_\_\_\_ Report Period : \_\_\_\_\_

District : \_\_\_\_\_ Date Submitted : \_\_\_\_\_

Order Date	Order Details	# Tests Ordered	Receipt Date	Receipt Time	Order Sent From	Order Sent To	# Tests Received	Carry over from previous stock	Stock in hand	Stock check comments	Other comments	Initials
							A	B	C=A+B	K		
	Requisition # :											
	Voucher # :											

Test Issue Date	Test Issue Time	Test Issue From	Test Issue To	# Tests Issued	Tests expired	Stock balance	Stock check comments	Other comments	Initials
D	E	F	G	H	I	J=C-(H+I)	K	L	

### Instructions

- Each time an order is made, a new stock card should be started, recording Order Date, Order Details, and # Tests Ordered. Until the order is received, the previous card should be used to record stock issue.
- Once the order is received, complete the first row and record the ending balance from column (J) of the previous card in Box (B) of the new card.
- The stock should be regularly checked, noting discrepancies in column (K). Record expired stock in column (I), comment as such in column (L), and safely remove and destroy it and document the same.

Signature of Chief Technician : \_\_\_\_\_ Signature of Laboratory charge : \_\_\_\_\_

## Consumption Record and Inventory Management

### Quarterly inventory tracking form

Lab Site : \_\_\_\_\_ Reorder Level : \_\_\_\_\_  
 District : \_\_\_\_\_ Date Submitted : \_\_\_\_\_  
 State : \_\_\_\_\_ Date Submitted : \_\_\_\_\_

Month Start Date	Opening Balance	Total# Tests Received	Total# Tests Used	Losses/ Adjustments (+/-)	Closing Balance	Months of Stock on Hand (at month end)	Earliest Expiry	Quantity Needed in Next Month	Total # Days of Stock Out	Comments
A	B	C	D	E	F=B+C-D+/-E	G=F/D	H	I	J	K
Month 1				e w a o			# Tests : Expiry Date :			
Month 2				e w a o			# Tests : Expiry Date :			
Month 3				e w a o			# Tests : Expiry Date :			
Quarter Total				e w a o			# Tests : Expiry Date :			

### Instructions

- In column (E): Enter positive or negative number for quantity of reagents removed from stock for any reason other than consumption by the lab (e.g. losses, expiry, damage, stock transfer, correction of accounting error). Circle 'e' for expired, 'w' for withdrawn, 'a' for stock adjustments, 'o' for other.
- Stock on hand refers to the quantity of usable stock available at all levels of the system at a point in time
- Closing balance of month one (F) should equal the opening balance (B) of month two.
- Minimum stock levels adequate for testing over 6 weeks (1.5 months) should be available at the time of placing the next order to prevent stock-outs.
- Each lab site would need to determine their individual average monthly test requirements based on the average inflow of patients for CD4 testing and also factor for any expected new patients to be able to estimate when to order and to establish the reorder level.

Signature of Chief Technician: \_\_\_\_\_ Signature of Laboratory in charge: \_\_\_\_\_

## Monthly Assay — Re Assay Format

Lab Site : \_\_\_\_\_ Report Period : \_\_\_\_\_

District : \_\_\_\_\_ Date Submitted : \_\_\_\_\_

Week Start Date	Accessions on Courier Specimens	Accessions on Walk-in Specimens	Cumulative Assessments	Controls	Re Assays	Total Tests Used	Re assay	No. of Re assays	Reason for reassays	Comments
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F=C+D+E</b>	<b>G=E/C * 100</b>	<b>H</b>	<b>I</b>	
Week 1										
Week 2										
Week 3										
Week 4										
Month TOTAL										

### Instructions

- Reasons for reassays are elaborated below and the symbols for each of these are in brackets preceding the reason. For purposes of simplicity the reasons for reassay may be recorded using symbols for the same , for example, in the week if 3 reassays have been done for reason (a) and 2 for reason(e), then it may be recorded as 5 in Column H and as 3a+2e in column I.
- Reasons for reassay: (a)Test re- run due to power disruption; (b)Test re- run due to instrument/ calibration/ control requirements; (c)Test re-run for confirming low or high values; (d)Test re- run for dilution requirement for verification in high positive levels; (e )Test re-run due to instrument failure; (f) Test re- run due to temperature variations; (g)Test re- runs for confirmation/ run failures; (h)Test re-run due to contamination; (i)Test re-run on client's request; (j)Test re-run for QC (k)Test re-run due to kit failure; (l)Positive samples to be repeated as per protocol; (m) New machine validation; (n)New kit validation; (o)Validations for QA & other purposes ; (p) New Technology Validations

Signature of Chief Technician: \_\_\_\_\_ Signature of Laboratory in charge: \_\_\_\_\_

**FORMAT FOR REPORTING TO NACO-SACS (National Reference Lab)**

To, \_\_\_\_\_

Date : \_\_\_\_\_

Name of HIV testing lab \_\_\_\_\_

Sr. No.	Lab No.	Name/IL	Age/Sex	Risk group	Screening Tests			Confirmatory test Kit Used		Interpretation	
					Test-I Kit Used ( Principle (	Test-II Kit Used ( Principle (	Test-III Kit Used ( Principle (	HIV-1	HIV-2	HIV-1	HIV-2

**Head / Director**

**Officer-In-Charge**

**Laboratory Technologist**

**CC :**

Additional Director (Technical) NACO, Ministry of Health & Family Welfare, Chandralok Building, 9th Floor, 36-Janpath, New Delhi-110 001  
Project Director, State AIDS Control Society.

**FORMAT FOR REPORTING TO SACS (ICTC / PPTCT)**

To, \_\_\_\_\_

Date : \_\_\_\_\_

Name of HIV testing lab \_\_\_\_\_

Sr. No.	Lab No.	Name/IL	Age/Sex	Risk group	Screening Tests			Interpretation	
					Test-I Kit Used ( ) Principle ( )	Test-II Kit Used ( ) Principle ( )	Test-III Kit Used ( ) Principle ( )	HIV-1	HIV-2

**Head / Director**

**Officer-In-Charge**

**Laboratory Technologist**

**CC :**

Additional Director (Technical) N.A.C.O., Ministry of Health & Family Welfare, Chandralok Building, 9th Floor, 36-Janpath, New Delhi-110 001  
Project Director, State AIDS Control Society.

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### HIV TEST REQUISITION FORM

**Name and address of the facility**

1 Patient ID : \_\_\_\_\_ Sex : 

M	F
---	---

  
Name (optional) : \_\_\_\_\_ Age : 

--	--

2 Authorizing clinician name & signature :  
\_\_\_\_\_

4 Date and Time Blood Drawn (dd/mm/yy) :

		/			/		
--	--	---	--	--	---	--	--

3 Referring clinic name :  
\_\_\_\_\_

		:			(hh:mm)
--	--	---	--	--	---------

5 Relevant clinical details :

**For laboratory use only :**

6 Date and Time sample received (dd/mm/yy) :

		/			/		
--	--	---	--	--	---	--	--

		:			(hh:mm)
--	--	---	--	--	---------

8 Sample Lab no.

--	--	--	--	--	--	--	--

7 Sample received in the right condition :

--	--

If No, list the state in which sample received : |

Unlabelled / Mislabeled / Hemolysed / Turbid  
Insufficient / Leakage / Inappropriate container

Please tick

\_\_\_\_\_

Receiving technician's initials and signature



+

**Work Sheet for ELISA**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Sample Ref. No. \_\_\_\_\_ Referred by \_\_\_\_\_ Lab No. \_\_\_\_\_ Well No. \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

Details of Kit used  
 Name & Lot No. \_\_\_\_\_  
 Expiry Date : \_\_\_\_\_

**Validity of Controls**

**Kit Controls**  
 \_\_\_\_\_  
 OD Value Validity  
 Blank \_\_\_\_\_  
 Negative Control \_\_\_\_\_  
 Cut off control \_\_\_\_\_  
 Positive Control \_\_\_\_\_

**External Controls**  
 \_\_\_\_\_  
 OD Value OD Ratio <ISD> ISD  
 <2SD> 2SD Validity

**VALIDITY OF INSTRUMENTATION (COMMENTS)**

(1) Washer \_\_\_\_\_  
 (2) Fitter \_\_\_\_\_  
 (3) Reader \_\_\_\_\_

VALIDITY OF RUN : \_\_\_\_\_ VALID/INVALID

Comments : \_\_\_\_\_

Signature of officer incharge \_\_\_\_\_ Signature of technologist \_\_\_\_\_

## HIV TEST REPORT FORM

### Name and address of the testing laboratory

1 Patient ID : \_\_\_\_\_ Sex :  M  F

Name (optional) : \_\_\_\_\_ Age :

2 Authorizing clinician name & signature :

\_\_\_\_\_

3 Referring clinic name :

\_\_\_\_\_

4 Date and Time Blood Drawn (dd/mm/yy) :

/   /

:   (hh:mm)

5 Sample lab number :

6 Was a result produced for the sample

Y  N if no state reason :

\_\_\_\_\_

\_\_\_\_\_

7 Date and Time sample received (dd/mm/yy) :

/   /

9 Test Result

HIV-1  HIV-2

8 Date test conducted (dd/mm/yy) :

/   /

10 Kit Details :

Name and type of kit	Principle	HIV-1	HIV-1
i)			
ii)			
iii)			

Interpretation \_\_\_\_\_

Technician initials  
and signature

Lab incharge initials  
and signature

Counselor initials  
and signature

## List of Contributors

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**NACO**

**National AIDS Control Organisation**