सत्यमेव जयते

National Guidelines for HIV Testing

NACO

National AIDS Control Organisaiton
Ministry of Health & Family Welfare, Government of India

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July 2015

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IAS
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Ministry of Health & Family Welfare
National AIDS Control Organisation
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Message

Accurate and prompt laboratory diagnosis of HIV is a critical component of HIV prevention program and is managed by the Laboratory Services Division of NACO. As an integral part of the programme implementation, NACO has coordinated the revision of a HIV testing manual to facilitate high quality diagnosis throughout the laboratory network.

It is very important that from time to time guidelines are revised so that upto date information is available to all the users in the field. And considering that there are more than 18000 centres where the testing is done, a national guidelines to maintain standards and quality is of optimum importance.

It is noteworthy that a comprehensive HIV testing manual inclusive of operating procedures for diagnosis of HIV has come through with the coordinated and concerted efforts of various organizations, individuals and professional bodies, who have put in months of dedicated inputs towards it, and whose expertise is greatly acknowledged.

I congratulate Lab Services Division and all the experts who have taken up to revise this guidelines and this will go a long way in further improving the quality of testing at the laboratories.

(K.B. Agarwal)

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Preface

The Human immunodeficiency Virus (HIV) and AIDS continue to be issue public health concern in spite of containment of HIV epidemic in recent times. National AIDS Control Organization (NACO), Government of India had promptly and adequately responded to this epidemic through creation of HIV laboratory network across the country. As a result, a decentralized approach of the laboratory network starting from one apex laboratory supporting the national and state reference laboratories which in turn provide technical and monitoring support to over 18000 Integrated Counseling and Testing centers throughout the country, has been created.

For a uniform and standardized approach the guidelines have been revised to be followed by all workers in an HIV laboratory.

The vision and constant encouragement of Mr. N.S. Kang, Additional Secretary and Director General, NACO and of Mr. K.B. Agarwal, Joint Secretary, NACO has greatly helped in undertaking this important activity. Sincere appreciation is due to Dr. A. R. Risbud, Scientist G, NARI, Pune who coordinated the whole process along with team of technical experts.

A special thanks to NACO team for their constant effort and hard work in providing direction to structure these guidelines. I would also like to acknowledge the group of national experts who jointly reviewed and revised the technical contents of this manual along with the Laboratory Services Division, NACO.

I sincerely appreciate the Centers for Disease Control and Prevention-Division of Global HIV/AIDS (CDC-DGHA), India and Project Concern International (PCI), India for providing technical assistance and support in the compilation of this document.

(Dr. Naresh Goel)

एड्स का ज्ञान : बचाए जान
TALK AIDS : STOP AIDS

Abbreviations

ADCC	Antibody Dependent Cell Mediated Cytotoxicity
AIDS	Acquired Immuno Deficiency Syndrome
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
CTL	Cytotoxic T- Lymphocyte
DAC	Department of AIDS Control
DBS	Dried Blood Spot
DC	Dendritic Cells
EDTA	Ethylene Diamine Tetra Acetic Acid
EIA	Enzyme Immune Assay
ELISA	Enzyme Linked Immunosorbent Assay
FDC	Follicular Dendritic Cells
GALT	Gut Associated Lymphoid Tissue
GOI	Government of India
HBV	Hepatitis B Virus
HCP	Healthcare Personnel/Provider
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLD	High Level Disinfection
IATA	International Air Transport Association
ICTC	Integrated Counselling and Testing Centre
IEC	Information Education and Communication
LRT	Long Terminal Repeat
LTNP	Long Term Non Progressor
MSDS	Material Safety Data Sheet
NAA	Nucleic Acid Amplification
NACO	National AIDS Control Organization
NASBA	Nucleic Acid Sequence Based Amplification
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NPV	Negative Predictive Value
NRL	National Reference Laboratory
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NSI	Non Syncytium Inducing
OD	Optical Density
OI	Opportunistic Infection
OPA	O' Phthalaldehyde
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PI	Protease Inhibitor
PLHA	People Living with HIV and AIDS

PPE	Personal Protective Equipment
PPTCT	Prevention of Parent to Child Transmission
PPV	Positive Predictive Value
RT	Reverse Transcriptase
RNA	Ribonucleic Acid
SACEP	State AIDS Clinical Expert Panel
SI	Syncytia Inducing
SRL	State Reference Laboratory
STI	Sexually Transmitted Infection
TCR	T Cell Receptor
Th1 & Th2	T Helper Subset 1 & 2
TMB	Tetramethylbenzidine
UN	United Nations
UP	Universal Precautions
WB	Western Blot
WHO	World Health Organization

Table of Contents

Overview of HIV Infection

Introduction

This chapter describes the history and present status of the HIV epidemic in India. It further elaborates on the structure, multiplication, and classification of HIV virus. It also discusses the immunopathogenesis and factors affecting the progression of HIV disease.

On 5 June 1981, the U.S. Centers for Disease Control and Prevention (CDC) [1] reported the identification of a new clinical entity called "Acquired Immunodeficiency Syndrome" among men having sex with men in New York and California. These men presented with rare Opportunistic Infections (OI), Pneumocystis (carinii) jiroveci pneumonia and Kaposi's sarcoma – a rare skin cancer that is usually seen only in immuno-compromised persons.[2] The causative agent of AIDS was identified two years later. In 1986, the International Committee on Taxonomy of Viruses recommended a separate name for the virus isolated from AIDS patients, the Human Immunodeficiency Virus (HIV).

HIV is transmitted from one infected person to another through penetrative sexual acts, both heterosexual and homosexual, through a contaminated blood transfusion or the sharing of needles and syringes, and from mother to child. Due to these restricted routes of transmission, the HIV epidemic was initially concentrated among high-risk groups. These 'high-risk groups,' the population most vulnerable to HIV, are comprised of sex workers (male and female), men having sex with men, Trans Genders, and injecting drug users.

Current Epidemic Status: Global and National

By the end of 2013, an estimated 35 million (33.1 million - 37.2 million) people were infected with HIV globally.[3] The average adult prevalence was 0.8 percent. Of the total number of infections 3.2 million occurred in children (individuals less than 15 years of age). Of the total number, 2.1 million were new infections – illustrating an overall decline in the number of new infections. Over the decade, thirty-three countries reported a decline in the incidence of HIV; two thirds, of the remaining countries, showed a stable incidence rate; and the remaining third showed an increasing incidence rate. Over the past five years, there has been a 19 percent decline in AIDS related deaths[3].

The HIV/AIDS epidemic in India began in 1986-1987 with the detection of the first HIV infection in Chennai and the first AIDS Case in Mumbai. Since then the HIV epidemic has spread to rural and urban areas, infecting high-risk groups as well as the general population. However, nearly 25 years since the epidemic appeared in India, the disease has not reached the proportions predicted by experts across the world. The Indian epidemic is still a concentrated epidemic with

high HIV prevalence remaining in the high-risk group. In 2012, the estimated adult prevalence, in the general population, was 0.27 percent and the total number of people living with HIV/AIDS (PLHA) was estimated to be 20.89 lakh[4]. Children less than 15 years of age accounted for 7 percent (1.45 lakh). India is estimated to have around 1.16 lakh annual new HIV infections among adults and around 14,500 new HIV infections among children in 2011[4].

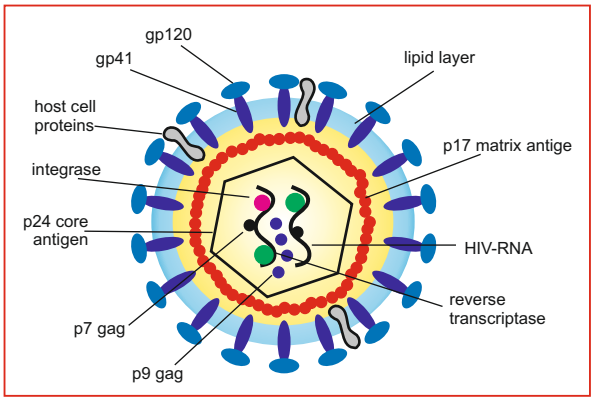
Virus: Classification, Structure and Genome

HIV is a lentivirus that infects and destroys cells in the immune system. Lentiviruses are in turn part of a larger group of viruses known as retroviruses. The name 'lentivirus' means 'slow virus.' They are thus named because they take a long time, often many years, to produce adverse effects in the body. There are two HIV types, HIV-1 and HIV-2. HIV-1 is the most prevalent type throughout the world. HIV-2 has limited geographic distribution. HIV-1 is closely related to Simian Immunodeficiency Virus (SIV), prevalent in populations of wild chimpanzees in West-Central Africa.

HIV is an enveloped virus (Figure 1.1). The virus envelope is composed of two phospholipid layers derived from the host cell membrane. The envelope also contains the trimers of an envelope coated protein, glycoprotein (gp) 160. Gp160 is composed of two subunits, gp120 and gp41. While gp120 has external protein and contains sites that bind CD4 cells and co-receptors on the surface of human CD4 T cells, gp41 is membrane bound protein. Inside the viral envelope there is a layer called the matrix, which is made from the protein p17.

The viral core (or capsid) is usually bullet-shaped and is made up of protein p24. Inside the core are three enzymes required for HIV replication: Reverse Transcriptase (RT), integrase and protease. Also held within the core is HIV genetic material which consists of two positive strands of single stranded Ribonucleic Acid (RNA).

Figure 1.1. The structure of HIV

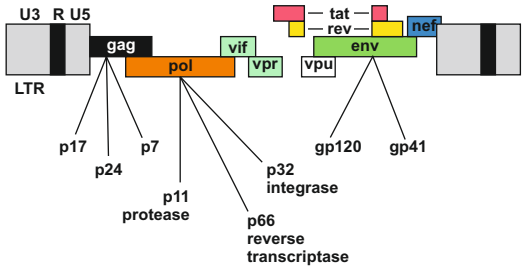


- HIV genome is approximately 9.1Kb in size and carries nine genes and long terminal repeat (LTR) regions at either end of the genome. There are three structural genes that code structural proteins of virions. These genes are known as Envelope (env) coding for envelop of HIV; groups specific antigens (gag) coding for capid and matrix; and DNA polymerase (pol) coding for integrase, protease and reverse transcriptase enzymes.
- Regulatory genes essential for virus replication are trans activator of transcription (tat),

regulator of expression of virion proteins (rev) and negative factor (nef).

- Accessory genes that are important in virus replication, regulation and a variety of host-cell manoeuvres are viral infectivity factor (vif), viral protein R (vpr), and viral protein unique (vpu) or vpx in the case of HIV-2.
- The ends of each strand of HIV's RNA contain LTR, an RNA sequence. Regions in the LTR act as switches to control the production of new viruses and can be triggered by proteins from either HIV or the host cell.
- For diagnostic testing, the detection of antibodies and viral proteins (Env, Gag and Pol) are often used. During the window period of detection, the p24 (Gag) protein is used as a diagnostic for HIV infection.

Figure 1.2. HIV genome

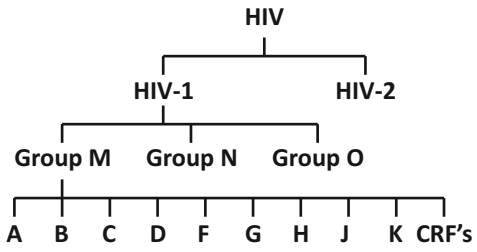


(Source: HIV Medicine 2007)

HIV Types, Subtypes and Genetic Diversity

HIV has undergone numerous genetic divergence leading to a number of subtypes as a result of a high mutation rate, high replication rate, lack of proof reading ability of reverse transcriptase enzymes and a propensity for recombination. Based on the phylogenetic analysis of numerous isolates from different geographical regions HIV is divided into types, groups, subtypes and sub-subtypes (Figure 1.3). There are two types of HIV; HIV-1 and HIV-2. HIV-1 is further divided into three main groups; major (M), outliers (O) and new groups (N). An additional fourth group, 'P,' has been documented in a Cameroonian woman living in Paris. Group M is most common type of HIV in the world, where as groups N and O are mainly confined to West and Central Africa and form very small minorities.

Figure 1.3. Classification of HIV



Group M is further classified into subtypes A-K based on phylogenetic relatedness. Occasionally, two viruses from different subtypes infect the same cell in an infected person and result in recombinant virus strains that may transmit within a population and establish as "circulating recombinant forms" (CRFs)[5]. Secondary recombinations of CRFs lead to the appearance of unique recombinant forms (URFs). The subtypes E and I have been reclassified as CRF01_AE and CRF04_cpx. Subtypes A and F are further classified into sub sub-types A1, A2 and F1, F2 respectively.

HIV-1 subtypes are distributed geographically based on the introduction of the virus and genetic divergence. HIV-1 subtype C is the most predominant subtype that is present in India, South Africa and China. Subtype B is predominantly seen in North America and Europe. Subtype A and other subtypes are distributed throughout different nations in Africa. Thailand has an epidemic

of CRF01_AE that was earlier classified as subtype E.
HIV-2 is endemic in West Africa and has spread in the last decade to India and Europe. HIV-2 is much less diverse but subtypes A-H have been proposed[6].

Life Cycle & Cell Tropism

HIV-1 utilise CD4 proteins on the surface of T lymphocytes as the receptor for the HIV virus. The virus may also infect other cell types with CD4 expressed on the surface (e.g. dendritic cells, macrophages, microglial). HIV-1 also requires second or co-receptors for entry into host cells. The surface membrane glycoprotein of HIV (gp120) binds to the CD4 receptor and interacts with two key players in HIV infection, co-receptor X4 (also known as CXCR4) and the C-C chemokine receptor R5 (CCR5). These receptors are embedded in target host cell membranes. The virus uses the receptors for chemokines (CCR5 or CXCR4) as second receptors. The viruses may use other chemokine receptors too. However, depending on the co-receptor used by the virus, it may be phenotyped as a R5 - CCR5 tropic virus or X4- CXCR4 tropic virus. HIV target cells displaying specific receptors and co-receptors.

The virus' lifecycle has a number of stages (Figure 1.4).

- ***Binding and Fusion:*** The first step in fusion involves the high-affinity attachment of viral gp120 to a CD4 molecule. This leads to conformational changes that expose the co-receptor (CCR5/CXCR4) binding sites present on the surface of a CD4 T lymphocyte. This leads to the fusion of the virus envelope with host cell membranes and releases the viral genetic material (RNA) in the protoplasm of the host cell.
- ***Reverse Transcription:*** In the protoplasm, the viral reverse transcriptase enzyme transcribes the single-stranded HIV RNA into a double-stranded c-DNA that subsequently moves to the nucleus.
- ***Integration:*** The HIV enzyme "integrase" integrates the HIV DNA with the host cell's DNA. The integrated HIV DNA is called provirus. The provirus may remain inactive for several years, producing few or no new copies of HIV.
- ***Transcription:*** When the host cell is activated, the provirus integrates with the host genome and transcribes with the host's RNA polymerase to create copies of HIV RNA, as well as HIV mRNAs. These are then exported from the nucleus to protoplasm.
- ***Protein Synthesis and Assembly:*** mRNAs code for viral polyproteins in the protoplasm. An HIV enzyme "protease" cuts the long chains of HIV proteins into smaller individual proteins. As the smaller HIV proteins come together with copies of HIV's RNA genetic material, a new virus particle is assembled.
- ***Budding:*** The newly assembled virus pushes out "buds" from the host cell. During budding the HIV envelope also acquires host membrane proteins and a lipid bi-layer.

Antiretroviral drugs target many steps in HIV's life cycle, e.g. reverse transcriptase activity, integrase activity, protease activity, inhibition of binding to CD4 receptors and inhibition of

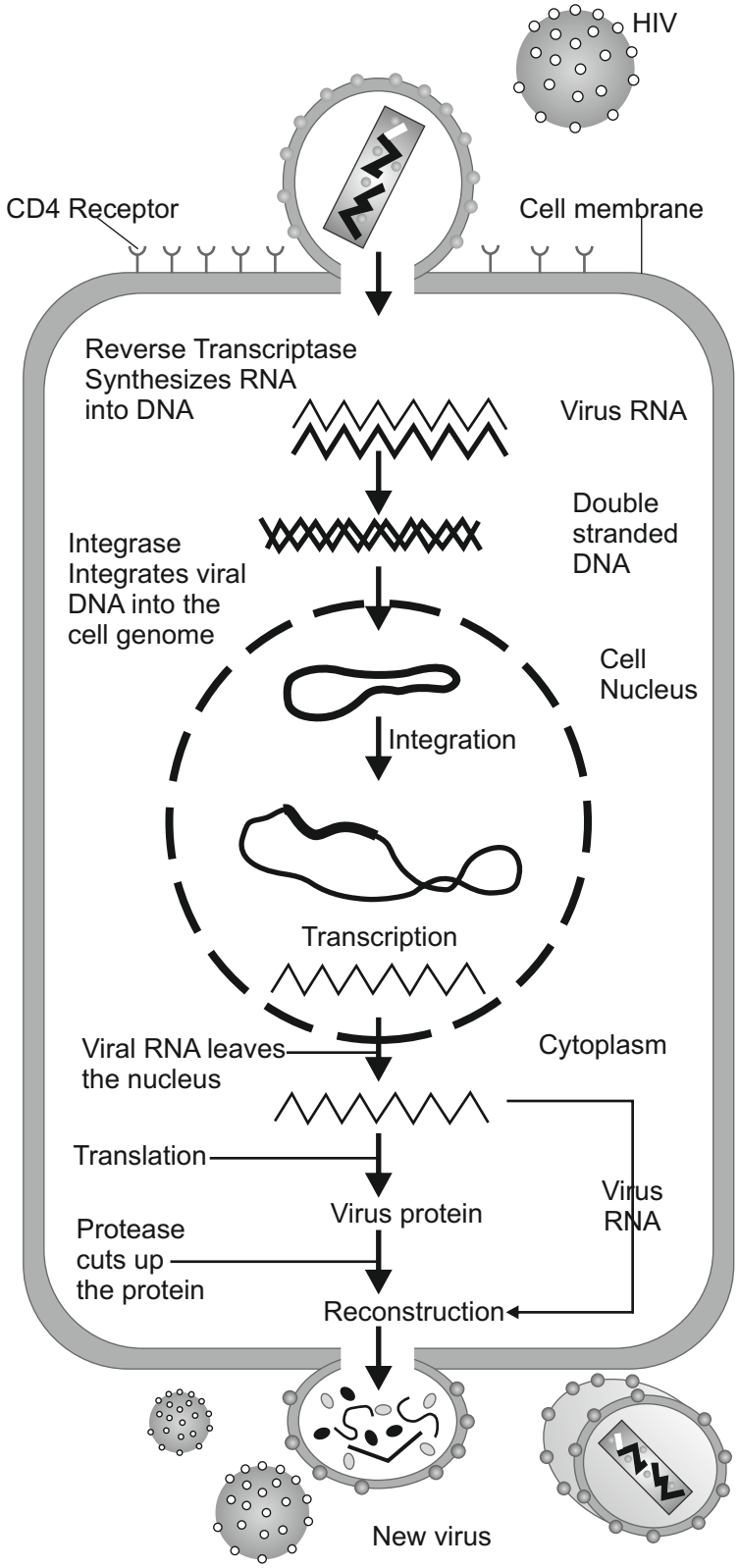


Figure 1.4 Lifecycle of HIV

Immunopathogenesis of HIV Infection

Pathogenesis is the process of development of a disease more specifically, cellular reactions and pathologic mechanisms occurring in development of disease. The immunopathogenesis of HIV infection can be understood by identifying and characterizing the virus, which has a high daily turnover of virions when actively multiplying. The host organism contributes to the course of HIV infection through host defence mechanisms and genetic factors.

Cells Involved in Immunopathogenesis

The virus resides inside the infected immune cell and causes disease by subverting the host's immune system. Two major cells, which play a key role in the immune mechanism, are T and B-lymphocytes. Subsets of T lymphocytes known as CD4 T (helper) cells and CD8 T (cytotoxic) cells are the principal effector cells in HIV infection. They express different T cell receptors (TCR) on their surface, which enables them to bind to antigen presenting cells. The main antigen presenting cells are the dendritic cells (DC), follicular dendritic cells (FDC), macrophages, and B cells. Besides these, HIV-1 can infect monocytes, microglia, oligodendrocytes, and astrocytes of the brain and retinal cells and several other cells in the body.

Viral Entry and Cellular Immune Response

Transmission occurs through sexual intercourse (vaginal, anal, or oral), parental route (IV drug use, unsafe blood transfusion, or organ transplantation), and vertical transmission from mother to child (during pregnancy, child birth, or by breast feeding). HIV infection is facilitated by the presence of ulcerative and non-ulcerative Sexually Transmitted Infections (STI). The virus can be found in blood and body fluids like semen, vaginal secretions, breast milk, cerebrospinal fluid, amniotic fluid and synovial fluid containing blood.

HIV enters the body as either a cell-free or cell-associated virus, through one of the several routes mentioned above. The virus targets cells displaying specific receptors and co-receptors. The surface membrane glycoprotein of HIV (gp120) binds to the CD4 receptors and co-receptor X4 (CXCR4) and R5 (CCR5). HIV has the ability to induce syncytium formation. Strains that do so are known as syncytium inducing (SI) strains and those that do not are non-syncytium (NSI) strains. HIV-infected macrophages and dendritic cells can form multinucleated syncytia with uninfected T-cells, thus transmitting the virus. The functional ability of monocytes and macrophages, including tissue macrophages, are thus compromised. Fusion of viral membrane with host cell membrane is mediated by the viral transmembrane glycoprotein gp41.

Following membrane fusion, the viral core uncoats in the cytoplasm. Two identical strands of viral RNA and two molecules of the enzyme reverse transcriptase (RT) enter the cell. The viral RNA is converted into proviral DNA by reverse transcriptase, through the many steps shown in Figure 1.4. This results in a double stranded HIV DNA with long terminal repeats (LTR) at each end.

Viral replication starts, at both the site of entry and at the draining lymph nodes, within 72 hours of entry into the cells.

Lymphocytes are activated on account of the infection. HIV replicates better in these activated cells. The spread of the virus throughout the lymphoid tissue and an increase in the number of virus-expressing cells precedes an increase in plasma viraemia and genital shedding of the virus. During the first two to three weeks of infection, virus load increases exponentially with a doubling time of 0.3 days.

Active immune response to viral antigens occurs at the same time as intense replication of the virus in activated T lymphocytes. Secondary to viral infection, and to co-receptor-dependent cytotoxicity leading to apoptosis and immune-mediated killing, a drastic depletion of infected CD4 and effector memory T cells occurs between days 10 and 21. This is pronounced in the gut associated lymphoid tissue (GALT).

HIV-2 is less easily transmitted (i.e. less infectious), and has a longer incubation period between infection and manifestation of illness. Immunosuppression in HIV-2 infected persons is significantly slower than in HIV-1 infected persons. The progression of HIV-2 is slower and infection leads to a significantly lower plasma viral load.

Humoral Immune Responses

In active infection, both effective cell-mediated immune response – by HIV specific cytotoxic T lymphocytes (CTL), and humoral response – carried out by complement fixing and neutralizing HIV specific antibodies, come into play. HIV antibodies first become detectable by IgM ELISA typically 3 weeks after infection. Early low-titer antibodies exert minimal selective pressure on the circulating virus populations. They also fail to slow the increase in viraemia.

The period from the time the virus enters the host until detectable levels of HIV specific antibodies appear is called the **'window period' or 'acute infection phase.'** During this period, an individual is infected and is also infectious to other individuals via the previously mentioned routes of transmission. Antibody levels are not detectable during this phase of the infection, rendering the person sero-negative, i.e., tests for detecting HIV antibodies are negative. The timeframe of this period ranges, on average from 3 weeks to 3 months.

Disease Progression and Mechanisms of Immune Cell Destruction

HIV infection progresses through three stages: acute infection phase, chronic asymptomatic phase, and AIDS.

Acute Infection Phase / Primary Infection or Window Period: Immediately after the infection, there is wide virus dissemination and seeding of lymphoid organs (e.g., GALT), culminating in

the destruction of CD4 cells.

As viraemia reaches its peak, the resting CD4 T-cell populations are depleted (in the second to third week of infection) and the immune system transitions to a state of hyper-activation. This is followed by a decrease in viraemia. The decrease in virus levels occurring at this time may be due to CCR5 CD4 target cell exhaustion. It may also be due to the first appearance of specific anti-HIV cytotoxic CD8 T lymphocytes, which accumulate in significant numbers at mucosal sites after the viral titers peak. Subsequently, virus levels drop significantly (in both blood and genital secretions) and attain their lowest levels by the 10th week post infection. There may be an associated clinical disease that is largely self-limiting. This period usually lasts for three to six weeks and terminates with the appearance of an adaptive immune response to HIV.

Chronic Asymptomatic Phase: Although the immune response succeeds in down regulating the viraemia, HIV is never completely eliminated and the progression to the chronic phase of the HIV disease occurs.

During this phase, HIV-specific antibody response increases further, exerting a selective pressure that results in the continuous evolution of the virus that may increase the genetic diversity and result in the development of mutant viruses.

Following HIV-1 infection, the progression of the disease to AIDS depends on several variables. These variables include factors like the host's susceptibility to the virus, genetic makeup, immune function and the presence of co-infection(s)/OI.

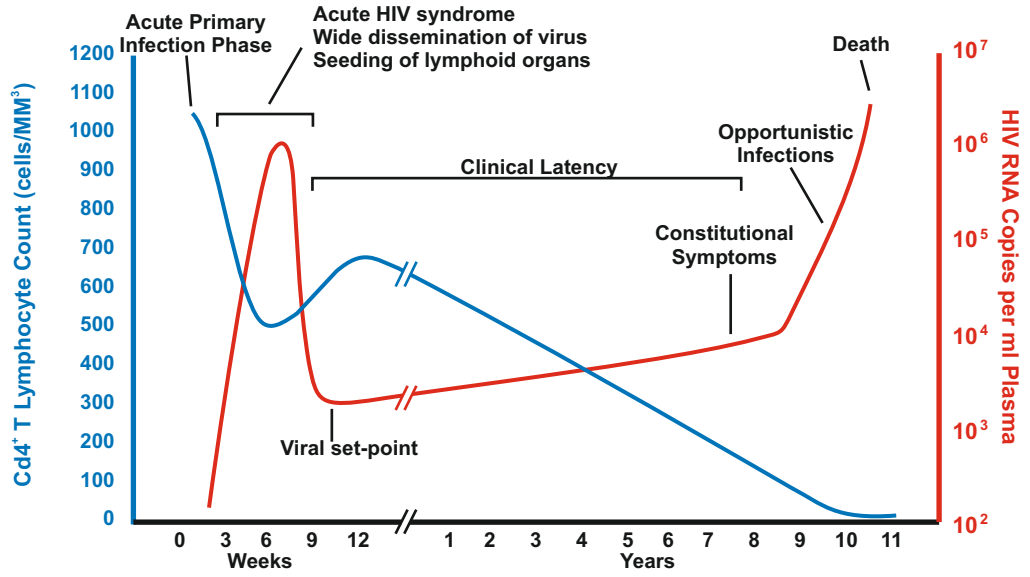
The host immune response is able to control viral infection during the early chronic phase.

Between six to twelve months after infection, the host's immune response establishes the plasma virus load set point. The plasma virus load set point is an important determinant in the progression of the HIV disease. The virus becomes largely sequestered in lymphoid tissue and continues to replicate there during the years of clinical latency. HIV brings about the destruction of HIV infected as well as uninfected bystander CD4 T cells through multiple mechanisms. Immune activation is the basic cause of CD4 cell destruction and hence, plays a major role in the progression of the disease. As the lymphoid architecture becomes disrupted and the host immune defences become exhausted, the plasma virus burden increases and the disease progresses towards AIDS. The chronic infection phase may last for seven to 10 years.

Acquired Immune Deficiency Syndrome: The advanced stage of HIV infection is characterized by an increase in the plasma viral load. Dysfunction in the immune system results from the reduction in both the quantitative and qualitative function of the T lymphocytes and other immune system cells, e.g., DCs, natural killer (NK) cells, and macrophages. As a result, there is

profound immune suppression, frequent opportunistic infections, and malignancies. The CD4 count is usually less than 200 cells/cmm and declines progressively. Clinical AIDS is defined as a CD4 cell count of less than 200/cmm and/or the appearance of AIDS defining illnesses. This stage may last for one to three years and results in death without the intervention of ART.

Figure 1.5. Graph showing HIV copies and CD4 counts in a human over the course of a treatment-naive HIV infection



Patterns of Disease Progression

The disease progression may vary from patient to patient depending on various factors. Certain patient groups have been identified based on the pattern of disease progression, described below.

Typical Progressors: The typical course of HIV infection includes three stages: primary infection (Acute sero-conversion), clinical latency, and AIDS (Figure 1.5). Eighty to ninety percent of HIV infected individuals are "typical progressors," with a median survival time of approximately 10 years.

In 50-70 percent of HIV infected individuals, the primary phase may be totally unapparent or may be associated with acute flu-like or mononucleosis like syndromes. These symptoms occur within 3-6 weeks of infection and may last for 9-12 weeks. During this phase there is a high level of the virus in the blood. The course of HIV immunopathogenesis is described in Figure 1.5. On an average, the progression of the HIV infection to AIDS takes approximately 8-10 years.

Rapid Progressors: Rapid progressors are individuals who develop AIDS symptoms or end stage HIV disease within 2-3 years after primary HIV-1 infection. About 5-10% of PLHA fit this profile. Some individuals are known to progress within a year of primary infection.

Long-term Non-progressors (LTNP): These are rare cases involving people infected with HIV, whose infection do not progress to AIDS in the absence of ART. They show <5000 HIV RNA copies

/ml. About 5 percent of PLHA fall into this category and do not experience disease progression for an extended period of time. A high percentage of LTNPs have been shown to have inherited mutations of the CCR5 receptor of T cell lymphocytes. In LTNP the immune responses seem to keep the virus in check.

Elite Controllers: Also known as natural controllers, are a subset of LTNPs. Their immune system, despite being infected with HIV, has been able to successfully suppress the virus to an undetectable level (HIV RNA below 50 copies/ml) for many years in the absence of ART [7].

Factors Affecting Disease Progression: Host, Viral and Environmental

The progression of the HIV infection from the asymptomatic stage to AIDS depends upon viral factors (e.g., viral fitness, co-receptor usage, generation of escape mutants, latency) as well as host factors. Host factors that influence the course of disease progression include genetic factors, such as HLA polymorphism & chemokine receptor genes polymorphism. HLA types A24, B35, B8 and C4 are known to be associated with rapid disease progression while B27 and B57 are known to be associated with slow disease progression [8-10]. Mutations in the co-receptor genes CCR5, CCR2, SDF and CX3CR1 are also known to influence the progression of HIV [11]. Environmental factors can also influence the progression of the disease. These include nutrition and co-infections. Vitamin A deficiency is linked with HIV susceptibility [12]. Tuberculosis [13] and Hepatitis B (HBV) [14] accelerate the disease progression.

Clinical Features and Antiretroviral Treatment

HIV infection causes the destruction of CD4 cells leading to immune suppression. Depending on the extent of immune suppression (as measured by CD4 cell counts, plasma virus loads, and the incidence of opportunistic infection) the patients are usually classified as per criteria set by the CDC, the World Health Organisation (WHO), etc.

Opportunistic infections (OIs) lead to significant morbidity and mortality and grossly affect the health and quality of life for PLHA. There are variations in the profile of OIs, depending on the prevalence of infections seen in different parts of the country, Tuberculosis is the most common OI reported in India. Other commonly reported OIs are candidiasis, cryptosporidiosis, toxoplasmosis, and pneumocystis jirovecii pneumonia. Early diagnosis and appropriate treatment of OIs can slow down disease progression.

Antiretroviral Treatment

Highly active anti-retroviral treatment (HAART) leads to complete suppression of plasma viraemia, brings down the frequency of OI and improves the quality of life for PLHA. Strict adherence to treatment can delay the development of drug resistance and the need for second line treatment.

There are three major classes of antiretroviral drugs available in the NACP:

1. Nucleoside/Nucleotide reverse transcriptase inhibitor (NRTI): acts as a DNA chain terminator
2. Non-nucleoside reverse transcriptase inhibitor (NNRTI): inhibits the HIV reverse transcriptase enzyme by binding to it
3. Protease inhibitors (PI): binds to the active site of the protease and prevents maturation.

Newer classes of anti-HIV drugs include fusion inhibitors that prevent the binding of the virus to susceptible cells and integrase inhibitors that stop the integration of virus DNA with host DNA.

The National AIDS Control Organization (NACO) initiated free anti-retroviral treatment in April 2004. It has established 425 ART centres all over India. As on March 2014, 7,26,799 adults and 42,015 children are initiated on ART.

Diagnosis of HIV Infection

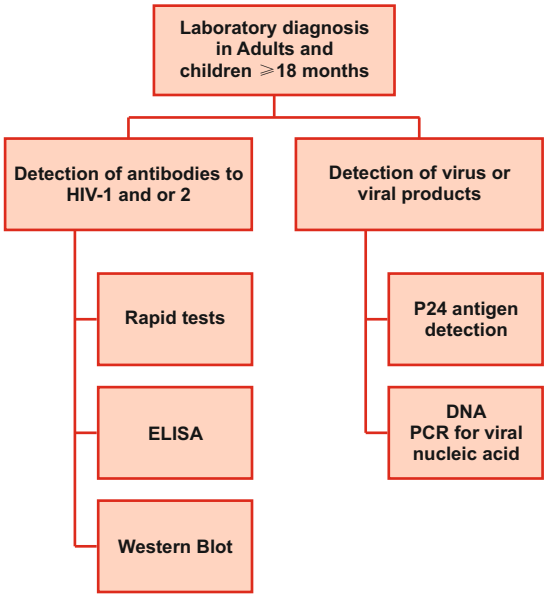
Like other infectious diseases, HIV diagnosis is made by either demonstrating the presence of virus or viral products in the host, alternatively by detecting host response to the virus. An HIV diagnosis is commonly made through serological assays to detect HIV specific antibodies or by Nucleic Acid Amplification Test (NAAT) to detect HIV nucleic acids.

Serological Tests: Enzyme linked immunosorbent assays (ELISAs), rapid tests and western blots (WBs) are the common tests for detecting HIV antibodies. To accurately diagnose an HIV infection, these tests are used in a specific sequence or algorithm. Additionally, Chemiluminescence Immunoassays (CIA), Immuno Floresent Assays and Line Immunoassays are also available for specific HIV antibody detection. Commercial assays are also available for P24 antigen detection.

NAAT: These are sensitive tests for diagnosis of HIV infections. They use polymerase chain reactions (PCRs) for the detecting various HIV structural genes (usually gag, pol and env). PCRs are the test of choice in certain situations, such as early infant diagnosis and during window period. Branch DNA (bDNA) assays based on signal amplifications are also used.

Diagnosis in a child less than 18 months cannot be done using antibody based assays as maternal antibodies may be present in the infant's circulation. Therefore, up to the age of 18 months, the diagnosis of HIV infection can only be reliably made by DNA PCR. [14]

Figure 1.6. Tests used for HIV diagnosis in individuals above 18 months of age



The window period is another situation where the HIV diagnosis can be made by detecting p24 antigens or by PCR. However, PCR is the test of choice since the p24 antigen detection test is relatively less sensitive.

Monitoring of Antiretroviral Therapy (ART)

HIV disease progresses in an individual at different rates which necessitates accurately staging of infection in an individual, predicting its course and monitoring its progression. The laboratory tests are used for monitoring progressing of HIV infection and ART.

- Immunologic tests
  - CD4 T cell enumeration
- Other Virological tests
  - HIV RNA load assays
  - Others - Measurement of HIV p24, Reverse Transcriptase (RT) activity assay.

Prevention and Control

Despite an array of ARTs it has not been possible to cure HIV. Hence prevention of HIV infection must continue to remain a priority for the national programme.

Transmission of HIV infection is primarily the result of risk behaviour. Hence, the National AIDS Control Programme places an emphasis on risk reduction – through Information, Education, and Communication (IEC) – within the general population and through Targeted Intervention (TI) programmes among high-risk groups. Important components of TI programmes include the promotion of condom use, peer educators/outreach workers working with IEC, and treatment of STIs.

In clinical trails among high-risk groups – pre-exposure prophylaxis, male circumcision, and vaginal microbiocides have shown promise in reducing HIV transmission . However, these are still not part of NACP. Scientists continue to search for possible HIV vaccines. Although, a large-scale trial in Thailand showed promise, the vaccine for HIV prevention will not be available in the near future.

Hence intensified detection of HIV infection, ART treatment for PLHA, and prevention through risk reduction programmes will continue to play a pivotal role in HIV prevention and control.

Collection, Storage, and Transport of Specimens for HIV Testing

Introduction

Currently, the diagnosis and monitoring of HIV infection is performed on blood specimens. Generally for serological tests (antigen and antibody detection) serum/plasma/whole blood is used, whereas for CD4 enumeration tests only whole blood collected in K2/K3 ethylene diamine tetra acetic acid (EDTA) evacuated tubes is used. For DNA/RNA PCR, Dried Blood Spots (DBS) or whole blood collected in K2/K3 EDTA is used. Hence, the procedure for collection, transport, and storage of blood specimens for the above tests is described here.

Blood Collection, Storage, and Transport for HIV Antibody Test

Blood Collection

Ensure pre-test counselling is done and informed consent has been obtained.

Identify the person using at least 2 identifiers. (e.g., name, identification number (ID),age, gender). [15]

Requirements for venous blood collection:

- Sterile disposable needle and syringe with plain test tube or bulb / plain evacuated tube (red top) with holder and needle
- Tourniquet
- Dry cotton swab
- Spirit swab
- Absorbent material (e.g. blotting paper)
- Facility for appropriate waste disposal

Assemble the supplies. Choose sterile blood collection device and needle based on the individual's age and size of the available vein. Evacuated tubes are preferred for the safety of the healthcare worker. For adults, evacuated tube and 21-gauge eclipse needle is commonly used. For children or adults with small, fragile veins, a butterfly needle (Sizes available: 23, 21, 19 gauge) and a 3-5 ml syringe is used. Selection is based on vein size.

- Label the tube for blood collection with at least two patient identifiers.
- Wash hands or disinfect with an alcohol based hand sanitizer.
- Put on gloves to comply with standard precautions.
- Place the individual's in a supine or sitting position with arm supported under good light.
- For the avoidance of soiling, place absorbent material below the forearm before commencing venepuncture.

- Explain the procedure briefly to the person and inform that a sterile blood collection device is being used.
- Assess the individual's veins to determine the best puncture site for venepuncture. Ensure a vein has been selected. Observe the skin for the vein's blue colour and palpate the vein for a firm rebound sensation with no pulsation.
- Apply the tourniquet to the individual's arm 3 to 4 inches above the venepuncture site and ask the individual to clench their fist. Avoid applying the tourniquet too tight or leaving it in place longer than one minute to eliminate hemo-concentration.
- Clean the venepuncture site with 70 percent alcohol or povidone-iodine using a circular motion, spiralling outward from the site. Allow the site to air dry before performing the venepuncture. Do not palpate the site after cleansing. If touching the site is necessary after cleansing, clean the site again before performing the venepuncture.
- Immobilize the vein by pressing 1 inch to 2 inches below the venepuncture site, drawing the skin taut. Position the needle bevel up, the shaft of the needle parallel to the path of the vein and at an angle of 30-45 degrees from the surface of the site (if using a holder and needle), or at an angle of 5-30 degrees, depending on vein depth, if using a butterfly. Insert the needle into the vein and withdraw blood slowly.

a. Syringes

Gently pull the plunger of the syringe to create a steady suction. Do not collapse the vein. Do not withdraw forcibly; this creates excessive pressure, foaming of blood occurs and the specimen is likely to be haemolysed. Collect 2-5 ml of blood. Do not recap the needle. Burn/cut the used needle in a needle destroyer. Immediately transfer the blood into a collection tube gently along the tube wall without squirting. Remove the tourniquet, put a dry cotton ball over the puncture site, gently remove the needle and simultaneously apply pressure. Instruct the patient to keep his/her arm straight and to continue pressure until bleeding stops. A bandage may be applied to the puncture site if required.

b. Evacuated tube, tube holder, and needle:

Grasp the holder securely and push down the collection (evacuated) tube until the needle punctures the rubber stopper. Blood will flow into the tube automatically.

Remove the tourniquet, put a dry cotton ball over the puncture site, gently remove the needle, and simultaneously apply pressure. Instruct the patient to keep his/her arm straight and to continue pressure until bleeding stops. A bandage may be applied to the puncture site if required. **Do not recap the needle**. Burn/cut the used needle in a needle destroyer.

Dispose-off the cut needle and/or syringe into a puncture proof sharps container. Discard cotton balls and spirit swabs into an infectious waste container. Discard wrapper/cover/cap of the

needle into a non-infectious waste container. Discard gloves into an infectious waste container and wash hands. Document and inform any adverse effects such as hematomas.

Separation of Sera

The blood collected is allowed to clot for 30 minutes. The serum should be separated as soon as possible and should be refrigerated. The test tube/red top evacuated tube, containing collected blood, should be centrifuged at 2000 to 3000 revolutions per minute (rpm) for ten minutes to separate the serum. The serum should then be aliquoted in pre-labelled screw-capped, sterile, O-ring storage vials using micropipette tips for testing/storage. No preservatives should be added as they interfere with the testing.

Storage of sera

The sera can be stored at 2 to 8^{o} C in the refrigerator for only up to a week. For longer storage, specimens need to be kept frozen at -20^{o}C. Repeated freeze-thawing should be avoided.

Specimen Transport

Shipment of infectious agents is permitted as per the International Air Transport Association's (IATA) 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 Figure 2 for a diagrammatic representation):

- The specimen tube, in which serum is to be transported, should not have cracks/leaks. Preferably, it should be made of plastic and be screw capped. The outside of the container should be checked for any visible contamination with blood that 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). Pack this container inside a cardboard canister/box containing sufficient material (cotton gauze) to absorb the blood in case the tube breaks or leaks.
- Cap the canister/box tightly. Fasten the request slip securely to the outside of this canister. This request slip should have all of the patient's 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 zip lock bag to prevent smudging on account of spillage. For mailing, this canister/box should be placed inside another box containing the mailing label and a biohazard sign.

Figure 2. UN class 6.2 specifications for the shipment of HIV testing specimens

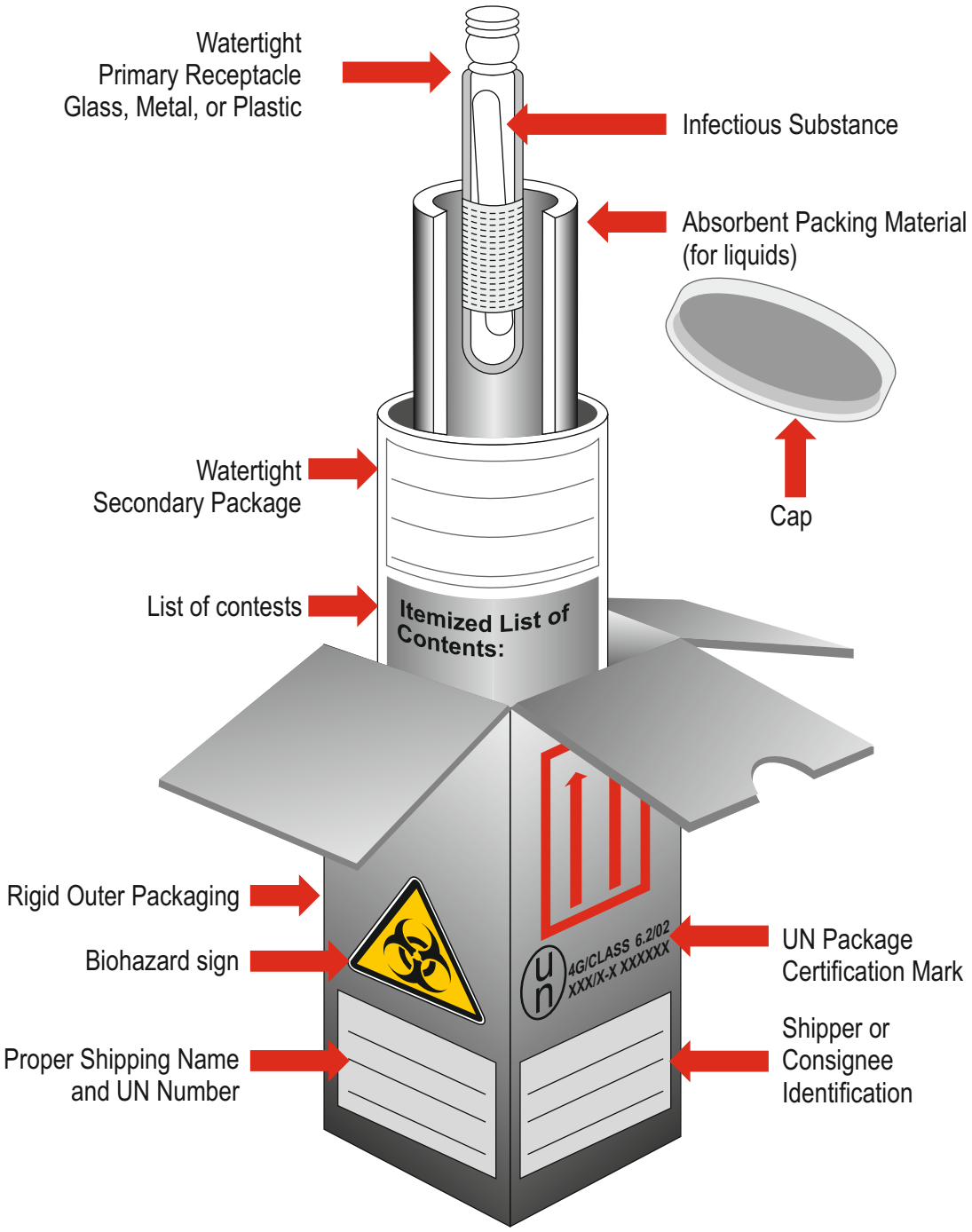


Figure 2 depicts the method of sample transport for a single or few (2-3) sample(s) that could fit into the secondary container shown in the diagram. The size of the primary sample container will vary with the number of samples being transported. For a larger number of samples, a tube rack (or some such container) may be used, wherein the samples can be transported in the upright position and at appropriate temperatures. 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 temperatures.
- 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 the 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 (use whichever pack necessary to maintain the sample at the appropriate 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. The cool box required for transportation could be a plastic breadbox 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 the purpose of shipping it 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 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.
- Unless prior arrangements have been made with the receiving laboratory, no transport should be done during weekends, holidays, or non-testing days for the testing laboratory.

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

Blood Collection, Storage and Transport for CD4 Enumeration

For CD4 enumeration, 3 millilitres of blood is collected in a K2/K3 EDTA evacuated tube. Once collected, the blood specimen needs to be processed immediately and definitely within a maximum of 48 hours. During this time the blood specimen needs to be kept at ambient temperature and not in the refrigerator.

It is important to note that blood should be collected from the same person at similar times of the day to avoid variations in results due to diurnal fluctuations.

Further Reading: *National Guidelines for the Enumeration of CD4,* NACO 2015.

Blood Collection, Storage and Transport for HIV-1 DNA PCR

Individuals who are appropriately trained in dried blood spots (DBS) collection technique and in standard work precautions should collect DBS specimen.

Further Reading: *Laboratory Guidelines for HIV Diagnosis in Infants and Children < 18 months,* NACO 2010.

Blood Collection, Storage and Transport for HIV-1 Viral Load

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. Although total quantification includes cell-free virus, virus in infected cells in all compartments of the body, as well as integrated provirus, the easiest measurement of a viral load is of the cell-free virus in an individual's plasma.

For HIV-1 Viral Load estimation, a whole blood specimen is collected in K2/K3 EDTA. The plasma is separated within six hours of collection and stored at -20^{o}C till further use.

Further Reading: *National Guidelines on Second-line and Alternative First-line ART for Adults and Adolescents,* NACO May 2013

Serological Diagnosis of HIV Infection

Introduction

Laboratory diagnosis by HIV testing is the only method of determining the HIV status of an infected individual's infected blood, blood products, organs, and tissues. HIV diagnosis at ICTCs and other laboratories is based on the demonstration of antibodies. Antibody detection can be done using an ELISA test, rapid test, and western blot test. These tests are used as screening tests and/or confirmatory tests. All tests should be performed and interpreted as per test instruction manuals that are supplied with the kit. HIV testing should be based on testing strategy and algorithm.

A number of moral, legal, ethical, and psychological issues are related to a positive HIV status; hence, any laboratory attempting to assess the HIV status of an individual should be conversant with these issues. Testing laboratories should ensure pre and post-test counselling for every individual and confidentiality to be maintained.

Objectives of Testing

- Transfusion and transplant safety
- Diagnosis of HIV infection in symptomatic and asymptomatic individuals
- Prevention of parent to child transmission
- For Post-Exposure Prophylaxis (PEP)
- Epidemiological surveillance using unlinked anonymous HIV testing
- Research

Pre-test Counselling

HIV testing when undertaken for assessing the status of an individual, should always be done after the pre-test counselling and after an informed consent by client. Testing without informed and explicit consent has proven to be counterproductive and has driven HIV positive individuals underground. Pre-test counselling along with post-test counselling prepares the individual to cope with the HIV test results. It is the responsibility of all blood collection centres to ensure that pre-test counselling is done before collection.

Confidentiality

The confidentiality of HIV test results should be maintained for both positive and negative reports. This is essential for ensuring respect for the privacy and rights of an individual and to protect them from victimization, discrimination, and stigmatization. The results should be handed over directly to the person concerned, to a person authorized by the patient, or in a sealed envelope to the clinician requesting for the test. No results, under any circumstances, should be communicated via telephone, fax, email, etc. The records must be kept secure.

Detection of Anti-HIV Antibodies

The central component in the diagnosis of HIV infection is the detection of anti-HIV antibodies in serum, plasma, or whole blood. Urine and saliva may be tested using specific kits. HIV antibody assays are commercially available in various formats.

Some of these assays can differentiate between HIV-1 and HIV-2 infections. However, the occurrence of antibody-cross reactivity makes differentiation difficult between HIV-1 and HIV-2. Differentiation between HIV-1 and HIV-2 is required since the treatment varies for the two types.

Technical errors and interference from other medical conditions may compromise the accuracy of HIV tests. Antigens used in HIV diagnostic tests must be appropriately specific and are usually purified antigens from viral lysates or antigens produced through recombinant, or synthetic, peptide technology. Such antigens helps to improve the sensitivity (true positives) and specificity (true negatives) of HIV assays (Ref. Chapter 6 for details on sensitivity and specificity). Along with the testing process, there is the requirement for a dedicated quality system in the laboratory to ensure accuracy and reproducibility of test result.

Screening Tests

Serological tests for the detection of HIV are classified as first to fourth generation tests based on the type of antigens used and principle of the assays (Table 3.1). NACO recommends the use of rapid test kits, which detect >99.5% of all HIV-infected individuals and have false-positive results in <2% of all those who are tested.

Table 3.1. Generation of anti-HIV antibody tests

Generation	Antigens/Antibodies	Comment/Characteristic
First	Antigens from HIV lysates	Lack of sensitivity and specificity
Second	Recombinant proteins and/or synthetic peptides	Improved sensitivity
Third	Recombinant proteins and/or synthetic peptides in an antigen sandwich configuration	Very high sensitivity and able to detect IgM antibody in addition to IgG antibody; reduces the window period considerably. Detects HIV-1 and HIV-2 simultaneously.
Fourth	Detection of both HIV antigen (p24) and both antibodies, IgG and IgM	Further reducing the window period

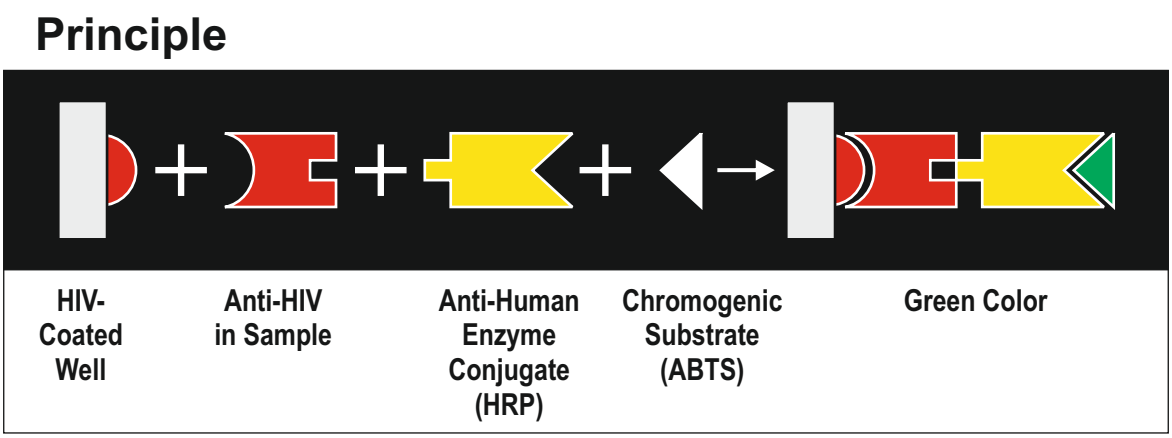
Commonly used screening tests are:

- Enzyme Linked Immunosorbent Assay (ELISA)
- Rapid tests
  - Immunoconcentration/Dot Blot assay (vertical flow)
  - Agglutination assay
  - Immunochromatographic assay (lateral flow)
  - Dipstick and comb assay based on Enzyme Immune Assay (EIA)

Enzyme Linked Immunosorbent Assay (ELISA)

All ELISAs consist of either HIV antigens or antibodies (depending upon the principle), attached to a solid phase (matrix or support), and incorporated with a conjugate and substrate detection system. Viral antigens may be whole viral 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 (lgG, sometimes lgM and IgA) coupled to enzymes (alkaline phosphatase or horseradish peroxidase), fluorochromes, or other reagents that will subsequently bring about a reaction that can be detected. 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-phenylenediamine dihydrochloride (OPD) and TMB – for horseradish peroxidase, which produce colour on being acted upon by the respective enzymes. The colour can be measured on an ELISA Reader as optical density (OD) values (Figure 3.1). ELISAs are suitable for use in laboratories where the specimen load is high.

Figure 3.1. Illustrates the principle of ELISA



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

- Indirect
- Competitive
- Sandwich
- Capture

Indirect ELISA

This is the most commonly used principle. HIV antigens are attached covalently to the solid phase support. This allows HIV antibodies present in the specimen to bind. These bound antibodies are subsequently detected by enzyme labelled anti-human immunoglobulin and a specific substrate system. If the test specimen contains anti-HIV antibodies, a colour reaction will take place.

Procedure: The instructions in the kit insert are to be carefully followed. All specified controls should be included with each test run to validate the test result.

- Appropriately dilute the specimen, add to the solid phase, and incubate for a specified time and 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 is added
- Colour change produced is measured after the specified time has passed using an ELISA reader at the specified wavelength
- The result is interpreted as detailed in the kit insert from the various OD values obtained

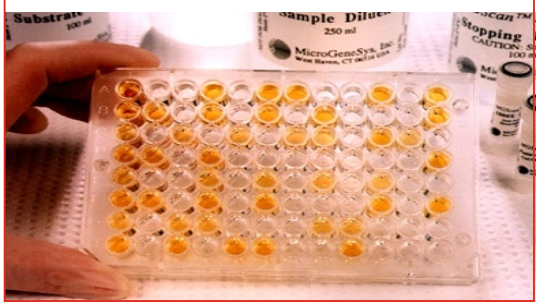
The indirect ELISA produces a colour change directly proportional to the concentration of specific antibodies in the specimen as depicted in Figures 3.2 & 3.3.

Figure 3.2. Microplate ELISA for HIV antibody: coloured wells indicate reactivity

Competitive ELISA

In this assay, the HIV-antibodies present in the specimen compete with the enzyme-conjugated antibodies in the reagent to bind to the solid phase antigen (Figure 3.4). In a competitive ELISA both the specific antibodies to HIV in the serum of an infected person and the antibody in the conjugate are added at the same time. They then compete for reactions on the antigens that are immobilized in the solid phase. In the absence of a specific anti-HIV antibody (in a non-infected person), the conjugate will bind unimpeded. In the presence of an anti-HIV antibody (in an infected person), only a little conjugate binds. This occurs because anti-HIV

Figure 3.3. Indirect ELISA



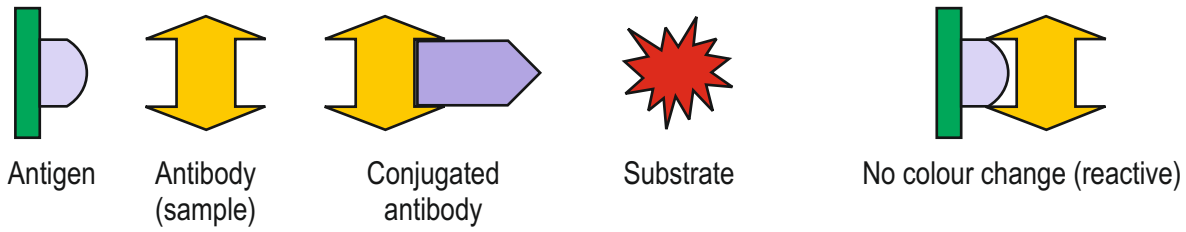
antibodies in the serum of an infected subject bind more firmly and have a higher affinity for the solid phase antigen. Consequently in the sample from an infected person there will be less or no colour development because the conjugate cannot bind and thus is not available to react with the substrate. Conversely, with specimens containing little or no HIV antibody, more conjugate will bind to the solid phase antigen and the subsequent addition of substrate will cause more colour development. Hence, the amount of anti-HIV antibody in the specimen is inversely proportional to the amount of colour produced and the OD value, i.e., low OD readings are associated with infection and high OD readings are produced when testing a person who is not infected.

Procedure: Follow the instruction given in the kit insert:

- Simultaneously mix the test specimen and the appropriate amount of the enzyme labelled HIV antibody conjugate to the solid phase antigen. Support and incubate at specified temperature.
- Plate is washed and substrate is added.
- The colour change that develops is measured with an ELISA reader and expressed as an OD value.

A competitive ELISA takes less time than indirect ELISA and no pre-dilution of test specimen is required.

Figure 3.4. Competitive ELISA

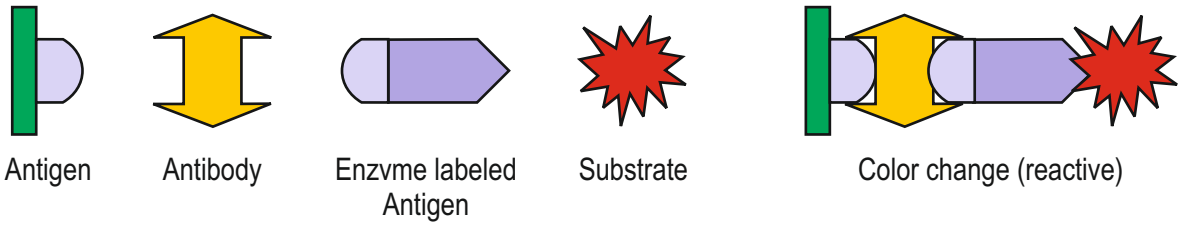


Antigen and Antibody Sandwich ELISA

This is a modification to improve sensitivity and specificity of the indirect ELISA. During the first step, antigens bound to the solid phase bind to antibodies in the test specimen. Since antibody molecules are bivalent/multivalent, they are still able to bind to another antigen. The next step includes the addition of similar enzymes labelled HIV antigens, i.e., same as the solid phase antigen. This will attach to the antibody molecule, which is already bound to the solid phase antigen with one arm. This forms a sandwich of antigen + antibody + enzyme labelled antigen complex (Figure 3.5). The next step is the addition of a specific substrate, which results in the development of colour if the complex is formed. The colour is measured with an ELISA reader. One advantage of the sandwich ELISA is that all classes of HIV antibodies can be detected. Antibody sandwich ELISAs that detect p24 antigens are available.

Procedure: The procedure for the sandwich ELISA is the same as the indirect ELISA. The only difference being that in this case, enzyme labelled antigen is added in place of enzyme labelled anti-human immunoglobulins.

Figure 3.5. Sandwich ELISA



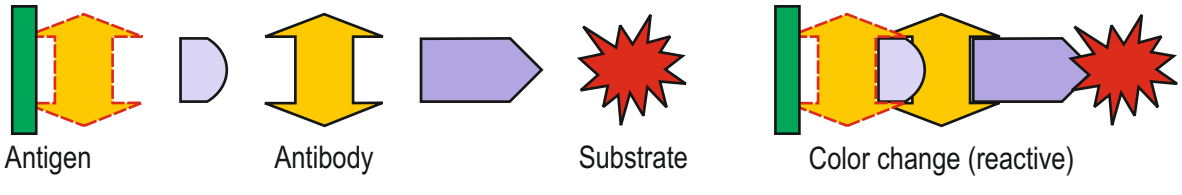
Antigen and Antibody Capture ELISA

Antigen and Antibody capture ELISAs are based on the principle of indirect or competitive ELISAs (Figure 3.6). The only difference occurs during the initial step of attaching an antigen to the solid phase.

Procedure: A monoclonal antibody directed against an HIV antigen is bound to the solid support. The next step is the addition of the HIV antigen supplied as a reagent. This antigen is captured by the monoclonal antibody bound to the solid phase. The test specimen, which has been appropriately diluted is added next. HIV antibodies, if present in the specimen, bind to the solid support HIV antigen. The remaining steps are the same as the steps for an indirect ELISA. An advantage of the antigen capture ELISA is that it is more specific than an indirect assay. Antibody capture assays were developed to test specimens with low concentrations of HIV antibodies (e.g., urine and saliva) or to detect a specific class of antibodies (e.g., lgG, lgM or lgA).

Procedure: In this test an anti-human immunoglobulin (anti-lgG, lgM or lgA) is attached to the solid support. The patient's specimen is added. The concentrated immunoglobulin in the patients' specimen binds to the solid phase anti-globulins. Next, the labelled antigen is added. This binds to the HIV antibodies in the specimen, which in turn bind to solid support. Next, the substrate is added and the OD value is read on the ELISA Reader.

Figure 3.6. Antigen and Antibody Capture ELISA



Kit controls (internal controls) and previously known positive and negative controls (external controls) should be used irrespective of the type of ELISA used.

Interpretation of ELISA Results

Each test must be validated according to the validation criteria given in the kit insert.

Cut-off-value

- Each kit manufacturer has devised a method of calculation that produces a cut off value, to classify a test specimen as positive or negative.
- Thus, a 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 the assay.

Performance Variables of ELISA

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

To validate the test, all kit controls (as specified in the kit insert) must be included with each test run. To ensure quality results, external controls must also be included with each run.

Factors which may affect the test results

Pre-analytical:

- Haemolysed sample
- Grossly lipaemic samples
- Repeated freezing and thawing
- Contaminated samples and reagents
- Improperly stored, expired and deteriorated reagents

Analytical:

- Pipetting errors
- Improper incubation time and temperature
- Improper washing procedure
- Carry over from the adjacent specimen
- Equipment malfunction
- Glove -powder aerosol
- Calculation errors

Post-analytical:

- Transcription errors

False Positive and False Negative ELISA Results

There are a number of conditions that can give rise to a false positive or false negative ELISA result. Common causes of a false positive result:

- Autoimmune diseases
- Alcoholic hepatitis
- Primary biliary cirrhosis
- Leprosy
- Multiple pregnancies

Common causes of a false negative result:

- Technical errors
- The test may be negative during the window period and during the end stage of the disease

Rapid Anti-HIV Tests

Several rapid tests have been developed using recombinant and/or synthetic antigens. The most commonly employed rapid anti-HIV tests are based on the principle of Immunoconcentration/dot blot immunoassay (vertical flow), Immunochromatographic (lateral flow), particle agglutination (e.g., gelatine or latex), and Dipstick and Comb assay based on EIA.

Rapid tests are visual point of care tests that do not require any special equipment. These tests are available in smaller test packs. These are therefore, suitable for a laboratory that tests small number of specimens. They are technically simple to perform. Most of them have sensitivity and specificity comparable to an ELISA. Moreover, some rapid test kits can be stored at an ambient temperature (20°C to 25°C).

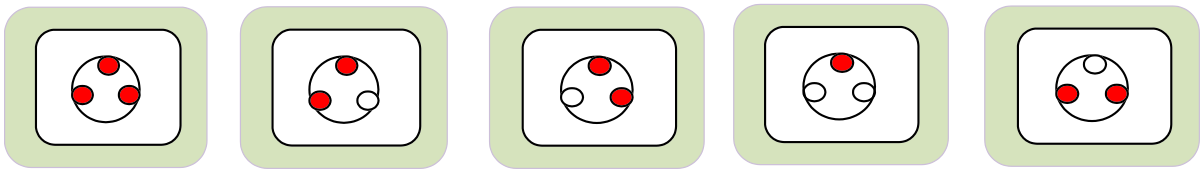
Immunoconcentration / Dot Blot immunoassay (vertical flow)

This is a type of solid phase immunoassay where HIV antigens are immobilized on a porous membrane. Specimen & reagent pass through the membrane & are absorbed into the underlying absorbent pad. As the specimen passes through the membrane, HIV antibodies if present, bind to the immobilized antigens. The conjugate binds to the Fc portion of the HIV antibodies and produces a distinct coloured dot against a white background. Figure 3.7 depicts the possible immunoconcentration results.

Procedure: This technique uses a small flat cup like device with a pad at the bottom (flow through). A few drops of the sample are added into the cup, as per the instruction manual. The added sample is quickly absorbed and runs down the pad at the bottom. A couple of reagents are added sequentially to complete the test. After the recommended time interval has passed, the test should be checked for the presence of a coloured spot at the pre-designated site on the pad. A control spot should always be verified before looking for test spots. Some kits are designed with one coloured test spot plus the control spot, whereas others are designed with

two coloured spots (to distinguish between HIV-1 and HIV-2) plus the control spot. In this test, the absence of a control spot makes the test invalid.

Figure 3.7. Immunoconcentration



Interpretation of Results:

- **Negative test result:** Appearance of only one dot, corresponding to control region 'C.' Specimen is non-reactive.
- **Positive test result:** Appearance of two dots, one for the control and the other shows the presence of HIV-1/HIV-2. If the test has the ability to differentiate between HIV-1 and HIV-2, then separate dots for HIV-1 and HIV-2 may be observed. The specimen can be reactive to HIV-1 antibodies, HIV-2 antibodies, or both as applicable.
- **Invalid test result:** The test should be considered invalid if neither the test DOT nor the control DOT appears. In the case of an invalid test, repeat the test using a new device.

Immunochromatography Tests

The strips/cards incorporate both the antigen and signal reagent into the nitrocellulose strip. The specimen (usually followed by a buffer) is applied to the absorbent pad on the kit. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where the HIV antigen has been incorporated. A procedural control is usually incorporated into the strip

The test device is incorporated with distinct bands of purified gp120 and gp41 synthetic peptides, specific to HIV-1 at test region '1' and gp36 synthetic peptide specific to HIV-2 at test region '2.' The third band incorporated at region 'C,' corresponds to the assay performance control. If present, antibodies to HIV-1 and/or 2 are captured by the respective antigens. After washing with a buffer, the Protein A conjugated reagent is added to reveal the presence/absence of bound antibodies. Post-final wash, a positive reaction is visualized by the appearance of coloured bands at specific sites. The absence of bands at test region '1' & '2' is a negative test result. The appearance of a control band validates the test. Figure 3.8 shows the principle of Immunochromatography and Figure 3.9 depicts the results.

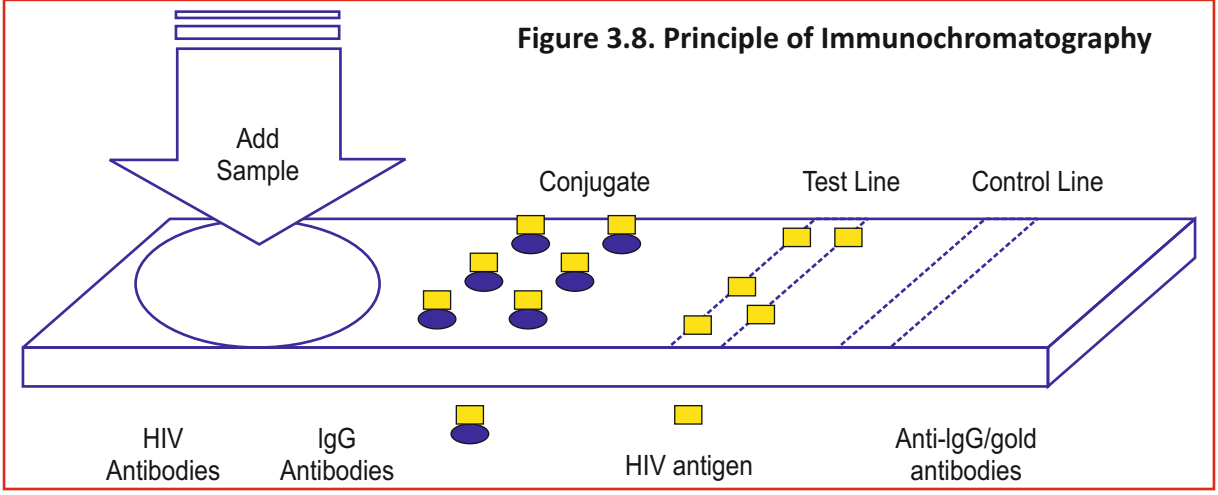


Figure 3.8. Principle of Immunochromatography

Interpretation of Results:

- **Negative test result:** Appearance of only the control band, corresponding to control region 'C'
- **Positive test result:**
  - In addition to the control band 'C,' appearance of reactive band at test region '1' Specimen positive for antibodies to HIV-1.
  - In addition to the control band 'C,' appearance of reactive band in test region '2' Specimen positive for antibodies to HIV- 2.
  - In addition to the control band 'C,' appearance of reactive bands at test region '1' and test region '2' Specimen positive for antibodies to HIV-1 and HIV-2.
- **Invalid test result:** The test should be considered invalid if neither the test band nor the control band appears. In case of invalid test, repeat the test using a new device.

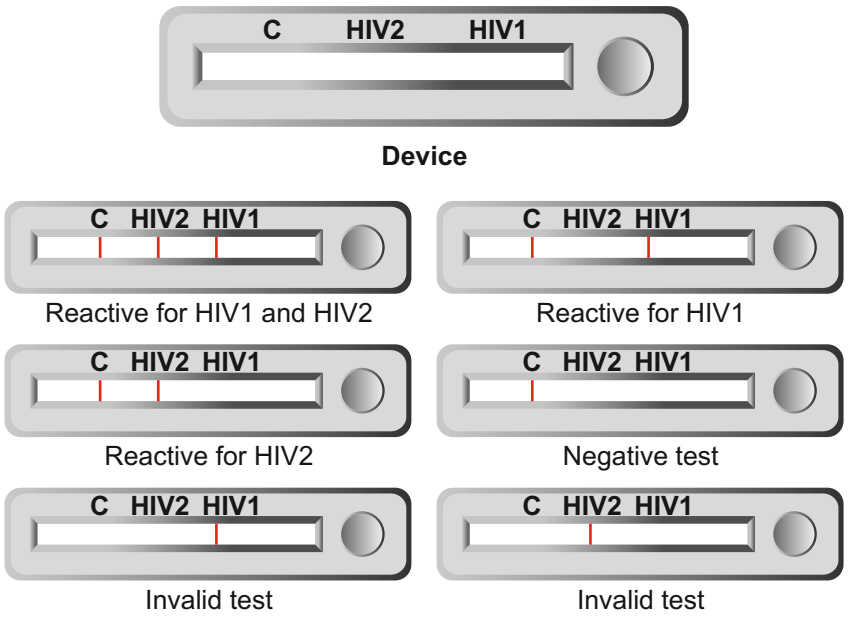


Figure 3.9. Interpretation of Immunochromatography tests

Particle Agglutination Tests

Agglutination assays are used for antibody detection, where the antigen is coated on a carrier particle and the antigen antibody reaction is observed in clumps. These assays incorporate a variety of antigen-coated carriers, e.g., red cells, latex particles, gelatine particles and micro beads. During the agglutination reaction, an HIV antibody combines with the HIV antigen on the carrier particles. Since all antibodies are multivalent, a sort of lattice network is formed (Figure 3.10). This structure can be visualized macroscopically per the directions in the kit insert.

Figure 3.10. Lattice formation in particle agglutination assay

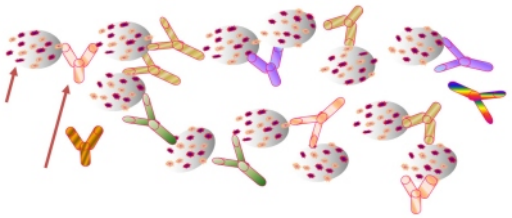
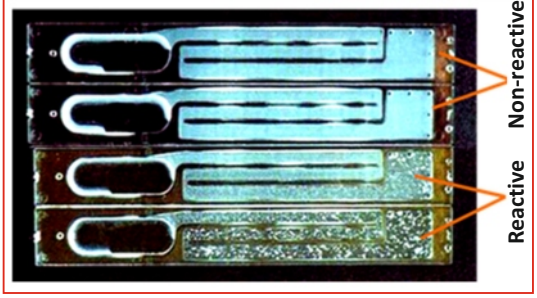


Fig. 3.11. Interpretation of particle agglutination test



Interpretation of Results:

Figure 3.11 depicts the possible results of an agglutination test. They are:

- **Reactive:** If a test specimen contains HIV antibodies, a lattice network will form between the antigen carrying particles and HIV specific antibodies. It will appear as the formation of clumps.
- **Non-reactive:** Absence of the agglutination denotes non-reactive result.

Dipstick and Comb Assay

These tests incorporate antigens “spotted” on a solid support. They are based on typical EIA methods, with enzyme substrate reactions occurring and resulting in the production of colour at the site of antigen spotting.

Immunocomb Assay

This is a rapid assay intended to differentiate between HIV-1 and HIV-2 antibodies in human serum or plasma. The comb test consists of a comb like device with projections. Each tooth represents the solid phase and has three spots for adsorption of a specific antigen/antibody. HIV-1 and HIV-2 antigens are immobilized as circular spots at two sites. The third spot acts as an antibody control containing goat anti-human IgG. The test is carried out by sequentially immersing the comb in wells with ready to use reagents. When the comb is incubated with a sera containing HIV antibody, these antibodies bind to the antigen on the comb. The complex is then visualized after the addition of antibody enzyme conjugate and substrate.

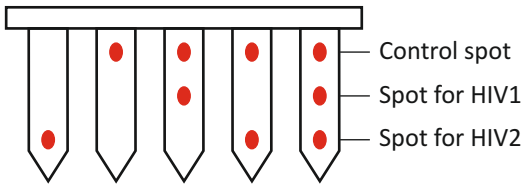
Procedure: A developing plate with six rows of wells, each containing a ready to use reagent, is supplied along with the kit. The test sample is added to the first row and the teeth of the comb

are sequentially transferred from the first row to the next, as per instruction manual. During the first step of the assay, the first spot on the tooth, picks up immunoglobulins and the second and the third spots pick up specific HIV antibodies from the sample. Subsequent steps are intended for the completion of an EIA reaction and for the development of coloured spots (Figure 3.12).

Interpretation of Results:

- Invalid test: Absence of upper spot
- Non-Reactive: Appearance of upper spot only
- HIV-1 Reactive: Appearance of upper and middle spot
- HIV-2 Reactive: Appearance of upper and lower spot
- HIV-1 and HIV-2 Reactive: Appearance of all three spots

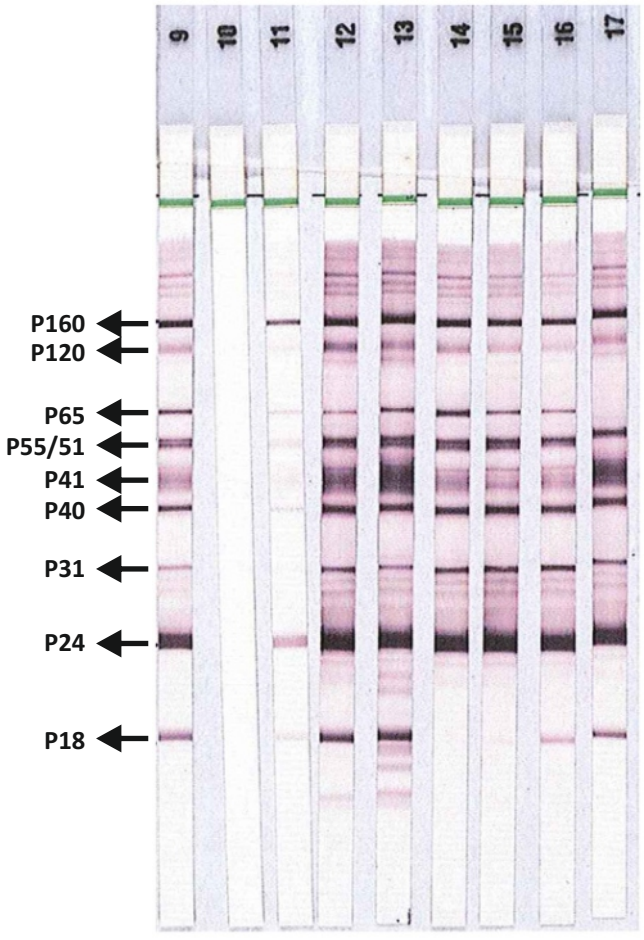
Fig. 3.12. Comb assay (Dot EIA)



Actual interpretation should be performed per the instruction manual supplied with the kit

One disadvantage of these types of rapid antibody tests is that each test device cannot be quality controlled with an external quality control sample.

Fig 3.13. Western Blot



Accuracy of Rapid Kits

Many commercially available rapid kits provide good specificity and sensitivity. Different kits should be used in combination based on testing strategies/algorithm. Every kit used should be monitored, from time to time, for its performance using internal and external controls. Any fallacies encountered in the performance of the kits should be immediately brought to the notice of local and regional authorities.

Western Blot Test

In the western blot, the various HIV specific recombinant or synthetic antigens are adsorbed onto nitrocellulose paper. The antibody, when

present, attaches to the antigen on the strip and the antigen antibody complex is then detected using enzyme conjugate and substrate. This is similar to what is done in an ELISA test, except that the product is insoluble. The test procedure should be carried out as per the kit insert. WB tests detect the presence of antibodies against specific HIV proteins, which are seen as bands on the test strip (Figure 3.13). The test results are interpreted as per kit instructions.

WB tests are a highly specific conformational test. NACO is presently providing it at the National Reference Laboratory level for resolving indeterminate results.

Rapid Anti-HIV Tests and IQC

All HIV tests should be performed adhering to protocol, taking into account the national guidelines and testing strategies. It is also imperative to incorporate external controls along with the test run. External controls (known positives and negatives) should be incorporated along with every batch of 15 samples or on a daily bases – in case the turn over of samples is more than 15 per day.

Limitations of Antibody Assays

Antibodies are not detectable in the window period. Therefore, antibody detection tests are of no use during this period. Diagnostic tests based on antibody detection are also not useful in the diagnosis of infection in children below 18 months of age. Babies born to HIV positive mothers may have passively acquired maternal antibodies. In this situation, tests that detect the viral genome may be done for early diagnosis (see Chapter 4). NACO is now promoting the use of the DBS technique for early infant diagnosis, based on the detection of HIV-1 DNA viral nucleic acid. This test is discussed in detail in chapter four.

Quality Assurance in HIV Testing

Assuring the quality of laboratory results is the core objective of any health laboratory and is a continuous on-going process. Participation in External Quality Assessment (EQA) / Proficiency Testing (PT), inter-laboratory comparison and Quality Control (QC) procedures ensure accurate and reliable results thereby increasing the credibility and acceptance of the laboratory. QC measures should be practiced daily. The goal of QC is to detect errors and introduce corrective measures before patient results are reported.

Chapter 4

Molecular & Other Assays for the Diagnosis of HIV Infection

Introduction

Serological assays for the detection of HIV antibodies are predominantly used for the diagnosis of HIV infections. In certain situations, such as patients in the window period and infants born to HIV positive mothers antibody detection assays cannot be relied upon. In these situations, the diagnosis of HIV infections is established using molecular assays to detect viral genomes. This chapter describes molecular assays, assays for virus isolation, and detection of virus core proteins (p24).

Diagnosis of Paediatric HIV Infection (< 18 months)

The standard diagnostic method for HIV infection in adults (i.e., testing for antibodies) has limited utility in newborns, infants, and children less than 18 months of age. This is due to the transplacental transfer of maternal IgG (including HIV-specific antibodies) from infected mothers to their babies during pregnancy. HIV antibody tests are reactive in most infants born to HIV positive mothers, though the infection is transmitted to less than half of such infants (even in the absence of ART). HIV antibodies can also be transferred through the breast milk of infected mothers. Maternal antibodies may persist in an infant's blood until 18 months after birth, and are difficult to differentiate from those produced by an infected infant. Therefore, antibody tests cannot produce a definitive diagnosis of HIV infection until 18 months of age. Waiting until this time delays specific treatment. In this situation, Nucleic Acid Testing (NAT) can facilitate early infant diagnosis. NACO recommends the use of a qualitative HIV-1 DNA PCR.

Further Reading: *Laboratory Guidelines for HIV Diagnosis in Infants and Children < 18 months,* NACO 2010

Detection of Acute HIV Infection

Virological tests can be used for the detection of acute HIV infection during the "window period," before HIV antibodies become detectable. Though positive NAT results confirm the HIV diagnosis, the NAT result may turn out negative if tested within 7 to 10 days of exposure. NAT tests may be successfully employed for the detection of HIV infection if appropriate infrastructure and technical expertise is available. At present, NACO does not recommend the use of NAT for the diagnosis of acute HIV infection.

NATs include tests for the qualitative detection of HIV-1 DNA or RNA, as well as the quantitative detection of HIV-1 RNA (viral load determination) through various assays.

Qualitative Polymerase Chain Reaction for HIV DNA

In infants, the sensitivity of a traditional PCR test – for the diagnosis of HIV infection and the qualitative detection of HIV DNA – is as high as 90 to 100 percent by the age of 4- 6 weeks. An example of a commercially available test, approved by the Drug Controller General of India (DCGI), is the qualitative AMPLICOR HIV-1 DNA PCR Test, ver. 1.5 (Roche), which can be used to test dried blood spots or whole blood collected in EDTA. This test has a reported 99.3 percent sensitivity and 96 percent specificity. NACO's first choice for the diagnosis of HIV-1 infection in infants and children less than 18 months of age (starting at 6 weeks of age or at the earliest opportunity thereafter) is the HIV-1 DNA PCR test. However, this test is likely to be phased out by the manufacturer in 2015.

Principle and Steps

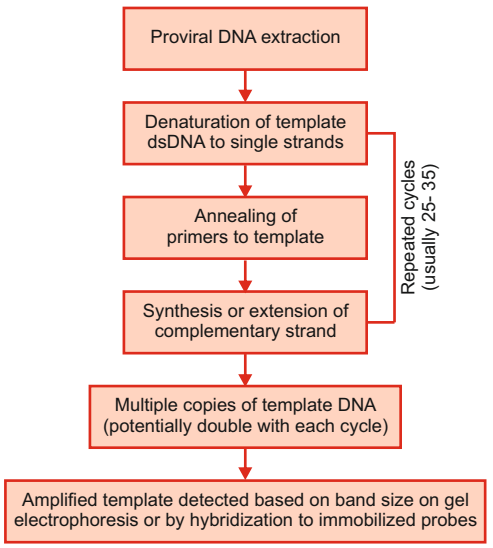
HIV reverse transcriptase (RT) produces DNA from the RNA genome of the virus. A complementary strand of DNA is then produced through viral DNA polymerase, to form double-stranded viral DNA. This DNA, which may integrate into the host cell genome or remain un-integrated in the cytoplasm, can be amplified by a PCR and detected. A PCR test for HIV DNA involves the following steps (Figure 4.1):

1. The extraction of DNA from blood (whole blood, dried blood spots, or peripheral blood mononuclear cells – PBMCs).
2. The denaturation or separation of the two strands of double-stranded viral DNA by heating to 95^{o}C.
3. Annealing of the two single strands of HIV DNA with complementary HIV-1 specific nucleotide primers, by cooling to (usually) 50 to 55^{o}C. Primers are specific oligonucleotides that are chemically synthesised and are complementary to DNA flanking the target sequence of interest (template). One primer is designed to anneal to the sense strand and the other primer to the anti-sense strand. The annealing temperature is derived from the melting temperature (Tm) of the primers. The temperature should be sufficiently low for the hybridization between primers and the template to occur but high enough to prevent mismatch hybridization of the primers to partially similar sequences elsewhere in the genome. A high concentration of the primers is maintained to ensure that this reaction is favoured over re-annealing of the complementary template DNA strands.
4. The extension or the synthesis of new complementary DNA strands through the incorporation of deoxynucleotide triphosphates (dNTPs) in the building blocks or bases of DNA, i.e., adenine (A), thymine (T), guanine (G) and cytosine (C). These are included in the reaction mixture, at a temperature of 72^{o}C. This synthesis starts from the 3' end of each primer, by the action of a heat-resistant polymerase enzyme (e.g., Taq polymerase from the bacterium Thermus aquaticus). Heating the solution to 95^{o}C for the second time stops the synthesis reaction. Often, a nested PCR reaction is used. Here, the PCR product can be re-amplified with a second set of primers. These primers are located internally 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's instructions must be followed for all steps of test performance and quality control.

Further details on sample collection, algorithms, test procedures, documentation and quality assurance procedures, related to HIV DNA testing, can be found in the NACO's *Laboratory Guidelines for HIV Diagnosis in Infants and Children < 18 months.* The laboratory design and procedural precautions for PCR testing are described in Annexure XI.

Figure 4.1: The steps of PCR



Qualitative Transcription-mediated Amplification Assay for HIV RNA

The APTIMA HIV-1 qualitative RNA assay (Gen-Probe) is licensed by the U.S. Food & Drug Administration (FDA) for use as an aid in the detection of acute HIV-1 infection in plasma specimens. In this test, the specimen is first incubated with a target capture reagent, which solubilizes the HIV-1 RNA genome and separates it from plasma. Amplification is then performed using transcription-mediated amplification (TMA), which is an isothermal process that utilizes Moloney Murine Leukemia Virus (MMLV) reverse transcriptase and T7 RNA polymerase. Amplicons are then subjected to a hybridization protection assay using chemiluminescent-labeled probes that are specific for the selected HIV-1 genomic region. A selection reagent differentiates between hybridized and unhybridized probes, by inactivation of the label on unhybridized probes. Results are read on a luminometer. An internal control RNA transcript is added to each reaction at the target capture step to control for proper capture, amplification and detection.

Quantitative HIV-1 RNA Assays

Quantitative HIV-1 RNA assays detect plasma (cell-free) viral RNA by using various techniques. The methods of amplification of HIV-1 RNA include:

1) Target amplification by
    a. Reverse transcriptase PCR (RT-PCR)
    b. Real time PCR (qPCR)
    c. Nucleic acid sequence-based amplification (NASBA)
2) Signal amplification by branched-chain DNA technique (bDNA)

Quantitative NAT are mainly used to determine viral load. These are discussed in greater detail in chapter 5. The sensitivity ranges from 25 percent to 50 percent within the first few days of life,

but increases to 100 percent by 6 to 12 weeks of age. Quantitative HIV-1 RNA assays, as sensitive among young infants as HIV-1 DNA, are more expensive than a qualitative PCR for HIV-1 DNA. Additionally, there is a certain lack of consensus on the cut-off for labelling a sample as positive in the HIV-1 RNA assays. Though most HIV-1 infected infants have a viral load of >100,000 copies/mL, the recommended cut-off is >10,000 copies/mL for making HIV diagnosis. However, this does not always indicate a definitive positive and may require re-testing, thus adding to the cost. More stringent precautions and quality control needs to be taken with RNA assays than with DNA assays. However, in many countries, HIV-1 RNA assays are commonly used to diagnose HIV-1 infection in infants due to the ease of availability. This can be attributed to their more common use as a follow-up test for infected individuals during therapy.

Other Assays

Virus Isolation

HIV isolation requires co-cultivation of peripheral blood mononuclear cells (PBMCs), from an infected individual and mitogen stimulated PBMCs from an HIV-uninfected individual. These are cultured together for up to 6 weeks in a medium containing interleukin- 2. The cultures are maintained at 37°C in a 5 percent CO_2 atmosphere for up to 28 days. They are fed with freshly activated PBMCs at regular intervals. The replication of HIV can be detected by measuring the p24 antigen by ELISA, or reverse transcriptase activity in culture supernatant. The disadvantages of a viral culture as a diagnostic test far exceed its advantages. It is labour-intensive, time consuming, expensive and requires containment facilities. It has limited use, except as a research tool.

Antigen Detection

The HIV-1 p24 antigen is present as either an immune complex, with anti-p24 antibodies, or as a free p24 antigen in the blood of infected individuals. The positive p24 test confirms diagnosis of HIV infection; however, a negative test does not rule out HIV infection. The test is based on the ELISA. The sensitivity of the test increases with the use of techniques to dissociate the p24 antigen from its antibody, as in immune complex-dissociated (ICD) tests. However, despite this advance, the diagnostic usage of p24 antigen assays is much less frequent than that of NAT. This is due to their relatively lower sensitivity. HIV p24 antigen assays, with increased sensitivity, are now commercially available and under evaluation, e.g., the ultrasensitive p24 assay (Perkin-Elmer). However, they have not yet been recommended for diagnosis.

Chapter 5

National Strategies and Algorithms For HIV Testing

Introduction

The HIV status of an infected individual can be reliably determined only by laboratory testing. A reliable, accurate, and reproducible result using uniform testing procedures is the most desirable outcome in the HIV testing laboratory. Well-defined strategies and diagnostic algorithms are necessary in view of the varying prevalence of HIV infection in different populations and the availability of a variety of different diagnostic kits in the market. In general, the following testing policies need consideration:

- Testing should be part of the overall comprehensive prevention programme.
- Testing should be technically sound and appropriate.
- Testing procedures must be field appropriate.
- Testing procedures must be cost effective.
- Laboratory procedures must be monitored to ensure quality.

A testing strategy refers to selecting the best type of test, or more than one type of test, for identifying and confirming HIV infection in a particular testing situation like:

- Blood /Organ donation safety
- Surveillance
- Diagnosis

HIV testing strategies involve a logical sequence of performing two or more tests, one after the other (serial) or simultaneously (parallel) to arrive at a conclusion on the HIV status of a person being tested. A testing algorithm refers to the combination and sequence of specific tests that are used to fulfil the testing strategy.

National HIV testing strategies are defined by the national program and are aimed at obtaining an accurate result. India's strategy is based on serial testing and includes repeat testing on initially reactive specimens. When issuing the final report, testing limitations (e.g., a negative result does not always rule out infection if the individual has been recently infected) should be communicated to the individual during post test counselling.

The type of strategy to be adopted would depend on the ultimate purpose for which HIV testing is being carried out. One of the essential prerequisites for the use of this algorithm is that the first, second, and third tests (A1, A2 and A3) employed are based on different serological principles and/or use of different HIV antigens in the assay. Samples with indeterminate results are to be sent to SRLs/NRLs for confirmation (e.g., Western Blot). Results obtained from SRLs/NRLs are to be communicated to the individual. The follow-up specimen from patients

with indeterminate result, should be collected two weeks after the first specimen collection. However, if the confirmatory test fails to resolve the serodiagnosis, follow up testing should be undertaken at four weeks, three months, six months, and 12 months. After 12 months, such indeterminate results should be considered negative. However, the molecular assays (HIV-1 and HIV-2 NAT) can be used to resolve specimens repeatedly (>2 times) giving indeterminate results.

HIV Testing Strategies

The type of strategy used for HIV testing depends on the specific purpose for which HIV testing is being carried out. Each testing situation is different, and not all testing strategies are appropriate for all testing situations. Testing strategies and testing algorithms almost always involve at least two tests and most testing strategies include repeat testing on initially reactive specimens. A serial testing strategy is recommended for use for NACP.

Serial Testing Strategy

Serial testing strategy usually is configured as two or three test algorithms and refer to the use of one screening test followed by one or more other ELISA / Rapid (E/R) tests, depending on the results of the first. A non-reactive result from the initial screening test completes the testing, i.e., report is given out as HIV negative. If the initial test is reactive, it is followed by a single test (in a two test algorithm), or two more tests (in a three test algorithm) for resolution.

Objectives of HIV Testing

- Blood and blood products safety. This is achieved by mandatory testing of all donated blood units and blood products.
- Screening of sperm, organs, and tissue donors.
- Diagnosis of HIV infection in clinically suspected cases.
- Voluntary testing after counselling.
- Epidemiological surveillance using unlinked anonymous HIV testing. Here the result of the test cannot be linked with the identity of the person.
- Research.

Choice of the Screening Assay

Before choosing a particular kit/strategy for HIV testing one has to be clear about the objectives of testing. The following parameters should be considered before testing:

- Sensitivity of the test kit
- Specificity of the test kit
- Prevalence of HIV infection in the population
- Cost effectiveness
- Appropriateness of testing to national guideline strategies and to the infrastructure facilities available.

Sensitivity of HIV Tests

The sensitivity of a diagnostic test describes its ability to detect all positive samples as positives. Tests with high sensitivity may show a few false positives but will not miss any true positives.

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

Specificity of HIV Tests

It is the accuracy with which a test can confirm the absence of an infection or identifying true negative. Tests with high specificity show less number of false negatives and are to be preferred for the diagnosis of HIV infection in an individual.

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

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, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value of the test (PPV)). The negative predictive value (NPV) describes the likelihood that a person showing a negative result is truly uninfected.

HIV Testing Strategies

The safety of blood and blood products is of paramount importance because of the enormous risk involved in the transmission of HIV through blood. Since the PPV is low in populations with low HIV prevalence, the WHO/Government of India 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 (E/R) used in strategy I, II, & Ill
2. Confirmatory tests with high specificity, like WBs and line immunoassays, are used in problem cases, e.g., in cases of indeterminate/discordant result of E/R.

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

Strategy 1 (for blood transfusion/transplant safety)

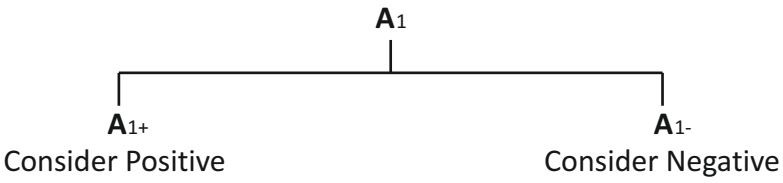
The specimen is subjected to one test for HIV reactivity. The test used in strategy 1 must have high sensitivity. If non reactive, the specimen is to be considered free of HIV (negative) and if reactive, the specimen is considered as HIV popsitive. This strategy is used for ensuring donation safety (e.g., blood, blood products, organs, tissues, sperms etc.). The unit of blood that tests reactive (positive) is discarded. If the donor is to be notified of his result, based on his prior consent, it becomes a matter of diagnosis (in which case strategies II & III must be used after proper counselling) and the donor should be referred to an ICTC for the confirmation of the

result. Figure 5.1 is a flow chart depicting strategy 1

Figure 5.1 Strategy 1

(For transfusion/transplant safety)

One test required



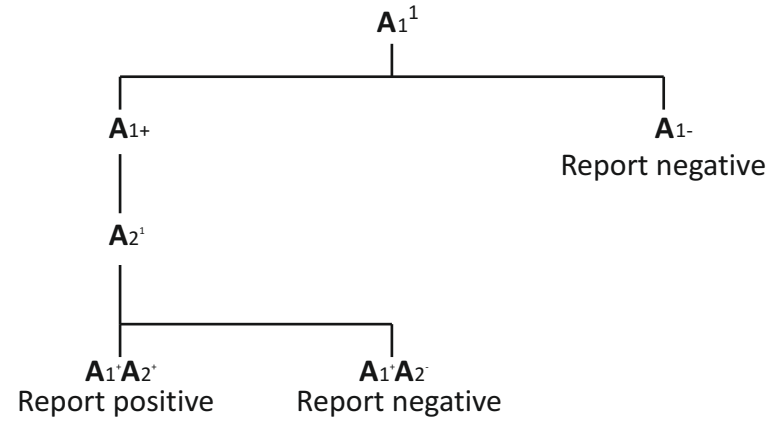
Strategy 2 A (used in sentinel surveillance)

A specimen is tested as above and if it is reactive it is tested further. A specimen is considered negative for HIV if the first ELISA or rapid test reports it so. In case it is 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 the test and/or the antigen used is different). It is reported positive only if the second ELISA/rapid test also gives a reactive report like the first test. In case the second E/R is non reactive, the result is taken as negative for sentinel surveillance purposes. This type of HIV testing is anonymous and unlinked. Figure 5.2 is a flow chart depicting strategy 2 A.

Figure 5.2. Strategy 2 A

(For surveillance)

2 tests required



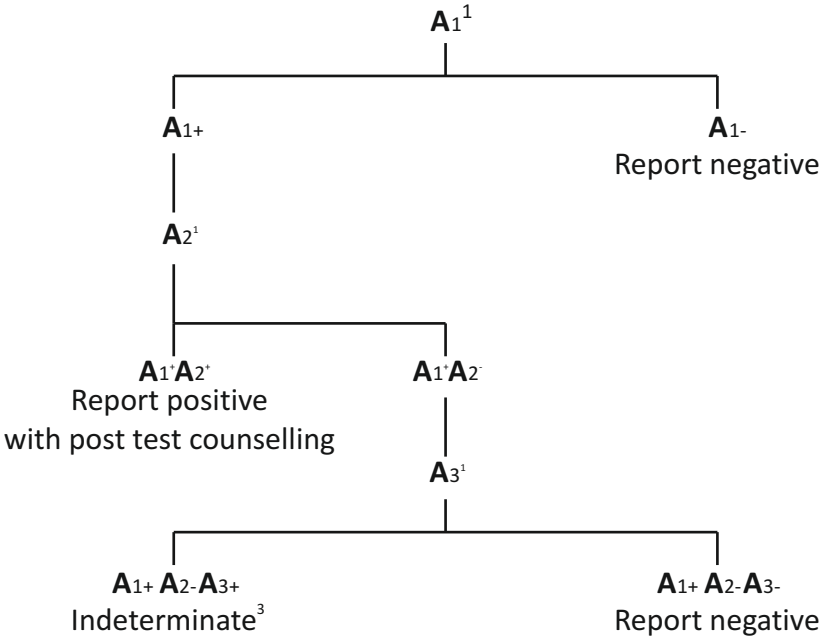
Strategy 2 B (used for diagnosis in symptomatic patients)

This strategy is used to determine the HIV status of a clinically symptomatic suspected AIDS cases in which blood/serum/plasma is tested with a highly sensitive screening test. The specimen is considered negative if the test gives a non-reactive result. In case the test result is reactive the specimen is tested with another test kit (based on a different principle of test or having a different antigen as compared to the first test). If the result is also reactive with the second test kit, the specimen is considered to be positive for HIV 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 reactive test results. In case a specimen is reactive with the first test kit and non reactive with the second test kit, the specimen is subjected to a third tiebreaker test. If the third test is reactive, the specimen is reported as indeterminate and follow up testing is undertaken after 2 to 4 weeks. In case the third tiebreaker test is non-reactive, the specimen is reported negative. Counselling, informed consent, and confidentiality are a must in all these cases. Figure 5.3 is a flow chart depicting strategy 2 B.

Figure 5.3. Strategy 2 B

(Diagnosis of an individual with AIDS indicator disease symptoms)



Strategy 3 (used for diagnosis in asymptomatic patients)

In strategy 3 the HIV testing done is similar to strategy 2, with the added testing of a third test for a positive result. Positive confirmation of a third reactive E/R test is required for a specimen to be reported HIV positive. If the specimen gives a reactive result with two E/R and non-reactive result with the third assay, it is reported as "indeterminate" and the patient is called again for repeat testing after 2-4 weeks. The test utilized for the first screening should be the one with the highest sensitivity 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 the diagnosis of HIV infection in asymptomatic individuals at ICTCs and PPTCT centres. Counselling, informed consent, and confidentiality are a must in these cases. Three different kits, with different antigen systems, and/or different principles of testing are required to follow this strategy.

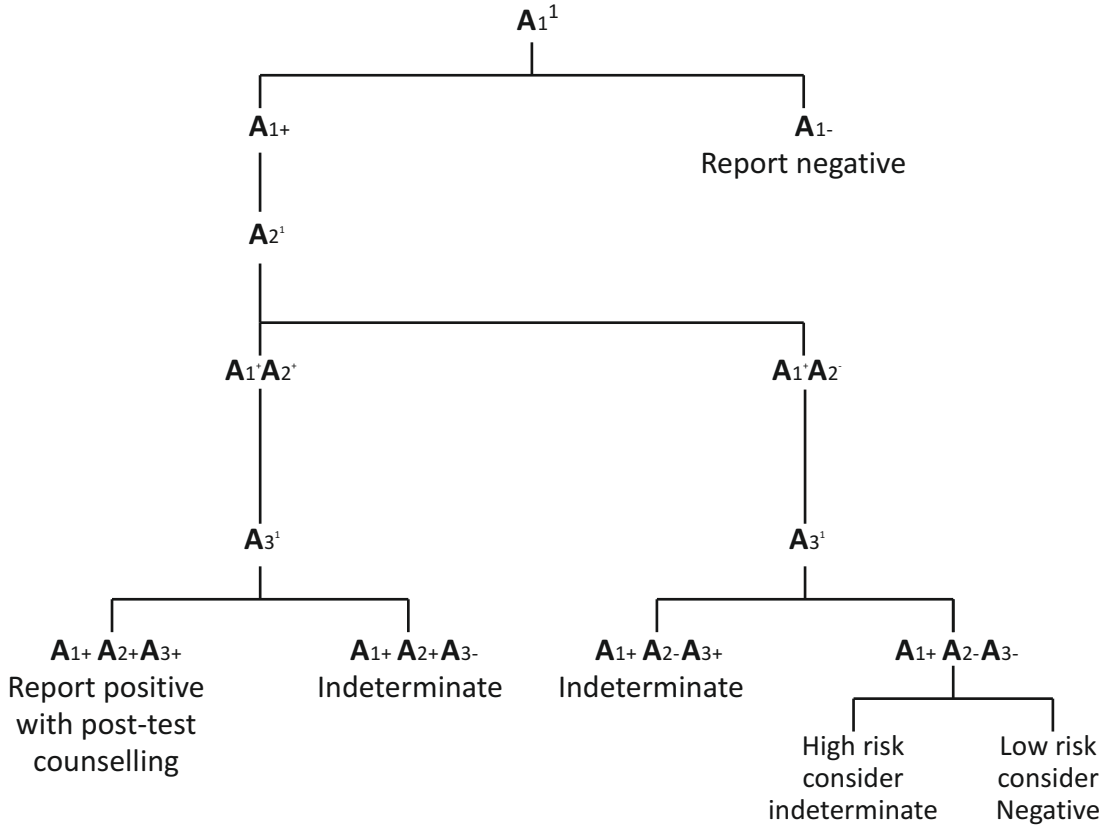
Strategy III is used for the diagnosis of HIV infection. If the specimen gives a reactive result with two assays and a non-reactive with the third assay, it is reported as "indeterminate" and

followed up as mentioned earlier. Figure 5.4 is a flow chart depicting strategy 3.

Figure 5.4 Strategy 3

(To detect HIV infection in asymptomatic individuals (ICTC's, PPTCTC's)

3 tests required



1. Assays A1, A2, A3 represent 3 different assays based on different principles or different antigenic compositions. Assay A1 should be of high sensitivity and A2 and A3 should be of high specificity. A2 & A3 should also be able to differentiate between HIV 1 & 2 infection. Such a result is not adequate for diagnostic purposes: use strategies 2B or 3.
2. 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.
3. Testing should be repeated on a second specimen taken after 14-28 days. In case the serological results continue to be indeterminate, then the specimen is to be subjected to a WB/PCR if facilities are available or refer to the NRL for further testing.

Testing Approaches

Unlinked Anonymous Testing

This testing approach is used for HIV surveillance purposes. All the identifiers are removed from the specimen before they are sent to the laboratory for testing, so that the test results cannot be linked to the individuals.

Voluntary Confidential Counselling and Testing

This approach is followed for the diagnosis of HIV infection in an individual. This testing is done after pre test counselling is provided and after obtaining informed consent from the individual. The test result is disclosed to the individual only after post test counselling. Confidentiality needs to be maintained throughout the process.

Mandatory Testing

Mandatory testing is recommended in India, only for the screening of donated units of blood, blood products, and donors of semen, organs, or tissues in order to prevent the transmission of HIV to the recipient.

The national HIV 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 healthcare services and facilities.
- Any HIV testing must be accompanied by pre test and post test counselling services and informed consent. Confidentiality of result should be maintained.

Operational Guidelines for HIV-2 Diagnosis

The natural history studies of HIV in adults indicate that HIV-2 is less pathogenic than HIV-1. Those infected with HIV-2 have slower disease progression, a much longer asymptomatic stage, slower decline in CD4 count, lower rates of vertical transmission, lower viral loads while asymptomatic and smaller gains in CD4 count in response to ART.

It is observed and well documented that infection with HIV-2 does not protect against HIV-1 and dual infection. Dually infected patients tend to present at a more advanced stage of disease than those with HIV-2 only. Infection with both HIV-1 and HIV-2 generally carries the same prognosis as HIV-1 single infection.

Information on the epidemiology of HIV-2 and dual infection in India is limited. However, a few cases of HIV-2 infection have been reported. In order to provide for treatment alternatives for HIV-2 and dual infected persons, it is important to assess the characteristics as well as response to ART for HIV-2 and dual infections and this would require accurate diagnosis of HIV-2.

Although HIV-1and HIV-2 are related, there are important structural differences between them. Accurate diagnosis & differentiation of HIV-1 & HIV-2 is crucial for treatment, as HIV-2 is intrinsically resistant to NNRTI, the pillar of national first line ART regimen. This information is important for treatment of infected individuals as well as for understanding extent of HIV-2 infections in India. Discriminating rapid kits are being used at ICTCs. However, HIV-2 positivity shown in these tests needs confirmation which cannot be done at ICTCs.

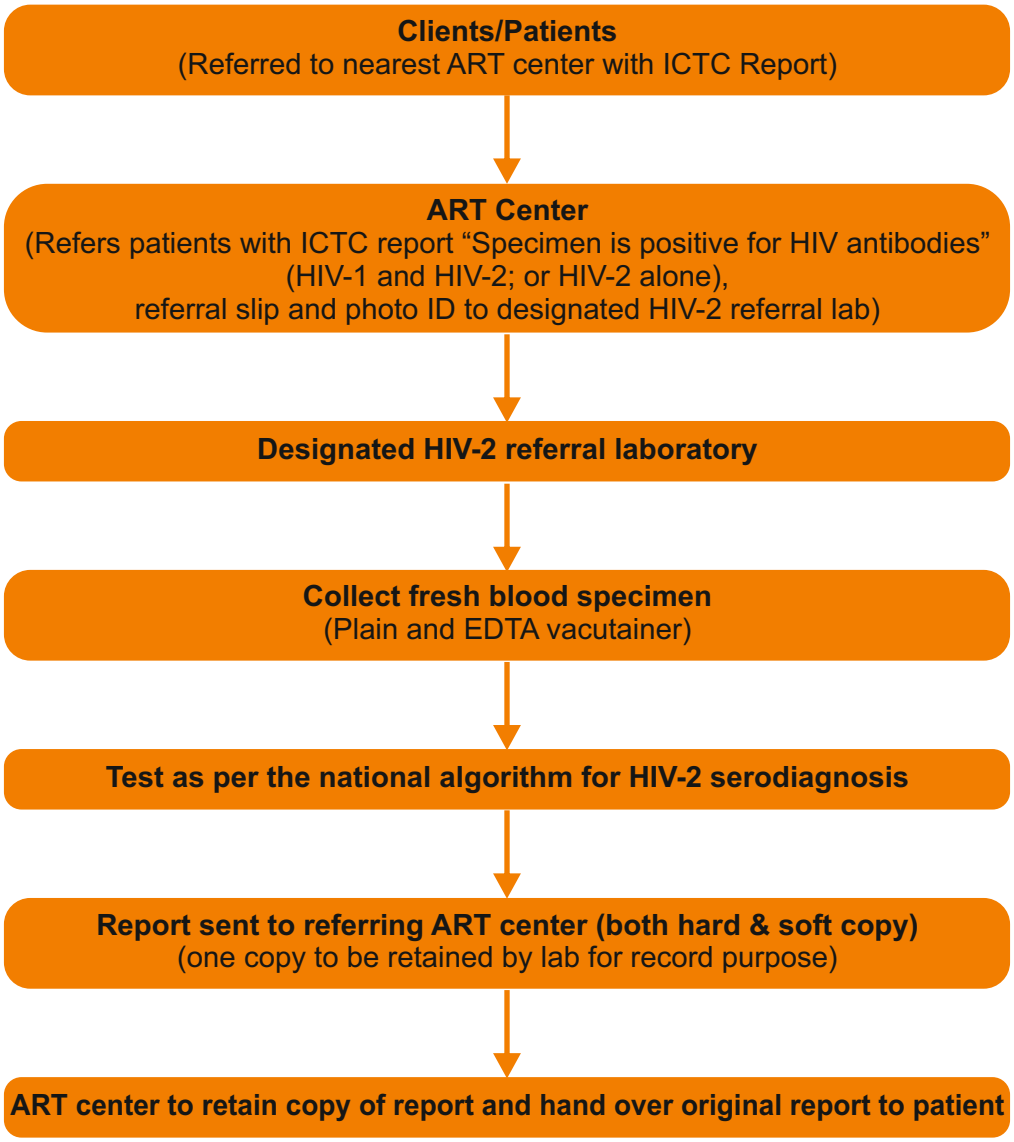
NACO has established a network of laboratories which includes ICTCs, SRLs and NRLs. Designated NRLs and SRLs will be responsible to confirm the presence of HIV-2 infection.

Patients with a HIV Positive report must be referred to the nearest ART centre for care, support and treatment by the ICTC. The ART centre will refer patients with the following HIV report from the ICTC **"Specimen is positive for HIV antibodies (HIV-1 and HIV-2; or HIV-2 alone)"** to the designated HIV-2 referral laboratory for accurate diagnosis & differentiation of HIV-1 & HIV-2; thus enabling the ART centre to select the appropriate treatment regimen.

Instructions to be followed by ART centers for referring clients/patients for HIV-2 diagnosis (Refer to Flow chart):

I. Clients/Patients with the following report from the ICTC **"Specimen is positive for HIV antibodies (HIV-1 and HIV-2; or HIV-2 alone)"** will be referred to the nearest ART centre for registration by ICTC.
II. The ART centre will then refer the said client/patient to the designated HIV-2 referral laboratory with the referral slip for HIV-2 testing. (Annexure-4)
III. The patient must carry ICTC report and a referral slip duly signed by the ART Medical Officer along with a photo ID to the referral lab (Refer Annexure-2 for designated HIV-2 referral laboratories) on any working day from Monday to Friday between 9:00 AM to 2:00 PM.
IV. The HIV-2 referral lab will collect fresh blood specimen for HIV sero-status confirmation.
V. Specimen will be tested by referral laboratory as per the national algorithm for HIV-2 sero diagnosis.
VI. Two copies of report will be sent to the referring ART centre (both hard & soft copy) within 4 weeks.
VII. One copy of the report to be retained by the referring ART centre & original to be handed over to patient/client.

Flow chart for referring the Patient for HIV-2 Testing



Laboratory Tests for Monitoring Progression of HIV Infection and the Response to Antiretroviral Therapy

Introduction

HIV infection may progress to AIDS at different rates in different individuals. This variability makes accurately assessing the stage of infection in an individual, predicting its course, and monitoring its progression essential. Laboratory tests that can meet these needs are invaluable during the period of clinical latency. Subsequently, they supplement various clinical parameters, which are also extremely important in categorizing the infection/disease stage. The response to ARTs is also monitored using these prognostic tests.

Laboratory Tests

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

- Immunologic tests
  - CD4 T cell enumeration
- Virological assays
  - HIV RNA load assays
  - Other Assays - Measurement of HIV p24, Reverse Transcriptase (RT) activity assay

Cd4 T cell enumeration and HIV RNA load tests are well-established assays, with many techniques having gained FDA approval. Assays, based on quantitating p24 or viral reverse transcriptase, are newer techniques that are under development.

- Increased HIV replication leads to the progressive depletion of CD4 cells, HIV's main target. CD4 T cell enumeration is a very useful marker and is the most commonly used laboratory test for assessing the stage of HIV infection and monitoring its progression.

Determining the increased rate of HIV replication, reflected in an increase in plasma viral RNA load, is presently considered one of the primary tests for monitoring the progression of HIV infection. However, due to its relative ease of performance and lower cost, CD4 T cell enumeration continues to remain the mainstay for monitoring the progression of infection in HIV positive individuals in resource limited settings. In its 2010 guidelines on *"Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach"*, the WHO advises that though viral load assays may not be considered necessary for the initiation of first-line ARTs, a viral load, when available, should be used to confirm suspected clinical or immunological failure as treatment failure. This would allow better preservation of

the efficacy of second-line regimens by ensuring that these are used only when there is a clearly documented virological failure.

NACO ART centres use CD4 T cell enumeration as the routine method to monitor HIV infected individuals, to initiate ART, and for follow-up. NACO provides HIV-1 plasma viral load assays, to ART plus centres and centres of excellence. The tests are used to confirm treatment failure in HIV infected individuals who are suspected to have failed first line ART. Its results are used to decide if second-line ART should be initiated (for further details, refer to the NACO *National Guidelines on Second-line ART for Adults and Adolescents*).

Immunological tests

CD4 T cell enumeration

Assays for the enumeration of CD4 T lymphocytes have been recognized as the hallmark clinical surrogate marker for assessing the stage of HIV disease progression. A CD4+ T cell count < 350 cells/ul is considered to start ART. Further details on the test performance and technology options are provided in the NACO document entitled, *"National Guidelines on the Enumeration of CD4 Lymphocytes".*

Virological Assays

HIV Viral (RNA) Load Assays

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. Total quantification includes cell-free virus, virus in infected cells in all compartments of the body, and integrated provirus. The usual measurement of viral load is that of cell-free virus in the plasma of an infected individual. Monitoring HIV-1 viral load has become a critical standard of care for monitoring and managing the response to the ART in HIV-infected individuals and their progression toward AIDS. However, due to cost and technical feasibility, NACO has included use of Viral Load assay to PLHIV, who are recommended by SACEP to identify first line ART failure cases.

Because these tests are different and results vary between them, only one kind of test should be used to measure the viral load in an individual over time.

Viral loads are usually reported as copies of HIV in one millilitre of blood. The best viral load test result is "undetectable". The sensitivity of commercially available viral load tests cannot detect copy numbers below 20 per ml. Samples with a smaller viral load may not be picked up in the assay.

Techniques

The techniques currently available for HIV RNA load estimation are as follows:

- Target amplification assays

- Quantitative reverse transcriptase PCR (RT-PCR)
  - Amplicor HIV-1 Monitor Test, version 1.5 (Standard/ Ultrasensitive/ COBAS) (Roche)
- Real time PCR (qPCR)
  - COBAS TaqMan HIV-1 Test (Roche)
  - RealTime HIV-1 Amplification (Abbott)
  - VERSANT HIV kPCR v1.0 (Siemens)
- Nucleic acid sequence based amplification assays (NASBA)
  - NucliSENS HIV-1 QT assay (bioMerieux)
- NASBA with real time detection
  - NucliSENS EasyQ HIV-1 v2.0 (bioMerieux)
- Signal amplification
  - Branched DNA (bDNA) assay
    - VERSANT HIV-1 RNA3.0 assay (Siemens)

All these methods can quantify HIV RNA in plasma samples. Dried blood spot protocols have also been developed for some of the newer assays (e.g. COBAS TaqMan HIV-1, RealTime HIV-1, NucliSENS EasyQ HIV-1 v2.0, and Versant HIV kPCR v1.0), which could be useful in resource limited settings where viral load assessment may be limited to specific centres and samples may have to be transported.

The various performance characteristics of the FDA-approved tests are summarised in Table 5.1. The principles of the various techniques available for viral load determination are described below. Of these, the plasmaviral load assays in use at NACO designated centres in 2010, at the time of the second-line ART rollout, were the Amplicor HIV-1 Monitor Test version 1.5, the Cobas Amplicor HIV-1 Monitor Test, version 1.5, and the COBAS TaqMan HIV-1 Test (for further details regarding the NACO strategy, algorithms and methods, refer to the *National Guidelines on Second-line ART for Adults and Adolescents*).

Quantitative Reverse Transcriptase PCR

Amplicor HIV-1 Monitor Test version 1.5 (Standard, Ultrasensitive and COBAS) (Roche)

The Roche Amplicor HIV-1 Monitor Version 1.5 and RocheAmplicor 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. The amplified DNA is hybridized to specific oligonucleotide probes, followed by a colorimetric detection assay. The dNTPs include uracil instead of thymine to minimize amplicon contamination. This allows for uracil DNA glycosylase (AmpErase), which has been added to the enzyme mix, to destroy the amplicon. The quantification of HIV RNA is achieved through the extrapolation of a standard curve. The curve is generated by introducing a known number of Quantitation Standard (QS) RNA molecules into each specimen, along with the lysis reagent.

The Amplicor HIV-1 Monitor version 1.5 (v1.5) is programmed on, and approved for use on Applied Biosystems Gene-Amp PCR system 9600/9700 thermal cyclers. In Amplicor HIV-1 monitor, the specimen preparation is manual. The amplification is automated on the ABI 9600/9700 and detection is by manual ELISA or automated ELISA reader. In addition, version 1.5 has a variation in design (the Cobas Amplicor HIV-1 Monitor Test, version 1.5), which allows for the capture of amplicon on magnetic beads rather than on a microwell. Alternatively, it uses a Cobas Amplicor robotic analyser to facilitate high throughput. In Cobas Amplicor the specimen preparation is manual and the amplification and detection steps are automated. The Amplicor HIV-1 Monitor v1.5 performs equally well with all HIV-1 group M subtypes. The test can quantitate HIV-1 RNA across the range of 50-750,000 copies/ml by using a combination of two specimen preparation procedures, the Standard (dynamic range 400-750,000 copies /ml) and UltraSensitive (dynamic range 50-1,00,000 copies/ml) procedures.

Real Time PCR

Real time PCR can detect amplified DNA during the process of amplification in real time, rather than at the end of the process. It does so by using specifically designed and labelled fluorescent probes. In their native state, these probes adopt a folded structure, positioning the quencher next to the fluorescent dye. In this condition, most of the fluorescence of the dye is absorbed by the neighbouring quencher, minimizing the emitted fluorescence. When amplicons are generated, fluorescent dye-labelled probes uncoil as they hybridize to the amplicons. This separates the fluorescent dye from the quencher, thereby increasing fluorescence. Fluorescence is proportional to the original amount of HIV-RNA in the sample. This ensures a more accurate and precise quantification of nucleic acid.

COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche)

The COBAS TaqMan HIV-1 assay was the first real time PCR detection method available. The targeted viral genome is a highly conserved region of the gag gene. Quantification of HIV-RNA is made using a second target sequence, the HIV-1 QS, a known concentration of which is added to each test specimen. The QS amplicons have the same length and base composition as HIV-1 target amplicons. Detection of the QS binding region has been modified to discriminate it from the target. The use of dual-labelled fluorescent probes (the proprietary TaqMan probes) allows for the real-time detection of the accumulated PCR products, by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The amplification of HIV-RNA and the QS are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each one effectively increasing the emission intensity of the individual reporter dyes, allowing a separate recognition of HIV-1 and the QS. The exponential growth decay in the curve of the PCR amplification directly correlates with the baseline amount of genetic material. The test is able to quantify HIV-RNA over a dynamic range of 48-10,000,000 copies/ml.

RealTime HIV-1 Amplification (Abbott)

The Abbott RealTime HIV-1 assay is developed for use on the automated Abbott m2000 system. The RealTime HIV-1 assay amplifies a part of the highly conserved 172- nucleotide region of the pol gene that codes for the integrase. An internal control (IC), unrelated to the HIV-1 target, is incorporated for each assay. Exponential amplification of the product is achieved through repeated temperature cycling as in PCR. Both targets, HIV-1 and IC, are amplified simultaneously in the same reaction. The products hybridize with HIV cDNA and IC-specific probes, labelled with a different fluorescent dyes (molecular beacon probes, similar to the proprietary TaqMan probes described in the previous method). The amplification cycle, at which a specific fluorescent signal is detected, is proportional to the amount of HIV-RNA present in the original sample. The assay has a low limit of detection (40 HIV-RNA copies/ml for 1 mL, 75 copies/ml for 0.5 ml, and 150 copies/ml for 0.2 ml volumes). The upper limit of quantification is 10-million copies/ml. This assay can quantify all HIV-1 variants, including groups M, N and O.

VERSANT HIV kPCR v1.0 (Siemens)

This is another automated amplification method based on reverse transcription and kinetic PCR (kPCR) or real time PCR technology. The RNA extraction module uses magnetic silica beads. In the amplification module, purified RNA is eluted and added to a PCR plate containing an HIV-1 primer/probe and enzyme mixes. The reverse transcriptase PCR step targets a highly conserved region of the HIV-1 pol gene. The dNTPs include uracil instead of thymine to minimize amplicon contamination. This allows for uracil DNA glycosylase, which has been added to the enzyme mix, to destroy the amplicon. Both HIV and internal control RNA molecules are reverse transcribed to make cDNA and are simultaneously amplified and detected using separate dual-labelled fluorescent probes specific for HIV and control amplicons. The test requires 0.5 ml of sample. The linear dynamic range of the assay is between 35 and 11,000,000 HIV-RNA copies/ml.

Nucleic Acid Sequence-based Amplification Assay

NucliSENS HIV-1 QT Assay (bioMerieux)

The NucliSENS HIV-1 QT assay (bioMerieux) is a nucleic acid sequence-based assay. It has been approved by the FDA for the quantitation of HIV-1 RNA in plasma. The assay is based on target amplification using the NASBA technology and instrument. The NASBA assay selectively and directly amplifies HIV-1 RNA, without a PCR, in a one-step sandwich hybridization procedure. To perform this procedure, it uses two oligonucleotide primers, three enzymes (simultaneous activities of avian RT, ribonuclease H, and bacteriophage T7 RNA polymerase), nucleoside triphosphates and the appropriate buffers. In this viral load test, the virion is disrupted 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 (i.e., at a single temperature, unlike the temperature cycling required for PCR). The nucleic acid is detected and

quantified directly by measuring electrochemiluminescence. Quantitation of HIV-1 viral load is accomplished by co-amplification of three internal RNA QS specific for HIV-1 *gag* and part of *pol.*

The RNA extraction technique used in this assay allows diverse samples (plasma, cerebrospinal fluid, lymph node 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 tests, e.g., sequencing.

NASBA with Real Time Detection

NucliSENS EasyQ HIV-1 (bioMerieux)

For the NucliSENS EasyQ HIV-1 assay, primers and probes have been designed based on a well-conserved region of the gag gene. The assay is based on NASBA (as above). The molecular beacons then detect the generated amplicons (hairpin probes with a fluorescent dye and a quencher at the end),, similar to the ones used in real time PCR for the detection of amplicon formation. Quantification can be done by taking a fixed amount of included calibrator RNA as a reference. The linear dynamic range of the latest version of the assay, the NucliSENS EasyQ HIV-1 v2.0, runs from 10 to 10,000,000 HIV-RNA copies/ml when 1 ml of plasma is examined.

Branched DNA Assay

VERSANT HIV-1 RNA3.0 assay (Siemens)

The Versant HIV-1 RNA3.0 assay differs from all the above methods because it uses signal amplification instead of target amplification to measure a 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 bDNA complex. The bound bDNA is incubated with an enzyme and then with a chemiluminescent substrate.

Other Virological Tests

The assays for other viral tests are not based on RNA detection and quantitation. Instead, they measure other components of the virus in order to estimate viral load. These assays were not approved by the FDA until 2010.

Ultrasensitive p24 Assay (Perkin Elmer)

For the Ultrasensitive p24 assay, a standard ELISA format is used to capture and detect HIV p24 coupled with a specific signal amplification, to increase the assay sensitivity. Heat denaturation of the plasma, prior to binding in the ELISA step, helps to dissociate immune complexes and denature the antibodies. Heat denaturation of the plasma means that they will no longer

compete for binding to the p24 antigen, a phenomenon that has plagued earlier versions of this assay. The assay also adds a kinetic readout, using the Quanti-Kin Detection System, to increase the linear range of the assay. HIV p24 concentrations are reported as femtograms of HIV-1 p24/ml of plasma.

Reverse Transcriptase Activity Assay (Cavidi)

In the ExaVir Load (Cavidi) RT assay, the RT enzyme is separated from the virus particle using a solid-phase extraction manifold. The amount of RT enzyme is quantified using a functional assay, whereby the RT incorporates bromodeoxyuridine (BrdU) monophosphate into the DNA, using a poly (A) template bound to a 96-well plate. BrdU is then quantified spectrophotometrically using anti-BrdU conjugated to alkaline phosphatase. This is followed by the addition of its substrate. The RT activity in the unknown sample is compared to that of a recombinant RT enzyme standard with a known concentration. The extrapolated result is reported as either fg of RT/ml of plasma or as HIV-1 RNA equivalents/ml, based on a conversion factor supplied by the manufacturer. The RT assay (Cavidi) v1.0 showed good detection rates for samples with viral loads of >10,000 copies/ml. The v2.0 assay improved this detection limit to 400 copies/ml and, recently, v3.0 has also been evaluated.

Table 5.1: Comparative performance characteristics of the FDA approved assays for viral load determination*

Method	AMPLICOR HIV-1 Monitor 1.5	COBAS TaqMan HIV-1	Real Time HIV-1	NucliSENS HIV-1 QT	VERSANT HIV-1 RNA 3.0
Manufacturer	Roche	Roche	Abbott	BioMerieux	Siemens
Principle	Target amplification (quantitative PCR)	Target amplification (real time PCR with TaqMan probes)	Target amplification (real time PCR with molecular beacon probes)	Target amplification (NASBA)	Signal amplification (branched DNA)
Dynamic range (copies/ml)	Standard 1.5 (400-750,000) Ultrasensitive 1.5 (50-100,000)	48-10,000,000	40-10,000,000	80-3,470,000	75- 500,000
Specimen type	Plasma in ACD or EDTA tube	Plasma, DBS	Plasma, DBS	Plasma in ACD, EDTA, or heparin tube	Plasma in EDTA tube
Specimen volume	Standard 1.5: 0.2 ml Ultrasensitive 1.5: 0.5 ml	0.5 ml -1 ml	0.2 ml -1 ml	1.0 ml	1.0 ml- 2.0 ml
Detected subtypes	Group M (A-G)	Group M (A-D, F-H; CRF01_AE)	Group M (A-D, F, G, H; CRF01_AE, CRF02_AG), Group N, Group O	Group M (A-G)	Group M (A-G)
Area of HIV genome selected for amplification	Gag	gag	pol	gag	Pol

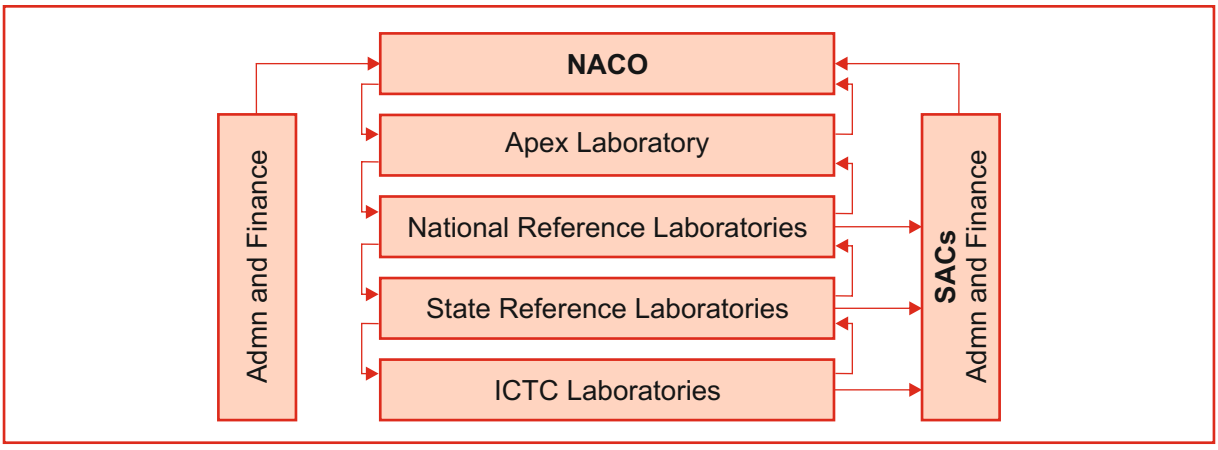
** Brand names used in this document are only for references.*

The Management of HIV Testing and Reference Laboratories

HIV testing in the National AIDS Control Programme (NACP) is performed by the Integrated Counselling and Testing Centres (ICTCs) and Blood Banks. Quality control for HIV testing is implemented through a chain of SRLs, NRLs and Apex Laboratory.

Table 7.1. Roles and responsibilities of laboratories under the NACO laboratory network

Level	Functions
Tier 1: Apex Laboratory: NARI	To provide technical assistance to the NACP To provide leadership to the laboratory network through NRLs Mentoring and monitoring of NRLs Training and capacity building of NRLs Implementing proficiency testing (EQA) for NRLs
Tier 2: National Reference Laboratories (13)	Mentoring and monitoring of SRLs Training and capacity building of SRLs Implementing proficiency testing (EQA) for SRLs Resolving the retesting discordant results from SRLs
Tier 3: State Reference Laboratories (117)	Mentoring and monitoring of ICTC Labs Training and capacity building of ICTC Labs Implementing proficiency testing (EQA) for ICTC Labs Quarterly rechecking of samples tested (20% positive and 5% negative) at ICTC labs as quality control Resolving the retesting discordant results from ICTC Labs
Tier 4: ICTC Labs (15,606)* Stand alone ICTC: 5694 Mobile ICTC: 124 F-ICTC : 8810 PPP (Public and Private Partnership) ICTC: 1977 * Till March 2014.	Perform HIV tests as per national guidelines Participating in proficiency testing and quality control activities Participate in training activities conducted by SRLs



Chapter 8

Operational Management of NACO Laboratories (National Reference Laboratory, State Reference Laboratories & ICTC)

Introduction

This chapter underlines the policies and procedures for proper design, execution and uninterrupted operations of the NRL, SRL and ICTC laboratories to ensure quality services on a day-to-day basis.

Scope

The scope of the laboratory defines its operating unit and its activities. In the case of an ICTC it includes counselling, sample collection, sample processing and testing for the diagnosis of HIV infection; whereas NRLs and SRLs facilitate the quality of testing at the ICTCs, along with training and surveillance activities. The laboratories should define their scope accordingly.

Organisation

The laboratory should have an organogram with the designation, name, and contact details of every member of the staff associated with the laboratory. The staff should be aware of the organogram, which should be designed in such a way that staff backup is available at every level in their absence.

Laboratory Arrangement

The laboratory must have separate designated areas for counselling, sample collection, washing, workstation, storage, and documentation. Steps must be taken to ensure privacy in the counselling and collection rooms. Each area must be properly labelled with biohazard symbols displayed in the workstation area and there restricted access to the laboratory.

Role and Responsibility

The role and responsibility of each member of the staff must be defined and documented. Staff must be aware of their individual, as well as collective role and responsibility. The following are some of the responsibilities of the laboratory staff:

- Daily assignment of work to technician (by laboratory in-charge)
- Monitoring of the laboratory environment, including temperature log and cleaning log
- Ensuring proper biosafety including PPE
- Stock verification (physical) - at the beginning and the end of each day
- Preparation of fresh 0.1 percent and 1 percent sodium hypochlorite solution every morning.

Client Flow

The laboratory must have the client flow displayed in the form of signage. The client should be received at reception, or the counselling room, where the list of tests done in the laboratory with the turn around time are clearly displayed. The client is then directed to the sample collection room after proper pre-test counselling and informed consent. The reports are given to the client within the turnaround time after post- test counselling.

Services, Complaints and Redressal

The clients must have access to the feedback forms where s/he can write or comment on the service(s) provided, including complaints. These forms can be deposited / posted in the feedback/complaint box, which should be locked. The ICTC in-charge will open the box at regular intervals to take the necessary actions for the redressal of complaints, if any. A record of all the complaints/feedbacks received and actions taken must be maintained.

Testing Workflow

The laboratory must have the SOPs for each and every procedure undertaken in the lab. The SOPs must be approved by the in-charge. All the SOPs must be reviewed at least annually and revised in case of any change in procedures. All staff must be familiar with the SOPs. SOPs should be made available/displayed in the work area.

- **Collection/ Receipt of Specimen**

  Specimen collection tubes should be labelled before withdrawing blood. A specific PID number, assigned by the counsellor, along with another identifier (e.g., age, sex) should be written on the tube.

  Clients should be made to sit comfortably and explained the procedure of specimen collection. All standard precautions should be observed at all times. Whole blood specimens should be collected by venepuncture after taking all the aseptic precautions (refer to Chapter 2).

  Specimens should be received in the laboratory only after ensuring that they meet the specimen acceptance criteria of the laboratory.

- **Maintenance of Records and Register Entry**

  Entry of the client details is done in the counsellor's room in the PID register. This includes the serial number, PID number, date of visit, name, address, district, and phone number. The counsellors should also maintain the ICTC register, which is for general clients. The details of the pregnant women visiting ICTC should be recorded in a separate register. All the details are entered in the form of the codes given.

  The ICTC technician maintains the laboratory register. It includes the serial number, date of receipt of sample, the PID number, name of the referring ICTC, and the sample number given

by the laboratory. The results of HIV tests (1, 2, and 3) and the final test report should be recorded and given with signatures and names. The counsellor should then enter the results into the PID register. A separate register must be maintained for specimen sent to other laboratories (SRL/NRL) for quality control or confirmation. Records of proficiency testing should also be maintained.

- **Separation of sera** (Refer to chapter 2)

- **Test kits taken out of the refrigerator and brought to room temperature**
  FEFO (i.e., "first expiry first out policy") is to be followed while using stored kits. All the kits required for HIV testing should be taken out half an hour before performing the tests to allow them to reach room temperature. Check the availability of relevant testing SOPs on the workbench, match kits with SOPs and go through them. Perform rapid tests, including the retesting of reactive samples (as per the strategy/algorithm). All the tests should be performed according to the manufacturer's guidelines, following the NACO strategy/algorithm.

- **Reading and Interpretation of Test Results**
  The test results should be read within the time limit specified by the manufacturer and the interpretation should also be done according to the manufacturer's guidelines. All the results should be recorded in the appropriate laboratory register. Testing devices and tested materials should be discarded in 1 percent sodium hypochlorite solution and allowed a minimum contact period of half hour.

- **Storage and Archiving of Samples** (as and when required)
  Samples may be stored in the refrigerator at 2-8^{o}C for a maximum period of up to 7 days. A deep freezer (-20^{o}C) is required to store the samples for a longer duration. It should be ensured that the samples are not placed along with the kits in the same shelf of the refrigerator. The laboratory should store an aliquot of the specimen, sent for quality control or confirmation, until the final report is received.

Troubleshooting

Despite following the policies and procedures stringently, a test run may fail. Troubleshooting refers to the measures undertaken to determine why a run has failed. The laboratory should have specific protocols laid down for troubleshooting

Further Reading: *National Guidelines on Quality Management System In HIV testing laboratories*

Disinfection of work area and waste management and disposal

The disinfection and waste management protocols in the laboratory must be as per guidelines (refer to chapter 10).

Record and Document Archiving

Proper recordkeeping of client results is vital for providing quality service, tackling the medico-legal issues, and operational research. As per the guidelines, all documents must be stored for at least 5 years or as per local guidelines whichever is longer. Documentation for ICTCs and SRLs should be done separately in those centres which have both facilities.

The following test details should be documented in the test register on the same day the tests is completed:

- Name of kit
- Lot and batch number
- Date of expiry
- Date of opening
- Recording of readings of the test, internal and external controls
- Name and signature of the laboratory technician and the laboratory in-charge.

Preparation of Reports in the Prescribed Format

- Report is prepared and signed by the technician in the prescribed format
- Report is reviewed and signed by laboratory in-charge
- Report should be handed over to counsellor (with receiving signature).

Other Responsibilities of NRL, SRL and ICTC Laboratories

- **Preparation and Regular Review of SOPs**

  SOPs should be prepared by the laboratory technician/technical officer and be approved by the in-charge. Revision of the SOPs should be done at least once a year. However SOPs should also be revised as and when required, especially if kit procedure or a kit is changed. The revision and version number must be documented, dated, and signed by the in-charge. out dated version of SOPs must be removed and archived.

- **Panel Sera**

  An NRL is to prepare panel sera for the proficiency testing of SRLs and ICTCs. The SRL has to carry out the testing of the panel sera and send the report to the NRL within a turnaround time. The SRL also has to aliquot and distribute the bulk panel to their respective linked ICTCs and then send the compiled report to the NRL. An ICTC carries out testing on panel sera and sends report to SRL within the stipulated turnaround time.

- **Collection of Kits from SACS/ NACO Designated Storage Centres**

  The technician has to bring the kits from the designated centre, ensuring that a proper cold chain is maintained, and store them in the refrigerator. It must be ensured that the kits are

stored in a separate refrigerator or on a separate shelf. Requirements have to be written in the indent book and signed by the In-charge.

- **Inventory Control**

The laboratory should have a well-defined inventory management system designed to ensure the following:

- An uninterrupted reagent supply to prevent reagent stock outs
- A three month buffer stock should be maintained and the re-order levels chalked out accordingly.
- Availability of other consumables (e.g. pipette tips, gloves, needles, syringes, vacutainers, sodium hypochlorite solution)
- Details of all the kits, consumable items like gloves, hypochlorite, tissue paper, stationary, detergents, needle, syringes, test tubes, blotting sheets etc. should be entered in the respective stock register.

- **Calibration and Preventive Maintenance of Equipment**

  The laboratory in-charge must ensure that all equipment used in the ICTC laboratory/SRL, including thermometers (for temperature logging of refrigerators), centrifuges, pipettes etc. must be calibrated. The required SOPs should be in place and strictly adhered to for the calibration of equipment.

  Annual maintenance contracts must be in place for the equipment. All equipment should be entered into the equipment stock register. Separate files should be maintained for each piece of equipment with all the necessary information (e.g., date of purchase and installation, validation certificate, annual maintenance contract, service reports).

- **Participation in the EQA Programme, Root-cause analysis, and Corrective Action for Erroneous Results**

  The laboratory must have a QA program including participation in the National EQA and QC. In case of erroneous results, the root-cause must be identified and necessary corrective action taken and documented to prevent recurrence.

- **Participation in Workshops (ICTC)/Organization of Workshops (SRL)**

  The ICTC technicians must participate in the workshop organised by the respective SRL and also undergo refresher trainings from time to time. The evidence of participation must be documented in their personnel files.

- **Collection of DBS for HIV Diagnosis in Infants and Children <18 Months**

  Designated centres assigned this task must refer to and follow the Laboratory Guidelines for HIV Diagnosis in Infants and Children <18 months, formulated by NACO.

- **Participation in Sentinel Surveillance Cycles**

  The laboratory in-charge must ensure participation of the ICTCs/SRLs designated as sentinel sites/laboratories in sentinel surveillance activities. They should follow the current guidelines laid down by the programme.

- **Management and Recording of Adverse Incidents**

  The staff must be familiar with the protocols to be followed in the laboratory in the event of

any adverse incident (e.g., spillage, needle-stick injury, splash, fire) All such incidents, including how they were managed, must be documented. The contact details of the person responsible for providing the preventive dosage of ARTs should be known by all the staff and displayed in the laboratory.

- **Indeterminate, Discordant, HIV-1/2 and HIV-2 Samples**
  Indeterminate and discordant samples must be sent by ICTCs to SRLs. The SRL should confirm these from the NRL and give necessary feedback to the client and ICTCs respectively. All HIV-2 positive samples and HIV-1/2 cross-reactive samples must be sent to HIV-2 referral laboratories (Refer Operational Guidelines for HIV2 Diagnosis: 2013).
- **Redressal of Complaints Regarding Kit Quality**
  Complaints regarding kit quality must be communicated to SACS and a copy should be sent to NACO. The complaint, along with the particular kit, should be sent to the SRL. The SRL should test 20 to 25 specimens, with known HIV status, with that kit and communicate the report to the ICTC, SACS, Apex laboratory and NACO. SACS/NACO will take appropriate action and communicate the same to all concerned.
- **RPR Testing**
  All the patients referred from the STI/RTI centre are to be screened for syphilis along with HIV testing. The STI/RTI counsellor must send the patient with the filled up referral form (annexure III) to the ICTC. The collection of samples for both tests (i.e. RPR and HIV) must be done at the same time in the ICTC. RPR test reports must be given to the patient separately in the prescribed format (Annexure IV & V).

Audit and Review

Regular pre-scheduled review meetings, with all the staff, should be held by the laboratory In-charge to discuss all issues with the ultimate aim of providing quality service to the clients. Internal and external audits and management review meetings (MRMs) with the senior management should be done once or twice a year. The minutes of all meetings should be documented.

Further Reading: *National Guidelines on Quality Management System In HIV testing laboratories*

Chapter 9

Occupational Exposure and Post Exposure Prophylaxis

Introduction:

This chapter deals with: the occurrence of occupational exposure of healthcare workers to some blood-borne viral pathogens (e.g., HIV, HBV and hepatitis C virus – HCV); ways of minimizing/preventing such exposures; and the need for prompt institution of Post Exposure Prophylaxis (PEP) after such exposure. In the occupational settings, there is a risk that healthcare providers will be exposed to blood borne pathogens like HIV, HBV, and HCV during working hours. An exposure that may place a HCP at risk of blood-borne pathogens is defined as: a percutaneous injury (e.g., needle stick or cut with a sharp instrument); contact with the mucous membrane 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 the intact skin when the contact duration is prolonged (e.g., several minutes or more) with blood or other potentially infectious body fluids.

Potentially infectious body fluids include blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid or other body fluids contaminated with visible blood. The following are not considered potentially infectious, unless visibly contaminated with blood: Faeces, nasal secretions, saliva, sputum, sweat, tears, urine and vomitus. Transmission through intact skin has not been documented. Any direct contact (i.e., contact without barrier protection) with the concentrated virus in a research laboratory or production facility requires clinical evaluation.

Factors that influence Risk of Infection

Various epidemiological and laboratory studies have shown that the risk of infection, following exposure, varies with the type of exposure:

- 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 (viral load) in the exposed blood/body fluid at the time of exposure
- Timely (<2 hours and up to 72 hours) availability and efficacy of the PEP.

Practices that Reduce Risk of Exposure

Prevention of exposure to the virus remains the mainstay of occupational infection prevention. Some simple procedures can limit the risk of infection to HCPs. Guidelines formulated/published by the CDC, known as the Standard Precautions (1996; formerly

Universal Precautions, 1987), should be followed for handling blood and body fluids – including all secretions and excretions (serum, semen, vaginal secretions) – by all HCPs at all times.

The Essentials of Standard Precautions and Safe Laboratory Practices

- Blood, blood products, all body fluids, and materials contaminated with them are considered as infectious for HIV, HBV, HCV, and other blood borne pathogens.
- Use appropriate barrier precautions to prevent exposure to skin and mucous membranes. Wear gloves, gowns/aprons, masks, and goggles, while handling all potentially infectious material.
- Take special care of handling sharp objects (like needles, lancets, scalpels, etc.) to avoid injuries:
  - Avoid unnecessary use of sharps and needles
  - Disposable needles should be used
  - Handle hollow bore needles with care as it may lead to deep injuries
  - Never recap needles
  - Never break/bend needles by hand
  - Needles/sharps should not be left on trolleys and bed side tables and must be disposed of immediately
  - Never pass used sharps from one person to another directly
  - Dispose sharps in a puncture resistant container containing one percent sodium hypochlorite solution
- Thoroughly wash hands with water and soap after removing gloves, handling infectious materials, before leaving the laboratory area, and immediately after any contamination of skin surfaces.
- Work surfaces should be decontaminated with 0.1 percent sodium hypochlorite solution.
- Laboratory personnel should refrain from mouth pipetting, eating, drinking, or smoking in the work area.
- Access to the laboratory should be limited to trained personnel only.
- Food and drink must be stored in refrigerators in areas other than the work area.
- All HCPs must be immunized against HBV.

Dos and Don'ts for the Exposed Individual

Don'ts

- Do not panic
- Do not place the pricked finger into the mouth reflexively
- Do not squeeze blood from wound
- Do not use bleach, alcohol, iodine, antiseptic, detergent, etc.

Do's

- Stay calm

- Remove gloves, if appropriate
- Wash exposed site thoroughly with running water and soap. Irrigate thoroughly with water, if splashes have gone into the eyes or mouth
- Consult the designated physician/personnel immediately as per institutional guidelines, for management of the occupational exposure.

Steps of Post Exposure Management

Steps to be followed after accidental exposure to blood/other potentially infectious materials:

1. First aid
2. Risk assessment
3. Informed consent and counselling
4. Decision on prophylactic treatment for HIV and HBV
5. Monitoring and follow up of HIV, HBV, and HCV status
6. Documentation and recording of exposure

First Aid: Management of Exposed Site

For skin: if the skin is broken after a needle stick or sharp instrument, immediately wash the wound and surrounding skin with water and soap, and rinse. Do not scrub. Do not use antiseptics or skin washes (e.g., bleach, chlorine, alcohol). Wash the area immediately after a splash of blood or bodily fluid on unbroken skin. Do not use antiseptics.

For the eye: Immediately irrigate the exposed eye thoroughly with water or normal saline. If wearing contact lenses, leave them in place while irrigating. Once the eye is cleaned, remove the contact lens and clean them in a normal manner. Do not use soap or disinfectant on the eye.

For mouth: Spit fluid out immediately. Rinse the mouth thoroughly using water or saline and spit again. Repeat the process several times. Do not use soap or disinfectant in the mouth.

*The exposure must be reported immediately to the concerned authority, so that, if required, prophylaxis can be started as soon as possible. Consult the nearest resource/ART centre for PEP, evaluation and follow up (as per National Guidelines on PEP).

Assessment of Infection Risk

A designated person/trained doctor must assess the risk of HIV and HBV transmission following an accidental exposure to blood (AEB). This assessment must be quick so as to start treatment/prophylaxis without any delay.

After an AEB (for percutaneous exposure) an HIV seroconversion rate of 0.3 percent is an average rate. The risk of infection transmission is proportional to the amount of HIV transmitted, which depends on the nature of exposure and the status of the source patient.

Assessing the Nature of Exposure

Three categories are described, based on the amount of blood/body fluid and the entry port (Table 9.1.). These categories are intended to help in assessing the severity of the exposure, but may not cover all the possibilities.

Table 9.1. Assessing the nature of exposure

Category	Definition and example
Mild Exposure	Mucus membrane/non-intact skin with small volume, e.g., a superficial wound (erosion of the epidermis) with a plain or low calibre needle; contact with the eyes or mucous membranes; subcutaneous injections following small bore needles.
Moderate Exposure	Mucus membrane/non-intact skin with large volumes or percutaneous superficial exposure with solid needle (e.g., a cut or needle stick injury penetrating gloves).
Severe Exposure	Percutaneous with large volume, e.g., an accident with wide bore needle (>18G) visibly contaminated with blood; a deep wound (haemorrhagic wound and/or very painful); transmission of a significant volume of blood; an accidental injury with material, which has previously been used intravenously or intra-arterially.

Assessing the HIV Status of the Source of Exposure

Evaluation of the Source (Table 9.2)

- If the HIV status of the source person is known and confirmed as negative, PEP is not required.
- 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 upon the severity of the wound and how much is known about the individual's HIV risk history.
- If the status of the patient is unknown, and the patient is available, he is to be counselled and consent obtained for testing.
- If the patient refuses testing but a sample of blood is available, it is the right of the exposed person that the blood sample be tested but the source patient may decline to be informed of the result.
- 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.
- 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 naive
  - Whether he/ she is on ARTs 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< 400 copies /ml
- High risk: Symptomatic with OI or AIDS, acute seroconversion, high viral load.
- In the case of a high-risk exposure from a source patient on ARTs, consult an expert to choose the appropriate PEP regimen if drug resistance is high.

Table 9.2. Assessing the HIV status of the source of exposure

HIV status of the source	Definition of risk in source
HIV negative	Source is not HIV infected (but consider HBV and HCV)
Low risk	HIV positive and clinically asymptomatic
High risk	HIV positive and clinically symptomatic
Unknown	Status of the patient is unknown and neither the patient nor his/her blood is available for testing

Informed Consent and Counselling: Almost every person feels anxious after exposure. They should be counselled and psychological support provided. They should be informed about the PEP. Exposed persons should receive appropriate information about the risks and benefits of PEP medications. It should be clear that PEP is not mandatory. Exposed persons should, however, be made to understand that a few cases of transmission have been seen in cases given prophylaxis.

Documentation of exposure is essential. For prophylactic treatment the exposed person must sign a consent form. Informed consent also means that if exposed, the person has been advised on PEP. If the individual refuse to initiate PEP, it should be documented. The designated officer for PEP should keep this document. An information sheet covering the PEP and the biological follow up after any AEB must be given to the person under treatment.

Decision on PEP Medications/Regimen: Because PEP has its greatest effect if started within 2 hours of exposure it is essential to act immediately. Ideally, therapy should be started within 2 hours and definitely within 72 hours of exposure. (Table 9.2).

Never delay starting therapy due to uncertainty. Re-evaluation of the exposed person should be considered within 72 hours post exposure, especially if additional information about the exposure or source person becomes available. If the risk is insignificant, PEP could be discontinued, if already started. Exposed individuals who are known or discovered to be HIV positive should not receive PEP. The decision to start this type of regimen depends on the type of exposure and the HIV status of the source person mentioned earlier.

Dosages of the Drugs for PEP for adults and adolescents- FDC of Tenofovir (TDF) 300 mg plus Lamivudine (3TC) 300 mg plus Efavirenz (EFV) 600 mg once daily for 4 weeks. If the source is already on ART, start the exposed person the above mentioned regimen at the earliest with proper counseling and then refer for an expert opinion.

Seek an expert opinion in case of:

- Delay in reporting exposure (> 72 hours)
- Unknown source
- Known or suspected pregnancy, but initiate PEP
- Breastfeeding mothers, but initiate PEP
- Source patient is on ARTs
- Major toxicity of PEP

While the Source Patient is on ART: The physician should consider, the comparative risk represented by the exposure, taking into account the source's history of and response to ARTs (based on clinical response), CD4 cell count, viral load measurements (if available), and current disease stage. If the source person's virus is known or suspected to be resistant to one or more drugs considered for PEP regimen, then the exposed person needs to be given alternate PEP drug regimen and referred for expert opinion.

Pregnancy and PEP: If the concerned HCP is pregnant at the time of occupational exposure to HIV, she should get the regimen for primary management of the exposure, like non-pregnant persons. Pregnant women, who sustain occupational exposure, should also be offered anti-retroviral chemoprophylaxis, if required. The designated authority/physician must be consulted about the use of ARTs for post-exposure management. For a female HCP considering PEP, a pregnancy test is recommended in case of a doubt.

Side effects and Adherence to PEP: Studies have indicated more side effects, most commonly nausea and fatigue among HCPs taking PEP than PLHAs taking ARTs. These side effects occur mainly at the beginning of the treatment and include nausea, diarrhoea, muscular pain and headache. The person taking the treatment should be informed that these may occur and should be dissuaded from stopping the treatment as most side effects are mild and transient, though possibly uncomfortable. Anaemia and/or leukopenia, and/or thrombocytopenia may occur during the month of treatment.

Adherence information and psychological support are essential. More than 95 percent adherence is important to maximise the efficacy of the medication in PEP. Side effects can be reduced through medications. A complete blood count and liver function test (transaminases)

may be performed at the beginning of treatment (as baseline) and after 4 weeks.

Post-exposure Prophylaxis Against HBV and HCV: Post exposure prophylaxis regimens against HBV depend upon the HBV status of the source, type of exposure, and previous immunization status of the exposed person.

Management of Individuals Exposed to HBV and HCV: There is no PEP regimen recommended for HCV; however, there are specific steps that can be taken to reduce the risk of infection for those exposed to HBV as described below.

Post-exposure Prophylaxis for HBV

A person's response to HBV exposure depends on his or her immune status, as determined by the history of hepatitis B vaccination and vaccine response, if tested 1 to 2 months after vaccination (see Table 9.3), and whether the exposure poses a risk of infection. HBV PEP is safe for women who are pregnant or breastfeeding.

Monitoring and Follow-up of HIV, HBV and HCV Status

Follow up of exposed HCPs:

- Long term follow up, counselling, and education
- Testing for at least 6 months after exposure (6 weeks, 12 weeks and 6 months)
- If PEP is used: drug toxicity monitoring at base line and after 2 weeks.

Table 9.3 Recommendations for HBV post-exposure prophylaxis, according to immune status

HBV immune status	Post-exposure prophylaxis
Unvaccinated	HBV vaccination and HBIg
Previously vaccinated, known responder (Anti-hepatitis B surface antigen positive)	None
Previously vaccinated, known non-responder	HBV vaccination and HBIg
Antibody response unknown : Test; if antibody response is < 10 IU/ml,	HBV vaccination and HBIg

The person should be provided with pre-test counselling and PEP should be started as discussed above. Before starting PEP, 3-5 ml of the person's baseline 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. Otherwise, it may be difficult to attribute the infection acquired due to exposure in the occupational setting. This may

have bearing on the claims for compensation from the health authorities. **The first sample for HIV testing is collected immediately after exposure, the 2nd at 6weeks, the 3rd at 12 weeks, and the last at 6 months after exposure.**

During the follow up period, especially the first 6-12 weeks, the following measures are to be adopted by the HCP: refraining from blood, semen, organ donation and abstinence from sexual intercourse. In case, sexual intercourse is undertaken, a latex condom must be used to reduce the risk of HIV transmission. Women should not breast feed their infants. The exposed person is advised to seek medical evaluation for any febrile illness that occurs within 12 weeks of exposure.

Table 9.4 Laboratory follow up after exposure

Timing	Tests done (irrespective of PEP intake)
Baseline	HIV, Anti-HCV and HBsAg
Month 3	
Month 6	

Documentation and Recording of Exposure:

The exposure report details should include:

- Date, time, and place of exposure
- Type of procedure done
- Type of exposure: percutaneous, mucus membrane, etc.
- Duration of exposure
- Exposure source and volume; type of specimen involved

N.B: All persons at risk must be informed of the importance of immediate exposure reporting to ensure that preventive care can be initiated in time to be effective and to remember that, a casual approach leads to casualty.

Occupational Exposure and Rights of the Exposed Persons: If transmission of a blood-borne infection occurs after occupational exposure has been documented, the HCP has a right to receive treatment and care for this illness. It is also the right of all infected persons to be free from discrimination at their workplace. Such persons are entitled to have all their human rights respected, beginning firstly with the right to confidentiality regarding their healthcare.

Chapter 9

Summary

In an occupational setting, there is risk that HCPs will be exposured to HIV, HBV and HCV. Accidental exposure to these pathogens may occur through injury by sharps and needles and exposures to mucous membrane by blood and other potentially infectious body secretions and excretions.

Such exposures can be minimized by HCPs following standard precautions at all times; by treating all patients/specimens as potentially infectious; by avoiding unnecessary invasive intervention wherever possible; by using appropriate barriers and PPE to prevent exposure to blood and body fluids; through the safe handling of sharps and their proper disposal to avoid injuries; by thoroughly washing hands after removing gloves; and by properly disinfecting work places and sterilizing used up articles, etc.

If exposure occurs, despite these measures, immediate care for the exposed part and the person; its documentation and reporting; the starting of appropriate PEP medication regimens after the proper assessment of such exposure; counselling, if indicated per national guidelines; and subsequent follow-up will be beneficial.

For the management of such occupational exposures, each institution should have an infection control team and policy arrangements for the prompt and uninterrupted (round the clock) supply of PEP drugs as per national guidelines. All HCPs should be vaccinated against Hepatitis B.

Legal and Ethical Issues In HIV Testing

Introduction

A draft bill, regarding the legal and ethical issues surrounding HIV/AIDS, has been prepared by NACO. Additionally, Goa has enacted the 1986 Goa Public Health Amendment Act which applies to the state of Goa. There are fundemental, legal and ethical issues regarding HIV testing, confidentiality, consent, discrimination, etc. which need to be discussed at the national level. Many countries, as well as the World Health Organisation, have put forward certain recommendations on these issues. These issues may be relevant to India as well and are discussed below.

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

Ethics of Prevention and Care

HIV is most commonly transmitted during sexual intercourse, both homosexual and/or heterosexual, through contaminated needle sharing, as in IV drug use, by a pregnant mother to her infant and through accidental exposure while providing care to PLHA. AIDS is also primarily a behaviourally transmitted disease and the transmission of HIV can only be interrupted through modifications in behaviour, e.g., using a condom during sexual intercourse, not sharing needles, and using therapeutic intervention to reduce mother to child transmission of HIV.

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

The issues which need to be debated to prevent the 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 respect must also extend to cover their culture values. 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. An example of the conflicting views which exists involve the education of MSMs and IDUs to modify their behaviour (use of condoms and provision of sterile needles through needle exchange programmes) to protect themselves

and others. Conservative view these strategies as "legitimizing" homosexuality, extramarital sex, and encouraging drug use. This opposition from conservatives on the basis of moral limits the state's first ethical responsibility, i.e., to protect the vulnerable.

Sex education, the empowerment of women (so that they are able to control their sexual life), and the provision of condoms challenge the social norms of societies where women are subordinate and subjugated for the control of AIDS, the consensus is to use voluntary public health strategies which emphasize mass education, counselling, and respect for privacy.

Discrimination/ Right to Work

The realization that HIV/AIDS was a disease of the socially marginalised led to the discrimination of HIV-infected by employers, landlords, school personnel, and even some healthcare professionals. Some other areas where PLWHA face discrimination include education, insurance, and travel. Community education programs, legislation, and public health policies can help in reducing this discrimination. HCPs should require anti-discrimination education. All health service providers should have policies in place which prevent discrimination of PLWHA from HCPs. If this does not happen, ICTC services may be limited due to a fear of discrimination and it may reduce the rate at which people return to collect their results.

In the context of employment discrimination on the basis of HIV status, there have been several progressive judicial pronouncements upholding an HIV positive person's right to work. The most notable among these being the honourable Bombay High Court's decision that an otherwise qualified person cannot be terminated from service unless he is medically unfit to perform the job's functions or poses a significant risk to others at work.

In this context, the relevant/central message from the CDC guidelines was that: "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 the healthcare setting, standard blood and body fluid precautions would protect the healthcare workers from not only HIV but from far more infectious diseases (e.g., hepatitis B and C). Thus, there is no ground for mandatory HIV testing and no possible ethical foundation for discriminating against PLWHA.

The US Centers for Disease Control and Prevention (CDC) has estimated the risk of transmission from an HIV infected surgeon during an operation to be between 1 in 42,000 to 1 in 420,000. Despite these estimates there is no documented transmission from an infected healthcare worker to a patient.

Confidentiality and HIV/AIDS

Confidentiality arises when there is a confidential relationship dependent on factors of trust, knowledge, and skill (e.g., doctor- patient relationships). ICTCs have an enormous responsibility to protect the confidentiality of clients due to the stigma attached to the disease. Maintaining the confidentiality of individual patients is crucial to protecting public health. People, particularly those at high risk, will only access ICTC services if they are assured of the confidentiality of those services. The confidentiality of physician-patient encounters is a basic medical ethic, 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 ethical codes 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, for the need to protect the welfare of the individual, or for public interest. Civil and criminal penalties may ensue for unlawful disclosure of HIV positive status due to 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 other individuals.

When necessary, exceptions to confidentiality are appropriate for protecting public health and individuals (including healthcare workers) who are endangered by persons infected with HIV. If a physician knows that a HIV seropositive individual is endangering a third party, the physician should, within the constraints of the local law: 1) attempt to persuade the infected patient to cease endangering the third party; 2) if persuasion fails, notify authorities, and 3) if the authorities take no action, notify the endangered third party.

Some state statutes make exceptions to confidentiality laws with regard to the spouse and sexual partners of the patient. Other state statutes make no such exceptions. The decision about whether to breach the confidentiality remains with the physician and are not 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 in the community's public health system.

Disclosure: Ideally, the disclosure of a person's HIV status should not in any way affect their

rights to employment, their position at the workplace, their right to medical care, and other fundamental rights.

No person shall be compelled to disclose his HIV status except by an order that the disclosure of such information is necessary in the interest of justice for the determination of issues in the matter before it.

No healthcare provider, except a physician or a counsellor, shall disclose the HIV positive status of a person to his or her partner as per law, time to time.

Courts have permitted disclosure in the following situations:

1. Required by law (statutory requirement)
2. Administration of justice
3. In the best interest of the patient (disclosure to a medical team if necessary for the treatment of the patient)
4. To protect another person (partner notification)
5. Necessary for public interest

Informed Consent for HIV Testing

ICTCs require a patient's informed consent. This policy on consent is important because HIV is not curable, its implications are life threatening, and the stigma attached to HIV is unprecedented. In addition, counselling (a confidential dialogue between a client and a counsellor, aimed at enabling the client to cope with stress and make a personal decision related to HIV/AIDS testing) is needed. A physician who performs any invasive procedure on a patient can do so only 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 1) the fiduciary relationship between a patient and a physicians, and 2) protection to the concept of patient autonomy. Informed consent applies to HIV testing and must be real informed consent and not implied consent.

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

Testing should always be accompanied with counseling.

Informed consent for testing and disclosure must be in writing. "If it is not documented, it did not happen," literally applies to informed consent for HIV testing.

Specified exceptions 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 of a minor or of an incompetent patient can be undertaken with a guardian's consent. Proxy consent could be given when a client is incapacitated (physicall ill / mentally unsound) and is unable to give consent.

HIV testing should not be undertaken without written informed consent due to the issues of confidentiality, discrimination, victimization, and psychological harms and burdens raised by an HIV positive result.
Informed consent is also a pre requisite for conducting research.

HIV Testing

HIV/AIDS involves issues of privacy, communal health, social and economic discrimination, coercion and liberty. HIV testing outside the context of blood banking, evokes a great deal of controversy and debate regarding issues such as; should individuals at high risk be required to be tested; how and who will counsel those planning to be tested regarding the implications, for themselves and others, of a positive test; what about confidentiality; and 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. With the exception of clearly defined circumstances, HIV testing has to be done under conditions of voluntary, informed consent and results need 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 only be undertaken voluntarily after counseling for behavior change, for clinical purposes, for seroprevalence studies, for ensuring safety and for research.

There has been and is a lot of debate on routine mandatory testing. Mandatory testing is not cost-effective and is rather counterproductive. Voluntary screening after counseling of identified high risk groups is more effective and productive for behavior change and case management. The safety of healthcare workers does not improve with routine mandatory HIV testing. Instead, the practice of upholding standard work precautions is far more beneficial due to the restrictive window period for HIV testing and the possibility of contracting other blood transmitted infections, like hepatitis B and C. UNAIDS/WHO does not support the mandatory testing of individuals, even when it comes to employment or for providing healthcare facilities.

General Medical Council (GMC), UK in bibliography - *Ethical Aspects on HIV/AIDS*

- The doctor/patient relationship is founded on mutual trust and respect.
- Doctors will extend the same high standard of medical care and support to PLWHA that they offer 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.

- Doctors should seek advice from competent local consultants regarding the limits of their clinical practice so they do not put their patients at risk of HIV infection.
- Doctors infected with HIV have the same rights to confidentiality and support as offered to other patients.
- Explicit informed consent is a must for HIV testing. Only in the most exceptional circumstances can testing without explicit consent be justified.
- Confidentiality of results must be maintained. Only under extreme circumstances, when the clinicians feels that withholding the test results will put either his/her colleagues or others at risk, can confidentiality be breached. When breaching confidentiality, test results should only be shared in a limited manner with relevant individuals.

List of Annexure

Annexure 1

List of National Reference Laboratories (NRLs)

1 All India Institute of Medical Sciences (AIIMS), Delhi

2 Christian Medical College (CMC), Vellore, Tamil Nadu

3 Institute of Preventive Medicine (IPM), Hyderabad, Andhra Pradesh

4 Madras Medical College (MMC), Chennai, Tamil Nadu

5 National AIDS Research Institute (NARI), Pune, Maharashtra

6 National Centre for Disease Control (NCDC), Delhi

7 National Institute of Biologicals (NIB), Noida, Uttar Pradesh

8 National Institute of Cholera and Enteric Diseases (NICED), Kolkata, West Bengal

9 National Institute of Immunohaematology (NIIH), Mumbai, Maharashtra

10 National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, Karnataka

11 Regional Institute of Medical Sciences (RIMS), Imphal, Manipur

12 School of Tropical Medicine (STM), Kolkata, West Bengal

13 Tamil Nadu Dr M G Ramachandran (TN Dr MGR) University, Tamil Nadu

Annexure 1

List of NRLs and related State Reference Laboratories (SRLs)

1 All India Institute of Medical Sciences (AIIMS), Delhi

i Government Medical College, Amritsar, Punjab
ii Government Medical College, Patiala, Punjab
iii Indira Gandhi Medical College, Shimla, Himachal Pradesh
iv Postgraduate Institute of Medical Education and Research, Chandigarh

2 Christian Medical College (CMC), Vellore, Tamil Nadu

i Government Medical College, Kottayam, Kerala
ii Government Medical College, Kozhikode, Kerala
iii Government Medical College, Thiruvananthapuram, Kerala
iv Government Medical College, Thrissur, Kerala
v Tirumala Devaswom Medical College, Alappuzha, Kerala

3 Institute of Preventive Medicine (IPM), Hyderabad, Telangana

i Andhra Medical College, Visakhapatnam, Andhra Pradesh
ii Gandhi Medical College, Secunderabad, Telangana
iii Government Medical College, Anantapur, Andhra Pradesh
iv Guntur Medical College, Guntur, Andhra Pradesh
v Kakatiya Medical College, Warangal, Telangana
vi Kurnool Medical College, Kurnool, Andhra Pradesh
vii Osmania Medical College, Hyderabad, Telangana
viii Rangaraya Medical College, Kakinada, Andhra Pradesh
ix Siddhartha Medical College, Vijayawada, Andhra Pradesh
x Sri Venkateswara Medical College, Tirupati, Andhra Pradesh

4 Madras Medical College (MMC), Chennai, Tamil Nadu

i Chengalpattu Medical College, Chengalpattu, Tamil Nadu
ii Government Medical College, Thanjavur, Tamil Nadu
iii Government Hospital of Thoracic Medicine, Tambaram, Tamil Nadu
iv Government Medical College, Coimbatore, Tamil Nadu
v Madurai Medical College, Madurai, Tamil Nadu
vi Stanley Medical College and Hospital, Chennai, Tamil Nadu
vii Theni Government Medical College, Theni, Tamil Nadu

5 National AIDS Research Institute (NARI), Pune, Maharashtra

I Armed Forces Medical College, Pune, Maharashtra
ii Byramjee Jeejeebhoy Medical College, Ahmedabad , Gujarat
iii Byramjee Jeejeebhoy Medical College, Pune, Maharashtra

iv Dr. Vaishampayan Memorial Government Medical College, Solapur, Maharashtra
v Goa Medical College, Panaji, Goa
vi Government Medical College, Aurangabad, Maharashtra
vii Government Medical College, Akola, Maharashtra
viii Government Medical College, Kolhapur, Maharashtra
ix Government Medical College, Latur, Maharashtra
x Government Medical College, Nagpur, Maharashtra
xi Government Medical College, Sangli, Maharashtra
xii Government Medical College, Surat, Gujarat
xiii Medical College, Baroda, Gujarat
xiv Shri Bhausaheb Hire Government Medical College, Dhule, Maharashtra
xv Shri Meghji Pethraj Shah Medical College, Jamnagar, Gujarat
xvi Shri Vasantrao Government Medical College and Hospital, Yavatmal, Maharashtra
xvii Smt. N.H.L. Municipal Medical College, Ahmedabad, Gujarat
xviii Swami Ramanand Teerth Rural Medical College, Ambajogai, Maharashtra

6 National Centre for Disease Control (NCDC), Delhi

i Dr. Sampurnanand Medical College, Jodhpur, Rajasthan
ii Government Medical College, Jammu, Jammu and Kashmir
iii Government Medical College, Kota, Rajasthan
iv Government Medical College, Srinagar, Jammu and Kashmir
v Jawahar Lal Nehru Medical College, Ajmer, Rajasthan
vi Lady Hardinge Medical College, New Delhi
vii Maulana Azad Medical College, New Delhi
viii Post Graduate Institute of Medical Sciences, Rohtak, Haryana
ix Rabindranath Tagore Medical College, Udaipur, Rajasthan
x Sardar Patel Medical College, Bikaner, Rajasthan
xi Sawai Mann Singh Medical College, Jaipur, Rajasthan
xii University College of Medical Sciences, New Delhi
xiii Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi

7 National Institute of Biologicals (NIB), Noida, Uttar Pradesh

i BRD Medical College Gorakhpur, Gorakhpur, Uttar Pradesh
ii Ganesh Shankar Vidhyarthi Memorial Medical College, Kanpur, Uttar Pradesh
iii Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand
iv Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh
v Jawaharlal Nehru Medical College, Aligarh, Uttar Pradesh
vi King George's Medical University, Lucknow, Uttar Pradesh
vii Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh
viii Maharani Laxmi Bai Medical College, Jhansi, Uttar Pradesh

ix Moti lal Nehru Medical College, Allahabad, Uttar Pradesh
x Sarojini Naidu Medical College, Agra, Uttar Pradesh

8 National Institute of Cholera and Enteric Diseases (NICED), Kolkata, West Bengal

i Assam Medical College, Dibrugarh, Assam
ii Govind Ballabh Pant Hospital, Port Blair, Andaman and Nicobar
iii Guwahati Medical College, Guwahati, Assam
iv Mahatma Gandhi Memorial Medical College, Jamshedpur, Jharkhand
v North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya
vi Patliputra Medical College, Dhanbad, Jharkhand
vii Rajendra Institute of Medical Sciences, Ranchi, Jharkhand
viii Shri Ramachandra Bhanj Medical College, Cuttack, Odisha
ix Silchar Medical College, Silchar, Assam
x The Maharaja Krishna Chandra Gajapati Medical College, Behrampur, Odisha
xi Veer Surendra Sai Medical College, Burla, Odisha

9 National Institute of Immunohaematology (NIIH), Mumbai, Maharashtra

i Gajra Raja Medical College, Gwalior, Madhya Pradesh
ii Gandhi Medical College, Bhopal, Madhya Pradesh
iii Grant Medical College and Sir Jamshetjee Jejeebhoy Hospital, Mumbai, Maharashtra
iv Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College, Sion, Mumbai, Maharashtra
v Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh
vi Regional Medical Research Centre for Tribal, Jabalpur, Madhya Pradesh
vii Seth Gordhandas Sunderdas Medical College & King Edward Memorial Hospital, Mumbai, Maharashtra
viii Topiwala National Medical College & Bai Yamunabai Laxman Nair Hospital, Mumbai, Maharashtra

10 National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, Karnataka

i Bangalore Medical College and Research Institute, Bengaluru, Karnataka
ii Bidar Institute of Medical Sciences, Bidar, Karnataka
iii Jagadguru Sri Shivarathri Medical College, Mysore, Karnataka
iv Jawaharlal Nehru Medical College, Belgaum, Karnataka
v Jaya Jagadguru Murugharajendra Medical College, Davangere, Karnataka
vi Karnataka Institute of Medical Science, Hubli, Karnataka
vii Kasturba Medical College, Mangalore, Karnataka

viii Kasturba Medical College, Manipal, Karnataka
ix Shri B. M. Patil Medical College, Bijapur, Karnataka
x Vijayanagar Institute of Medical Sciences, Bellary, Karnataka

11 Regional Institute of Medical Sciences (RIMS), Imphal, Manipur

i Arunachal State Hospital, Naharlagun, Arunachal Pradesh
ii Civil Hospital, Aizwal, Mizoram
iii District Hospital, Dimapur, Nagaland
iv Government Medical College, Agartala, Tripura
v Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur
vi Naga Hospital Authority, Kohima, Nagaland

12 School of Tropical Medicine (STM), Kolkata, West Bengal

i Burdwan Medical College, Burdwan, West Bengal
ii Calcutta National Medical College, Kolkata, West Bengal
iii Dr. Radha Gobinda Kar Medical College and Hospital, Kolkata, West Bengal
iv Midnapore Medical College and Hospital, Midnapore, West Bengal
v North Bengal Medical College, Darjeeling, West Bengal
vi Patna Medical College, Patna, Bihar
vii Pt. Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh
viii Sri Krishna Medical College, Muzaffarpur, Bihar
ix STNM Hospital, Gangtok, Sikkim

13 Tamil Nadu Dr M G Ramachandran (TN Dr MGR) University, Chennai, Tamil Nadu

i Government Mohan Kumarmangalam Medical College, Salem, Tamil Nadu
ii Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry
iii K.A.P. Viswanathan Government Medical College, Trichy, Tamil Nadu
iv Kanyakumari Government Medical College, Kanyakumari, Tamil Nadu
v Thoothukudi Government Medical College, Thoothukudi, Tamil Nadu
vi Tirunelveli Medical College, Tirunelveli, Tamil Nadu

Annexure 2

Designated HIV-2 referral laboratories

Sr. No.	Referral States / UTs	Name of laboratory & Address
1	Maharashtra, Mumbai, Dadra & Nagar Haveli, Daman & Diu, Goa	NACO Program Laboratory National AIDS Research Institute Plot No.73, "G "Block, MIDC, Bhosari, Pune-411026 Ph. No: 020-27331200 Ext. E mail: nrl.nari1@gmail.com
2	Bihar, West Bengal, Jharkhand, Sikkim,	Virology Unit, Department of Microbiology 4th Floor, School of Tropical medicine, 108, C.R. Avenue, Kolkata-700073. Ph. No: 033-22123693 Email: nrl.stm1@gmail.com
3	Delhi, Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, Chandigarh, Rajasthan	Centre of AIDS & Related Diseases, National Centre for Disease Control, 22- Shamnath Marg, New Delhi-110054. Tele /Fax: 011- 23934517 Email: nrl.nicd@gmail.com
4	Andhra Pradesh	Institute of Preventive Medicine, BSQC Department, Narayanguda, Near YMCA Hyderabad-500029 Ph. No: 040-27568167 Email: nrl.ipm@gmail.com
5	Uttar Pradesh and Uttaranchal	National Reference Laboratory (NRL) Dept. of Microbiology, All Indian Institute of Medical Sciences (AIIMS) New Delhi-110029 Ph. No: 011-26593673 Email: nrl.aiims@gmail.com
6	Assam, Meghalaya, Arunachal Pradesh	Dept. of Microbiology, Gauhati Medical College & Hospital, Guwahati – 781032 Ph. No: 0361-2529457 Email: srl.assam.gmc@gmail.com
7	Odisha	SCB Medical College & Hospital, Cuttack, Orissa-751007 Ph. No: 0671-2410041 Email: srl.orissa.scbmc@gmail.com
8	Gujarat	Dept. of Microbiology, B.J.Medical College, Asarwa , Ahmedabad, Gujarat-380016 Ph. No: 079-22683721 Email: srl.gujarat.bjmc@gmail.com

Designated HIV-2 referral laboratories

Sr. No.	Referring ART centers	Name of laboratory & Address
9	Kerala, Lakshwadweep	State Reference Laboratory (SRL) Department of Microbiology. Govt. TD medical college, Alapuzha, Kerala-688005 Ph. No: 04772282015 Email: srl.kerala.mc2@gmail.com
10	Madhya Pradesh & Chattisgarh	State Reference Laboratory (SRL) Department of Microbiology, Gandhi Medical College, Barakktullah Vishwavidyalaya, Sultania Road, Bhopal - 462001. Ph. No: 0755-4050160 E-mail: srl.mp.gmc@gmail.com
11	Karnataka	Dept. of Neuro-virology, NIMHANS, Hosur Road, Bangalore-560029. Ph. No: 080-26995778 Ext. Fax : 080-26564830 Email: nrl.nimhans8@gmail.com
12	Manipur, Nagaland, Tripura, Mizoram	National Reference Laboratory (NRL) Department of Microbiology, Regional Institute of Medical Science, P.O. Lamphelpat, Imphal (west), Manipur-795004 Ph. No: 0385-2414750 Ext-241 Email: nrl.rims1@gmail.com
13	Tamil Nadu & Pondicherry, Andaman & Nicobar islands	HIV NRL Laboratory, Tower Block-1, Room No. 106, Madras Medical College, Chennai-600003 Ph. No: 044-25383445 Ext. Email: nrl.mmc@gmail.com

HIV TEST REPORT FORM

(Form to be filled in duplicate)

Name and address of ICTC centre: .....

.....

.....

Name: Surname..... Middle name.....First name.....

Gender: M / F / TG Age:..... Years PID # ..... Lab ID #.....

Date and time blood drawn: .....(DD/MM/YY) .....(HH:MM)

Test Details:

Specimen type used for testing: Serum / Plasma / Whole Blood

Date and time specimen tested: .....(DD/MM/YY) .....(HH:MM)

Note:

- Column 2 and 3 to be filled only when HIV 1 & 2 antibody discriminatory test(s) used
- No cell has to be left blank; indicate as NA where not applicable.

Column 1	Column 2	Column 3	Column 4
Name of HIV test kit	**Reactive/Nonreactive (R/NR) for HIV-1 antibodies**	**Reactive/Nonreactive (R/NR) for HIV-2 antibodies**	**Reactive/Nonreactive (R/NR) for HIV antibodies**
Test I:			
Test II:			
Test III:			

Interpretation of the result : Tick (✓) relevant

☐ Specimen is negative for HIV antibodies

☐ Specimen is positive for HIV-1antibodies

☐ *Specimen is positive for HIV antibodies (HIV-1 and HIV-2; or HIV-2 alone)

☐ Specimen is indeterminate for HIV antibodies. Collect fresh sample in two weeks.

* Confirmation of HIV 2 sero- status at identified referral laboratory through ART centres

Name & Signature
Laboratory Technician

Name & Signature
Laboratory In-charge

---End of report---

Page (1 of 1)

Referral Slip for HIV-2 testing

To be filled in duplicate by ART I/C/SMO/MO. Original copy to be sent to HIV-2 referral laboratory. Client/patient to carry ICTC HIV report & photo ID

Name: Surname:..... Middle name: .....
First name: .....

Date: ...../...../.....(DD/MM/YY) Gender: M / F / TG Age: .....Years

ICTC PID #..... Pre ART Reg. #.....

Name and postal address of referring ART center: .....
.....
.....

Email ID of referring ART center/MO in charge: .....
.....
.....

Name and postal address of the HIV-2 referral laboratory :.....
.....
.....

Name & Signature of Medical Officer ART center: .....

Annexure 5

PID Register for ICTC (Clients excluding Pregnant Women)

ICTC Code:..... ICTC Name: .....

Block:..... District:..... State:.....

S. No.	PID No.	Date of visit	Name	Address	District	Phone
1	2	3	4	5	6	7

Laboratory Register for ICTC

Sl No.	Name of the centre	PID No.	Name of referring ICTC	Lab sample no.	HIV test results			Final test result given	Quality control no. Sent to SRL/NRL	Confirmation result received from lab	Signature
					Test 1	Test 2	Test 3				
1	2	3	4	5	6	7	8	9	10	11	12

Referred sample from the ICTC/PPTCT/BB Centre																		
ICTC/ PPTC/ BB Centre										SRL								
Sl No.	Name of the centre	PID No.	Date of Testing	Details of kits used (Name, Exp date, Lot No.)			Results			SRL No.	Date of Testing	Details of kits used (Name, Exp date, Lot No.)			Results			Remarks
				1st Test	2nd Test	3rd Test	1st Test	2nd Test	3rd Test			1st Test	2nd Test	3rd Test	1st Test	2nd Test	3rd Test	

Signature of LT

Signature of Incharge

STI/RTI Referral Form

(To be filled and handed to the client by STI/RTI Counsellor)

Referral to

ICTC/Chest & TB Laboratory .....

The Patient with the following details is being referred to your center.

Name:..... Age:..... Sex:.....

STI/RTI-PID No.:.....

Kindly do the needful

Referring Provider

Name:..... Designation:.....

Contact Phone:..... Date of referral:.....

(To be filled and retained site so as to be collected by STI/RTI Counsellor weekly)

The above patient referred has been provided

ICTC/TB/RPR/VDRL/..... services and the patient has been tested/Diagnosed/Treated for.....

The test/result of

RPR/VDRL/is/are.....

Signature of the Medical Officer / Couunselor / Lab. In-Charge

Annexure 9

Reporting format for Syphilis test					
Name:		Age:		Sex:	
ICTC PID No:					
Name of the kit/test:					
Test Result Qualitative:					
Quantitative:					
Date:		Signature of Lab Technician/In-Charge			

Integrated Counselling and Testing centre,.....Hospital

Laboratory Reporting form (RPR Test)

(To be completed at site of testing)

1. STI Registration no. (if any)..... 2. PID no.....
3. Age / Sex: ..... 4. Referred from.....
5. Sample Received on ..... 6. Test performed on: .....
7. Kit Used (With Expiry & Lot no.).....
8. Result of RPR Rapid test:..... (Reactive/Non-Reactive)

   (Write in space provided)

9. Referred to nearest STI Clinic (If found positive).....
10. Remarks if any .....

Signature with Date: Signature with Date:

Annexure 11

Laboratory Design and Procedural Precautions for PCR

Because of the sensitivity of the PCR test, it is important that care is taken to avoid cross-contamination of samples (during extraction) and even more importantly, the carry-over of amplicon that can result in false positive results. For all amplification techniques, greatest attention is directed towards the prevention of contamination. Once contamination has occurred, it is very difficult to remove and testing may have to cease. Without exception, test results must be rejected, even if only one of the accompanying negative or reagent (blank) controls reveals amplicon contamination.

Setting up a molecular diagnostics laboratory has its own unique requirements, and those should be carefully considered during laboratory design. The PCR laboratory needs to be designed as per 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, the laboratory should be divided into four separate work areas, each having dedicated equipment:

- Reagent storage and set-up
- Sample preparation and nucleic acid extraction
- Assembly of reaction mixture and amplification
- Product analysis

Specifically, and at the very least, the pre-amplification activities (reagent storage and sample preparation) should be strictly segregated from amplification and, especially, from the post-amplification activities (product analysis), in both time and space. Ideally, in the pre-amplification laboratory there should be a slight positive pressure as compared to the air in the connecting hallway, while the post-amplification laboratory should be at a slightly reduced pressure to ensure an inward flow of air, preventing the amplicons from escaping outside.

In addition to ensuring strict workflow measures as described above, the following precautions also need to be taken in a PCR laboratory.

- All clinical samples must be handled using standard precautions, to avoid the possibility of transmission of HIV, including the use of personal protective equipment like laboratory gowns and gloves
- Separate biosafety laminar flow cabinets (class II) should be used to ensure safety and to prevent cross-contamination
- Barrier tips or positive displacement pipettes should be used for dispensing samples
- Measures should be taken to prevent aerosolization (e.g., avoiding vigorous Pipetting; briefly centrifuging tubes with samples, extracted DNA and especially amplicon prior to opening the caps)

- Reagents should be aliquoted and stored
- Master mixes (including reagents other than the template) should be prepared to minimize handling of reagents.
- Equipment, gowns, gloves and work instructions should not be moved from one work area to another in the PCR laboratory

Chemical safety in the Laboratory

- Occupational safety and health administration published guidelines the hazard communication standard which provide certain institutional education practices to ensure that all laboratory personnel have thorough working knowledge of hazardous chemicals with which they work.
- All the hazardous substances in the work place be identified and clearly marked with label stating health risks such as whether carcinogen, mutagen or teratogen and hazard class, whether corrosive, poison, flammable or oxidising. Each laboratory should have a chemical hygiene plan which include guidelines for proper labelling of the containers Material safety data sheets (MSDS), and other chemical hazard information available from chemical manufacturers and/or suppliers, and written chaemical safety training program. These should be accessible in laboratories where these chemicals are used, e.g. as part of a safety or operations manual.
- Manufacturers of laboratory chemicals issue charts describing methods for dealing with spills.in MSDS. Spillage charts and spillage kits are also available commercially.
- Appropriate charts should be displayed in a prominent position in the laboratory.
- Only amounts of chemicals necessary for daily use should be stored in the laboratory.

Common chemical exposed in laboratory

Chlorine

- Chlorine solution is corrosive to eyes respiratory tract and skin. Inhalation cause pneumonitis even though not combustible enhance combustibility of other substances to cause fire. Strong acidic solutions violently react with bases. Attacks many metals, plastic and rubber.
- Wear utility gloves protective clothing, masks and goggles while preparation of Chlorine solution

Alcohol

- Alcohol is volatile and flammable, harmful if ingested and affect central nervous system, irritate eyes. Always keep the container tighly closed and keep away from ignition source.
- Iodine solution is irritant to respiratory tract, and eyes, avoid vapours and contact with eyes

Gluteraldehyde

- Gluteraldehyde cause severe irritation of respiratory tract and eyes. Work in well ventilated area and wear gloves , eye protection and masks.

Phenol

- Phenol compounds are corrossive to respiratory tract, eyes and skin and cause severe burns

and it is absorbed through skin to cause central nervous system involvement and coma. Continuous contact at low concentration cause dermatitis.In case of contact with skin remove any contaminated clothing and contaminated skin is swabed with glycerol and flush with water. In case of eye contact immediately rince with water and seek medical advice.

Electrical safety

All electrical installations and equipment are to be inspected and tested regularly, including earthing / grounding systems.

Miniature Circuit-breakers (MCB) and Earth Leakage Circuit Breaker (ELCB) should be installed in appropriate laboratory electrical circuits. MCB do not protect people; they are intended to protect wiring from being overloaded with electrical current and hence to prevent fires. ELCB is intended to protect people from electric shock. All laboratory electrical equipment should be earthed/grounded, through three-pin plugs.

Laboratory should have dedicated sockets for each equipment. Avoid extension cords and multipoint sockets.

All laboratory electrical equipment and wiring should conform to national electrical safety standards and codes.

Fire evacuation plan should be displayed in the laboratory showing the nearest fire escape route. Exit way should always remain clear of obstructions. Employees should have knowledge to use extinguisher. Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, buckets (of water or sand) and a fire extinguisher. Fire extinguishers should be regularly inspected and maintained, and their shelf-life kept up to date.

Types and uses of fire extinguishers

TYPE	USE FOR	DO NOT USE FOR
Water	Paper wood fabric	Electrical fires flammable liquids burning metals
Carbon dioxide (CO_2) extinguisher gases	Flammable liquids and gases, electrical fires, Alkali metals	Paper
Dry powder	Flammable liquids and gases alkali metals electrical fires	Reusable equipment and instruments as residues are very difficult to remove
Foam	Flammable liquid	Electrical fires

Sodium hypochlorite preparation

Formula for dilution of Stock solution of Sodium hypochlorite to working concentration of Sodium hypochlorite

$$\text{Amount of stock required} = \frac{\text{Working Conc. Required}}{\text{Stock Conc.}} \times \text{Working solution volume required}$$

Water Required = Working solution volume required – Amount Stock Required

Preparation of different concentration of Sodium Hypochlorite Solution.

Required Strength (Available solution of chlorine)	Stock/commercially available Sodium Hypochlorite		
	4 %(40g/L); dilute	5 %(50g/L); dilute	6%(60g/L); dilute
0.1%(1 g/L)	1:39*	1:49	1:59
0.5%(5 g/L)	1:7	1:9	1:11

*parts of stock solution: parts of water

Supervisory Check List for Visit by SRL Staff to ICTC Laboratory			
Visited by:..... Date & time:			
GENERAL INFORMATION			
Laboratory name and address			
Lab telephone			
	No. Available	**Trained**	**Vaccinated (Hep B)**
Lab In- charge / Medical Officer			
Lab technician (NACO / Institution)			
Lab counselor			
Lab assistant			
Follow up of Action required as per the previous visit:			
Status of Equipments:			

S. No	Equipment name	No. Available	Working Condition (Yes/No)	Name of Manufacturer	Date of Purchase (as per Equipment Register)	Calibration done on	AMC (Yes/No)
1	Refrigerator						
2	Pipettes						
3	Centrifuge						
4	Needle destroyer / Needle cutter						
5	Refrigerator Thermometer						
6	Room Thermometer						

Status of Consumables:

S. No	Consumables	Number available	Adequate quantity and functioning (Y/N)	Remarks
1	HIV Test Kits			
a	Kit 1 (.....)			Exp date.....
b	Kit 2 (.....)			Exp date.....
c	Kit 3 (.....)			Exp date.....
2	Test tube racks			
3	Marker pens			
4	Discarding Jar			
5	Color coded bins			
6	Color coded bags			
7	Sodium Hypochlorite solution			
8	Disposable syringes and needles			
9	Spirit			
10	Cotton swabs, tourniquets			
11	Disposable gloves			
12	Disposable masks			
13	Plastic/ water resistant apron			
14	Goggles			
15	Hand washing soap			
16	Lab aprons			
17	Spillage kit			
18	Eye wash kit			
19	PEP kit			
20	First Aid Kit			

Status of Records, Reports and Registers:

S. No	Records, reports and Registers	Available and or Adequate (Y/N)	Remarks/ suggestions
1	SOPs for		
A	Pre-analytical procedures (Specimen collection, sample rejection & Processing i.e. Serum separation, Specimen pre -test storage, Blood/ body fluids precautions)		
B	Analytical procedures (3 tests: Comb Aids, Tridot, EIA Comb)		
C	Post Analytical procedures (Post test Specimen storage, specimen transport, Biohazard waste disposal, Needles stick injury, Accidental spills and PEP)		
D	Floor chart and work station labeling		

S. No	Records, reports and Registers	Available and or Adequate (Y/N)	Remarks/ suggestions
E	SOPs available at workstations		
2	**Log sheets/ workbook**		
A	Test Workbook		
B	Workstation disinfection log sheets		
C	Temperature monitoring log sheet ▸ Refrigerator ▸ Room temperature		
D	Documented reviewed by lab supervisor		
3	**Registers**		
A	Specimen Collection and rejection Register and Specimen Requisition Slip		
B	Lab Register		
C	EQAS Register and File (with records) ▸ Retesting ▸ Panel Testing ▸ Corrective action report (if any)		
D	Lab Consumables register and indent book		
E	Equipment/ Stock Register		
F	Accident and Incident Register		
G	Complaint / Suggestion Register		
H	Attendance and Movement Register/s		

Status of Records, Reports and Registers:

S. No	Observations	As per guidelines (Yes/No)	Remarks/ Suggestions
1	**Cleanliness** in the Lab (work area and storage area)		
2	**Work stations** identified and organized		
3	Washbasin / sink with continuous water supply		
4	Display of **IEC material** in Lab		
5	**Pre-Analytical procedures** ▸ Specimen collection ▸ Specimen Processing ▸ Specimen Storage ▸ Kit Storage (including cold chain maintenance)		
6	**Analytical Procedures** ▸ HIV Test 1 ▸ HIV Test 2 ▸ HIV test 3		

S. No	Observations	As per guidelines (Yes/No)	Remarks/ Suggestions
7	**Post Analytical Procedures** ▸ Specimen Storage ▸ Specimen Disposal		
8	**Turn Around Time for Tests performed**		
9	**Knowledge and practice about** ▸ Cold Chain maintenance ▸ Infection Control measures including Universal Work Precautions ▸ Handling of bio-hazard emergencies ▸ FEFO principle ▸ Transport of samples ▸ EQAS ▸ Planning and procurement of consumables		
10	**Furniture** for Lab staff (for record keeping and reporting)- cupboard, table, chairs		
11	**Maintenance of Records** ▸ Test results legible, technically verified and confirmed against patient identity ▸ All records, reports and registers well maintained		

Overall observations:

Overall Recommendations:

Signature of Laboratory Technician **Signature of the Supervisor visiting the Lab**

Annexure 14

List of SOPs and Records at ICTCs

1. SOP on Collection, Processing and Storage of Samples
2. SOPs for HIV Rapid Tests.
3. SOP for IQC and EQA
4. SOP on Documentation
5. SOP for Equipment Management
6. SOP for Bio-Medical Waste Management
7. SOP for Preparation and Release of Reports
8. Personnel Files
9. Equipment File
10. Adverse Incidence Records
11. EQAS records
12. Kit Inventory Records
13. Corrective Actions

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