LABORATORY MANUAL FOR TECHNICIANS
(ICTCs, PPTCTCs, BLOOD BANKS & PHCs)

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National AIDS Control Organization
Ministry of Health & Family Welfare

CONTENTS

LIST OF ABBREVIATIONS

BTC	:	Blood testing centre
cDNA	:	Complementary DNA
CD4	:	Cluster determinant 4
CSF	:	Cerebrospinal fluid
CV	:	Coefficient of variation
DNA	:	Deoxyribonucleic acid
EIA	:	Enzyme immuno assay
ELISA	:	Enzyme linked immunosorbent assay
EQA	:	External quality assessment
E ratio	:	ELISA ratio
gp	:	Glycoprotein
HBIg	:	Hepatitis B immunoglobulin
HBV	:	Hepatitis B virus
HCP	:	Health care personnel
HCV	:	Hepatitis C virus
HIV	:	Human Immunodeficiency Virus
HIV – 1	:	Human Immunodeficiency Virus Type -1
HIV – 2	:	Human Immunodeficiency Virus Type -2
HIV -I M	:	HIV -1 Major group
HIV -I O	:	HIV -1 Outlier group
HIV – I N	:	HIV -1 New group
HTLV	:	Human T lymphotropic virus
IB	:	Immunoblot

ICTC	:	Integrated counseling and Testing Centre
NPV	:	Negative predictive value
PPV	:	Positive predictive value
NRL	:	National Reference Laboratory
SRL	:	State Reference Laboratory
OD	:	Optical density
PCR	:	Polymerase chain reaction
PEP	:	Post exposure prophylaxis
PHA	:	Passive haemagglutination
PPTCTC	:	Prevention of parent to child transmission centre
QA	:	Quality assurance
QAP	:	Quality assurance programme
RIA	:	Radio immunoassay
RNA	:	Ribonucleic acid
SD	:	Standard deviation
SOP	:	Standard operative procedure
TQM	:	Total quality management
UV	:	Ultra violet
VCTC	:	Voluntary Counseling & Testing Centre
WB	:	Western blot

CHAPTER 1
BACKGROUND OF HIV EPIDEMIC IN INDIA

In India, the first case of HIV/AIDS was reported in 1986 from Chennai in a commercial sex worker. But today, as per data available from NACO, the number of infections stand at a staggering 5.2 million.

At the end of 2004, world over about 14,000 new infections were being acquired every day. Of these more than 95% infections are in developing, low and middle income countries including India. Children below the age of 15 years account for almost 2,000 infections per day and among the adults 50% of the infections are seen in women.

Most of the infections in India are acquired through unsafe sexual practices. The spread of infection within the country is not uniform but heterogeneously spread across states and districts. The states are categorized as high prevalence, vulnerable and low prevalence states depending on the intensity of infection. The most common HIV sub type seen in India is HIV 1 sub type C. The hetero-sexual mode of transmission is pegged as the most common route of infection accounting for 86% of total infections. The other routes through which HIV gets transmitted are from infected mother to child and/or perinatal transmission, transmission due to contaminated blood and blood products and transmission through infected syringes and needles as seen commonly among injecting drug users and very occasionally among health care professionals.

HIV is not transmitted through casual social contact.

Solutions against the epidemic are based on direct interruption of transmission of HIV and also by tackling the contributing factors. Solutions are listed below:

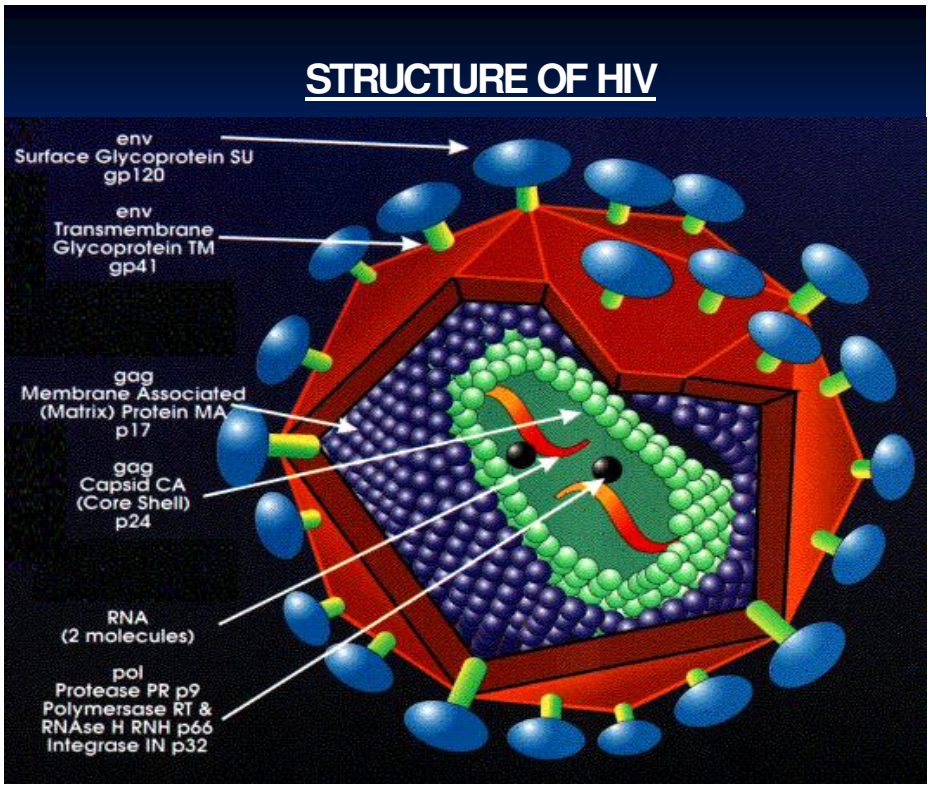
- Awareness about transmission modes
- Practice of safe sexual behaviour
- Mandatory screening of blood/blood products
- Using sterilized needles/syringes
- Reducing infection from mother to child (PPTCT)
- Political commitment (for implementation of HIV/AIDS control measures)
- Care and support for the infected and affected persons
- Reducing stigma and discrimination so that more and more people access the VCTC / ICTC and PPTCT services.
- Voluntary confidential counselling and testing

CHAPTER 2
VIROLOGY

HIV is the human immunodeficiency virus belonging to class of retroviruses and sub family lentivirinae. It is a rapidly mutating virus. There are mainly two types of HIV; HIV-1 and HIV-2. The HIV is transmitted through infected blood and body fluids. Sexual transmission is the most common route of transmission. It is important to understand some basic facts associated with the entry of HIV into body, manner in which it causes the disease, strategies employed by the virus to kill the cells and the ways by which it can be controlled. In order to do so, it is critical to have knowledge about the nature of virus, its life-cycle , and the mechanisms of replication and onward transmission. . The HIV is believed to have originated from sub human primates, monkeys and chimpanzees in Africa.

Manifestation of AIDS (Acquired Immunodeficiency Syndrome) is regarded as the final stage of HIV infection and may lead to the death of the afflicted individual in the event of unavailability of treatment

Structure of HIV



HIV is a 120 nanometer double stranded RNA virus. The presence of surface protein gp120 is important for the attachment of virus to the host cell, transmembrane protein gp41 is important for fusion and the core containing RNA and enzymes is required for replication. HIV has three enzymes namely reverse transcriptase, integrase and protease – all very important for perpetuation of HIV.

Virus replication

- HIV virus acquired through any route of transmission whether through blood, body fluid or unsafe sexual contact targets the T- lymphocytes with CD4 receptor (CD4 T-lymphocytes), dendritic cells, macrophages and monocytes. CD4 T- lymphocytes cells are however considered as the primary target of HIV.
- Thereafter, gp120 surface protein of the HIV attaches itself to the complimentary receptor CD4 on T cell just like a key fitting into a lock.
- Then gp41, the transmembrane protein of HIV fuses with the host cell membrane followed by the entry of viral core comprising of the RNA, enzymes and protein into the cytoplasm.
- Next the viral RNA is transcribed into complementary DNA under the influence of viral enzyme reverse transcriptase. Subsequently a double stranded DNA is formed.
- The viral DNA then translocates into the nucleus and with the help of the viral enzyme integrase, gets integrated into the host cell DNA. This is called provirus.
- The viral RNA and proteins are synthesized, assembled into virion which matures and is released from the cell by budding.
- This virus now infects other cells. As the infection progresses more and more CD4 cells are affected and finally the HIV infected individual develops AIDS.

Window Period (WP) is the time that elapses between entry of HIV into the body and the detection of HIV specific antibodies. Usually WP is three weeks, but may vary from three weeks to three months. At times, the same may get extended up to six months.

Modes of transmission

The major mode of transmission of HIV has been found to be through sexual contact. The frequency and the efficiency of transmission are determined by various factors like presence of sexually transmitted infections, practice of unsafe sexual behaviour, acute and advanced HIV disease, high viral load and low CD4 count. All these factors favour HIV transmission.

Sexual transmission (unsafe sexual practices)

- Heterosexual
- Homosexual

Blood contact (contaminated blood)

- Blood transfusions
- Intravenous drug use
- Occupational exposure (needle stick, percutaneous -through cracks in skin, etc.)

Mother-to-child (infected mother to newborn)

- In-utero
- During delivery
- Breast feeding

Transmission of HIV through blood and blood products has been reduced through mandatory screening of blood. Inactivation of viruses in blood products has minimized transmission of the virus through blood transfusion mode. The Supreme Court of India has made it mandatory to screen every unit of blood for HIV. Transfusion of blood is rationalized; blood is used only when necessary. It is safer to use blood components rather than using the whole blood.

Targeted interventions have reduced transmission in IDUs (intravenous drug users). The other effective method for reducing transmission of HIV, especially in cases of occupational exposure is through post-exposure prophylaxis (PEP). Drugs used for PEP are supplied free of cost by the government.

The unsafe sexual practices still continue to be the main mode of transmission in the country and world over. It is very important to know how to prevent or stop HIV transmission through sexual route. It is important that counseling is done to motivate the client to take personal responsibility for his sexual health and practice safe behaviour (use of condom). A vast majority (more than 86%) of HIV infections in India have been acquired through unsafe sexual practices. Presence of ulcerative and non-ulcerative sexually transmitted infections increases the risk of acquiring HIV from an infected partner. Therefore, it is imperative that HIV testing is encouraged amongst clients at risk, through counseling and at the same time the use of condoms is promoted.

Another way of preventing transmission particularly through contaminated needles is to reduce the number of injections and substitute it with oral drugs. Bio-safety precautions should be practiced at all times while providing services and infectious waste should be disposed off as per the guidelines. Targeted interventions like needle exchange programme have also helped in reducing HIV transmission in IDUs.

Mother to child transmission of HIV from infected mother to child is minimized by use of antiretroviral treatment of the mother and the infant under the PPTCT programme of NACO.

HIV is not transmitted through casual social contact like handshaking, hugging, living at the same place as an HIV/AIDS patient, sharing of utensils , etc.

Though, treatment for AIDS patients is now available at government ART centres, there is still no cure for the disease and neither is any successful vaccine available for HIV. The antiretroviral treatment once started has to be adhered to and taken for the rest of the life. So, prevention remains the best way of staying away from HIV infection.
This can be achieved by:
- Practicing safe sex (use of condom, mutually faithful sexual partner)
- Use of disposable syringes and needles
- Use of safe blood and blood products
- Adhering to standard work precautions

Susceptibility of HIV

HIV is a very fragile virus. it needs living human cells to replicate and cannot survive outside the body**.** The following methods can be used to kill the virus.
Sterilization
- Autoclaving at 121^{0}C at 15 lb pressure for 20 minutes
- Dry heat 170^0 C for 1 hr. (holding time)
- Boiling for 20 minutes

Disinfection – minimum 30 minutes contact time
- Sodium hypochlorite 1%
- Ethanol 70%
- Povidone iodine (PVI)
- Formalin 4%
- Glutaraldehyde (activated) 2%

CHAPTER 3

IMMUNOPATHOGENESIS AND NATURAL HISTORY OF HIV INFECTION

An understanding of immune system is extremely important to understand how HIV causes AIDS.

Immune System

A healthy body teams with millions of different white blood cells that work together to scout and destroy external pathogens (viruses, bacteria, etc.). Because the white cells are so vital to the immune response, they are used as a measure of immune health.

Different types of white blood cells involved in protection include:

- T-cells (T-lymphocytes)
- B-cells (B-lymphocytes)
- Polymorphs/neutrophils
- Macrophages
- Natural killer cells

T-cells

T-cells organize and direct other white blood cells in the fight against infection. These cells confer what is known as cell mediated immunity. There are two types of T-cells, helper T-cells, which are known as CD4 T-cells and the cytotoxic T-cells (CD8 T-cells). In response to infections and antigens, these cells respond by secreting certain soluble proteins that help other types of immune system cells, such as B-cells and thus help to rid the body of invaders (viruses, bacteria, etc.). Cytotoxic T-cells / CD8 T-cells kill other cells infected with viruses and other germs. This killing of infected cells helps prevent spread of infection in the body by stopping the replication of pathogens.

B-cells

B-cells (lymphocytes) are a specialized type of white blood cells which help the body to fight the invader organisms by making specific proteins called antibodies, which recognize and bind to a particular invader. Each given antibody matches an antigen (part of invader) as a key matches a lock. Once attached to the invader, these antibodies act as signal for other immune cells to swarm and engulf, kill and quickly remove the offending invader/antigen. Once a set of B-cells has encountered a particular antigen/invader, they can remember it if it ever invades again. This helps so called sensitized B-cells to launch a quick, more intense and effective response against the invader. So, the invader/bacteria cannot cause disease e.g. immunization in children protects them against certain specific pathogens against which immunization is given (polio, tetanus, diphtheria, etc.). This is called humoral immunity.

Phagocytes

These are cells which engulf (eat) germs. These cells attack everything foreign and harmful from dust to viruses. Phagocytes are of two types: neutrophils which circulate in the blood and enter tissues which have become infected and macrophages which are present in healthy tissues where they lie in wait for invaders (bacteria, etc.) . The name macrophage literally means “big eater” as they capture, engulf and break down all types of micro-organisms. Macrophages also secrete special proteins called cytokines which direct a large number of immune cells to the infected area, including neutrophils to fight the invading bacteria.

Natural killer cells (NK cells)

These are large cells which contain toxic granules and circulate in the tissues. NK cells kill viruses and tumour cells. These cells fight the invader in the earliest stages of infection before CD4 and CD8 cells take over.

Antigens: These are molecules, mostly protein in nature which are foreign to the body and can harm the body. All parts of HIV are foreign and are antigenic e.g. p24 antigen (core portion), envelope (gp120/140), etc.

Antibodies: These are certain molecules which are produced by the immune cells (B-lymphocytes) of blood in response to the presence of foreign substances, with which the antibodies react to control infection.

A number of different types of antibodies (immunoglobulins) are produced against HIV antigens. These help in detection of HIV-infection in an individual. However, these antibodies cannot kill HIV.

Natural history of HIV

Infection with HIV leads to a progressive impairment of cellular immune function, characterized by a gradual decline in peripheral blood CD4 T – lymphocyte levels resulting in an increased susceptibility to wide variety of opportunistic viral, bacterial, protozoan and fungal infections and to certain malignancies also. The course of the disease is marked by increasing levels of viral replication, emergence of more virulent viral strains and progressive destruction of immune system. However, the natural history of HIV infection is changing with better diagnosis, ARV therapy, and early treatment and prophylaxis of various opportunistic infections. An HIV infected individual passes through different stages of infection. Initially there may not be any symptoms, however, as more CD4 cells get destroyed, symptoms start appearing in the form of infections like T.B., diarrhea, prolonged fever, Herpes zoster and others. When the immune system reaches a critical limit i.e. when the CD4 cells fall below 200 cells/mm^3, the patient may develop AIDS. Such patients can eventually die within 1-2 years if no treatment is provided. Stages of HIV are given below.

Stages of HIV disease

	Signs and clinical features	Typical duration	CD4 T-cell count Range/mm^3
1	Acute primary HIV infection	1-2 weeks	1000-500
2	Asymptomatic, no s/s other than lymphadenopathy	10 years	750-500
3	Early symptomatic (non life-threatening infections of chronic or intermittent symptoms)	0-5 years	500-100
4	Late symptomatic (increasingly severe symptoms, life threatening infections, malignancies)	0-3 years	200-50
5	Advanced AIDS (serious opportunistic infections, increasing hazard of death)	1-2 years	50-0

S/S= Signs and symptoms

CHAPTER 4

LABORATORY BIOSAFETY AND STANDARD WORK PRECAUTIONS

Common sense safety practices are the principal means by which laboratory personnel can avoid infection from biohazards. Gloves and skill are all that stand between laboratories and the infectious agents they handle all day.

The primary work-related dangers are parenteral exposure through accidental needle sticks, cuts from contaminated equipment, exposure of mucous membranes to aerosolized droplets, and exposure of chapped or broken skin wounds and scratches to contaminated specimens.

A safe working environment in the laboratory can be maintained by instituting biosafety practices that include careful measures for personal hygiene, for cleanliness in the work space, and proper handling of bio-hazardous materials. An understanding of these necessities and an awareness of potential biohazards while performing laboratory duties will help prevent accidents, injuries and infection.

Risk of exposure to HIV is significant but not as acute as exposure to hepatitis B virus (HBV) and hepatitis C virus (HCV). Recommend HBV vaccinations for lab workers.

Standard work precautions (SWP)

Practicing standard work precautions is the most effective and efficient method of preventing exposure to HIV and other blood borne pathogens.

- Standard precautions apply to these body fluids: blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid and breast milk.

- Standard precautions do not apply to these body fluids, **unless** they contain visible blood: feces, nasal secretions, sputum, sweat, tears, urine, vomitus, and saliva.

Proper laboratory hygienic practices to minimize exposure to pathogens associated with blood and body fluids.

- Standard work precautions must be practiced with all specimens at all times (i.e. treat all patients and specimens as if they are infectious).

- Gloves must be worn when handling blood or body fluids and when handling items or surfaces contaminated with blood or body fluids.

- Use mechanical pipetting devices for all pipetting. **Mouth pipetting** should be avoided.

- Hands must always be washed after removing gloves and before leaving the laboratory.

- Eating, drinking and smoking in the work area is a serious health risk and must not be practiced.

- Eye protection must always be worn when a chance exists of generating splashes, droplets or aerosols.

- Lab coats must always be worn while in the laboratory to ensure that clothes are kept free from infectious agents.

- Lab coats must be removed before leaving the laboratory and must be kept away from dining or food area.

- Cuts and scratches on hands and arms must be covered and protected with occlusive bandage.
- Activities such as applying make-up and combing hair must not be practiced in the laboratories. Long hair should be pinned up or covered.
- Protective shoes that completely cover feet must not be worn (open-toed shoes or sandals must not be worn).

Prevention of needlestick injuries

Although many potential routes of exposure to infected blood and body fluids exist, 80% of all exposures of health care workers occur as a result of needle sticks. Avoid the use of needles and syringes whenever possible.

- Needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes or otherwise manipulated by hand.
- Disposable syringes and needles, scalpel blades and other sharps should be placed in puncture resistant containers located as close as practical to the area of use or destroyed in the needle destroyer.

The laboratory work space

"Containment" of infectious agents is the principal means of providing a safe laboratory working environment.

General laboratory practices

- Reagents must be well labeled, in proper containers and properly stored
- Bench tops must be kept free from clutter.
- Material must not be placed near the edge of counters or shelves, but rather organized as related to need.
- Hazardous materials and reagents not in use must be safely stored out of the immediate work area.
- Unbreakable materials should be used.
- Eye wash station should be provided for accidental exposure from splashes.
- First aid kit should be provided in every laboratory.
- Use of sharp instruments should be avoided whenever possible (scalpel, needles, and scissors).
- Use of pest control measures that will prevent known and unknown vectors from carrying disease in or out of the laboratory must be encouraged.
- Disinfect, clean and decontaminate laboratory areas with 1% bleach and 70% ethanol.
- Access to laboratory testing areas should be limited to authorized personnel.

Cleaning and disinfection of work areas

- Counter tops, work surfaces, laboratory equipment, etc. must be cleaned with disinfectant 1% sodium hypochlorite solution before and after each work day.
- Spills must be immediately contained and properly cleaned with a disinfectant (See pictures).

Recommended disinfectants

Disinfectant	Final concentration
Sodium hypochlorite (household bleach) With available chlorine	0.5% - 10% Lab - 1% used in discards jars 10% to manage blood spills
Ethanol	70%
Isopropyl alcohol	70%
Formaldehyde	4%
Glutaraldehyde	2%
Hydrogen peroxide	4%
Povidone iodine	2.5%

Handling of biohazardous waste

Segregate infectious and non-infectious waste at the point of generation.

- Biohazard warning signs must be plainly pasted on discard containers for infectious waste.
- Household type waste (papers, leftover food, etc.) should be discarded in municipal waste bins.
- A separate puncture resistant container should be used for glass waste and needles.
- All laboratory waste must be properly decontaminated before disposal. This can be accomplished by autoclaving at 121^0 at 15 lbs. pressure for 45 minutes or by incineration. Plastic waste is not incinerated. It is either shredded or buried on the identified sites.
- Liquid wastes should be decontaminated by autoclaving or by adding sufficient sodium hypochlorite (liquid bleach) before discarding.
- Blood spills must be immediately decontaminated with 10% sodium hypochlorite solution freshly made as shown (listed in the above table).

Occupational Exposure and Post-Exposure Prophylaxis

An "exposure" that may place a Health Care Provider (HCP) at risk of bloodborne infection is defined as a percutaneous injury (e.g. needle-stick or cut with a sharp instrument), contact with the mucous membranes of the eye or mouth, contact with non-intact skin (particularly when the exposed skin is chapped, abraded, or afflicted with dermatitis), or contact with intact skin when the duration of contact is prolonged (e.g. several minutes or more) with blood or other potentially infectious body fluids.

Body fluids that are potentially infectious include - blood, semen, vaginal secretions, cerebrospinal fluid, synovial, pleural, peritoneal, pericardial and amniotic fluids or other body fluids contaminated with visible blood. Exposure to tears, sweat, urine, faeces, saliva of an infected person is normally not considered as an "exposure" unless these secretions contain visible blood.

Management of Exposure :

Steps to be taken on accidental exposure to blood (or body fluid containing blood) are:

- Wash wound immediately with running water and soap
- Inform the lab /hospital management and document occupational accident
- Consult with nearest ART centre/ resource for Post-exposure prophylaxis, evaluation, and follow-up (as per the National guidelines on PEP)
- Counselling and collection of blood for testing from the exposed HCW with written informed consent must be done.
- Whenever possible confidential counselling and testing of source for Hepatitis, HIV etc must be done. A history should be taken as well to ascertain likely risk of the source. (PEP should be provided to the exposed HCW until report of source is available and confirmed negative.).
- Risk of infection and transmission must be evaluated
- Never delay start of therapy due to debate over regimen. Begin with basic 2-drug regimen, and change if warranted, once expert advice is obtained
- Reevaluation of the exposed person should be considered within 72 hours post exposure, especially as additional information about the exposure or source person becomes available. The exposed person is advised to seek medical evaluation for any febrile illness that occurs within 12 weeks of exposure.
- Administer PEP for 4 weeks .PEP should be provided until result of the source's test is available and confirmed negative or until course completed ,if source positive or unknown .
- A repeat HIV test of the exposed individual should be performed at 6 weeks, 12 weeks and 6 months post-exposure, regardless of whether or not PEP was taken

Ideally, prophylaxis should be begun within 2 hours of exposure.

Do's	Dont's
Remove gloves, if appropriate	Do not panic!
Wash site thoroughly with running water. Irrigate thoroughly with water or saline if splashes have gone into the eye or mouth.	Do not reflexively place pricked finger mouth. Do not squeeze blood from wound, this causes trauma and inflammation, increasing risk of transmission Do not use bleach, alcohol, betadine, or iodine, which may be caustic, also causing trauma
	Do not use bleach, alcohol, betadine, or iodine, which may be caustic, also causing trauma

(Practice biosafety as shown in colour plates).

Standard work precautions

Do's	Dont's
General masures: Minimise splashing or the formation of droplets or aerosols in all procedures and while handling potentially infectious materials.	Do not eat, drink, smoke or apply cosmetics in the place of work.

Take extraordinary care to avoid accidental wounds from sharp instruments contaminated with potentially infectious material.

Avoid contact of open skin lesions with infectious material.

Discard all disposable articles contaminated with blood in plastic bags or in containers with sodium hypochlorite solution with CAUTION labels.

Disinfect all non-disposable and reusable items before sterilization.

Discard needles and other sharp instruments in puncture resistant containers containing 1% sodium hypochlorite solution.

Do not do any paper work on potentially contaminated surfaces.

Do not undertake direct patient care if you have weeping or exudative skin lesions.

Do not keep glutaraldehyde solution (CIDEX) beyond the recommended time after activation.

Hand washing

Thoroughly wash hands with soap and water.

- Before wearing gloves.
- After removing gloves.
- After completion of work.
- Before and after eating, preparing food or feeding.
- After using the toilet.
- After blowing nose, coughing or sneezing into the hands.
- Before invasive procedures.
- Before and after contact with wounds.
- After accidental contamination with blood or other body fluids.
- After each contact with patient.
- After handling soiled linen or waste.
- Before providing care to patients whose immune system is deficient.

Take care to clean the space in between the fingers and with a sterile scrub brush as shown in pictures.

Dry hands by either hand driers or use disposable sterile towels.

Barrier precautions

Gloves

Do's	Dont's
Wear gloves when there is to be contact with blood, body fluids, mucous membranes, non-intact skin, items or surfaces contaminated with body fluids, and for performing all vascular access procedures.	Do not wear gloves to examine a patient with intact skin.

If there is a breach in skin of hands then wear gloves in all situations.

Change gloves after contact with each patient, especially if soiled with body fluids.

A series of venipunctures can be done after washing gloved hands if gloves are not soiled.

If the gloves get soiled with blood, wash gloved hands with soap under running water before changing gloves for next venipuncture.

Discard gloves that are peeling, cracked, discolored or have visible tears or holes.

Do not touch your eyes, nose, mouth or skin with gloved hands.

Do not walk around the workplace wearing gloves.

Gowns

Wear a gown or apron of plastic or water resistant paper when splashes of blood or other body fluids are expected e.g.
- collection of blood sample
- processing of blood sample etc.

Masks and caps

Wear masks and caps during all routine and emergency surgical and invasive procedures.

Use deflector type of mask made of paper.

Discard them after one hour or at the end of a procedure.

Eye covers

Wear eye covers when droplets or splashes of blood or other fluids are expected.

Shoe covers

Wear shoe covers in all situations where feet/shoes are likely to come in contact with body fluids.

Needles and syringes

Use sterile/disposable syringes and needles.

Do not remove needles from disposable syringes.

Rinse reusable needles and place in puncture resistant containers with CAUTION label and send for sterilization

In the event of a needle stick injury, wash with soap under running water, let the blood flow. Do not squeeze the site.

Destroy the disposable needles and syringes by using needle destroyer.

Do not try to recap used needles or try to bend or break them with hands.

Collection of blood samples:

Do's

Observe all standard work precautions carefully.

Use gloves and take special care if there are cuts or scratches on the hands. Use gloves or water proof dressing in case of open skin lesions (band aid)

Use disposable/autoclaved syringes and needles.

Use thick dressing pad or absorbent cotton below the forearm when drawing blood.

Remove tourniquet before withdrawing the needles.

Place a cotton swab soaked in spirit and maintain pressure till bleeding stops.

Place used needles and syringes in a puncture resistant container containing disinfectant.

Label all bottles / vials before collection of blood.
- Tighten the cap of specimen container securely.
- Wipe off exterior of the container free of any blood with a disinfectant.

Dont's

Do not take blood samples if there are weeping or exudative skin lesions.

Do not spill blood.

Do not give extra skin pricks.
Do not spurt while transferring blood from syringes to the vial.

Do not recap used needles.

Do not remove needles from syringes.

Do not touch your eyes, nose, mouth or skin with gloved hands.

Do not walk around the work place wearing gloves.

Do not do paper work on potentially contaminated surfaces.

Use disposable screw capped vials, place them in plastic bags and tie securely before transportation.

Use plastic 'bread boxes' with proper caution label for transporting these specimens to the laboratory.

Wash hands following completion of blood collection.

Report all accidental exposure. Wash thoroughly with soap and water and let blood flow freely. Then apply Iodophor/tincture of iodine or 70% alcohol.

Decontaminate by autoclaving all potentially contaminated material before disposal or discard in a bucket containing disinfectant with proper 'caution' label.

CHAPTER 5

COLLECTION, TRANSPORT AND STORAGE OF SPECIMENS FOR HIV TESTING

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

Performing venipuncture:

- Gloves should be worn and sterilised /disposable syringes and needles should be used.

- For avoiding soiling, a piece of linen with a layer of dressing pad (a sheet of absorbent cotton between two layers of gauze piece) or simply a big piece of absorbent cotton may be placed below the forearm before commencing veni-puncture.

- After collecting 3- 5 ml of blood aseptically, it should be carefully transferred from the syringe without squirting into a sterile plastic, leak proof specimen container preferably screw capped. The containers should be labelled before commencement of venipuncture. The cap may be tightly screwed after the blood has been transferred to the vial.

- After blood is collected, the tourniquet is removed and the needle is withdrawn. The patient is given a dry sterile cotton swab to press over the site of venipuncture. Elbow may be flexed to keep the cotton swab in place till the blood stops. Any blood spill is carefully wiped with 70% ethanol/10% bleach solution. (As shown in the color plate)

- All the swabs and cotton pieces are placed in plastic bags for disposal. If the outside of the vial is visibly contaminated with blood, it should be cleaned with 10% freshly prepared sodium hypochlorite solution.

- The blood is allowed to clot for 30 minutes (not more than 2 hours) at room temperature. The clot may be gently broken if necessary using sterile Pasteur pipettes.

- Alternatively vacuum based blood collection systems (vacutainers) can also be used. These vacuum based collections are relatively safe for usage and harbour minimum risk of unwanted exposure to infected blood.

Separation of sera samples:

- The vial / vacutainer should be centrifuged at (3000 rpm) for 10 minutes to separate serum to avoid haemolysis. If no centrifuge is available, the blood with clot may be left in the refrigerator at $+4^0$C overnight. The clot will retract and get separated from serum.

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

Addition of preservative

- The usual preservatives should not be added since it inactivates conjugates and gives rise to false serological results.

- If necessary, 5 bromo, 5 nitro, 1-3 dioxane in propylene glycol (Bronidox) at a final concentration of 0.05% is recommended as preservative.

- Thiomersal at a final concentration of 0.01% is effective only for a few weeks as it loses activity when exposed to light.

Storage of serum specimens

- The sera samples are placed in leak proof plastic containers in the refrigerator at 2 to 8^0C for upto 48 hours for storage.

- The outside of the container is checked for visible contamination with blood which should be cleaned.

- All the specimen vials should be adequately labelled with patient details.

- Then the specimen vials are packed in a second tightly capped unbreakable container surrounded by adequate packing material (see figure 1 below).

- For storage for a long time, specimen need to be frozen at -20^0 C or deep-freezing at –70^0C is advised in labs where deep freezers are available.

Sample transportation

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

- The specimen tube, in which serum is to be transported, should not have cracks / leakage. It should preferably be made of plastic and be screw capped. The outside of the container should be checked for any visible contamination with blood which should be disinfected.

- Place the tube containing the specimen in a leak-proof container (e.g. a sealed plastic bag with a zip lock or alternatively the bag may be stapled and taped) and pack this container inside a cardboard canister / box containing sufficient material (cotton gauze) to absorb all the blood should the tube break or leak.

- Cap the canister/box tightly.

- Fasten the request slip securely to the outside of this canister. This request slip should have all details i.e. name, age , sex , risk factors, history of previous testing, etc. should accompany the specimen. The request slip should be placed in a plastic ziplock bag to prevent smudging on account of spillage.

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

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

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

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

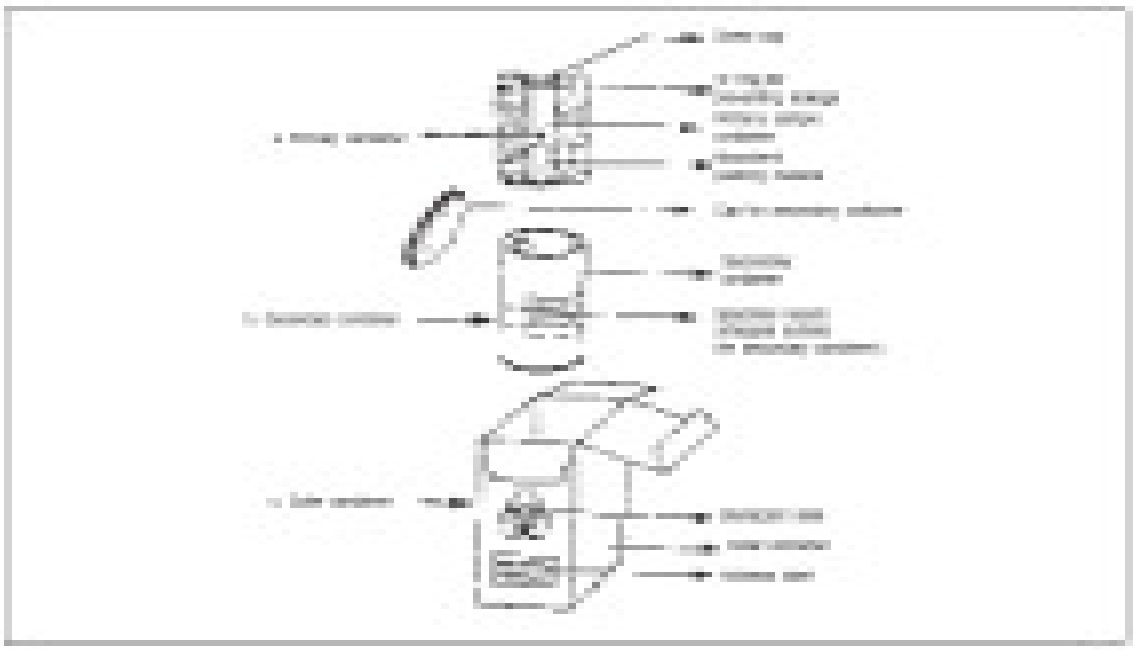


Figure 1 Packaging of specimen for transport to the laboratory.

Safe Handling and disposal of sharps:

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

In case suitable means of disposal of syringe / needles are not available, these disposable syringes hould be heated in dry ovens and be allowed to mutilate to prevent recycling of plastic syringes. Needles can be incinerated.

Biohazard Sign

Biohazard label should be put on packet/parcel containing infectious material for transport of samples.

CHAPTER 6

LABORATORY DIAGNOSIS OF HIV INFECTION

It is important to understand that HIV infection has a long asymptomatic phase and the patient may usually not be aware of the infection in the initial period. Individuals practicing high risk behaviour may be infected and be unaware of the fact. Such individuals in the community need to be counselled and tested after informed consent in a confidential and non stigmatizing way. Laboratory testing is the only way of diagnosing HIV infection. The following objectives are therefore important to arrive at a successful laboratory diagnosis.

- Whom to test- one needs to be able to correctly suspect and screen a client at risk for HIV status.
- How to test i. e. the principles, procedures and the methodology of testing conforming to the objectives of testing and the national policy.
- How to interpret the test result- This refers to the collation and interpretation of the test results followed by the categorization of tested individuals into either HIV positive or HIV negative individuals. This is done by following the national policy of testing as prescribed by the government (NACO guidelines on HIV testing).

The procedure of testing includes pre-test counselling during which the client is informed about ramifications of HIV infection, modes of acquisition, window period, practicing responsible behaviour to prevent further transmission, the remedies and support systems available and importantly, motivating the client to get tested. If the client agrees to undergo the test, a written informed consent is taken and blood is collected. The final interpretation of HIV status is based on the lab test results received. The test may be either positive, indeterminate or negative. The report is given to the client in person maintaining confidentiality and with post-test counselling . Post-test counseling emphasizes and reinforces the pre test counselling. The immune status of the client following a positive result is assessed by doing a CD4 cell count and the case is referred to an ART centre, if required. At every stage, counselling of the client is continued. This all should be done in accordance with the National guidelines.

Pre and post test counselling goes a long way to reduce the stigma and victimization of the individual and empowers the individual to take care of his life. It is important to understand that HIV is not just a biological condition but affects the psychological and emotional state of the client. Counselling involves one to one dialogue between the client and the counselor in a comfortable and conducive environment so that the client is able to confide in the counsellor. The counsellor should fully apprise the client about the treatments and services available such as ART and RNTCP. It is the counsellor's responsibility to explain in detail – the necessity of practicing safe behaviour (safe sex and safe syringes) so that secondary transmission of HIV is prevented. Post-test counselling, whatever the test result may be, should mainly reinforce the above points to make sure that the client is able to make informed and rationale choices in the future as regards financial, social and behavioural matters.

The main stay of diagnosis of HIV/AIDS in India is based on detection of HIV specific antibodies. Though, in certain circumstances, one might have to resort to detection of viral products for diagnosing infection. This could be in the case of a new born exposed to HIV, during the perinatal period in an HIV infected mother, during the window period and to resolve discordant serological results that is in cases where one or two tests are reactive and the third test is non reactive. There are different strategies/algorithms employed for the detection of antibodies.

In children below 18 months, detection of virus and/or viral products is done to diagnose HIV infection. This is done by either detecting p24 antigen using ELISA or conducting viral nucleic assays for detection of RNA in plasma and/or DNA in cells through PCR. DNA PCR is the recommended test for infant diagnosis.

A number of kits such as ELISA and rapid assays are available in the market to detect HIV antibodies and the kits should be selected carefully. Diagnosis of HIV infection requires two to three different assays based on different antigen systems and or different principles of tests. For the first i.e. the screening assay, the kit selected should be 100% sensitive and highly specific. The second and the third assays i.e. the supplementary or confirmatory assays selected should have high sensitivity as well as high specificity i.e. specificity of more than 98%. If the test comes out positive, then confirmatory testing is done i.e. the sample is then tested with two or three different kits one after another according to the strategy/algorithm of testing, adopted. The tests need to be done on the same sample. The strategies/algorithms are employed to detect and diagnose HIV as per the National Guidelines.

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

- Indirect ELISA
- Competitive ELISA
- Sandwich ELISA
- Immuno capture ELISA

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

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

When a serum sample tests reactive once by a system of ELISA / Rapid (E/R) test for detection of HIV for donated blood in blood banks the unit of blood testing reactive with screening HIV-test is discarded. In case the donor desires to know the result she/he is referred to VCTC /ICTC for further assessment of HIV status. Supplemental tests may be E/R and Western Blot, etc.

ELISA takes up to three hours to yield results. It has a major advantage of being economical, and is less labour intensive if a large number of samples are being tested every day. Although rapid tests give result within minutes these are relatively expensive per test. Commercial kits are available for ELISA and rapid tests. The recommended first line tests by NACO for blood banks are ELISA.

Rapid tests include:
- Dot blot assays (immunoconcentration, based on vertical flow)
- Particle agglutination (gelatin, RBC, latex, microbeads)
- Dip stick and comb tests, etc (ELISA technology based).
- Immunochromatography based tests(lateral flow)

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

Supplement tests

• Second and third ELISA /Rapid • Western blot	WB is done to resolve discordant results, in legal cases, problem cases and for research

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

False positive and false negative results

There are conditions which may give rise to false positive or false negative ELISA /Rapid test results. False positive results have been reported in cases of haematologic malignant disorders, DNA viral infections, autoimmune disorders, multiple myeloma, primary biliary cirrhosis, alcoholic hepatitis, chronic renal failure, positive RPR (Syphilis) test and due to technical errors. False negative results have been reported in cases of window period prior to seroconversion, immunosuppressive therapy, malignant disorders, B-cell dysfunction, bone marrow transplantation and due to technical errors.

Strategies/algorithms of HIV testing

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

- ELISA/ Rapid tests:
  Used in strategy I, II & III

- Supplemental test: E/R and Western Blot(WB) used in strategy II and III
  WB is used in problem cases where discordant serological results are obtained, if WB is not available than client should be retested all over again after 2-4 weeks or referred to the reference lab.

Strategy I, follow algorithm I

Blood/plasma/serum is subjected once to E/R for HIV. If negative, the sample is to be considered free of HIV and if positive, the sample is taken as HIV infected for all practical purposes. This strategy is used for ensuring donation safety (blood, organ, tissues and sperms etc.). Unit of blood testing positive is destroyed as per guidelines. A donor who gives consent to know the result of HIV test is informed about the result. In case the test is positive, donor is informed about the possible reactive nature of the result and is advised that the same requires confirmation. He/she is referred to ICTC for counseling, testing and confirmation of the test result.

Strategy IIA, follow algorithm II A

This strategy /algorithm is used for sentinel surveillance (anonymous, unlinked testing). A serum sample is considered negative for HIV if the first ELISA or rapid test is non-reactive, but if reactive, it is subjected to a second ELISA or rapid test which utilizes a system different from the first one. i.e. the principle of test and/or the antigen used is different. The sample is reported reactive only if the second ELISA/ rapid test confirms the positive report of the first test. In case the second E/R is non reactive, then we would take the result as negative for surveillance purposes. This is strategy II A.

Strategy IIB, follow algorithm Algorithm IIB

This strategy is used for detection of HIV infection in symptomatic individuals with symptoms of AIDS, clinically. The sample is processed as in strategy IIA, but a sample reactive with first assay and non-reactive with the second assay is subjected to the third tie breaker E/R. If the third E/R is reactive, sample is reported as indeterminate and patient is called back for repeat testing after 2- 4 weeks . If the third test is negative, it is reported as negative. Two to three different test kits with different antigen system or different principle of test are required to follow this strategy (II B) of testing. In this strategy, if the first two consecutive tests are positive for presence of HIV antibodies, a positive report can be given to the patient after post-test counselling.

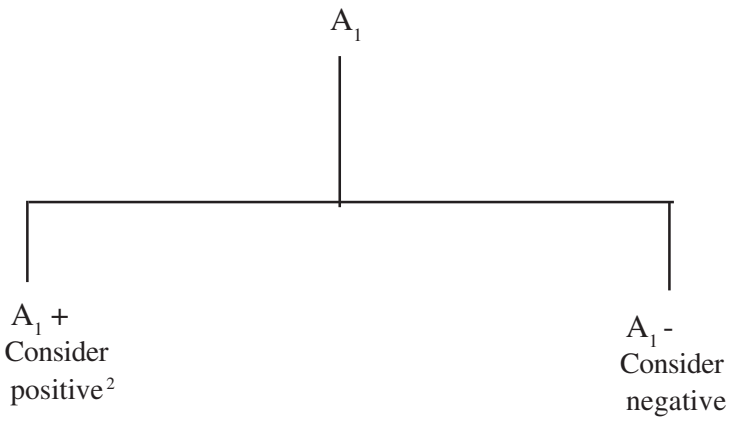
Strategy III, follow algorithm III

This strategy/ algorithm is used at ICTC/VCTC to diagnose HIV infections in asymptomatic individuals. It is similar to strategy IIB, with the added confirmation of a third reactive E/R test being required for a sample to be reported as HIV positive. If the sample gives reactive result with two E/Rs and non reactive with the third assay, it is reported as "indeterminate" and patient is called back for repeat testing after 2-4 weeks. A sample reactive in first assay and non-reactive in second assay is subjected to a third tie breaker assay. In case, the third assay also gives non- reactive result, sample is reported as negative for HIV infection. In such cases when a negative report has been issued (first test reactive and and the next two tests non- reactive) but, the client history is suggestive of high risk factor / behaviour he may be asked to come for follow up testing after 2-4 weeks.

The test to be utilized for the first screening test is one with the highest sensitivity (may give high number of false positives) and the second and third tests (supplemental) should be those with the highest specificity (to minimize false positive reactions). This strategy is used for diagnosis of HIV infection in asymptomatic individuals at ICTCs / VCTCs. Counseling and informed consent are a must in these cases. Three different kits with different antigen system and / or different principle of test are required to follow this strategy.

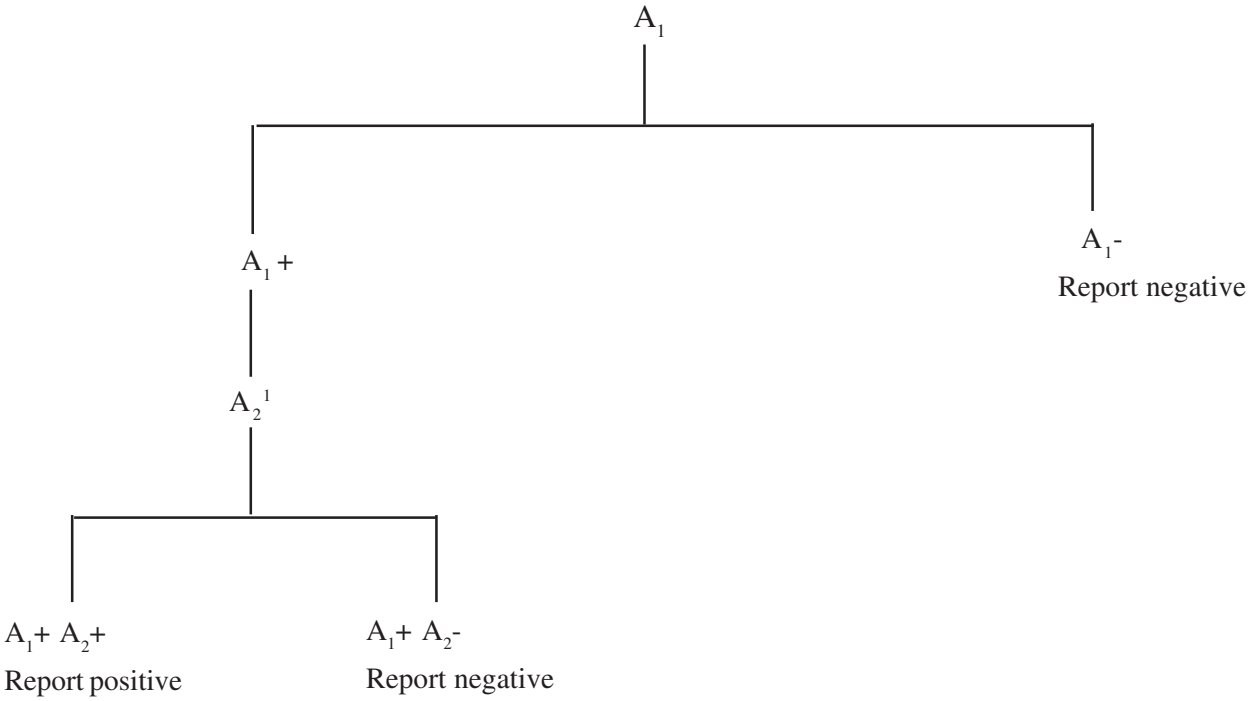
Use of improved, third and fourth generation serological assays demonstrates that seroconversion typically occurs 3-12 weeks post-infection, although significant delay can occur in some individuals.
In diagnosing HIV-1 infection, the specificity of ELISA is >99%, when properly performed and the sensitivity is >98%. In low-risk populations, the false-positive rate of combined EIA and WB testing is estimated to be <1 in 100,000. Highly sensitive and specific agglutination and EIA methods for detection of type-specific antibodies to HIV-2 are also available.

Strategy I
(For Transfusion/ transplantation safety)
One test kit required

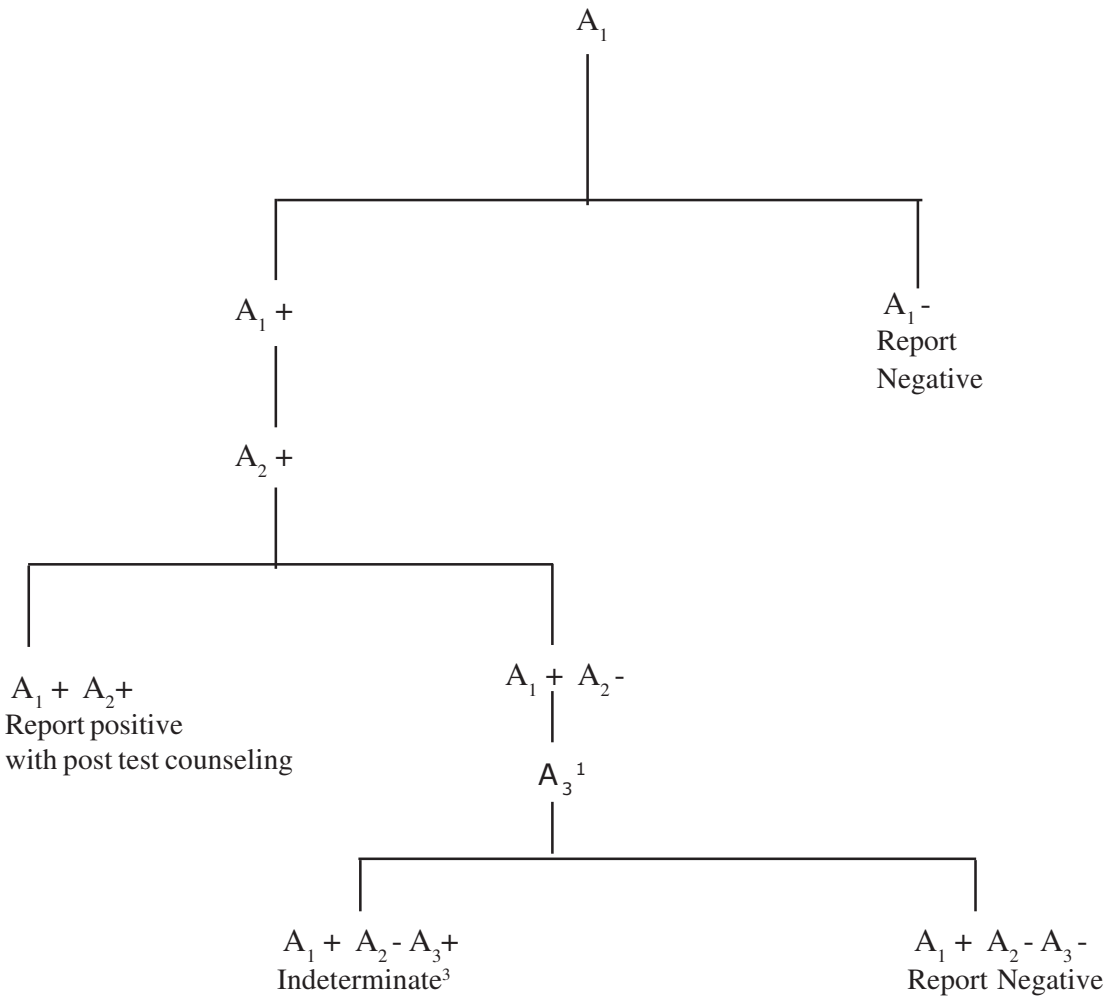


(Destroy the unit of blood
as per guidelines refer to VCTC/ICTC
for confirmation of status after consent

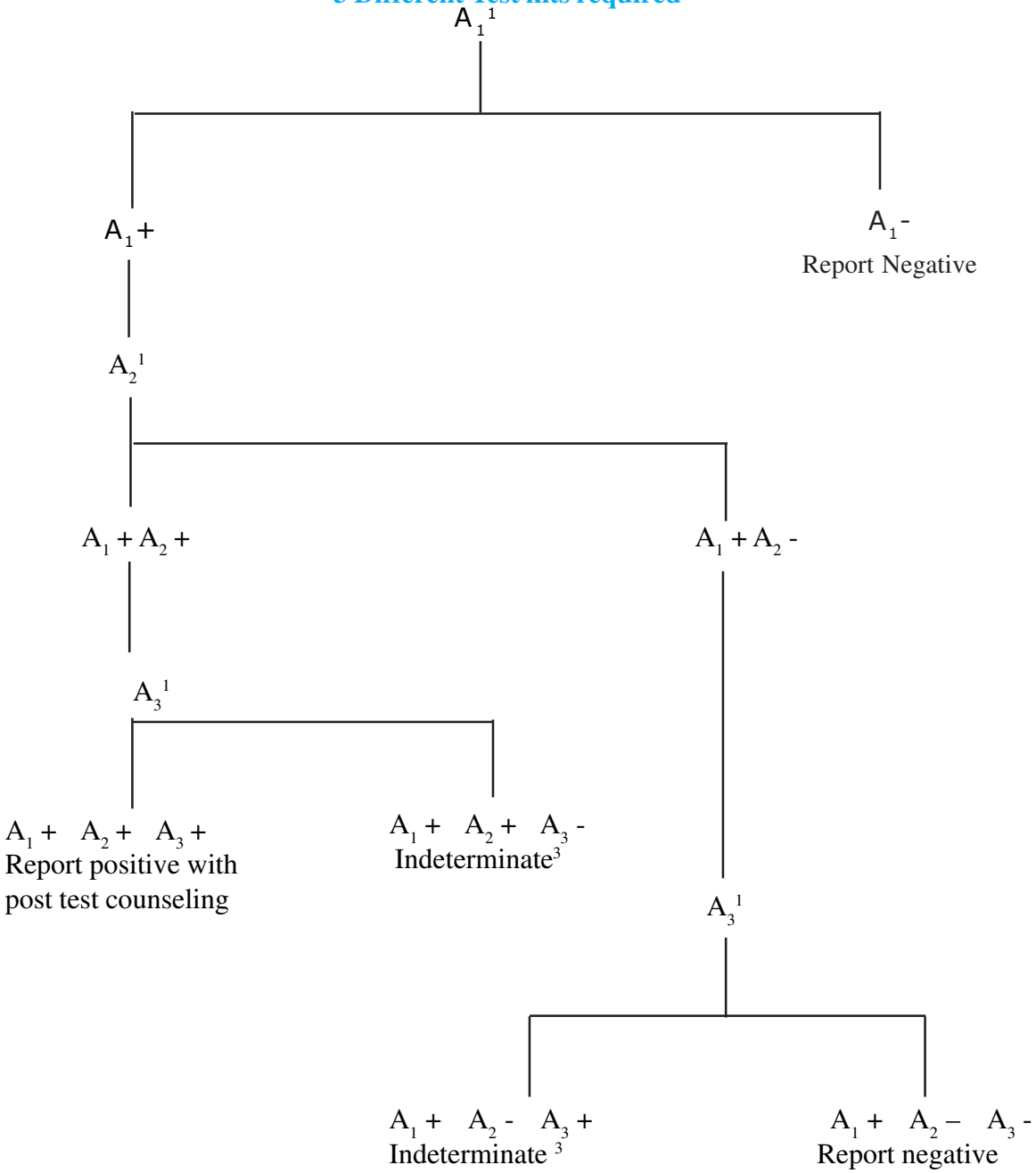
Strategy II A
(For sentinel surveillance)
2 Test kits required



Strategy II B
(Diagnosis of an individual with symptoms suggestive of AIDS clinically)
3 different kits are required.



Strategy III
[To detect HIV infection in asymptomatic individuals (ICTC, PPTCT)
3 Different Test kits required



[1]Assays A1, A2, A3 represent 3 different assays

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

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

CHAPTER 7

PERFORMANCE OF RAPID TESTS IN A HIV TESTING LABORATORY

Rapid tests are in vitro qualitative tests for the detection of antibodies to Human Immunodeficiency Viruses (HIV) types 1 and 2 in human serum, plasma, whole blood saliva and urine. Currently HIV testing in India is performed on serum/whole blood (fingerprick blood collected with anticoagulant), and plasma. This is because the HIV testing on urine and saliva samples has not been evaluated and validated in India In recent times, a large number and wide range of rapid tests of high quality have become available and are being currently used world wide in laboratories. Most rapid assays are in kit form that include all necessary reagents and require no other specialized equipment. The three most common assay formats that can be used with are given below :

Particle agglutination assays

These tests typically require 10 minutes or more to obtain a result. When a patient specimen containing HIV antibodies is mixed with latex particles coated with HIV antigens, cross-linking occurs and results in lattice formation seen as agglutination. Results are interpreted visually. Serum , plasma and whole blood can be used as possible samples in this category. An example is shown below

Fig 1. Capillus agglutination test

Reactive

Latex Aggregation – white clumping

Non-reactive

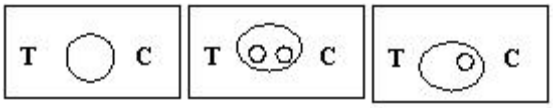
No Latex Aggregation – no white clumping

Other particles like gelatin beads and RBCs are also used to develop agglutination based rapid tests. These solid particles are coated with HIV antigens and when these in solution interact with antibodies present in an HIV positive sample a lattice is formed which is seen as clumps. The step by step performance of Capillus test is shown in the coloured plates.

Immunoconcentration (flow through) assays

These devices employ solid-phase capture technology, which involves the immobilization of HIV antigens on a porous membrane. The antibodies if present in (serum, plasma, whole blood) the specimen flow through the membrane during the performance of test and are absorbed on the antigen into an absorbent pad. A dot or a line visibly forms on the membrane when developed with a signal reagent. Tests usually include a procedural control dot or line. Some tests allow the differentiation of HIV-1 from HIV-2. These tests usually require several steps for the addition of specimen, wash buffers, and signal reagent. They can usually be performed in 5 to 15 minutes. The step by step performance of Tri dot test is shown in the coloured plates.

Fig 2 Immunoconcentration method



Non-reactive **Reactive** **Invalid**

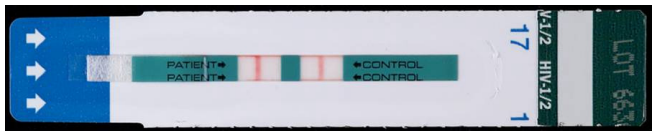
Immunochromatography (lateral flow) HIV tests

The strips / cards, incorporate both antigen and signal reagent into a nitrocellulose strip. These tests mostly require only a single step. The specimen (usually followed by a buffer) is applied to an absorbent pad. Alternatively, the specimen is diluted in a vial of buffer, into which the test device is inserted. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where HIV antigen has been applied. A procedural control line is usually applied to the strip beyond the HIV-antigen line. A visual line at both the test and control sites indicates a positive test result, a line only at the control location indicates a negative test result, and the absence of a line at the control site means the test is invalid. Examples include Determine (Abbott), Unigold (Trinity) and Trline tests. Step by step performance of test is shown in the color plates.

Fig. 3 Determine HIV 1 / 2

Reactive

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



Non-reactive

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

Fig 4. Unigold HIV

Reactive / Positive **Non-Reactive**

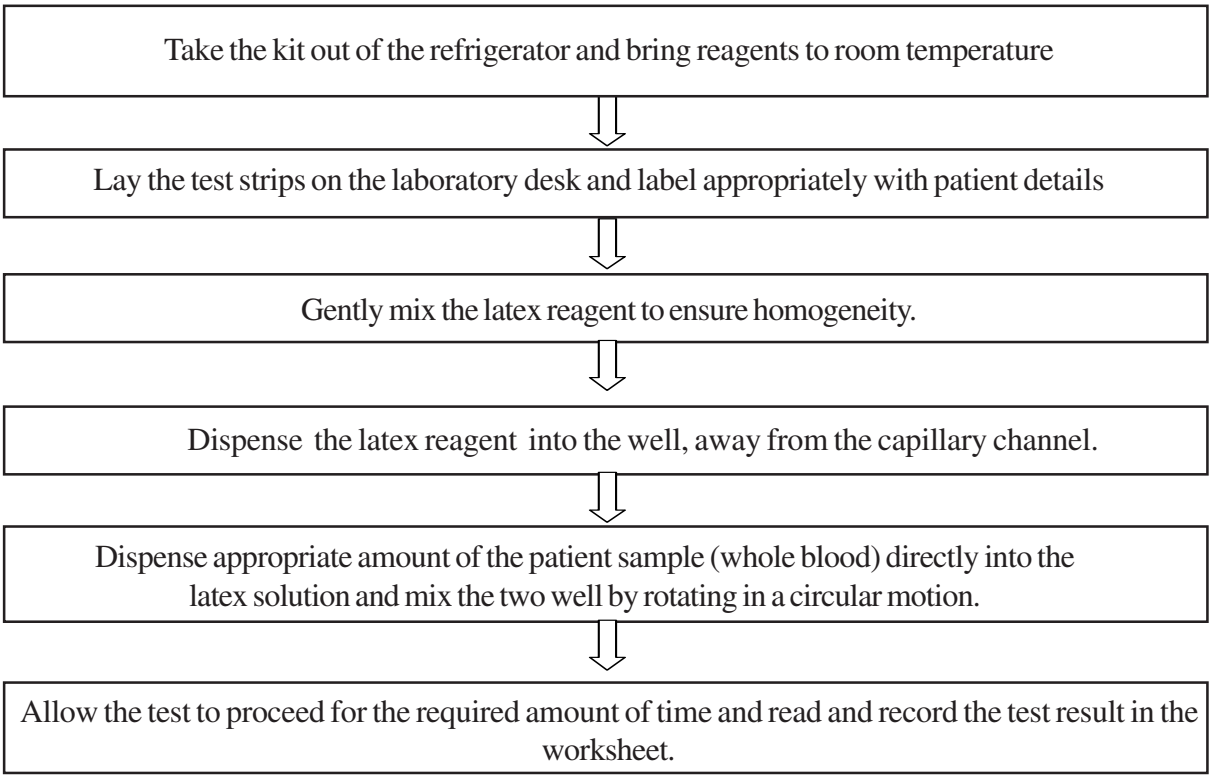
Standard operating procedures

Particle agglutination assay

Capillus HIV test is an example of this type of test and is explained briefly below.
Materials required:

- Autopipettes (5-50µl)
- Absorbent paper
- Hand gloves (powderless)
- Autopipette tips
- Discard jar with discard solution
- Wash bottles
- Stop watch

Test procedure:



Immunoconcentration assays

Tri-Dot rapid test works on the principle of immunoconcentration (flow through) and is briefly described below.

Materials required : Same as above

Specimen: Serum /plasma

Test procedure

Take the kit out of the refrigerator and bring reagents to room temperature

⇩

Take the test device out of the pouch and label with patient details

⇩

Load the test device with appropriate amount of buffer solution

⇩

Dispense appropriate amount of patient sample on to the test card

⇩

Add requisite amount of buffer solution to remove excess sample

⇩

Add required amount of chromogen (color developer)

⇩

Read and record the test flow results as negative or positive

Immunochromatography assays (lateral flow) (ICT)

Two examples of this type of kits are Tri line and Determine HIV 1/2

Materials required : Same as above

Specimen: Serum /plasma

Test procedure (HIV 1 /2 Tri-line test):

Take the kit out of the refrigerator and bring reagents to room temperature

⇩

Take the test device out of the pouch and label with patient details

⇩

Load the test device with appropriate amount of patient sample and discard the dropper into the discards jar

Add requisite amount of buffer solution to the test device

⇩

Read and record the test results as per manufacturers directions

Determine HIV 1 / HIV 2:

Specimen : Whole blood , serum, plasma

Test procedure:

Take the kit out of the refrigerator and bring reagents to room temperature

⇩

Take the test strip out of the pouch and label with patient details and pull out the protective foil cover

⇩

Take appropriate amount of specimen and apply on the absorbent pad onto the strip

⇩

In case the specimen used is whole blood apply 1 drop of chase buffer, the same not to be applied in case of serum / plasma

⇩

Read and record the results after an appropriate time.

* The test result of all rapid tests are interpreted as HIV positive or HIV negative following the instructions given in the literature.

CHAPTER 8

PERFORMANCE OF ELISA IN A HIV TESTING LABORATORY

Laboratory diagnosis is the only method of determining the HIV infection status of an individual. Detection of anti-HIV antibodies continues to remain the mainstay of testing for presence of HIV infection. This can usually be done by performing an ELISA (Enzyme Linked Immunosorbent Assay) or a rapid test.

Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily assayed enzyme that possesses a high turnover number. ELISAs can provide a useful measurement of antigen or antibody concentration.

It is usually the most commonly performed screening test at blood banks and tertiary care sites testing a large number of specimens a day. It is easy to perform, adaptable to large number of samples, is sensitive and specific and cost effective. The choice of test for conducting an ELISA should be made taking into consideration the available resource, storage facility, technical expertise, infrastructure, objective of testing, prevalence of infection and performance characteristics of test kits, etc.

ELISAs can be divided into the following 4 categories:

- Indirect
- Sandwich
- Competitive
- Immuno capture ELISA

Standard operating procedures[2]

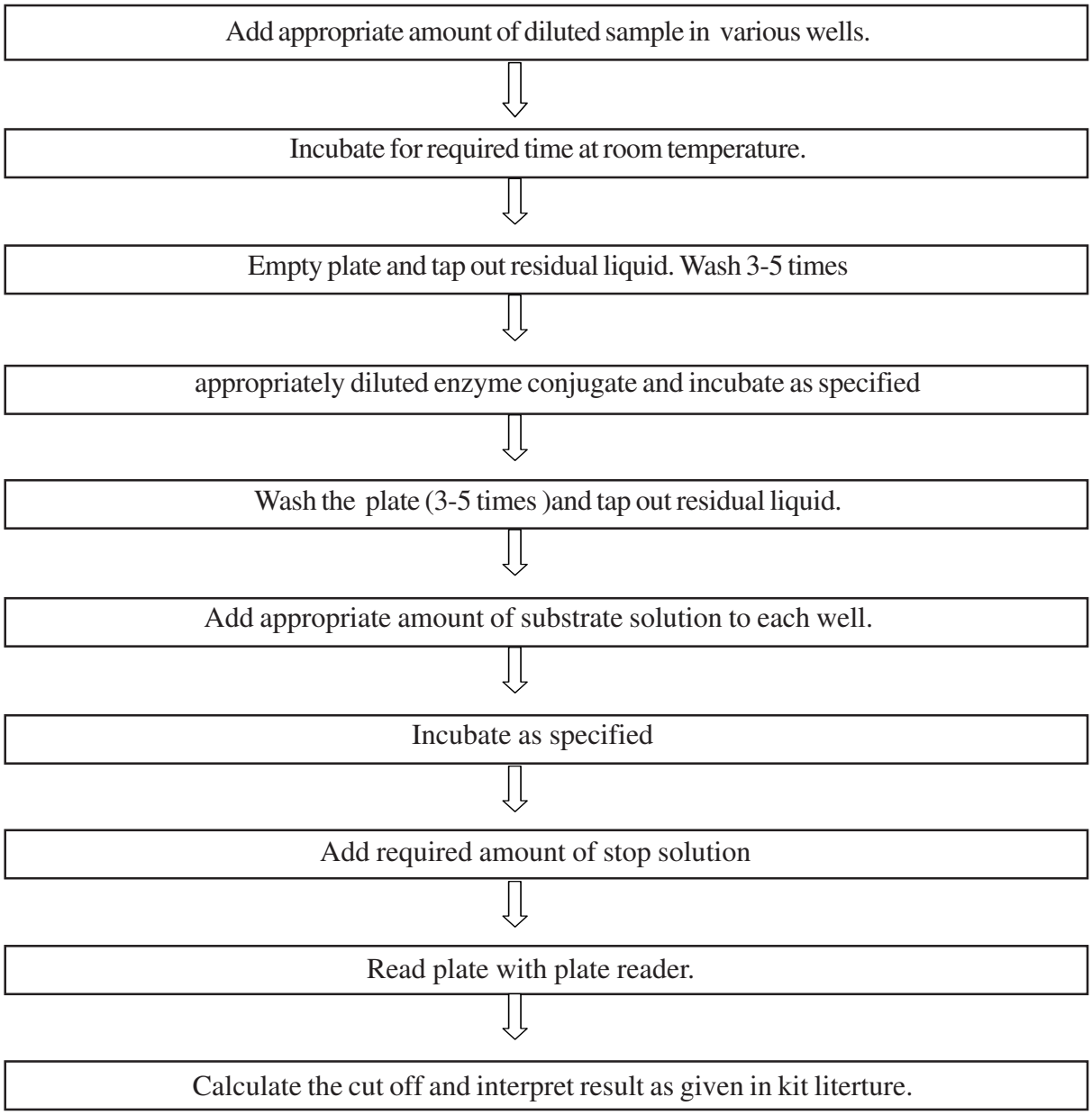
Indirect ELISA

Principle :
HIV antigens are attached covalently to the solid phase support allowing HIV antibodies present in the specimen to bind, and these bound antibodies are subsequently detected by enzyme labelled anti-human immunoglobulin and specific substrate system. If the test specimen contains antibodies colour reaction will take place. Most of the commercial assay available in the market are based on this principle.

Materials required:
- Single / multichannel pipettes with disposable tips: 5-50µl , 50-200µl
- Incubator (37 ± 2^0C)
- ELISA reader with or without washer
- Sodium hypochlorite solution (1 %)
- Powderless disposable gloves
- Absorbent paper
- Deionised water
- Discards jar with hypochlorite solution
- Wash bottles

[2]These procedures have not been provided as substitutes for product inserts. Exact instructions must be followed as provided in the product insert. These only serve as examples of the various test types.

Protocol



Sandwich ELISA assay

One of the most useful of the immunoassays is the "sandwich" ELISA. This assay is used to determine the antigen/antibody concentration in unknown samples. This ELISA is fast and accurate. However, in India most of the sandwich ELISA assays being used detect HIV antibodies and principle of the same is given below.

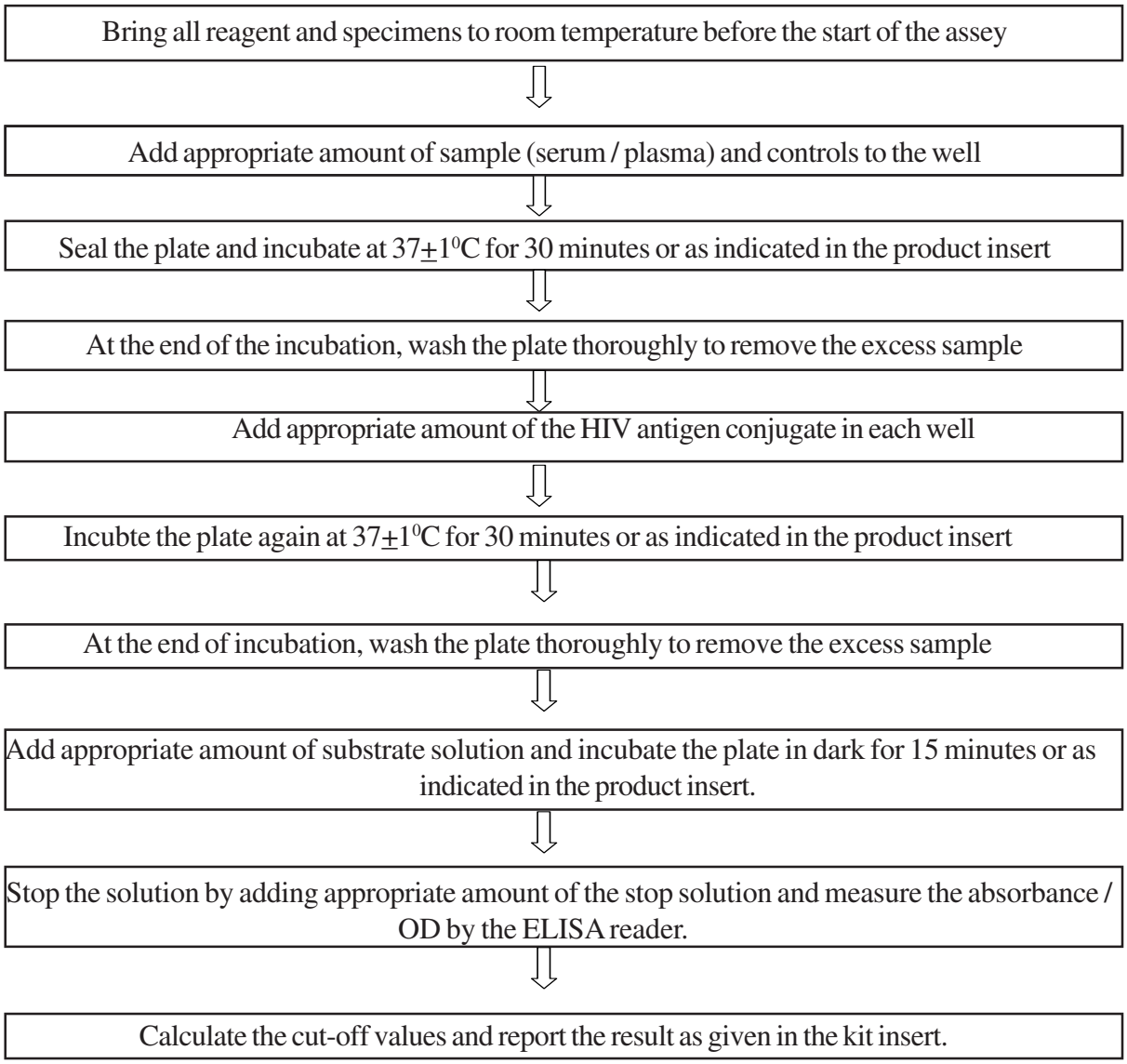
Principle

The principle involves the use of recombinant HIV antigens for detection of antibodies to HIV in human serum or plasma. The HIV antigens are attached to the solid phase. When the serum/ plasma is added to the wells, the HIV antibodies if present in the sample bind the antigens adsorbed on the plate to form an antigen-antibody complex. This is followed by the addition of the conjugate of recombinant DNA HIV 1/ 2 antigens attached to an enzyme such as horse radish peroxidase (HRPO), resulting in the formation of an sandwich -HIV antigen (1/2) : anti HIV antibody (1/2) : HIV 1/2-HRPO, which is detected by the addition of a substrate cum chromogen (color developer). The absorbance of the color that is developed is a measure of the antibodies present in the sample.

Materials required

Same as in Indirect ELISA.

Procedure



Competitive ELISA assays

Principle

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

Materials: These are the same as used in case of indirect / sandwich ELISA techniques

General Protocol

Add appropriate amount of standard / sample solution to the wells

⇩

Add appropriate amount of conjugate solution to the wells

⇩

Wash the plate with wash solution 3-4 times

⇩

Allow to incubate for required amount of time (as in kit insert)

⇩

Add substrate as indicated by manufacturer. After suggested incubation time has elapsed, optical densities at target wavelengths can be measured on an ELISA reader and test results interpreted as given in kit insert

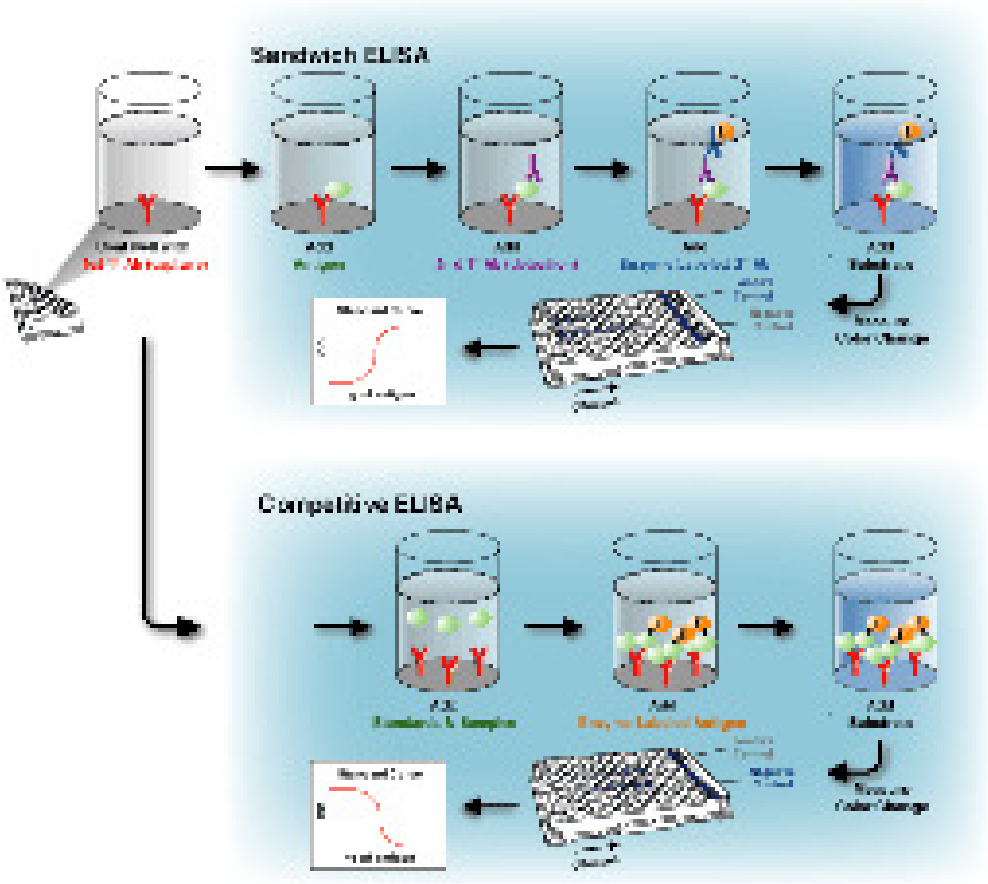


Fig 1 Sandwich and competitive ELISA techniques

Immuno capture ELISA

This ELISA may be used to determine the presence of antigen/ antibody in unknown samples, However for purpose of HIV testing by this ELISA, we detect antibodies to HIV in the patient samples.

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

The antigen capture ELISA for detecting HIV antibodies in the patients sample is described here.

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

Troubleshooting ELISAs assays

- Interpret the control results.
- If the negative controls are giving positive results, there may be contamination of the substrate solution, or contamination of the enzyme-labeled antibody, or of the controls themselves.
- If no color has developed for the positive controls or for the samples, check all reagents for expiry date, concentration of reagents, and storage conditions. Check the integrity of the antibody reagent.
- If very little color has developed for the positive controls and the test samples, check the dilution of the enzyme labeled antibody, and the concentration of the substrate.
- If color has developed for the test samples but not the positive or negative controls, check the source of the positive controls, their expiration date and their storage.
- If color can be seen, but the absorbance is not as high as expected, check the wavelength setting of the ELISA reader.
- When rerunning an assay while troubleshooting, change only one factor at a time.
- Other errors that may lead to low absorbance may include improper washing of the plates, improper pipetting (operator related / calibration related).
- Errors may also occur because of improper calibration of reader.

CHAPTER 9

STANDARD OPERATING PROCEDURES OF SOME ROUTINE INVESTIGATIONS TO BE UNDERTAKEN AT PHCS & PPTCTCS

Hemoglobin estimation

Hemoglobin (Hb) is a porphyrin iron protein compound that transports oxygen from the lungs to the body tissues where it is utilized for energy metabolism. It is usually advised in the diagnosis of anemia. Different methods in use include:

1. Drabkin's method
2. Automated cell counters

Drabkin's method

Principle

Haemoglobin is oxidised to methemoglobin by potassium ferricyanide, which reacts with cyanide ions of potassium cyanide to form cyanmethemoglobin. The haemoglobin is estimated with the help of cyanmethemoglobin curve. It is the most commonly employed method for the estimation of hemoglobin. The advantages of this method are i) error due to subjective visual matching is avoided as spectrophotometer is used and hence reading is precise and reliable, ii) measures all forms of haemoglobin except sulphaemoglobin. iii) single step procedure using single reagent. iv) cyanmethemoglobin formed produces broad absorbent band at 530 nm v) good stable haemoglobin standards are available.

Materials required:

- Glass test tubes with K_2/K_3 EDTA anticoagulant (10% solution) / K_2-K_3 EDTA vacutainers
- Autopipette with 5-50 µl and 200-1000 µl capacity
- Glass pipette (5 ml / 10 ml capacity)
- Autopipette tips
- Filter paper
- Tissue paper
- Semi-autoanalyser / photocolorimeter
- Hemoglobin standard (commercially available).
- Drabkin's reagent.

Specimen collection

Draw 3-5 ml of blood in the vacutainer (depending upon the capacity) or alternatively draw the requisite amount of blood in a tube with K_2/K_3 EDTA as the anticoagulant. Mix the blood with the anticoagulant by gently inverting the tube 5-6 times. The blood should not get clotted under any circumstances.Finger prick method can also be used and blood can be directly taken with an autopipette from the finger in case only Hb has to be estimated.

Method

- Add 20 µl (0.02 ml) of whole blood into 5ml of the Drabkin's solution (commercially available) and wait for 5 minutes.
- Read absorbance on a photometer at 530 nm and calculate the result with the help of a hemoglobin standard curve prepared by using the standard hemoglobin.

References

Wintrobe MM. 'Clinical Hematology' 7th Edition. Philadelphia: Lea and Febiger; 1975, pp 114-115.

Glucose estimation in blood

Glucose is the major carbohydrate found in blood and a chief source of energy in the human body. The blood glucose levels are perfectly maintained under the influence of hormones like insulin and glucagons. However, the hormonal imbalance at times may result in abnormalities of glucose metabolism and result in diseased conditions. Thus the detection of blood glucose levels can provide a basic understanding of the malfunctioning of the tissues and body.

Glucose oxidase – peroxidase method

Principle: As a part of this method, glucose is first oxidized into gluconic acid and hydrogen peroxide. The hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoamine dye complex.

Materials required:

- Glass test tubes
- Sodium fluoride vacutainers
- Autopipette with 5-50 µl and 100-1000 µl capacity
- Autopipette tips
- Filter paper
- Tissue paper
- Centrifuge (table top)
- Semi-autoanalyser / photocolorimeter / glucometer
- Glucose estimation kits / strips

Specimen collection

Draw 3-4 ml of blood in the vacutainer / tube with sodium fluoride as the anticoagulant. In case vacutainer is not being used usually a pinch of sodium fluoride is sufficient for 3 ml of blood. Mix the blood with the anticoagulant by gently inverting the tube 5-6 times. The blood should not get clotted under any circumstances.

Method:

- Separate the plasma by centrifuging the blood specimen at 3000 rpm for 10 minutes.
- Take 1.0 ml of the glucose reagent (commercially available kit) in a test tube and add 10 µl (0.01 ml) of plasma to the tube. Vortex and incubate at 37^0C for 5-10 minutes (depending upon the kit). The reagent should be brought to room temperature before use.
- Read at 500-520 nm and calculate the result with the help of a glucose standard curve prepared by using the standard hemoglobin.

Estimation of glucose by a glucometer

Blood glucose can also be estimated by rapid methods with the help of glucometers. This usually involves the use of a strip that has the reagents pre-loaded on to it. The process involves whole blood (from finger prick) that is applied on to the strip which is then inserted into the glucometer to get a reading. Following the instruction of the manufacturer.

Precautions

- Improper anti-coagulant to blood ratio might effect the results.
- The blood should not get clotted under any circumstances.

- The substrate (glucose reagent) should be checked for any color development before subjecting to testing.
- The pipettes must be calibrated before use.
- In case of estimation by a glucometer, the strips should be sealed properly to ensure that they do not catch moisture.

Reference

Trinder, P. Determination of glucose in blood using glucose oxidase with an oxygen receptor. Ann. Clin. Biochem., 1969, 6: 24-27.

Urine analysis

Urine analysis may be carried out to diagnose urinary tract infections, other diseases associated with renal functions (proteinuria, hematuria) as well as for the diagnosis of certain metabolic disorders (diabetes etc.). It consists of the following:

Physical and chemical examination: Urine color, appearance, specific gravity and odor, pH, proteins, glucose/ sugar, bile salts, bile pigments, ketones and nitrites etc.
Microscopic examination: Pus cells, RBCs, crystals, casts (granular / hyaline) and bacteria.

Materials required:

- Urine containers (sterile)
- Test tubes (3-5 ml capacity)
- Glass micro-slides
- Covers slips
- Centrifuge (table top)
- Urine strips (3 parameter – pH, protein, glucose)
- Microscope

Specimen collection

Minimum (first morning, mid-stream) sample of 3 ml (1 ml in case of children) should be collected by the clean catch technique. Ideally the sample should be analysed within 1-2 hours of collection, alternatively the sample may be refrigerated.

Method:

- Transfer appropriate volume of urine from the container into the tube. Take the urine strip out of the
- strip container. Lid must be replaced immediately on the strip container as the strips can get spoilt by catching moisture.
- Dip the strip into the urine for 2-3 seconds. All 3 test pads should get adequately dipped into the sample. Take out the strip and compare it with the color matrix provided with the packing after 1 minute (or as indicated in the product insert).
- Subsequently centrifuge the specimen for 10 minutes at 3000 rpm. Remove the supernatant and transfer the sediment on to a glass micro-slide. Cover it with a cover slip and examine the sediment under the 40X objective of a microscope and report the findings.

Precautions

1. A mid-stream preferably early morning urine sample should be collected.
2. In case the urine cannot be analysed immediately, it should be kept at 4^0C till analysed.
3. Sterile containers should be used for sample collection.
4. The urine testing strips must be sealed properly during storage

VDRL / RPR test for syphilis

Principle

Syphilis tests are used for the detection of reagin and other antibodies in blood or body fluids produced against the spirochaete bacteria that cause this disease. The causative organism of the disease is *Treponema pallidum*. The tests may be used to screen or to confirm the infection. It is usually done by the following methods

Venereal disease research laboratory (VDRL) test. The VDRL test is done to screen for syphilis. It checks for an antibody called reagin that can be produced in people who have syphilis. The VDRL test may be done on a sample of serum.

Rapid plasma reagin (RPR) test. The RPR test also detects reagin and is commonly used as a rapid screening test.

Treponema pallidum haemagglutination assay (TPHA). TPHA is the specific test used to confirm a syphilis infection after screening test results indicate possible syphilis. This test detects antibodies to the spirochaete that causes syphilis and can be used to detect syphilis at all stages, except during the very early stage.

The current text describes the VDRL / RPR tests.

Materials required:

- Red top vaccutainers / plain glass tubes,
- Centrifuge (table top),
- VDRL/RPR kit, droppers (as supplied with the kit or otherwise),
- VDRL rotator (optional).
- Mixing/ Applicator Sticks

Method:

Draw 3-4 ml of blood into the vacutainer / glass tube. Allow the blood to clot at room temperature (ambient). This is followed by centrifuging the sample to separate the serum. Bring the kits to room temperature. Take 1 drop (50-100 µl) of serum sample and place it on the RPR/VDRL plate. Add to it the requisite amount of the VDRL/RPR reagent. Mix the sample and the reagent thoroughly with the help of applicator sticks (provided in the kit) and keep rotating the plate either by uniform motion of hands or by putting on a VDRL rotator for the stipulated time (see product insert) usually 8-10 minutes. Read the result as per the instructions in the insert.

Quality control: The positive and negative controls must be used with every batch of tets.

Interpretation Clumping / agglutination of the particles amounts to a reactive/positive result.

Gram Staining

It is one of the most important staining techniques in microbiology and is almost always the first test performed for the identification of bacteria and fungi. The primary stain of the Gram's method is crystal violet. Crystal violet is sometimes substituted with methylene blue, which is equally effective. The microorganisms that retain the crystal violet-iodine complex appear purple/violet under microscopic examination. These microorganisms that are stained by the Gram's method are commonly classified as Gram-positive or Gram non-negative. Others that are not stained by crystal violet are referred to as Gram negative, and appear red/pink.

Materials

- Crystal violet
- Iodine solution
- Decolorizer
- Safranin
- Water (preferably in a squirt bottle)
- Glass slides
- Staining rack

Method

Gram-staining is a four step procedure which uses certain dyes to make a bacterial cell stand out against its background. Make a thin smear of the sample and heat fix the smear before staining.

- Place slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds. When the time has elapsed, wash slide for 5 seconds with water. The specimen should appear blue-violet when observed with the naked eye.

- Now, flood the slide with the iodine solution. Let it stand for about a minute.When time has elapsed, rinse the slide with water for 5 seconds and immediately procede to the next step. At this point, the specimen should still be blue-violet.

- This step involves addition of the decolorizer, ethanol. Add the ethanol dropwise until the blue-violet color is no longer emitting from the smear. As in the previous steps, rinse with the water for 5 seconds.

- The final step involves applying the counterstain, safranin. Flood the slide with the dye as for crystal violet. Let this stand for about a minute to allow the bacteria to incorporate the saffranin. Gram positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.

- Having completed these steps, blot the slide gently with or allow it to air dry before viewing it under the oil emersion lens of the microscope.

Precautions

- The slides should be free of grease before the sample is applied .
- The sample must be applied and air dried on the slide properly.
- The stains must be filtered before use.
- The smears should not be rubbed.

Ziehl-Neelsen (ZN) staining

ZN staining is a key technique for the diagnosis of mycobacterial infections. The technique is applicable to most biological specimens. It is also known as acid fast (AFB) staining. Modified ZN staining is used to diagnose opportunistic protozoan parasitic infections.

Materials

- Glass slides
- Staining rack
- Carbol fuchsin
- Loeffler's methylene blue
- Sulphuric acid
- Binocular microscope

Method

- Mount the specimen on the slide and heat fix it before staining. The smear should be air dried and heat fixed by flaming.

- Place slide on a slide holder or a rack. Flood the slide with ZN carbol fuchsin. Heat the slide from below till the stain just boils. Keep it for 5 min with intermittent heating. Do not allow the stain to dry, put more carbol fuschin if required to cover it. Wash the slide with tap water.

- Decolourise with 20% sulphuric acid (for modified AFB staining 5% sulphuric acid is used). After 1 minute, wash with water and pour more acid. Repeat this again and keep it for 5-10 minutes or till red colour comes out. Wash slide well with water.

- Counter stain with Loeffler's methylene blue for 30 seconds. Wash and dry.

- Examine under 10X to localize the field and then look for acid fast bacilli (AFB) under 100X and parasites under 40X/100X objective

Acid fast smear reporting (RNTCP guidelines)

Number of AFB seen per oil immersion field	Report
No AFB/100 Fields	No AFB seen
1-9 / 100 fields	Scanty
10-99 / 100 fields	1+
1-10 / field	2+
> 10 / field	3+

Precautions

- The slides should be free of grease before the sample is applied.
- The sample must be applied and air dried on the slide properly.
- The stains must be filtered before use.
- The smears should not be rubbed

Anti-A, anti-B and anti-AB blood grouping

Principle

The procedure makes use of Monoclonal Anti-A, Monoclonal anti-B, Monoclonal Anti-AB antibodies and the test is based on the principle of haemagglutination. Red blood cell antigens A, B or AB, when mixed with their respective antibodies, agglutinate. Presence of haemagglutination determines the group of the tested blood.

Composition

Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-AB, are mouse monoclonal IgM antibodies raised against human blood group antigens A and B.

Specimen

Properly stored anti-coagulated blood or 10% RBC- saline suspension should be used.

Materials

- Glass microslides
- Autopipette with 100-1000 µl capacity
- Autopipette tips
- Filter paper

- Tissue paper
- Applicator / mixing sticks (usually provided with kit)

Procedure

Bring the reagents and samples to room temperature.

- Place 1 drop of Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-AB, in separate areas on glass slide.
- Label the respective area as A, B & AB, and also with name or code number of the patient.
- Add 1 drop of whole blood sample or RBC-saline suspension adjacent to each drop of Monoclonal Anti-A, Monoclonal Anti-B, Monoclonal Anti-AB reagents.
- Mix the reagent drop and the sample with an applicator stick and spread over an area of about 1 square inch within the circle.
- Gently tilt the slide forward and backward at room temperature for a maximum period of 2 minutes.
- Read the slides for haemagglutination. Do not interpret fibrin strands as agglutination.

Interpretation

Agglutination of red blood cells within two minutes indicates the presence of the corresponding antigens in the patient's red blood cells. Absence of agglutination indicates the absence of such antigens on the red blood cells.

Agglutination results are interpreted as follows for phenotyping

Red cells sample reacted with				
Anti-A	**Anti-B**	**Anti-AB**	**Saline**	**Result**
+	-	+	-	'A' Group
-	+	+	-	'B' Group
+	+	+	-	'AB' Group
-	-	-	-	'O' Group
-	-	+	-	Weaker variants of 'A' / 'B' Grp
+	+	+	+	(*)

(*) Suggestive of auto-antibody in the blood giving a non-specific reaction.

Note :

Run positive and negative test controls for each batch of blood grouping sera every time before proceeding with the actual test samples.

Precautions

- The blood drop on the slide should not be allowed to dry, partial drying of the blood could be misinterpreted as agglutination.
- The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
- Haemolysed blood samples should not be used.
- Improper antigen-antibody concentration may cause false or delayed agglutination.

Anti-D blood grouping reagent

The test employs Monoclonal Anti-D IgM antibody and is intended for detection of Rhesus (RH) D antigen in Human Red Blood Cells.

Principle

The procedure used with this reagent is based on the principles of haemagglutination. Red blood cells with Rhesus D antigens agglutinate when mixed with anti-Rhesus (D) antibody. Phenotyping (grouping) of them is done by reacting the blood sample with Monoclonal Anti-D IgM. Presence of haemagglutination determines the positive Rhesus D antigen and the blood is categorized as Rh +ve.

Materials

same as in anti A / B / AB testing

Specimen:

Properly stored anti-coagulated blood.

Procedure

- Bring the reagents and samples to room temperature.
- Label the respective area as "D" and also with the patient particulars
- Place 1 drop of Monoclonal Anti-D IgM on a glass slide.
- Add 1 drop of whole blood sample or RBC-saline suspension adjacent to each drop of the Monoclonal Anti-D reagent.
- Mix the reagent drop and the sample with an applicator stick and spread over an area of about 1 square inch within the circle.
- Gently tilt the slide forward and backward at room temperature for a maximum period of 2 minutes.
- Read the slides for haemagglutination. Do not interpret fibrin strands as agglutination.

Interpretation

Rh D+ve: Red cells sample positive for haemagglutination with Monoclonal Anti-D IgM.
Rh D-ve : No agglutination of red cells with Monoclonal Anti-D IgM.

Precautions

Same as above

References

1. Vox sanguinis, 1989, 56, 122.
2. Bio test bulletin, 1998, 3, 177.
3. WHO Expert committee on Biological standardization, Technical reports sera 1977, 610, WHO Geneva.
4. American Association of blood banking technical Manual, 1990, 345.

Hepatitis B (HBsAg) Rapid Test

The most common agent of acute hepatitis is a virus. Of all the viruses that result in hepatitis, Hepatitis B Virus is responsible for the most serious form of the disease. This method describes a rapid, qualitative, one-step immunoassay based on the immunochromatographic principle. This method employs a combination of monoclonal-dye conjugate (colloidal gold) and polyclonal solid phase antibodies to selectively identify Hepatitis B surface antigen.

Materials

- Centrifuge machine
- Plain Glass tubes / red top vacutainers/purple top (K_2/K_3 EDTA) vacutainers
- Autopipette with 20-200 µl capacity
- Autopipette tips
- Filter paper
- Tissue paper
- filter paper
- gloves
- Droppers

Specimen:

Approximately 3-5 ml of blood may be collected in a plain glass tube / vacutainer. The sample may be allowed to clot followed by centrifugation at 3000 rpm for the separation of serum. Alternatively plasma may also be used.

Procedure

- Take the test card out of the pack and label it with the patient particulars.
- Add 1-2 drops of the specimen (serum/plasma) into the sample window. The sample must be brought to room temperature if refrigerated.
- Visually interpret positive results in 10 minutes and negative results in 30 minutes.

Interpretation of test results

- Negative: If only one line appears in the control zone, interpret the result as NEGATIVE/NON-REACTIVE.
- Positive: If two lines appear in the result Area, interpret the result as POSITIVE / REACTIVE
- Retest: If no lines appear in the result area, it is likely that not enough specimen was added or there was some other procedural mistake repeat the test.

Precautions

- Use only serum or plasma specimen.
- Refrigerated specimen must be brought to the room temperature before testing.
- Specimen should not be repeatedly frozen and thawed.
- Specimen with extremely high concentrations of red blood cells, fibrin should be re-centrifuged before using.

Hepatitis B (HBsAg) ELISA Test

The presence of HBV in the blood can also be established by ELISA technique.

Materials required:

- Single / multichannel pipettes with disposable tips: 5-50µl , 50-200µl
- Test tubes and racks
- Incubator (37$\pm$2^{0}C)
- ELISA reader with or without washer
- Sodium hypochlorite solution (1 %)
- Powderless disposable gloves
- Absorbent paper
- Deionised water

Specimen : Serum / plasma

Procedure:

1 Bring all reagents to room temperature before start of assay.
2 Take out the required no. of strips from the kit and label appropriately.
3 Add required amount of sample / control to the wells.
4 Incubate for the appropriate amount of time.
5 Wash all wells at least 5 times with wash buffer (provided in the kit). After final wash, invert the plate and blot dry by hitting on to the absorbent paper (3-4 times).
6 Add requisite amount of conjugate and incubate at 37^{0}C as indicated in the kit insert.
7 Wash as in step 5 above

8 Add required amount of substrate in all wells and incubate at 37^0C as indicated in the kit insert.
9 Wash as above, followed by the addition of required amount of substrate.
10 Incubate for the required amount of time.
11 Stop the reaction and read absorbance in the ELISA reader

Hepatitis C virus (HCV) Rapid test

Anti-HCV test is a direct binding test for the visual detection of hepatitis C antibodies (anti-HCV) in serum as an aid in the diagnosis of hepatitis C infection. Anti-HCV tests are based on the principle of double antigen sandwich immunoassay for determination of anti-HCV in serum. Purified recombinant antigens are employed to identify anti-HCV specifically. Test results are read visually without any instrument. Both immunoconcentration and immunochromatography methods are employed in the design of anti-HCV rapid tests

Materials

- Centrifuge machine
- Plain Glass tubes / red top vacutainers/purple top (K_2/K_3 EDTA) vacutainers
- Autopipette with 20-200 µl capacity
- Autopipette tips
- Filter paper
- Tissue paper
- gloves
- Droppers

Specimen:

Approximately 3-5 ml of blood may be collected in a plain glass tube / vacutainer. The sample may be allowed to clot followed by centrifugation at 3000 rpm for the separation of serum. Alternatively plasma may also be used

Procedure

- Take the test card out of the pack and label it with the patient particulars.
- Add 1-2 drops of the specimen (serum/plasma) into the sample window. The sample must be brought to room temperature if refrigerated.
- Visually interpret and record the results.

Interpretation of test results

- Negative: If only one line appears in the control zone, interpret the result as NEGATIVE / NON-REACTIVE.
- Positive: If two lines appear in the result Area, interpret the result as POSITIVE / REACTIVE
- Retest: If no lines appear in the result area, it is likely that not enough specimen was added or there was some other procedural mistake. Retest the sample.

Precautions

- Use only serum or plasma specimen.
- Refrigerated specimen must be brought to the room temperature before testing.
- Specimen should not be repeatedly frozen and thawed.
- Specimen with extremely high concentrations of red blood cells, fibrin should be re-centrifuged before using.

HCG pregnancy detection (rapid card test)

Principle

HCG is a glycoprotein hormone produced by the placental trophoblastic cells shortly after the fertilized ovum is implanted in the uterine wall. The primary function of the hCG is to maintain the corpus luteum during early pregnancy. The appearance of hCG in both urine and serum soon after conception and its rapid rise in concentration make it an excellent marker for detection and confirmation of pregnancy. The hormone may become detectable in both urine and serum as early as 5 to 10 days after conception.The immunochromatographic test is a solid-phase, two-site immuno-assay in which combination of monoclonal and polyclonal antibodies are used to detect the levels of hCG in urine. In the test procedure, sample is added to the sample well and the sample is allowed to soak in. If hCG is present in the specimen, it will react with the conjugate,which binds to the antibody on the membrane to generate coloured band.

Materials required

- Urine containers (sterile)
- Rapid hCG pregnancy detection cards
- Filter paper
- Gloves
- Droppers (plastic) – usually provided with the test kit

Specimen Collection:

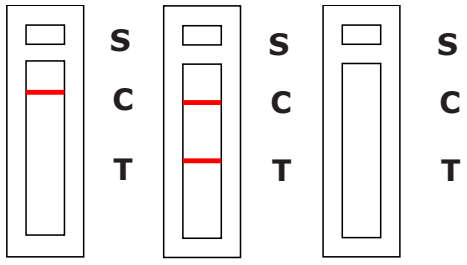
hCG pregnancy detection can be performed at any time of the day. Collect random urine sample in a clean container, first morning specimen is recommended for best results. If the testing is not performed immediately, the specimens should be stored at 2 to 8°C. Do not freeze. If urine has been refrigerated, let it warm to room temperature before using.

Procedure

- Remove the test device from its foil pouch and place it on a flat, dry surface. Test device must be allowed to stand at room temparature for atleast 30 minutes prior to testing
- Label the device with the patient name or control number
- Place the end of the dropper in the urine sample. Draw urine into the dropper.
- Hold the urine dropper atleast one inch above the test device, add two drops of urine into the sample well. Do not lift or shake the device.
- Read the result in the result window as indicated in the product insert (usually between 5-15 minutes)

Interpretation (see figure)

- Negative result: Only one straight line in the control window (C) means the patient is not pregnant.
- Positive result: Two straight lines, one each in test (T) and control (C) window, means the patient is pregnant.
- Inconclusive or invalid result: If there is no distinct straight line in the control window (C), the test is inconclusive or invalid. A control line (C) should always appear. If there is no line, it is recommended that, in this case the test be repeated or a fresh specimen is obtained and re-tested.



Non-reactive Reactive Invalid

CHAPTER 10

QUALITY ASSURANCE

What is "Quality"?

Quality is the ability of a product or service to satisfy the needs of a specific customer. It may be achieved by conforming to established requirements and standards.

Quality Management is having systems in place to continually evaluate:

- What is being done
- How it is being done
- What are opportunities for improvement
- How to make changes for improvement
- What is the impact of the change/improvement

Why is quality important?

Quality at a testing site will result in accurate and reliable test results, which are essential to all aspects of patient health, including prevention, care and treatment.

The approach taken to ensure lab quality is a systems approach. A systems approach examines all components in the system, not just focusing on any one component. It places as much emphasis on identifying and describing the connections between system components as on identifying and describing the components themselves.

Lab Quality System

A lab quality system is the organizational structure, responsibilities, processes, procedures, and resources for implementing quality management of the laboratory or testing site. In other words, it implies all activities which contribute to quality of tests, directly or indirectly.

A quality system to the HIV rapid testing sites has several benefits. It:

- Monitors all parts of the testing system
- Detects and reduces errors
- Improves consistency between testing sites
- Helps contain costs

A lab quality system has 12 components. These include:

- Organization
- Personnel
- Equipment
- Purchasing and inventory
- Process control
- Documents and records
- Information management
- Occurrence management
- Assessment
- Process improvement (trouble shooting)
- Customer services
- Facilities and biosafety

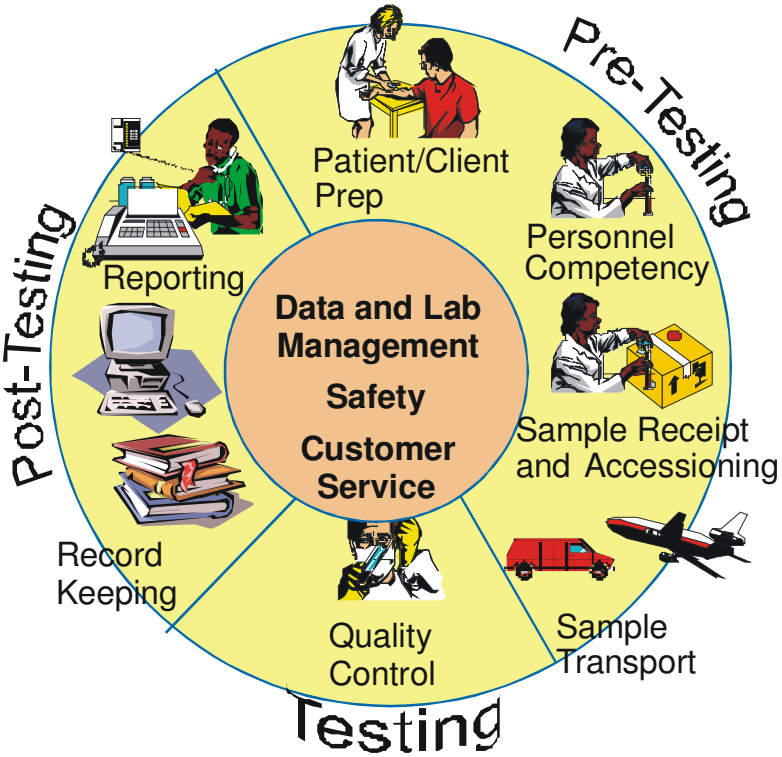
All individuals working in the laboratory are responsible for quality.

Quality Assurance vs. Quality Control

Quality assurance (QA) includes activities which ensure that processes are adequate for a system to achieve its objectives. Quality control (QC), on the other hand, includes activities that evaluate a product or work result (ensure that the result is accurate).

Examples of QA include establishing standard procedures for sample collection and defining criteria for acceptable samples. Examples of QC include analyzing a known QC sample to determine if a test is valid, and deciding if a sample is acceptable for testing. This shows that QC is part of QA. External quality control sample (in addition to kit controls) should be included for every run (in case of ELISA) and once daily in case of rapid tests. The value of this external control is known as the range [$\pm$ 2 SD]. The value of this control must always lie within this range to validate the test result.

The Quality Assurance Cycle



Quality assurance is applied throughout the testing process at all testing sites. It is not a one time event. As seen in the graphic above, this is a continual process encompassing 3 phases with multiple activities associated with each phase of testing.

Why Do Errors Occur?

Errors can occur throughout the testing process. Some causes include:

- Individual responsibilities unclear
- No written procedures are in place
- Written procedures are not followed
- Training is not done or not completed
- Checks not done for transcription errors
- Test kits not stored properly
- QC, EQA not performed
- Equipment not properly maintained

The table below provides the examples of errors that may occur during the three phases of Quality Assurance cycle, and what can be done to prevent them.

Every individual is responsible for preventing and detecting errors before, during, and after testing.

	Before Testing	During Testing	After Testing
Common Errors	• Specimen mislabeled or unlabeled • Specimen stored inappropriately before testing • Specimen transported inappropriately • Test kits stored inappropriately	• Algorithm not followed • Incorrect timing of test • Results reported when control results invalid • Improper measurements of specimen or reagents • Reagents stored inappropriately or used after expiration date • Dilution and pipetting errors • Incorrect reagents used (i.e., using buffers from a different kit)	• Transcription error in reporting (error in transferring results from work register / instrument print-out on to the report form) • Report illegible • Report sent to the wrong location • Information system not maintained

Why is Quality System Important ?

Quality is the foundation of everything that is done in the lab. A quality system:

- Sets the standard for level of quality
- Meets/exceeds customer expectations
- Provides means to prevent, detect and correct problems
- Becomes the core of a monitoring, evaluation, and improvement system
- Reduces costs

External Quality Assessment Program in HIV testing

The National AIDS Control Organization, facilitates the conduct of EQAP providing funds to NRLs/SRLs and directives to SACS, NRLs and participating ICTCs, PPTCTs and blood bank labs, for compliance, emphasizing the importance of EQAP. The participating laboratories in the states test the EQA panel sent by NRL/SRL as routine test samples and send the results to the assigned NRL/SRL within 2 weeks. NRL/SRL analyse the data and provide feed back to the participating labs. In case of discordance of results experts from NRL visit the defaulting centre / laboratory for trouble shooting.

See flow charts below for quality control in ELISA and rapid testing

FLOW CHART FOR PERFORMING HIV TESTING USING QUALITY CONTROLALIQUOT

Performance of rapid test with external control (HIV)

Selection of rapid test	*	Licensed
	*	Quality checked
Performance of Rapid HIV test	*	As per manufacturer's guidlines
	*	Internal controls are checked
Inclusion of external control in daily run	*	Include one positive external control daily
Examine cartridge, comb, etc. for result	*	Examine for either coloured dot/dots, or coloured line/lines and/or agglutination.
Validation of rapid test result	*	Control spot should give positive result (coloured dot, line) and /or agglutation
Test result invalid	*	Control spot not seen
	*	Positive control result does not show as positive.

FLOW CHART FOR PERFORMING HIV TESTING USING QUALITY CONTROLALIQUOT

Performance of ELISA with external control (HIV)

Selection of EIA kit	▪ Licensed ▪ Quality checked
Performance of EIA test	▪ As per manufacturer's guidelines ▪ Internal controls are checked.
Inclusion of external control in daily run	▪ First external control in the first vertical row of ELISA plate ▪ Duplicate external control in the last vertical row of ELISA plate
E-ratio of external control	▪ Recorded O.D. value of external control / cut off value (kit)
Preparation of Levy - Jennings chart	▪ Mean is calculated from at least 20 external control runs ▪ $\pm$ 2 SD is calculated from 20 external control runs ▪ Mean is drawn on the Y axis of the chart ▪ Limit of $\pm$ 2 SD is drawn on either side of mean ▪ Daily run is plotted on X axis
Interpretation	▪ 'E' - ratio of external control is matched with Levy - Jennings chart. ▪ If E-ratio of external control falls within $\pm$ 2 SD limit the run is considered valid. ▪ If it falls beyond $\pm$ 2 SD limit the run is invalid.

CHAPTER 11

LABORATORY MANAGEMENT

There is always pressure on the laboratory to produce quality results, using current technology, while keeping up with increasing demand to aid clinicians and program managers. This can be ensured by instituting a proper lab management system that looks after all the various aspects of HIV testing. A well developed lab management system should work to increase staff efficiency and reduce reagent wastage. It should therefore cover pre-analytical, analytical as well as post-analytical stages of laboratory testing.

A well designed lab management system should consist of the following:

Laboratory Configuration

The key to a reliable HIV testing lab is for it to be optimally configured. This should involve the following:

- Equipment layout: The layout of the equipment should be in line with the testing requirements of the lab. This may vary for a lab with high or a low testing throughput. This would include layouts for refrigerator, water bath, incubator, centrifuge, ELISA reader, etc.
- Workflow staging and direction: Work areas should be arranged to allow uni-directional sample flow and defined space for each test step. Defined areas are needed for:
  - Specimen collection
  - Specimen receiving and storage
  - Specimen preparation
  - Specimen testing-instrument
  - Result production, validation and release
  - Reagent and consumable storage
- Electrical requirements: These should be in line with the testing requirements of the laboratory.
- Staffing requirements: Decisions on staffing should give due consideration to the required level of expertise in terms of the type of laboratory (rapid testing / use of ELISA systems). It should also take into account the number of trained staff required on the basis of the operational volumes.

Stock / inventory management of reagents and consumables

The laboratory should have a well defined inventory management system. The inventory or the stock management system could be manual or electronic depending upon the available resources. The system should be designed to ensure the following:

- Uninterrupted reagent supply to prevent reagent stock-outs.
- Clearly define the buffer stocks as well as the re-order levels
- Availability of other consumables, (e.g. pipette tips, gloves, needles, syringes, vacutainers, and other tubes).
- Stocks are in line with the testing demand/needs of the lab to prevent a situation where there is an excess stock of reagents / consumables. Any such situation would lead to wastage of precious resources on account of expiry of these goods.

Data management

Proper record keeping of patient results is vital for providing optimal patient care and gaining knowledge from patient data collected. A well defined data management system should have the following aspects:

- Ensure reliable and rapid delivery of results to clients / clinic sites
- Ensure clinics have systems for receiving and processing result data
- Ensure the laboratory maintains records of result data for defined periods
- Use standard reporting formats
- Ensure that dedicated human and other resources for data management are assigned

In addition, the data management system should work to ensure that:

- Laboratories examine all specimens accepted to ascertain that they meet the proper criteria for data
  - Results should be entered on both the **worksheet** and **patient result form**
  - **Worksheets** should be filed by date in the laboratory for easy result retrieval
  - **Patient report form** results should be entered and processed.
  - All patient reports should be signed by the concerned technologist / technician and the same should be verified by the lab head.

The release of results in the correct form is as important as conducting the test. The results should only be handed over to the concerned individual after confirming all the antecedents / particulars of the concerned individual. Confidentiality should be maintained in all respects.
Another important aspect of data management system is the archiving of results. Archives can be be electronic or paper-based. A consistent system for data storage based on one or more variables (collection date, clinic site, patient ID) that allows easy reference and retrieval of records.
The lab may choose to file the various lab forms for purposes of record keeping or alternatively enter the information on related lab registers. Sample formats of various lab registers are provided in the annexure.

CHAPTER 12

LABORATORY INFRASTRUCTURE

There has been a progressive increase in access to HIV antiretroviral drugs in the country. However, limitations on laboratory capacity can be an important barrier in the way of reaching country targets. These limitations can be in the form of both laboratory infrastructure as well as staff requirements. It is critical to improve the existing standards of lab infrastructure at local, regional and national levels so as to permit uniform availability of laboratory facilities for HIV testing, OI diagnosis as well as chemistry, and hematology for improved management of people living with HIV/AIDS.

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

Design of Clinical Laboratory

The challenge behind designing a laboratory consists of meeting the requirements of all its different sub-sections and dealing with the complex mix of routine and sophisticated equipment. The lab should be in an area of restricted access. This is required to minimize any unnecessary interference, which may affect the integrity and flow of operations within the lab. The laboratory is a highly sterile area where any sort of contamination is to be prevented. The laboratory should be divided into the following areas:

Sample collection (Phlebotomy)

The sample collection should ideally be done outside the testing area. This will ensure restricted access and prevent exposure of clients to needless infections. However, it can be located on the same floor as the laboratory. It should be close to the main reception for easy access to the patients. The phlebotomy room should have a minimum space allocation of 8 x 10sq feet. It should be well illuminated and be provided with at least a fan (if not air-conditioning) for patient and technician comfort. The toilets should be close to the phlebotomy room for the ease of collection of urine & stool samples.

It may consist of the following:

- Desk and chair
- Phlebotomy chair with adjustable arm support
- Couch for patients (in case they can't sit upright)
- Storage area for consumables
- Waste disposable bins – foot paddle operated. (3 nos)
- Needle destroyer

Laboratory area

A laboratory should be designed with the following in mind:

- Patient load
- Type of investigations to be carried out
- Level of automation, type and number of equipment to be placed in the lab.
- Number of technicians serving the laboratory

There should be adequate space for the staff to move around without crowding the available space. The various sections (biochemistry, microbiology & serology, clinical pathology, hematology etc.) should be well differentiated from each other. There should be sufficient space for the receipt of the samples. The arrangement of equipment inside the laboratory should be in sequence so that sample transfer from sample receipt area to the testing area is uni-directional. There should be sufficient storage space for samples

(both routine and refrigerated) and it should be clearly demarcated from the storage space designated for consumables and lab records.

Power backup and air-conditioning

All essential electronic equipment should have adequate power backup (preferably UPS). The available power backup should be in accordance with the frequency and duration of power cuts. The laboratory should have standard air-conditioning, which can exert effective temperature and humidity control. Autoclaving activity should be performed outside the laboratory work area

Biosafety

The lab should have a safety hood for the storage and use of volatile, inflammable and corrosive material. All the working benches should be provided with a laboratory sink preferable with elbow taps. Standard eye wash facility should be provided. The washing sink should be provided separately. The hand wash area should preferably have a hand drier and soap dispenser and should be located close to the entrance so that lab staff can wash hands before entering and while leaving the laboratory. Eating and drinking activities should be strictly prohibited in the lab and signs regarding the same should be displayed clearly.

CHAPTER 13

EQUIPMENT MAINTENANCE AND CALIBRATION

Preventive maintenance and regular calibration of laboratory equipment constitutes an important facet of laboratory management. It is an imminent requirement in all laboratories and should be followed strictly as per the laid down norms so as to ensure quality laboratory testing. The schedules for equipment maintenance and calibration need to be prepared in advance and circulated amongst all laboratory staff so that the same can be rigorously adhered to. Preventive maintenance schedules are usually prepared as per the manufacturers' guidelines and may be conducted weekly, fortnightly, monthly, biannually or annually. The process and steps associated with preventive maintenance need to be clearly defined to ensure that they can be carried out as required. However, preventive maintenance cannot serve as an alternative to the annual maintenance contracts (AMC) for the lab equipment, which must be drawn with the concerned manufacturer on a regular basis and without fail.

This chapter details the preventive maintenance steps associated with the various laboratory equipment related to HIV testing and other routine laboratory tests.

Centrifuges

Routine preventive maintenance of centrifuges may be undertaken as detailed below:

- The centrifuges must be positioned exactly horizontally and on rubber /polystyrene foam sheets to prevent the instrument moving away from its place when out of balance during centrifugation.
- Ensure that the rubbers should always be in the respective slots.
- Centrifuge load must be balanced at all times. The speed control knob should be regulated gradually.
- Stop the centrifuge immediately in case of abnormal noise.
- After use, the buckets should be inverted to drain dry.
- Any sample spillage inside the centrifuge chamber should be disinfected immediately.
- The centrifuge may be cleaned at short intervals (preferably daily) as it is one of the most frequently used instruments.
- Check mounting and brushes and replace if necessary.
- Calibration:
    - Set the centrifuge speed at one value
    - Record speed and preset value with a calibrated tachometer.
    - Take 4 readings for 1 speed
    - Repeat procedure for 3 different speeds, if variation $> \pm$ 1.5%, contact manufacturer

Refrigerators

The following general advice may be helpful for maintenance:

- Refrigerators must be so placed that sufficient air can pass the condenser (at the back of the refrigerator) for exchange of heat and also to facilitate cleaning of the condenser.
- The refrigerator door must seal perfectly to prevent warm outside air from entering the cool chamber. The gasket must be checked on a regular basis.
- Calibration: Use a pre-calibrated thermometer to ensure accuracy of temperature check.
- Check and record temperature daily.

Hot air oven

Hot air ovens may be mainly used for drying laboratory equipment as well as for heat sterilization. Sterilization in dry air is only effective when the material is exposed for 60 minutes to 160^0 C or for 40 minutes to 180^0 C.

- The thermostat must be checked and calibrated regularly with a pre-calibrated thermometer.
- If there is a fan, check if it is working.
- The inside of the oven must be cleaned regularly.

Autoclave

Autoclaves need careful handling and must be regularly inspected. They can be hazardous and can seriously injure a person with hot steam accidentally escaping from the instrument. The primary factors influencing perfect steam sterilization are saturated steam, temperature and time, etc.

- The operator must be properly trained in the use of an autoclave (refer to the HIV testing manual for various steps associated with the operation of autoclave).
- Wherever possible, the autoclave must be placed in open space.
- Inspect and clean interior chambers of autoclave regularly.
- Check the outlet valves regularly for leakage.
- Do not touch drainage tap, outlet or safety valve while heating under pressure.
- Check the autoclave tape (used in the preparation of the material to be sterilized) which must have turned black and the covering paper brown (not yellow or black).
- Calibration: use a pre-calibrated pressure gauge to check accuracy of pressure in the autoclave
- Adequate sterilization by autoclaves and dry ovens should be monitored by the weekly use of a biological (spore suspension) or chemical indicator

Analytical balance

The following steps may be undertaken :

- The balance should be placed on a solid vibration-free surface, free from dust and at even temperature, away from sunlight.
- Avoid weighing in vessels made of plastic, and use glass vessels or weighing paper, as applicable. The weighing vessel and the sample to be weighed should be at the ambient temperature.
- Place sample in a weighing vessel / butter paper in the middle of the weighing pan, to avoid corner-load error. Liquids or powders should never be directly weighed on the pan.
- The pans of the balance must be cleaned regularly. In case of spillage of biological material, disinfection can be done with 70% alcohol.
- After completion of weighing return the balance to zero weight.
- Weight of the material to be weighed should be within the range of the balance.
- Calibrate the weighing balance as per manufacturer's instructions or with the use of standard pre-calibrated weights, as per the range of balance.
- Six different readings should be taken and recalibration must be done if variation between readings > $\pm$1.5%.

Incubators and water baths

The incubator must maintain a constant temperature within a narrow range to ensure accuracy of measurement. The following are important for maintenance and proper functioning of the incubator:

- Temperature should be daily recorded in incubators.
- Incubators must be cleaned and disinfected routinely at short intervals (at least every fortnight) and after spillage of infectious material.
- The actual temperature must correspond to the thermometer control when the instrument is used.
- The level of water in the water bath must be maintained to protect the heating filament.
- The water bath must be refilled regularly to prevent growth of algae and bacteria.
- Calibration: use a pre-calibrated thermometer to ensure accuracy of temperature checks.

Pipettes

Maintenance and calibration of mechanical pipettes (autopipettes) must be done at a regular frequency to ensure accuracy of dispensation. For purposes of maintenance, the following steps may be taken:

- The autopipettes must never be used for dispensing volatile / corrosive material (acids, alkalis) as it will disturb the vacuum in the pipette.
- The volumes on the autopipette should be increased and decreased gradually.
- The pipettes must always be returned to the zero position after use.
- The pipettes should always be stored in a vertical / erect position and in a pipette holder.
- The pipette snout must be cleaned regularly with a moist filter paper / gauge after use.
- For steps on calibration, please refer to annexure X of the HIV quality assurance manual.
- The glass pipettes must be cleaned with appropriate detergents to ensure that the detergent being used should not disturb the volume markings etched on the pipette.

ELISA reader

Regular maintenance of the ELISA machine should be carried out according to the manufacturers' instructions and may vary between brands. The machine itself should not be opened.
The filters must be protected from moisture and fungal growth. Keep Silica gel packet in the filter box.

Plate washer

Plate washers are critical in ELISA assay performance. The washer works on a simple principle and comprises of wash fluid, waste fluid reservoirs, pressure and vaccum pumps, a dispense manifold and a plate carrier.
The wash fluid is pressurized and a valve opens to allow the fluid through a manifold and into assay microwells. The waste fluid under vaccum is aspirated back through the manifold to the waste container. A number of cycles of dispense and aspiration comprises the washing of a plate. At the end of the wash procedure the wells are empty of the fluid.

After use care

- Fill the rinse bottle with about 500 ml of distilled water.
- Dispose off the unused wash buffer. Rinse with distilled water, a couple of times and leave about 500 ml in the wash bottle. Fix the cap tightly.
- After using the washer switch off power.

ANNEXURE

LIST OF LABORATORY REGISTERS AND FORMATS TO BE MAINTAINED

The following should be maintained for keeping a track of various activities in the laboratory:

1. Specimen log-in register
2. Lab test result entry register
3. Daily temperature log (refrigerator, water bath and hot air oven)
4. General equipment maintenance log
5. Microscope maintenance log
6. Autoclave operation log
7. Complaint and corrective action log
8. Format for worksheet
9. Expired kits log
10. New reagent verification log
11. Stock card
12. Monthly assay-reassay format.
13. Consumption record and inventory management.
14. HIV consent form
15. HIV test requisition form
16. HIV test report form
17. Proforma for sending reports to NACO / SACS
18. Injury and Post-prophylaxis register

Sample formats for the above registers are provided ahead:

Specimen Log in register

Patient ID	Patient name	Age	Sex	referred by		Time test received	Required Investigation	sample requirements	If not, remarks	initials of the receiving person
				clinician (name) (name)	clinic					

LAB TEST RESULT ENTRY REGISTER

Patient ID	Patient name	Age	Sex	name of the investigation	test result	test valid / invalid	time test completed and entered	whether report generated	initials of the entering tecnician

Daily Temperature log (water bath, refrigerator and hot air oven)

Name of the laboratory_____

Temperature (degrees C)

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
January																															
Feb																															
march																															
April																															
May																															
June																															
July																															
Aug																															
Sept																															
Oct																															
Nov																															
Dec																															

MICROSCOPE MAINTENANCE LOG

Name of the laboratory _____

Daily	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	
Clean objectives, stage and condenser																																
Check fine & coarse adjustment																																
Check mechanical stage																																
check light alignment																																

NEW REAGENT VERIFICATION LOG

Name of the laboratory_____

	OLD REAGENT				NEW REAGENT			VERIFICATION		
DATE	**Reagent / kit**	**Cat No.**	**Lot No.**	**Expiry Date**	**Cat No.**	**Lot No.**	**Expiry Date**	**Analyte**	**Value with Old kit**	**Value with New kit**

Verified by lab head _____

EXPIRED KITS LOG

Name of the laboratory_____

Date	Reagent / kit	Lot No.	Expiry Date	Tick to confirm that kit is discarded after expiry	Technicians's signatures

Verified by lab head _____

Autoclave Operation Log

Name of the facility & dept			
Equipment		Serial Number of the Equipment	

DATE	Daily check Inlet PSI ✔	Daily check Clean Drain ✔	Items being sterilized	Lot # (if needed)	Indicator type used (sporestrips)	Time	Time/ Temp Attained	Time Off	Time Removed	Indicator Results	Acceptable or not	Initials

Verified by lab head _____

Equipment Maintenance Log

Health Department		City	
Equipment		Serial Number	

Scheduled Maintenance

Tasks	Interval	Vendor or Staff Initals

Unscheduled Maintenance Record

Date	Tasks	Initals

Verified by lab head _____

Complaint and Corrective Action Log

Name of the Lab / Department		
Date Reported :	Time:	Initiated By :
Source of Communication / Complaint		
Date of Occurance	Time	
Narrative of the Event (If Necessary)		
Immediate Corrective Action Taken		
Does the written procedure cover how to deal with this event? Yes No* Not Applicable *If No - Procedure must be updated within fifteen days from date of event.		
If Yes - Was the written procedure followed? **Yes**		**If No** - Why not? Elaborate Below
Follow Up Activities Required? Yes ☐ No	**If Yes** - Indicate what and date to be completed below.	
Who Completed - Signature		**Date :**
Signature		**Date :**

Stock Card

Regularly reported record of orders, receipts and issues of tests

Lab Site: _____ Report Period: _____

District: _____ Date Submitted _____

Order Date	Order Details	# Tests Ordered	Receipt Date	Receipt Time	Order Sent From	Order Sent To	# Tests Received	Carry over from previous stock	Stock in hand	Stock check comments	Other comments	Initials
							A	B	C=A+B	K		
	Requisition #: Voucher #:											

Test Issue Date	Test Issue Time	Test Issued From	Test Issued To	Tests #Issued	Tests Expired	Stock Balance	Stock Cheque Comments	Other Comments	Initials
D	E	F	G	H	I	J=C-(H+I)	K	L	

Instructions :

- Each time an order is made, a new stock card should be started, recording Order Date, Order Details, and # Tests Ordered. Until the order is received, the previous card should be used to record stock issue.
- Once the order is received, complete the first row and record the ending balance from column (J) of the previous card in Box (B) of the new card.
- The stock should be regularly checked, noting discrepancies in column (K). Record expired stock in column (I), comment as such in column (L), and safely remove and destroy it and document the same.

Signature of Chief Technician: _____ Signature of Laboratory in charge: _____

Consumption Record and Inventory Management

Quarterly inventory tracking form

Lab Site: _____ Reorder Level: _____

District: _____ Report Period: _____

State: _____ Date Submitted: _____

	Month Start Date	Opening Balance	Total # Tests Received	Total Tests Used	Losses/ Adjustments (+/)	Closing Balance	Months of Stock on Hand (at month end)	Earliest Expiry	Quantity Needed in Next Month	Total # Days of Stock Out	Comments
	A	B	C	D	E	F = B + C - D +/- E	G = F/D	H	I	J	K
Month 1					e			# Tests:			
					w						
					a			Expiry Date:			
					o						
Month 2					e			# Tests:			
					w						
					a			Expiry Date:			
					o						
Month 3					e			# Tests:			
					w						
					a			Expiry Date:			
					o						
Quarter TOTAL					e			# Tests:			
					w						
					a			Expiry Date:			
					o						

Instructions

- In column (E): Enter positive or negative number for quantity of reagents removed from stock for any reason other than consumption by the lab (e.g. losses, expiry, damage, stock transfer, correction of accounting error). Circle 'e' for expired, 'w' for withdrawn, 'a' for stock adjustments, 'o' for other.
- Stock on hand refers to the quantity of usable stock available at all levels of the system at a point in time
- Closing balance of month one (F) should equal the opening balance (B) of month two.
- Minimum stock levels adequate for testing over 6 weeks (1.5 months) should be available at the time of placing the next order to prevent stock-outs. Each lab site would need to determine their individual average monthly test requirements based on the average inflow of patients for CD4 testing and also factor for any expected new patients to be able to estimate when to order and to establish the reorder level.

Signature of Chief Technician: _____ Signature of Laboratory in charge: _____

Monthly Assay – Re Assay Format

Lab Site: _____ Report Period : _____

District: _____ Date Submitted: _____

	Week start date	Accessions on Courier specimens	Accessions on Walk-in specimens	Cumulative accessions	Controls	Re assays	Total tests used	Re assay %	No. of reassays	Reason for reassay	Initials
		A	B	C=A+B	D	E	F=C+D+E	G=E/C*100	H	I	
Week 1											
Week 2											
Week 3											
Week 4											
Month TOTAL											

Instructions :

- Reasons for reassays are elaborated below and the symbols for each of these are in brackets preceding the reason. For purposes of simplicity the reasons for reassay may be recorded using symbols for the same , for example, in the week if 3 reassays have been done for reason (a) and 2 for reason(e), then it may be recorded as 5 in Column H and as 3a+2e in column I.

Reasons for reassay: (a)Test re- run due to power disruption; (b)Test re- run due to instrument/ calibration/ control requirements; (c)Test re-run for confirming low or high values; (d)Test re- run for dilution requirement for verification in high positive levels; (e)Test re-run due to instrument failure; (f) Test re- run due to temperature variations; (g)Test re- runs for confirmation/ run failures; (h)Test re- run due to contamination; (i)Test re-run on client's request; (j)Test re-run for QC (k)Test re-run due to kit failure; (l)Positive samples to be repeated as per protocol; (m) New machine validation; (n)New kit validation; (o)Validations for QA & other purposes ; (p) New Technology Validations

Signature of Chief Technician: _____ Signature of Laboratory in charge: _____

FORMAT FOR REPORTING TO NACO-SACS (National Reference Lab)

To _____

_____

Date _____

Name of HIV testing lab_____

Sr. No.	Lab NO.	Name/ ID	Age/ Sex	Risk Group	ScreeningbTests			Confirmatiory Test Kit Used (.....) Principal (.....)		Interpretation	
								HIV-1	HIV-2	HIV-1	HIV-2
					Test-I Kit Used ( ) Principle ( )	Test-II Kit Used ( ) Principle ( )	Test-III Kit Used ( ) Principle ( )				

Head / Director **Officer-In-Charge** **Laboratory Technologist**

CC:

- **Additional Director (Technical) NACO, Ministry of Health & Family Welfare, Chandralok Building, 9th Floor, 36-Janpath, New Delhi – 110 001.**
- **Project Director, State AIDS Control Society.**

FORMAT FOR REPORTING TO SACS (ICTC / PPTCT)

To _____

_____

_____

Date _____

Name of HIV testing lab_____

Sr. No.	Lab NO.	Name/ ID	Age/Sex	Risk Group	ScreeningbTests			Interpretation	
								HIV-1	HIV-2
					Test-I Kit Used ( ) Principle ( )	Test-II Kit Used ( ) Principle ( )	Test-III Kit Used ( ) Principle ( )		

Head / Director **Officer-In-Charge** **Laboratory Technologist**

CC:

- **Additional Director (Technical) NACO, Ministry of Health & Family Welfare, Chandralok Building, 9th Floor, 36-Janpath, New Delhi – 110 001.**
- **Project Director, State AIDS Control Society.**

Consent form for HIV Testing

This is to state that I have been counselled about the HIV test to be conducted on me and have been explained about the implications of the test result- positive, negative or indeterminate. All the details pertaining to HIV, its transmission, and testing procedure have been explained to me. Its limitations and interpretation of results have been explained to me in a manner that I can understand.

I, hereby, give my consent for the test to be conducted on me in order to ascertain my HIV sero-status.

Signature:

Date:

Note:

1. It may be noted that general consent obtained for carrying out procedures in hospital does not include HIV consent.

2. In case of minor, the consent should be obtained from the parents.

3. In case of unconscious patients, where there is a need for diagnosis of HIV for management of the patient, consent should be obtained from the parents, spouse/closest relative available at that time.

In case no attendant is available, the test, if necessary for management of the patient, may be carried out on recommendations of two attending doctors.

HIV TEST REQUISITION FORM

Name and address of the facility

1 *Patient ID:* _____ *Sex:* M F

Name (optional): *Age* ☐☐

2 *Authorizing clinician name & signature:*

3 *Referring clinic name*

4 *Date and Time Blood Drawn (dd/mm/yy):*

☐☐ / ☐☐ / ☐☐

☐☐ : ☐☐ *(hh:mm)*

5 *Relevant clinical details*

For laboratory Use only :

6 *Date & time sample received (dd/mm/yy):*

☐☐ / ☐☐ / ☐☐

☐☐ : ☐☐ *(hh:mm)*

8 *Sample Lab no.*

☐☐☐☐☐☐☐

7 *Sample received in the right condition:*

Y N

If no, list the state in which sample received:
Unlabelled /mislabelled / Hemolysed / turbid

Insufficient/leakage /inappropriate container

Please tick

Receiving technician's initials and signature

Work Sheet for ELISA

		2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Sample Ref. No. **Referred by** **Lab No.** **Well No**

_____

_____

Details of kit Used

Name & Lot No : _____

Expiry Date: _____

VALIDITY OF CONTROLS

Kit Controls	External Controls
_____	_____
OD Value Validity	OD Value OD Ratio <1SD >1SD <2SD >2SD Validity

Blank _____
Negative Control _____
Cut off Control _____
Positive Control _____

VALIDITY OF INSTRUMENTATION (Comments)

(1) Washer ……..
(2) Filters ……..
(3) Reader ……..

VALIDITY OF RUN:-

VALID /INVALID

Comments...

Signature of officer incharge **Signature of technologist**

HIV TEST REPORT FORM

Name and address of the testing laboratory

1 *Patient ID:* _____ *Sex:* M F

Name (optional): *Age* ☐☐

2 *Authorizing clinician name & signature:*

_____

3 *Referring clinic name*

4 *Date and Time Blood Drawn (dd/mm/yy):*

☐☐ / ☐☐ / ☐☐

☐☐ : ☐☐ *(hh:mm)*

5 *Sample lab number*

☐

7 *Date & time sample received (dd/mm/yy):*

☐☐ / ☐☐ / ☐☐

8 *Date test condcuted (dd/mm/yy)*

☐☐ / ☐☐ / ☐☐

6 *Was a result produced for the sample*

Y N *if no state reason:*

_____

_____

9 *Test Result*

HIV -1 ☐ ***HIV-2*** ☐

10 **Kit Details**

Name and type of kit	Principle	HIV-1	HIV-2
i)			
ii)			
iii)			

Interpretation _____

_____

Technician initials and signature

Lab incharge initials and signature

Counselor initials and signature

INJURY AND POST PROPHYLAXIS REGISTER

INJURY AND POST PROPHYLAXIS REGISTER																			
Status of Source																			
Name, Age & Sex	Date	Type of Injury			HIV		Unknown		HBs Ag		HCV Antibody		PEP Given		HIV Testing of Exposed HCP*				Final Comment
		Mid	Moderate	Severe	Negative	Positive	High Risk	LowRisk	Negative	Positive	Negative	Positive	Basic	Expanded	0 Day	6 wks	12 wks	6 Months	T / NT

APENDIX-1
LIST OF CD4 CENTRES UNDER NACO

Andhra Pradesh

Osmania General Hospital
Deptt of Microbiology
Afzal Gunj
Hyderabad.

Nizam Institute of Medical Sciences
Microbiology
Punjagutta, Hyderabad-500 082.

Andhra Medical College
King George Hospital, Deptt of Microbiology
Vishakhapattanam-530 002
Government Medical College
Deptt of Microbiology
Kannavarithota
Guntur 522 002.

Assam

Guwahati Medical College
Deptt of Microbiology
Guwahati.

Bihar

Govt. Medical College
Patna
Bihar

Shri Krishna Medical College & Hospital
Deptt of Microbiology
Uma Nagar, Muzaffarpur – 842004

Calcutta

Calcutta School of Tropical Medicine
Deptt of Virology
110 C.R. Ave.
Kolkata 700 073

Chandigarh

Postgraduate Institute of Medical Education & Research
Deptt of Immunopathology
Research Block A, 4th Floor, Sector-12
Chandigarh 160 012.

Delhi

National Institute of Communicable Diseases
Advance Centre for AIDS and Related Diseases
22 Shammnath Marg
Delhi -110 054.

Dr. Ram Manohar Lohia Hospital
Deptt of Microbiology
Babar Kharar Singh Marg
New Delhi 110 001

Maulana Azad Medical College
Dept. of Microbiology
Bahadur Shah - Zafar Marg
New Delhi-110002

All India Institute of Medical Sciences
Deptt of Microbiology
Ansari nagar
New Delhi - 110 029

VMMC & Safdarjang Hospital
Regional STD Teaching, Training & Research Centre
Ring Road
New Delhi 110 029.

Goa

Goa Medical College
Microbiology
Bambolim
Panaji- 403 202.

Gujarat

B. J. Medical College
Deptt of Microbiology
Asarwa, Ahmedabad -380016

Haryana

Pt BD Sharma Postgraduate Institute of Medical Sciences
Deptt of Microbiology
Rohtak- 124001
Haryana

Himachal Pradesh

Indira Gandhi Medical College
Deptt of Microbiology
Shimla (Himachal Pradesh)
171 001.

Karnataka

National Institute of Mental Health and Neurosciences (NIMHANS)
Neurovirology
Hosur Road
Bangalore - 560 029.

Bowring and Lady Curzon Hospital
Dept. of Microbiology
Lady Curzon Road
Shivaji Nager
Bangalore - 560001

Karnataka Institue of Medical Sciences (KIMS)
Deptt of Microbiology
Vidyanagar
Hubli -580022

Govt. Medical College
Dept. of Microbiology
Irwin Road, Mysore-570 001.

Vijayanagar Institute of Medical Sciences (VIMS)
Bellary – 583104

Kerala

Medical College Hospital - Thiruvananthapuram
Dermatology and Venerology
Thiruvananthapuram - 695 011

Madhya Pradesh

Choithram Hospital and Res. Centre
Dept. of Microbiology & Immunology
Manik Bagh Road, Indore 452 014.

Maharastra

Govt. Medical College
Dept. of Microbiology
Nagpur.

Govt. Hospital Sangli
Deptt of Microbiology
Civil Hospital street.
Sangli.

National AIDS Research Institute (NARI)
Deptt of Immunology
73 Bhosari, Block G, MIDC
Pune – 411026.

B. J. Medical College
Deptt of Microbiology
Station Road
Pune-411 001.

Manipur

Regional Institute of Medical Sciences
Deptt of Microbiology
RIMS road,
Imphal West (Manipur)
795004.

Jawaharlal Nehru Hospital
Microbiology Deptartment
Porompat Road
Imphal-795010.

Mumbai

Seth G.S. Medical College & KEM Hospital
Deptt of Microbiology
Parel
Mumbai 400 012

Grant Medical College and Sir J.J. Hospital
Deptt of Microbiology
Byculla
Mumbai 400 008

Lokmanya Tilak Municipal Medical College
Dept. of Microbiology
Fourth Floor, College Building
Sion, Mumbai – 400022

BYL Nair Charitable Hospital of TN Medical College
Microbiology, VCCTC, 303-A, 3rd Floor, College Building
Al Nair Rd, Mumbai 400 008.

Nagaland

Naga Hospital
Deptt of Microbiology
Kohima.

Punjab

Govt Medical College
Deptt of Microbiology
Amritsar -143001

Rajasthan

S.M.S. Medical College
Dept. of Microbilogy & Immunology
Jawaharlal Nehru Marg
Jaipur – 302004, Rajasthan

Dr. SN Medical College
Deptt of Microbiology
Shastri Nagar
Jodhpur - 342003

Tamilnadu

Government Hospital of Thoracic Medicine
Tambaram TB Sanitorium, Immunology
G.S.T Road, Tambaram
Chennai 600 047.

Madras Medical College
HIV/AIDS Reference Center
Institute of Microbiology
Chennai – 600003

Kilpauk Medical College
Deptt of Microbiology
Chennai.

Madurai Medical College
Institute of Microbiology
Panagal Rd.
Madurai - 625 020.

Christian Medical College
Deptt of Clinical Virology
Ida Scudder
Vellore- 632 004.

Govt Medical College
Deptt of Microbiology
Tirunelveli.

Uttar Pradesh

Banaras Hindu University
Institute of Medical Sciences, Deptt of Micobiology
Varanasi-221 505.

King George's Medical College
Dept. of Microbiology
Lucknow