

NATIONAL TRAINING ON HIV AND STI LABORATORY DIAGNOSIS

PARTICIPANTS' MANUAL



Government of Nepal
Ministry of Health and Population
National Public Health Laboratory (NPHL)

December 2014

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Foreword

The diagnosis of HIV infections is solely based on laboratory findings. There is a need of scaling up of HIV testing and counseling services, for HIV infection identification, to reduce the gap between confirmed HIV infections and estimated HIV infections in the country. As of July 2013, 22,994 cases of HIV were reported to the National Center for AIDS and STD Control (NCASC), whereas the estimated number of HIV infections, in 2013, was 40,723 (NCASC 2014). The laboratory findings on HIV will be helpful for HIV prevention, care and treatment services which is a priority for the Government of Nepal (GoN).

Laboratory results play a vital role in HIV diagnosis, prevention, treatment, and management. The management of Sexually Transmitted Infections (STIs)/ Opportunistic Infections (OI) Prophylaxis, Antiretroviral Therapy (ART) initiation and monitoring is based on results generated at the laboratory. An accurate and reliable diagnosis is directly related to patient care and support. At present, there are more than 200 HIV testing sites (HIV Testing and Counseling) providing HIV diagnostic services. One of the major components in generating quality laboratory results is the availability of trained laboratory personnel.

We are pleased to introduce this laboratory training manual, "National Training on HIV and STI Laboratory Diagnosis" for conducting HIV and STI laboratory trainings. This training manual will be the guiding document to train laboratory staff on HIV and STI diagnosis. Through this training manual, laboratory staff will be able to accurately diagnose infections, which, in turn, will result in timely management of the infection. In addition, this manual will also be used as a reference and resource material for laboratory professionals in HIV testing sites. Lastly, I would like to express my sincere gratitude to all those who contributed their knowledge and provided technical expertise while developing this manual. I would like to thank the NCASC, WHO and UNICEF for their technical inputs and the United States Agency for International Development (USAID)-funded Saath-Saath Project for supporting us technically as well as financially in developing this manual. We look forward to the use of this training manual for HIV and STI diagnosis at HIV testing centers around the country. We hope that this manual will improve the proficiency in laboratory testing as well as enhance laboratory quality on HIV diagnosis and its management in Nepal.

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ACRONYMS

AIDS	Acquired Immuno Deficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretro Viral
ASCP	American Society for Clinical Pathology
CD	Cluster of Differentiation
DBS	Dried Blood Spot
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic acid
EIA	Enzyme Immuno Assay
EID	Early Infant Diagnosis
ELISA	Enzyme Linked Immunosorbent Assay
EQA	External Quality Assessment
EQAS	External Quality Assessment Scheme
FEFO	First-to-expire-first-out
HIV	Human Immunodeficiency Virus
HTC	HIV Testing and Counseling
IFBLS	International Federation of Biomedical Laboratory Science
IQC	Internal Quality Control
LMIS	Logistics Management Information Systems
MoHP	Ministry of Health and Population
NAAT	Nucleic Acid Amplification Test
NCASC	National Center for AIDS and STD Control
NPHL	National Public Health Laboratory
OI	Opportunistic Infection
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PITC	Provider Initiated Testing and Counseling
PMTCT	Prevention of Mother to Child Transmission
PrEP	Pre Exposure Prophylaxis
QC	Quality Control
RBC	Red Blood Cells
RNA	Ribonucleic Acid
RPR	Rapid Plasma Reagin
SDP	Service Delivery Point
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
TPPA	Treponema Pallidum Particle Agglutination
VCT	Voluntary Counseling and Testing
VL	Viral Load
WHO	World Health Organization

NATIONAL TRAINING ON HIV AND STI LABORATORY DIAGNOSIS

1. Background

Trained laboratory personnel for laboratory diagnosis of Human Immunodeficiency Virus (HIV) and Sexually Transmitted Infections (STIs)/ Opportunistic Infections (OIs) are prerequisite for accurate and reliable diagnosis of HIV, STIs and OIs. Quality laboratory diagnosis of HIV and STI/OI plays crucial role in HIV and STI prevention, treatment and effective management of infection.

This National Training on HIV and STI Laboratory Diagnosis Participants' Manual will be used to provide training on diagnosis of HIV and STI/OI to all levels of laboratory professionals. It is intended that the guideline will be used by laboratory personnel for HIV testing, standardization and diagnosis at both reference center and testing sites.

2. Title

The title of the manual is "National Training on HIV and STI Laboratory Diagnosis", Participants' Manual.

3. Purpose

The purpose of "National Training on HIV and STI Laboratory Diagnosis" is to provide the basic concept on HIV and STI/OI diagnosis and strengthen capacities of the participants to perform rapid tests of HIV and certain STIs/OIs accurately and reliably in a safe and professional manner.

4. Objectives

The objectives of the training are to enable the participants to

- Describe HIV and STIs/OI .
- Explain the procedure to diagnose HIV and STIs/OIs and interpret the results accurately in a safe and professional manner.
- Explain good clinical laboratory practices and safe healthcare waste management practices.
- Describe on logistic management at HIV Testing and Counseling (HTC) centers.
- Explain and adhere on laboratory quality system and participate in External Quality Assessment Scheme (EQAS) for HIV test using Dried Blood Spot (DBS) technique.
- Describe procedure to manage the documents and records in professional manner.

5. Overview of training modules

5.1 Learning modalities

This training comprises the theoretical and practical sessions with hands on practice.

Theory sessions:

The theory sessions will be delivered through power point presentations on different modules followed by discussion and question answers.

- Presentations will provide basic concept on the topic.
- Group discussions will allow participants to share experiences and ideas.
- Energizers/Games will help to refresh and make the learning environment lively.

Practical sessions:

The practical sessions will include demonstration on HIV testing and STI/OI laboratory diagnosis followed with hands on practice.

- Practical demonstrations will allow participants to follow Standard Operating Procedure (SOP) on HIV and STI/OI laboratory diagnosis.
- By “Hands-on” practice exercises, participants will gain laboratory skill on specimen collection, testing and interpretation on different laboratory test.

5.2 Participants (Target Audience)

This training package is designed for laboratory personnel working in HIV testing and counseling centers.

5.3 Number of workshop participants

For optimum learning environment and management of the training, it is recommended that the number of participants will be 20.

5.4 Evaluation/Assessment

The training will be assessed using different set of questionnaires and evaluation form as follows:

- Pre and Post-test at beginning and end of the training
- Questions after each session
- Evaluations at the end of training

5.5 Competency certification criteria

The following criteria are recommended for certification of individuals who perform HIV testing. A certificate of successful completion will be awarded to participants upon meeting the requirements outlined below:

Successful completion of the National Training on HIV and STI Laboratory Diagnosis will be based on:

- Daily attendance
- Passing score of 80% on written post-workshop examination
- Passing score of 80% on final practical examination

5.6 Outline of Training Modules

This manual has 17 modules. This first session provides an overview of training.

No.	Modules	Learning Objectives: After completion of the modules, the participant will be able to
	Overview of the Training	Understand the Goal, Objectives and Ground rules
1	Overview of HIV Infection	- Explain the basic concept of virus, HIV, and AIDS - Describe the progression of HIV infection - Describe the HIV epidemic in global and national scenario in terms of number of people affected - Explain “window period” and how it may affect HIV testing results
2	National HIV/ AIDS strategy and importance of Testing and Counseling	- Explain the need for HIV testing and counseling (HTC) in HIV prevention programs - Describe the role of HIV rapid testing in supporting prevention and counseling programs - Describe importance of counseling and confidentiality in HIV testing - Describe the programs/settings where HIV rapid tests are used in country
3	Overview of Laboratory Testing Technologies for HIV Progression	- Describe HIV testing technologies and applications - Explain the spectrum of testing technologies for HIV across the continuum of care
4	HIV Testing Strategies and Algorithms	- Explain the HIV rapid testing algorithm approved in the country - Determine HIV status following serial testing algorithm
5	Early Infant Diagnosis (EID): HIV	- Explain the basic concept on EID - Explain the importance of EID - Explain the testing algorithm for EID
6	Universal Precautions and Post Exposure Prophylaxis (UP/PEP) and Safe Health Care Waste Management and Safety at the HIV Testing Site	- Explain the universal precautions for laboratory safety - Describe on personal health and safety practices - Explain how to maintain a clean and organized workspace - Explain to safely manage the health care waste generated in testing site
7	Blood Collection and Dried Blood Spot (DBS) preparation for HIV EQAS and EID	- Describe the process to collect blood from a finger prick or/and venipuncture accurately and confidently - Explain the process for preparation of DBS specimens from blood for HIV EQAS - Explain the process for preparation of DBS specimens from blood for HIV EID
8	Performing HIV Rapid Tests	- Perform HIV rapid tests according to Standard Operating Procedure (SOP) - Explain to accurately interpret individual test results - Explain and accurately determine HIV status

No.	Modules	Learning Objectives: After completion of the modules, the participant will be able to
9	Quality Assurance at HIV testing site	- Describe the essential elements of a laboratory quality system and how they apply in HIV testing - Explain the key factors that may compromise the quality of HIV testing - Describe responsibilities in preventing and detecting errors before, during, and after testing
10	Quality Control in HIV Test (IQC and EQAS)	- Describe laboratory quality control - Explain Internal Quality Control (IQC) - Describe the Difference between internal and external controls - Explain External Quality Assessment and types - Explain the External Quality Assessment Scheme (EQAS) in HIV - Describe Dried Blood Spots (DBS) for HIV EQAS
11	Logistic Management at HIV Testing Site	- Explain and list all the supplies required for rapid HIV testing sites - Describe to maintain proper records of all consumables and non-consumables at site - Describe the logistic management practices
12	Use and Care of Equipment at HIV Testing Site	- Explain the rationale and function of equipment needed at HIV testing sites - Describe on proper use, maintenance and monitoring of the equipment available - Explain on CD4 T lymphocyte count and viral load test machines
13	Overview of Sexually Transmitted Infections (STI) and Opportunistic Infections (OIs)	- Explain STI - Describe Transmission mode and Common signs and symptoms of STIs - Explain OI - Explain the common OIs in HIV infection
14	Overview of Laboratory Diagnosis of Sexually Transmitted Infections and Opportunistic Infections	- Describe the spectrum of testing technologies for different STIs and OIs - Explain the role of the laboratory techniques in the diagnosis and management of STIs and OIs
15	Performing Laboratory Tests for STIs and OIs	- Perform different laboratory diagnosis of STIs and OIs according to SOP - Explain and accurately interpret individual test results
16	Documents and Records	- Explain the difference between a document and a record - Explain the rationale for following documents and keeping records - Explain the examples of documents and records kept at a test site - Describe importance of Standard Operating Procedures (SOPs)
17	Professional Ethics	- Describe ethical issues related to HIV testing - Explain the importance of professional ethics - Explain ethical conduct in HIV testing - Describe the appropriate actions to maintain confidentiality and integrity in HIV testing

MODULE 1

OVERVIEW OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

Purpose

To provide basic concept on Human Immunodeficiency Virus (HIV) infection.

Learning objectives

At the end of this module, participants will be able to:

- Explain virus, HIV and AIDS
- Describe the progression of HIV infection
- Describe the HIV epidemic in global and national scenario in terms of number of people affected
- Explain “window period” and how it may affect HIV testing results

Content outline

- What is virus?
- What are HIV and AIDS?
- HIV transmission
- Window period
- Stages of HIV infection
- Global and National epidemiology of HIV and AIDS

Time: 60 minutes

1.1 Virus

Virus is a unicellular, ultra microscopic particle that replicates only inside the living cells. Viruses can infect all types of life forms, from animals and plants to bacteria. Virus particles (known as virions) consist of two or three parts: i) the genetic material made from either Deoxyribo Nucleic Acid (DNA) or Ribo Nucleic Acid (RNA), long molecules that carry genetic information; ii) a protein coat that protects these genes; and in some cases iii) an envelope of lipids that surrounds the protein coat when they are outside a cell. Virus contains either RNA or DNA as genetic material but never both. Laboratory diagnosis is based on detection of either RNA or DNA or viral protein like p24 antigen or antibodies against viral component (antigen).

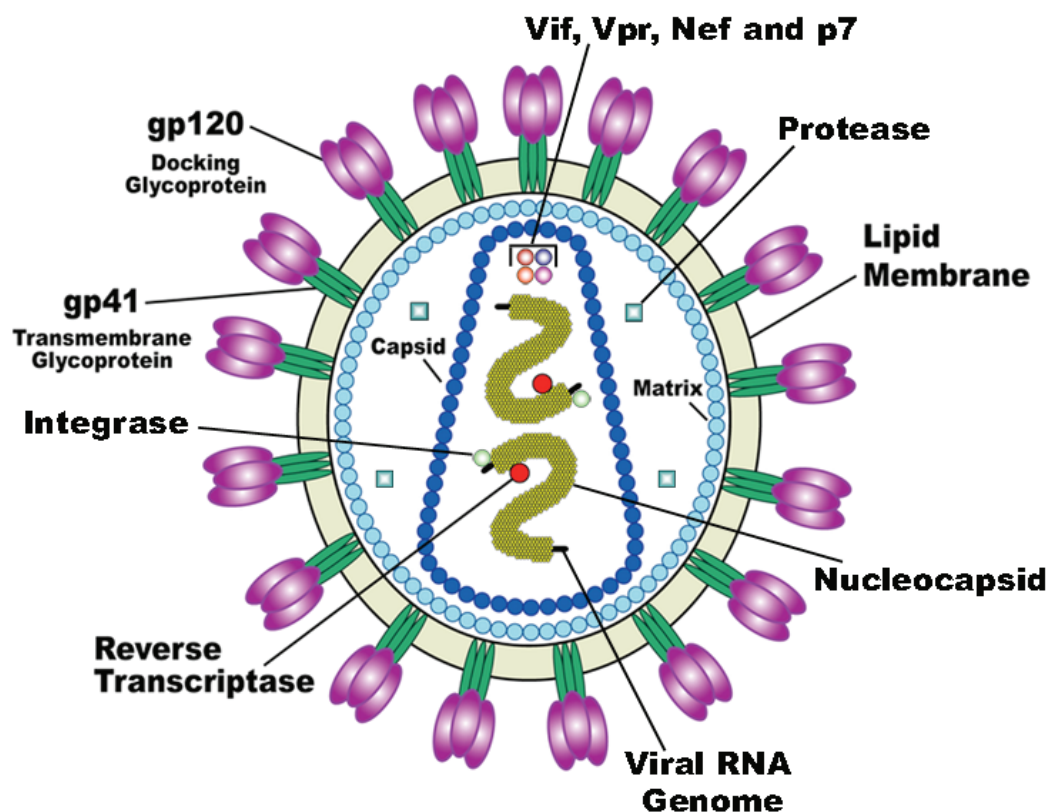
1.2 Human Immunodeficiency Virus (HIV)

HIV is a member of the genus Lentivirus part of the family Retroviridae. HIV infects CD4 T lymphocyte helper cell which is the vital cell of the immune system. When CD4 T cell count declines below a critical level, cell-mediated immunity is lost, and the infected person becomes progressively more susceptible to OIs and this leads to AIDS. CD4 T lymphocyte count and viral load test is done to assess the HIV progression and immune status of the person.

HIV is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell. It is composed of two copies of positive single-stranded RNA that codes for the virus nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins and enzymes needed for the development of the virion such as reversetranscriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein surrounds the capsid ensuring the integrity of the virion particle which is surrounded by the viral envelope.

The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of *tat* *env* and *rev*), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env* contain information needed to make the structural proteins for new virus particles. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.

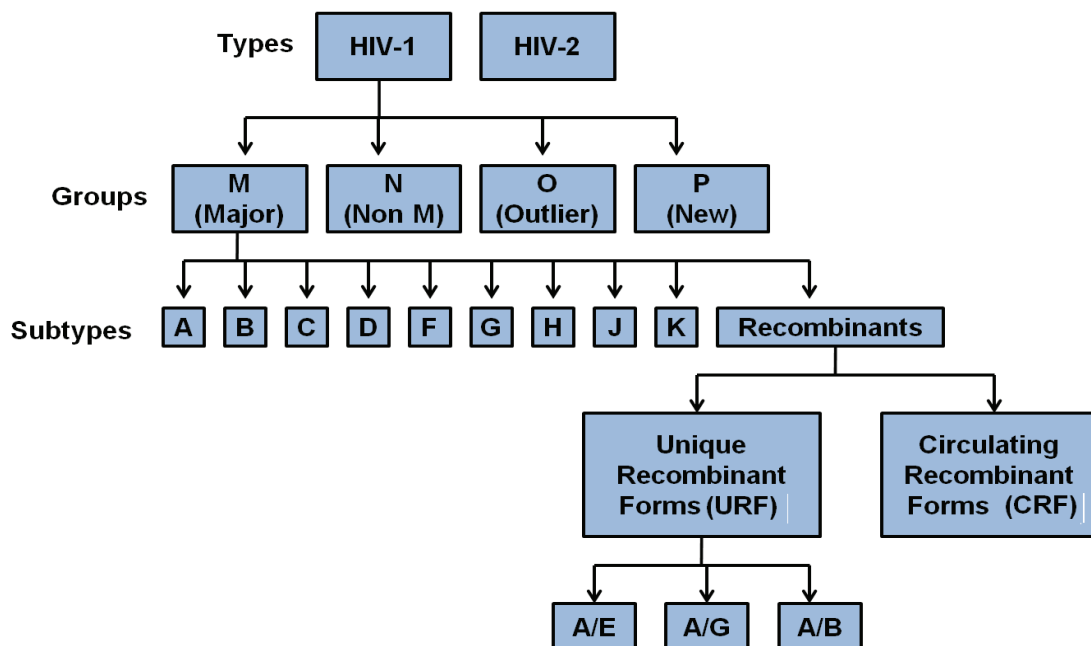
Figure 1.1: Structure of HIV virus



1.2.1 Subtypes of HIV virus

HIV can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). Both produce the same patterns of illness. HIV 2 causes a slower progression of disease than HIV 1. It is important for tests to detect the HIV subtypes that are present in the region.

1. HIV-1 is the most common and pathogenic strain of the virus. HIV 1 can be divided into groups M, N, and O and P. The pandemic is dominated by Group M, which is composed of subtypes A – K.
2. HIV 2 is most often found in West Central Africa, parts of Europe and India. Few cases have been confirmed in Nepal. In case of suspicion of HIV 2 in the country, the laboratory (test site) should contact NPHL for further investigation.

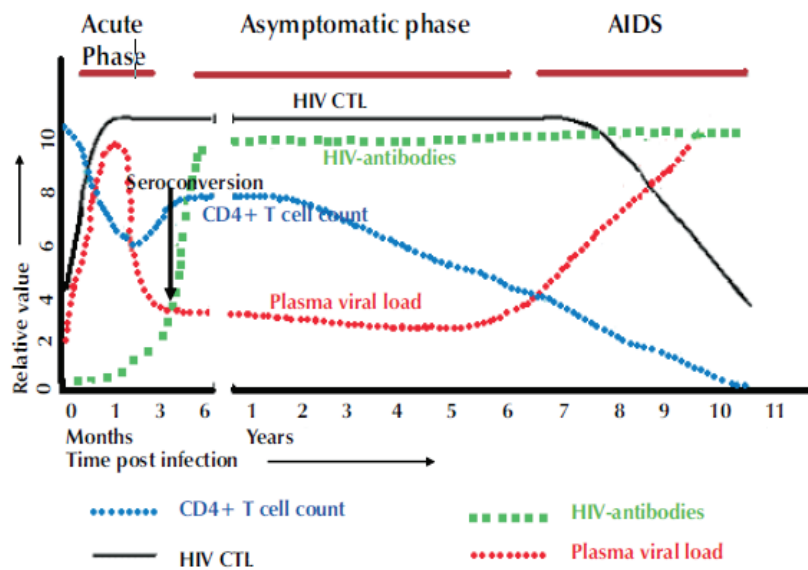
Figure 1.2: Distribution of HIV virus types and subtypes

1.2.2 HIV replication

HIV enters CD4 T lymphocyte cells by the absorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell. After HIV has bonded to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonucleases, and protease, are injected into the cell. During the microtubule-based transport to the nucleus, the viral single-strand RNA genome is transcribed into double-strand DNA, which is then integrated into a host chromosome.

Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and a host of co-factors. Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle.

In different phases of HIV progression, different laboratory markers can be identified using laboratory technique. Following figure provides information about laboratory markers identified during HIV progression.

Figure 1.3: Laboratory markers during HIV progression

HIV CTL=HIV Cytotoxic T Lymphocytes

1.3 Acquired Immunodeficiency Syndrome (AIDS)

AIDS is the stage or condition due to consequence of HIV infection. HIV infection leads to a weakened immune system (defense mechanism against infection). This makes a person with HIV vulnerable to infections. AIDS results when HIV infection progresses to an advanced stage, damaging the immune system to a point at which the body can no longer fight illness. AIDS is defined in terms of either a CD4 T lymphocyte cell count below 200 cells per μL or the occurrence of specific diseases in association with an HIV infection. The viral load level (HIV RNA) will be at peak with untreated client or virological failure with Antiretroviral Therapy (ART).

1.4 HIV vs. AIDS

HIV is the virus that can cause AIDS. Not everyone who is infected with HIV can develop AIDS. Everyone with AIDS is infected with HIV. AIDS is the result of progression of HIV Infection. Anyone infected with HIV, although healthy, can still transmit the virus to another person.

1.5 HIV transmission

HIV is transmitted by three main routes: sexual contact, exposure to infected body fluids or tissue and from mother to child during pregnancy, delivery, or breastfeeding (known as vertical transmission).

- Unprotected sexual contact: The most frequent mode of transmission of HIV is through sexual contact with an infected person, this may be heterosexual or homosexual. Risk of transmission increases in the presence of many sexually transmitted infections and genital ulcers.
- Blood and blood products: The second most frequent mode of HIV transmission is via blood and blood products. Blood-borne transmission can be through needle-sharing during intravenous drug use, needle stick injury, transfusion of contaminated blood or blood product, or medical injections with unsterilized equipment.
- Mother to child: HIV can be transmitted from mother to child during pregnancy, during delivery, or through breast milk.

1.6 Window period

Window period refers to the period between the HIV infection and formation of antibodies against HIV at detectable level. On average, the body's immune system takes three to eight weeks to develop

enough HIV antibodies to be detectable. Window period is the phase where humans have been infected with HIV, but antibodies are not produced at a detectable level. One may test false-negative for HIV antibodies, and can still pass the virus to others during this period.

1.6.1 Seroconversion during window period: “Seroconversion” is a term used to describe the change from non-detectable to detectable antibody levels. Specimen may test non-reactive initially, but change to testing reactive after a certain time period. Seroconversion occurs generally three-eight weeks after the initial infection.

1.7 Disease progression

Severity of illness is determined by amount of virus in the body (increasing viral load) and the degree of immune suppression (decreasing CD4+ counts). As the CD4 count declines, the immune function decreases.

After infection, the virus enters the blood and attacks the body’s immune system, specifically the important T-helper cell which co-ordinates the immune system’s response to infections. HIV gains entry to the T-helper cell by attaching itself to the CD4 protein on the surface of the cell. Once the HIV has gained entry, it takes over the cell and replicates, seeking new T-helper cells to infect. The infected cell dies after a couple of days.

The body’s natural response to an infection is to fight infected cells and replace the cells that have been lost. But gradually HIV overwhelms the immune system, leaving the body vulnerable to infections and other diseases. The time it takes to do this varies from person to person, but averages at about seven years. Without treatment, the viral load, which refers to the amount of free virus in the blood, will increase to the point where the body can no longer fight it and CD4 T cells decrease far below the normal count of 500-1500 cells per cubic milliliter of blood. This will make the body vulnerable to different opportunistic infections.

1.8 Acute HIV & HIV testing

Acute HIV infection is the first phase of HIV infection. It begins when a person is infected with HIV and lasts for about two months. When a person first gets HIV, the amount of HIV in his blood, semen, and other bodily fluids gets very high within a few days, as the HIV virus is replicating itself very quickly at this point. A person with acute HIV infection is much more likely to transmit HIV to others. The early HIV test can detect HIV during the acute infection phase. The best test for detecting acute HIV infection is the early HIV test through Nucleic acid amplification test (NAAT) which detects viral genome. Most people will have enough of the virus in their blood 10 to 12 days after being infected for the early test to detect HIV infection. After a week of the virus presence in blood, antigen testing (P24 antigen) can be used for HIV detection.

Can HIV progression be delayed?

HIV progression can be delayed by:

- Prevention and early treatment of OIs
- Antiretroviral therapy
- Positive living

1.9 HIV and AIDS: global epidemiology

With the first case of HIV AIDS reported in 1981 in USA, HIV now is a global pandemic. Most of countries are affected by the HIV pandemic. Advancements in technology and medicine have helped to control HIV prevalence. However in developing countries the infection is still not decreasing. Below are the key highlights of the HIV pandemic across the globe.

- About 6,000 new HIV infections a day in 2013.
- About 68% are in Sub-Saharan Africa.
- About 700 are in children under 15 years of age.

- About 5,200 are in adults aged 15 years and older, of whom:
 - Almost 47% are among women
 - About 33% are among young people (15-24 years)

Table 1.1 Number of people living with HIV in 2013

Total	i. 35 million [33.2–37.2 million]
Adults	i. 31.8 million [30.1–33.7 million]
Women	i. 16 million [15.2– 16.9 million]
Children less than 15 years	i. 3.2 million [2.9– 3.5 million]

Table 1.2 Number of new infection of HIV in 2013

Total	2.1 million [1.9– 2.4 million]
Adults	1.9 million [1.7– 2.1 million]
Children less than 15 years	240,000 [210,000 – 260,000]

Table 1.3 Number of deaths due to AIDS in 2013

Total	1.5 million [1.4 – 1.7 million]
Adults	1.3 million [1.2 – 1.5 million]
Children less than 15 years	190,000 [170,000 – 220,000]

Source: UNAIDS Global Report 2014

Table 1.4 Situation of HIV in Nepal

Estimated number of people living with HIV (2013) by age groups	
Total	40,723
Children (0-14 years)	3,282
Adults (15-49 years)	34,056
Adults (50+ years)	3,385
Adult (15-49) HIV prevalence	0.23%
Adult women (15-49) living with HIV	28%
Young people (15-24) living with HIV	9%
Estimated newly infected in 2013	1,408
Cumulative number of reported HIV infections (1988 - 16 July 2014)	25,222

Table 1.5 Cumulative HIV infection by sub-group and Gender (as of July 2013)

	Male	Female	TG	Total
Total	15,837	9,344	41	25,222

Source: NCASC Factsheet, 2014 [as of 15 July 2014]

Module review

1. What is HIV? What is AIDS? How does HIV relate to AIDS?
2. What are the means by which HIV is transmitted?
3. What is “window period?” How does it affect HIV test results?
4. How can the disease progression of HIV/AIDS be delayed?
5. What is the status of HIV in Nepal?

MODULE 2

NATIONAL HIV/AIDS STRATEGY AND IMPORTANCE OF TESTING AND COUNSELING

Purpose

To provide basic concept on HIV prevention using HIV tests combined with counseling.

Learning objectives

At the end of this module, participants will be able to:

- Describe the National HIV and AIDS Strategy 2011-2016
- Describe the role of HIV rapid testing in supporting prevention and counseling programs
- Describe the importance of counseling and confidentiality in HIV testing
- Describe the approaches of HIV counseling

Content outline

- National HIV and AIDS Strategy 2011-2016
- Testing and counseling as an integral part of HIV prevention, care and support services
- Approaches of HIV testing and counseling adopted by the country
- Importance of counseling and confidentiality in HIV testing

Time: 30 minutes

2.1 National HIV and AIDS strategy 2011-2016

The goal of National HIV and AIDS Strategy 2011-2016 is to achieve universal access to HIV prevention, treatment, care and support. This strategy has the objectives to reduce new HIV infections by 50%, reduce HIV related deaths by 25%, and reduce new HIV infection in children by 90% by 2016. The strategy's major focus will be on addressing all dimensions of the continuum of care, effective coverage of quality care along with strengthening the system integration of HIV services into public health system in a balanced way to meet the specific needs of target populations.

Strategically, HIV testing and counseling will be gradually taken up by public health system and will be expanded up to Primary Health Care center (PHC) and Health Post (HP) levels as an integral part of government health care service by 2015. As such, other divisions of Ministry of Health and Population (MoHP) will take increasing role in programming, implementation and monitoring. NGOs will be also engaged in testing and counseling where government service is not available or has not reached the targeted groups till the services are made available in the public health system. In all such operations, HIV related information, including HIV testing results should be kept confidential. Special focus on Public Private Partnership (PPP) for establishing testing and counseling services will be adopted. The testing and counseling services should be as per the national standard guidelines and protocols.

2.2 HIV testing and counseling (HTC)

There are over two hundred sites offering HIV testing and counseling (HTC) nationwide. There is need for harmonization and coordination in the HTC sites run by different programs, at the same time integrating into the health system. Provider initiated testing and counseling is being initiated from Anti natal Clinic (ANC), STI and Tuberculosis (TB) check up clinics.

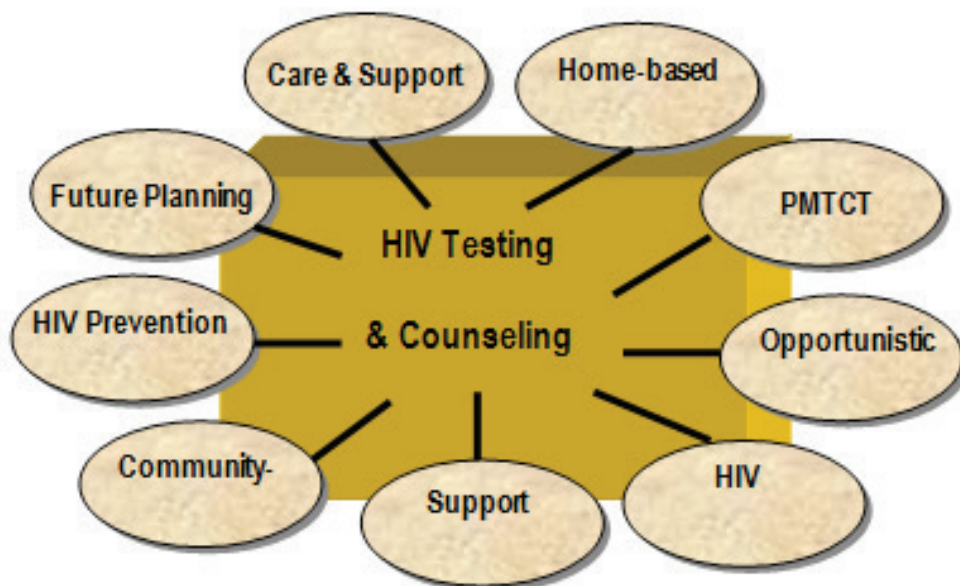
To address the national strategy, laboratory diagnosis has a vital role in HIV prevention, treatment and care. There is also need of scaling up on case detection to meet the national program, and expansion and strengthening of HTC with possible integration with existing health services. Linkages and integration

with other program services such as TB, HIV, Prevention of Mother to Child Transmission (PMTCT), ANC, Safe motherhood, family planning should be strengthened to assure comprehensive management of patients and future sustainability.

Key actions (from National HIV and AIDS strategy 2011-2016)

- Promote the uptake of counseling and testing among key affected population which includes people who inject drugs (PWID), Sex workers and their clients, Men who have Sex with Men (MSM) and transgender populations and male labor migrants and their spouses through targeted communications and linkages between community outreach and the counseling and testing service centers.
- Promote Provider Initiated Testing and Counseling (PITC) especially in STI, ANC, child birth, post-partum, FP and TB services including family and couple counseling but continue operating Client Initiated Counseling and Testing services. Scale up rapid HIV testing services and mobile HTC services in a non-duplicated manner in targeted locations in a cost effective way to ensure maximum utilization with strong referral linkage to higher level of treatment, care and support.
- Institutionalize HIV testing and counseling services as a part of PPP in health institutions (teaching hospitals, private hospitals, nursing homes, polyclinics) following the national testing and counseling guidelines and protocols and ensure reporting the services to the government as per the national standards.
- Expand couples counseling and serodiscordant couples counseling, drug-injecting partners through partner counseling, children and adolescents with HIV at HIV testing and counseling sites and by peer educators including providing active support for beneficial disclosure.

Figure 2.1 HIV T&C as an entry point to HIV prevention, care and support services



HTC is a crucial gateway for HIV prevention, care, and treatment services, which has vital role in reducing morbidity, mortality, and HIV transmission. Along with the uptake of HIV services, numerous approaches to HTC have emerged, including client-initiated HTC (or “voluntary counseling and testing”), and community-based HTC, as well as PITC. Such approaches to HTC is increasingly used to facilitate diagnosis and access to HIV-related services by engaging clients at health facilities, a key setting for reaching individuals in need of HTC services.

2.4 Approaches of HIV testing and counseling

There are different approaches for HIV testing and counseling which are described below.

2.4.1 Client initiated HIV testing and counseling

The primary model for providing HIV testing and counseling has been client-initiated HIV testing and counseling - also known as voluntary counseling and testing (VCT) - in which individuals must actively seek an HIV test at a health or community-based facility. Client-initiated HIV testing and counseling usually emphasizes individual risk assessment and management by counselors, addressing issues such as the desirability and implications of taking an HIV test and the development of individual risk reduction strategies. Current evidence also suggests many opportunities to diagnose HIV in clinical settings are being missed, even in places with serious HIV epidemics. While expanded access to client-initiated HIV testing and counseling is still necessary, other approaches are also required if coverage of HIV testing and counseling is to increase and, ultimately, universal access to HIV prevention, treatment, care and support is to be achieved.

2.4.2 Provider initiated HIV testing and counseling

Provider-initiated HIV testing and counseling (PITC) refers to HIV testing and counseling in which the health care provider specifically recommends for HIV test to patients attending health facilities. The PITC can help to increase uptake of HIV testing, improve access to health services for people living with HIV (PLHIV), and may create new opportunities for HIV prevention. With this approach, HIV test is recommended for all patients whose clinical presentation might result from underlying HIV infection or as a standard part of medical care for all patients attending health facilities in areas of high HIV prevalence.

2.4.3 Community-based testing and counseling

In community-based testing and counseling, outreach service is provided in different settings. This can be performed both at facility and community settings. The setting could be; (i) stand-alone or multi service HTC facilities within the community, or (ii) outreach services offered outside at fixed site such as work place (e.g factories) or mobile center during events (Musical events, Sport events, World AIDS day), or (iii) home based service in which HTC service provider physically goes to the home of potential client. A team including laboratory staff will provide the HIV testing and counseling at community level. This will be helpful to test more people and extend the service. Community people who are reluctant to visit the clinic will benefit from community based testing and counseling.

2.4.4. Couples testing and counseling

In couples testing and counseling, both sexual partners are counseled as a couple (e.g., HTC, PMTCT sites). Testing and counseling discordant couples (one person test positive and the other person test negative) is a highly effective preventive intervention. This facilitates disclosure and joint planning for risk reduction. It increases utilization of care and treatment if the partner knows about and supports the infected person. It allows for planning and care of children based on sero-status of both parents. This supports pre-exposure prophylaxis (PrEP) and condom use, which can help prevent HIV transmission. In addition to this, it creates an opportunity for couples to discuss, establish, or revise sexual agreements for their relationship and allows couples to prepare a risk-reduction plan based on the HIV status of both partners.

2.5 Importance of counseling and confidentiality in HIV testing

In HIV testing sites, three "C" principles (Counseling, Confidentiality and Consent) must be followed. Counseling is required for HIV prevention activity and to enroll in treatment, care and support (if diagnosed for HIV infection). Effective counseling helps to understand their situation more clearly and identify a range of options for improving the situation to cope better with the problem.

Confidentiality is linked with the stigma and discrimination which is associated with HIV. Record and identity should be adequately maintained and should not be shared with unauthorized persons. Consent is needed before doing the test to ensure that he/she is comfortable with testing.

2.6 HIV related stigma and discrimination

Stigma and discrimination is one of the major components related to HIV. The laboratory test result should not be disclosed to public. Rejection to services, education, social events and often delayed response to test and treatment are other stigma associated issue related to HIV. Evidences have suggested that in stigma and discrimination free setting (health care and others) utilization of services has improved substantially.

2.7 Positive prevention

Positive prevention is a key component in HIV prevention, care and support. Positive prevention should aim to support PLHIV to protect their health, to avoid new STIs and OIs, to delay HIV/AIDS disease progression and to avoid passing their infection on to others. Access to treatment and care through engagement of positive people should be promoted. Effective counseling for people living with HIV on safer sex interventions to prevent HIV transmission to others, avoiding contracting sexually transmitted infections and condom promotion including counseling on FP and reproductive health to couple and women living with HIV enabling them to make an informed decision should be provided. It is crucial to promote ongoing behavioral counseling and psychosocial support to HIV-discordant couples through couples counseling and support groups that cover topics such as HIV transmission risk reduction, reproductive health issues, couples communication and condom provision.

Module review

1. Explain the importance of HIV testing and counseling in HIV prevention program.
2. Describe the approaches of HIV testing and counseling.
3. Explain about Stigma and Discrimination and Positive prevention related to HIV?

MODULE 3

OVERVIEW OF LABORATORY TESTING TECHNOLOGIES IN HIV PROGRESSION

Purpose

To provide basic knowledge on HIV testing technologies in HIV progression.

Learning objectives

At the end of this module, participant will be able to:

- Explain about the HIV testing technologies and applications
- Explain about the spectrum of testing technologies for HIV across the continuum of care

Content outline

- Basic concept on various diagnostic techniques for HIV
- Laboratory test for HIV diagnosis and disease progression
- Laboratory test for ARV initiation and monitoring

Time: 60 minutes

3.1 Introduction

Laboratory service is essential for HIV diagnosis, monitoring and management. Diagnosis of HIV infection cannot be confirmed by any means other than blood or body fluid tests by the laboratory. CD4 T lymphocyte count is a prerequisite for the initiation of antiretroviral therapy (ART) and viral load for monitoring treatment outcome and also for monitoring the disease progression. Both immunological and microbiological monitoring of ART is, therefore, exclusively dependent on laboratory service. Therefore, laboratories will play a vital role in implementing HIV programs providing the diagnostic as well as ART monitoring.

3.2 Diagnostic service

The presence of HIV 1/2 infections in individuals can be ascertained only through the use of laboratory tests on body fluids such as blood, plasma, serum etc. The laboratory confirmation of HIV infection is needed at different settings to ensure status and safety.

In primary infection with HIV, the virus in the blood can be demonstrated by nucleic acid-based test (PCR for pro-viral DNA and RT-PCR for viral RNA), p24 antigen test or virus culture. Antibodies to HIV are detectable within three to eight weeks of infection by commonly employed tests and in virtually all infected individuals within six months. Once antibodies appear in the blood, they persist for a lifetime. However, there have been some isolated reports of antibodies being undetectable after successful treatment in infants. These reports are however rare.

3.3 Use of HIV testing technologies in the continuum of care

A variety of tests are performed for diagnostic as well as monitoring of HIV progression. HIV diagnostic tests play an important role in initially identifying those who are infected with the HIV virus. Other tests, e.g., CD4 T lymphocyte count and viral load, play an important role in determining whether therapy can be initiated, and once initiated, if the drugs are working or not and monitoring the disease progression.

3.4 Spectrum of laboratory test for HIV progression

The laboratory diagnosis of HIV can be confirmed through detecting the antibodies against HIV infection, or HIV antigen or HIV nucleic acid. The ARV initiation can be based on CD4 T lymphocyte count

and monitoring can be done through CD4 T lymphocyte count and/or viral load.

Diagnosis of HIV infection can be carried out by detecting any of the followings:

- Antibodies to HIV diagnosis
 - o Rapid tests; Enzyme Immunoassays (EIA/ELISA); Western blot (WB)
- HIV antigen
 - o P24 antigen test
- HIV Nucleic acid detection
 - o HIV DNA PCR

Initiation and monitoring of ARV can be carried out through immunological and virological monitoring:

- Initiation and monitoring of ART
 - o CD4 T lymphocyte count
 - o Viral Load (HIV RNA PCR)

Body fluids used for HIV testing and monitoring:

HIV tests could be performed on a wide range of body fluids. Serum, plasma, whole blood and oral fluids are used the most. The samples used for HIV diagnosis are whole blood, serum and plasma, and EDTA blood for CD4 T lymphocyte count, plasma/serum for viral load and whole blood or Dried Blood Spot (DBS) sample for HIV DNA PCR test in our context.

3.4.1 Antibodies to HIV detection

a. Rapid HIV tests

Rapid HIV antibody tests provide same-day results and do not require additional reagents or equipment than not contained in the kit. Current rapid tests are based on three immunologic formats (Immuno concentration, Immunochromatography, Particle agglutination). Most HIV rapid tests contain antigens to HIV-1 and HIV-2 and detect antibodies to both. A positive test result is indicated by clumping, a spot, dot, or line, depending on the test format. The sensitivity and specificity of the latest generation of rapid tests are similar to those of ELISA for HIV diagnosis.

b. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is the most commonly used test to screen for HIV infection because of its relatively simple methodology, inherent high sensitivity, and suitability for testing large numbers of samples, particularly in blood testing centers. A common feature of all varieties of ELISA is the use of enzyme conjugates that bind to specific HIV antibody and substrates/chromogens that produce color in a reaction catalyzed by the bound enzyme conjugate. The most popular ELISA involves an indirect method in which HIV antigen is attached to a well of a 96-well micro titer plate that detects antibodies against HIV infection. Most ELISA detect antibodies to HIV-1 and HIV-2 and are suitable for sites of large sample size where technically skilled laboratory personnel are available.

c. Western blot (WB)

The Western blot assays also detects the antibodies against HIV infection. It is based on using an electrophoretic technique to separate HIV antigens. This technique denatures the viral components, imparts a negative charge to the antigens, and separates them primarily on the basis of their molecular weights. The separation of antigens in the technique allows for the identification of specific antibodies to each of the viral antigens. In this assay, multiple antibodies against HIV-1 and HIV-2 are detected.

3.4.2 HIV antigen detection

a. P24 antigen test

The p24 antigen test detects the presence of the p24 protein of HIV, the capsid protein of the virus. Monoclonal antibodies specific to the p24 protein are mixed with the blood to be analyzed. Any p24

protein in the blood will stick to the monoclonal antibody and an enzyme-linked antibody to the monoclonal antibodies to p24 causes a color change if p24 was present in the sample. This is an ELISA-based technology, and is therefore less expensive than other nucleic acid technologies and, in principle, requires less stringent laboratory capacity. The p24 antigen test is not useful for general diagnostics, as it has very low sensitivity and only works during a certain time period after infection before the body produces antibodies to the p24 protein.

3.4.3 HIV nucleic acid detection

a. HIV DNA PCR test

HIV DNA PCR test is based on polymerase chain reaction (PCR) which is used for early detection of HIV infection and considered as gold standard technique. Through this technique HIV provirus is detected and used for early HIV infection in children (below 18 months) and/or with “indeterminate” antibody profile results. The presence of integrated HIV proviral DNA can be detected by PCR that targets a segment of the highly conserved gene. Clinical studies have indicated that detection of HIV proviral DNA in whole blood specimens by PCR is highly sensitive and specific. It is qualitative test unlike RNA PCR (Viral load). The detection of HIV nucleic acids (proviral DNA) by PCR can provide early evidence of HIV infection (approximately 10-14 days after infection), when results of routine diagnostic assays are inconclusive.

For those infected before and during birth, the sensitivity of the test is 40-50% in the first 48 hours of life, 90% at 14 days of age and >95% at six weeks of age. Hence, six weeks is considered to be an optimal age for testing. Samples for DNA PCR can be collected in DBS paper specialized for Nucleic acid. PCR testing is complex and requires specialized equipment and experience. It therefore is not suited for basic laboratories.

3.4.4 Initiation and monitoring of ART

Laboratory monitoring of people with HIV infection is essential for timely initiation of medication and impact of medication. Regular monitoring of disease progression plays the crucial role for the program. The monitoring can be done through immunological and/or virological test.

- Immunological monitoring
- Virological monitoring

a. Immunological monitoring: CD4 T lymphocyte count

Immunological monitoring refers to the CD4 T lymphocyte count which is the hallmark of the body's immune status. CD4 T lymphocyte count also provides the information whether to initiate the ARV or not and the impact of ARV.

CD4 T lymphocytes:

Cellular components of blood comprise red blood cells and white blood cells. Two populations of leucocytes constitute granulocytes and non-granulocytes, including the lymphocytes. Surface receptors of the lymphocytes provide identity to sub-populations of lymphocytes which differentiate into unique clusters. This property gives the subtypes of lymphocytes a nomenclature of clusters of differentiation followed by the number of the unique subtype (CD1, CD2, CD3, CD4...). CD stands for cluster of differentiation; CD numbers are now used to identify cell surface antigens that can be distinguished by monoclonal antibodies. CD4 T lymphocytes (CD4+ T-cells), commonly known as T helper cells, play a vital role in maintaining the integrity of the human immune system.

A primary target of HIV is CD4 T lymphocytes which are preferentially depleted during the course of the disease. It is well recognized now that accurate and reliable enumeration of CD4 T lymphocytes is very crucial for monitoring the rate of progression to AIDS, both for initiating prophylaxis for opportunistic infections as well as monitoring the impact of ART. Immunofluorescence analysis by Flow Cytometry

(FCM) is the gold standard for CD4 T lymphocyte count. FCM is a widely used method for estimation of CD4 T lymphocytes.

Available CD4 T lymphocyte count platforms in the country:

- BD FACS Count and BD FACS calibur
- Partec cyflow
- Pima (Point of care Testing)

b. Virological monitoring: HIV RNA PCR (Viral load test)

Once the ARV is initiated, regular viral load test provides the information on progression of HIV infection and the effect of ARV in HIV infected individuals. The number of viral load after ARV provides information on the outcome and efficacy of drugs and helps decision making for shifting to next line ARV treatment.

HIV viral load measurement is a useful tool for monitoring the disease progression, efficiency of treatment and predicting emergence of resistance in HIV against antiretroviral drugs. A baseline plasma viral load is established before starting ART and periodic monitoring is essential. It is predicted that with successful therapy a fall of 1.5 to 2 log in plasma viral load occurs within 4-6 weeks. With successful ART, it should become undetectable in four to six months of therapy. Viral load should be done after six months of initiation of ARV and after this regularly in every 12 months. However, based on the clinical condition of people living with HIV, experienced ART clinician can refer for viral load test.

Module review

1. Explain Rapid test, ELISA, P24 antigen test, DNA PCR, CD4 T lymphocyte count, and Viral load test.

MODULE 4

HIV TESTING STRATEGIES AND ALGORITHMS

Purpose

To provide basic concept on national strategies and algorithm for HIV diagnosis.

Learning objectives

At the end of this module, participants will be able to:

- Explain the HIV rapid testing algorithm approved in the country
- Explain how to determine HIV status following serial testing algorithm

Content outline

- Strategies and algorithms
- National HIV testing algorithm
- Interpreting HIV status
- Sensitivity and specificity

Time: 30 minutes

HIV test can be conducted for screening blood intended for blood transfusion and organ transplant, diagnosis of HIV, surveillance or research. The selection of HIV testing strategy for surveillance, blood screening and diagnosis depends on contextual factors, such as a country's policies and given epidemic state, appropriate population groups and settings for HIV testing.

4.1 Strategies and algorithms for HIV testing

Strategies are defined as the testing approach used to meet a specific need, such as for blood safety, surveillance, and diagnosis. For a given strategy, multiple algorithms may be used depending on the needs of test settings.

Algorithms are defined as the combination and sequence of specific tests used in a given strategy. The number of algorithms should be limited.

Testing strategies describe the generic testing approach, i.e. the sequence in which a combination of tests should be performed. The testing algorithm inform about the combination of specific HIV test kits (specific products) to be used in a given strategy.

In the context of Nepal, where sophisticated laboratories and trained human resources are not readily available; simple algorithms have been incorporated into the national program that is easily applicable throughout the country.

The choice of testing algorithm would also depend on the following factors:

- Objective of HIV testing, whether it is for diagnosis, surveillance or blood transfusion and organ transplant.
- Sensitivity and specificity of the test kit being used.
- Prevalence of HIV in the population.
- Resources available – human, financial, infrastructural.

For HIV testing strategies where more than one test may be required, the selection of test kits and the order in which they are in testing algorithm are important for obtaining accurate test results. Firstly, the tests should contain different antigens. Secondly, the first test (A1) should have high sensitivity. The first

is a screening test therefore it is ideal to have a more sensitive test to detect all positives. Because some false positives will occur, the second test (A2, confirmative test) needs to be highly specific to ensure that all truly negative test results are identified as negative. The third test should have comparable sensitivity and specificity like the first and second tests with different antigenic properties from previous ones to correct the disagreement between them.

4.2 HIV testing strategy and algorithm

HIV diagnosis can be done through parallel or serial testing strategy based on the national need.

- Parallel testing: Samples are tested by all the three different tests at the same time. If the outcome of the test is found to be reactive in at least by two different test kits, the sample is considered as positive.
- Serial testing: Samples go through a first screening test and results of the first test determine whether additional tests are required. There is no need for additional tests, unless the result is reactive.

4.3 Ideal algorithm

The ideal algorithm used, is one in which tests are highly sensitive and highly specific.

Both tests should not share the same false negatives and false positives, and a third test is performed, if needed.

Key factors in determining the country's algorithm:

- Test performance in a country, sensitivity and specificity of test kit, test kits availability in country, program needs, ease of use, type of specimen, cost, potential need to differentiate between HIV 1 & HIV 2.

4.4 National HIV testing algorithm: serial testing algorithm

Serial testing means samples are tested by a first test and its results determine whether additional testing from different test kit is required. If the first test shows a non-reactive result, the tested sample will be reported as "HIV Negative." If the first test shows a reactive result, the sample will be tested further by a second test; if the second test also shows a reactive result, the tested sample will be reported as "HIV Positive."

When two test results disagree (the first is reactive and second is non-reactive), the finding is called "discordant." In this case, a third test must be performed; the result of the third test will be the final test result.

The first test used in a serial HIV testing algorithm should be highly sensitive so that all positive samples will be identified as positive. The second test should be highly specific so that all true negative samples will be identified as negative.

Figure 4.1: National HIV testing algorithm: serial testing algorithm

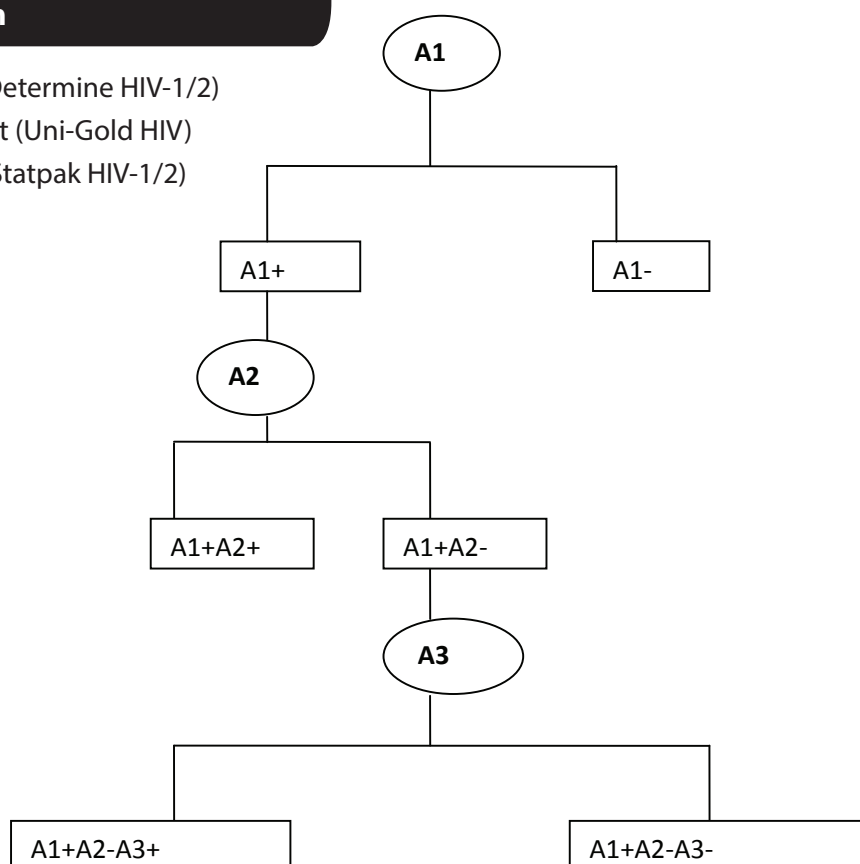
A1: Screening Rapid test (Determine HIV-1/2)

A2: Confirmatory Rapid test (Uni-Gold HIV)

A3: Tiebreaker Rapid test (Statpak HIV-1/2)

(+) = Reactive

(-) = Non Reactive



TEST 1 (A1)	TEST 2 (A2)	TEST 3 (A3)	HIV Status
Non-reactive			Negative
Reactive	Reactive		Positive
Reactive	Non-reactive	Non-reactive	Negative
Reactive	Non-reactive	Reactive	Positive

Facilitator will demonstrate lab result report form and explain how to fill the form

4.5 Sensitivity and specificity

Sensitivity and specificity of test kits play an important role in accurate diagnosis of the infections. Ideally 100% sensitive and specific test kits give zero false positive and false negative. However in reality no test is 100% sensitive and specific but many are >99%.

- Sensitivity (Se) of a test is its capacity to correctly identify people that are infected with HIV.
- Specificity (Sp) of a test is its capacity to correctly identify people that are not infected with HIV.
- Positive Predictive Value (PPV) is the probability that a person who tests reactive is indeed infected with HIV.
- Negative Predictive Value (NPV) is the probability that a person who tests negative is not infected with HIV.

Table 4.1 Calculation for sensitivity and specificity

Test result	Actual HIV result (Gold standard)		Total
	HIV infected	HIV not infected	
Positive	A (370)	B (2)	A+B (372)
Negative	C (4)	D (624)	C+D (628)
Total	A+C (374)	B+D (626)	1000

Sensitivity = $A \div (A+C) = 370 \div 374 = 98.9\%$

Specificity = $D \div (B+D) = 624 \div 626 = 99.7\%$

Positive Predictive Value = $A \div (A+B) = 370 \div 372 = 99.5\%$

Negative Predictive Value = $D \div (C+D) = 624 \div 628 = 99.4\%$

Accuracy = $(A+D) \div ((A+C)+(B+D)) = 994 \div 1000 = 99.4\%$

Points to note:

- Before any test is adopted in a country for use, a series of key steps must be taken to evaluate the tests before they are fully adopted for country-wide use.
- The ideal algorithm used is one in which tests are highly sensitive and highly specific.
- No test is 100% sensitive or 100% specific when compared to the “gold standard”.
- Always follow the sequence of the tests in the algorithm and never miss out a required step or test.
- Always perform and read the test results according to the manufacturer’s instructions.

Module review

1. Explain the testing algorithm adopted by MoHP in Nepal. What rapid tests are used and in what order?

MODULE 5

EARLY INFANT DIAGNOSIS (EID): HIV

Purpose

To provide information on early diagnosis of HIV infection in children.

Learning objectives

At the end of this module, participants will be able to:

- Explain the basic concept on EID
- Explain the importance of EID
- Describe test algorithm for EID

Content outline

- Early Infant Diagnosis
- Importance of EID
- Laboratory testing for EID
- Testing algorithms for EID

Time: 60 minutes

5.1 Early infant diagnosis

HIV can be transmitted during pregnancy, childbirth or breastfeeding. Without interventions, the risk of transmission from an infected mother to her child ranges from 30 to 45 percent. With the initiation of precautionary measures including infant feeding modifications, antiretroviral prophylaxis, following PMTCT measures, the risk is reduced to less than five percent.

Table 5.1 Estimated risk of Mother to Child Transmission (MTCT) of HIV in the absence of interventions

Activities	Estimated risk in Percentage
During pregnancy	5–10%
During labor and delivery	10–15%
During breast-feeding	5–20%
Overall without breast-feeding	15–25%
Overall with breast-feeding to 6 months	20–35%
Overall with breast-feeding to 18 to 24 months	30–45%

Source: de Cock et al, JAMA (2000)

5.2 Importance of EID

EID is crucial in identifying HIV-infected infants early in order to provide them with lifesaving ART. Early access to treatment and care will be helpful in reducing morbidity and mortality. Children who are diagnosed as HIV-infected need to be carefully monitored and provided with ARVs. Children who are treated successfully with ART may then be able to live healthy lives.

5.3 Laboratory diagnosis

Standard HIV-antibody testing, as is done with adults and older children, cannot be used to identify infection in infants below 18 months of age, as it also detects maternal HIV antibodies that are transferred to the baby during pregnancy and which subsequently decline slowly during the first year of life). Maternal HIV specific antibodies are detectable in the infant's blood for up to 18 months of age. Most uninfected infants lose maternal antibodies between six and twelve months of age. A few continue to test positive until 18 months of age. It is essential to distinguish between passively acquired antibodies and active HIV infection. More sophisticated technique such as nucleic acid amplification test (NAAT) that detects viral nucleic acid (DNA/RNA) or viral products like P24 antigen detection test are required for diagnosis of HIV infection in infants. Virological testing based on PCR for presence of HIV DNA has become the most widely used assay and is considered as gold standard for EID.

HIV DNA PCR tests can be reliably performed on whole blood specimens or blood collected and dried on specialized filter paper; these samples are known as dried blood spots (DBS). The use of DBS requires only a few drops of blood from an infant and does not require phlebotomy.

5.3.1 Virological testing of HIV exposed infants less than nine months of age

Most infants are seen at vaccine clinic for vaccination at six weeks of age and a majority of infected infants—particularly those infected in utero, intrapartum and during early breastfeeding, are likely to have detectable virus by six weeks of age. So, samples should be collected at six weeks of age for HIV DNA PCR testing to identify HIV infected children. However, sample can be collected earlier if any related symptoms are noticed in HIV exposed children.

5.3.2 Antibody and virological testing of HIV exposed infants between nine to eighteen months of age

In babies who are between nine and eighteen months of age, rapid antibody testing should be used as the first screening to exclude infection, followed by confirmation with HIV DNA PCR testing in children whose antibody test is positive.

- Repeat virological testing is done to confirm a positive first test.
- The HIV status of all the EID babies should be confirmed using antibody test at the age of 18 months
- Very rarely, an antibody test may be negative after early ART initiation even though the child is actually HIV infected. In this instance, continue ART, conduct a repeat HIV DNA PCR test, and consult an HIV specialist.

5.4 HIV diagnosis in babies less than 18 months

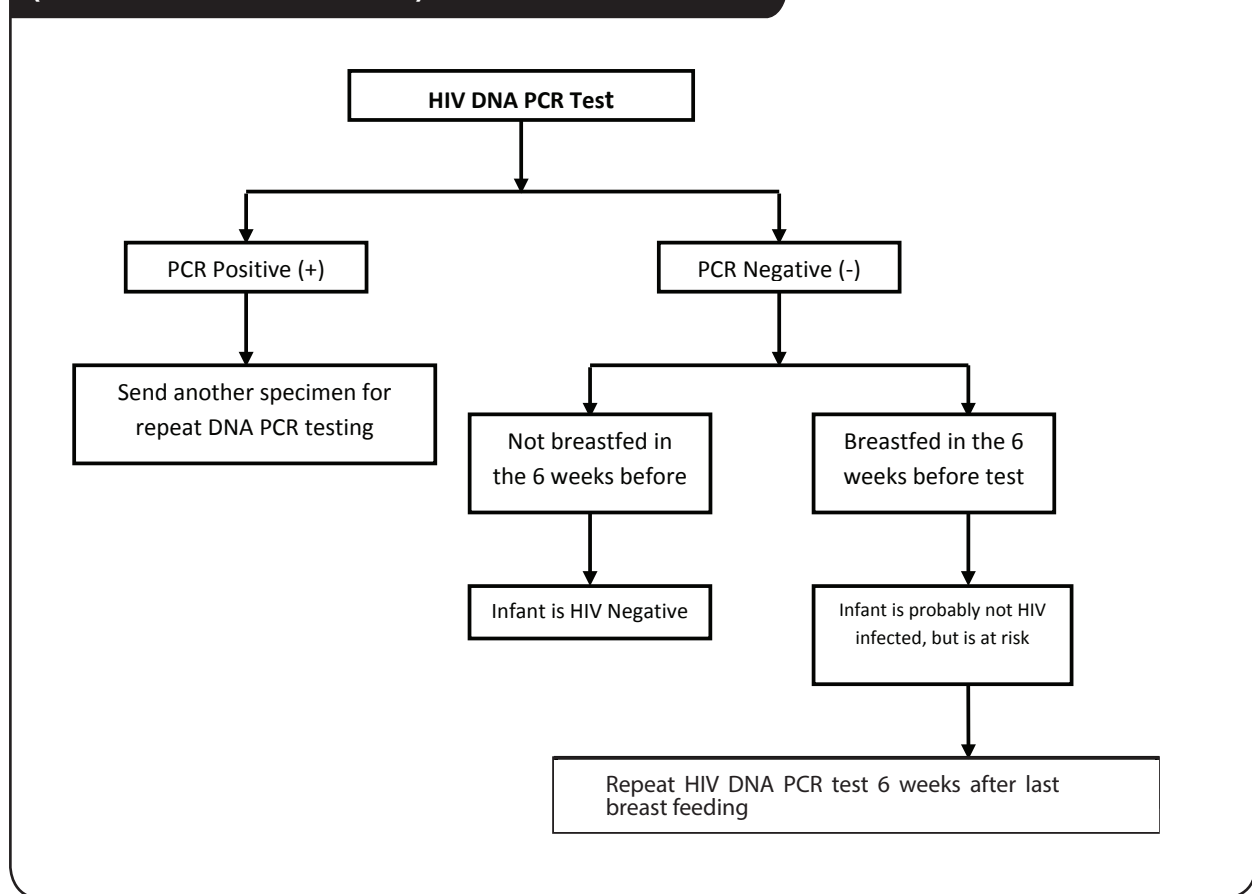
5.4.1 Diagnosis of HIV in infants six weeks to nine months of age

When an HIV exposed infant from six weeks to nine months of age is brought to a health facility, a whole blood specimen or DBS is collected and sent for HIV DNA PCR.

If the first DNA PCR test is positive, second PCR test is performed for confirmation and baby is referred for ART. If the second DNA PCR test is positive, it is concluded that the infant is infected and they are continued to the ART. If the infant is breast fed, continuation is encouraged for as long as possible. ART initiation in infants should not be delayed, while awaiting confirmatory results. After first positive PCR, ART should be started.

If the infant tests negative for PCR and has not been breast fed for six weeks prior to the test, infant is HIV-negative. If the infant has been breast fed in the six weeks before testing then the infant is probably not infected but is at risk. Repeat test six weeks after last breast feed. If the child remains negative, a repeat antibody test at 18 months is recommended to confirm continued negative status (to check for late breastfeeding transmission).

Figure 5.1: Strategy for diagnosis of HIV in infants (six weeks to <nine months) of HIV infected mothers

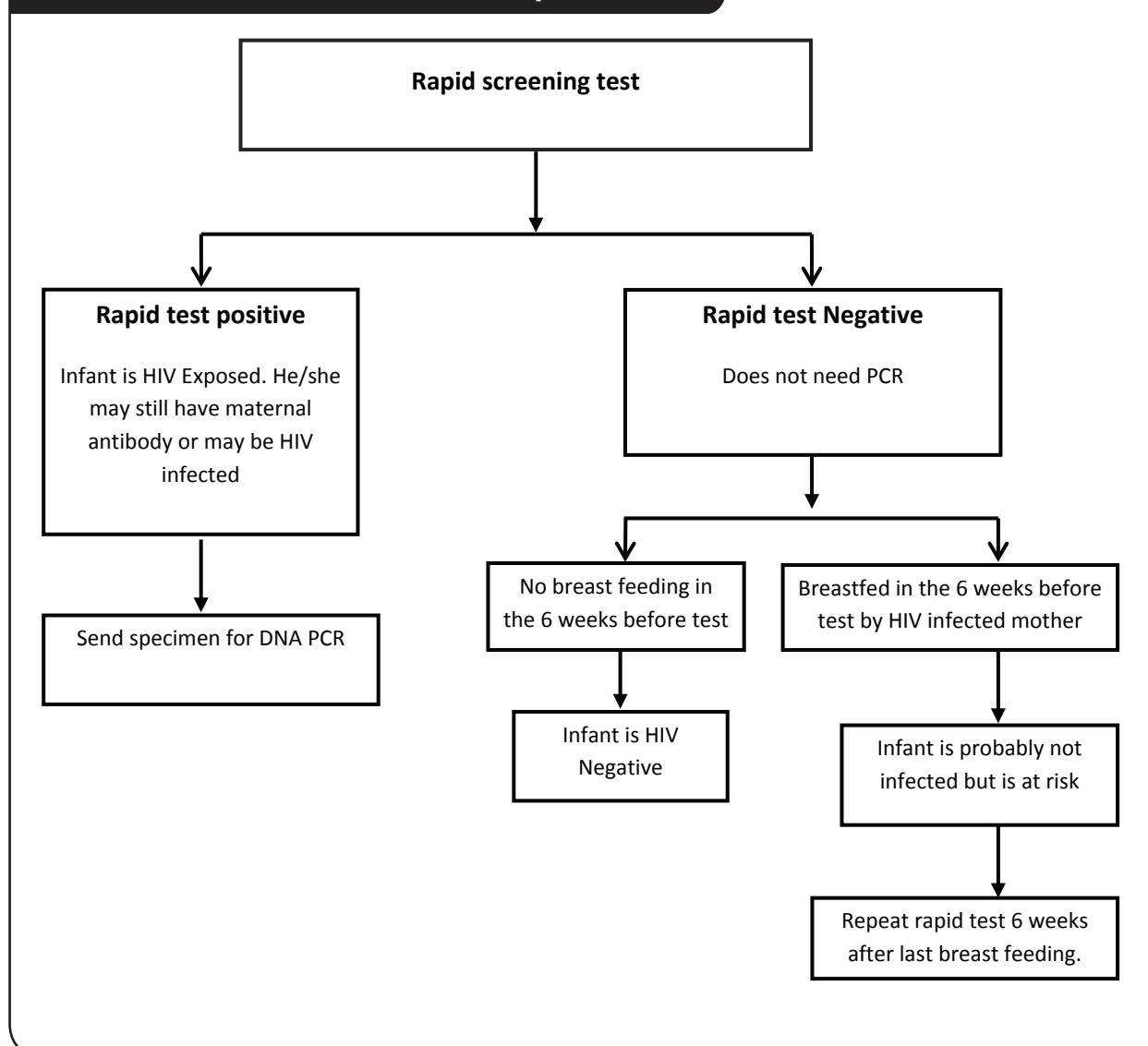


5.4.2 Diagnosis of HIV in babies 9-18 months old known to be HIV exposed

When a baby 9-18 month of age is brought for HIV testing, perform rapid test first. If the rapid test is positive, then collect and send DBS specimen or whole blood for the DNA PCR test. He/she may have maternal antibodies or may be HIV infected.

If the rapid test is negative, the DNA PCR test is not needed. If the baby has not been breast fed in the last six weeks before the rapid test, baby is HIV negative. If the infant has been breastfed in the six weeks before the rapid test, the infant is probably not infected but is at risk. Repeat the rapid test six weeks after last breast feed. All babies testing HIV negative before 18 months should have an 18 month antibody test to confirm status and check for late unsuspected breast milk transmission.

Figure 5.2: Strategy for Diagnosis of HIV in 9 - 18 months old babies known to be HIV exposed



Module review

2. Why is early diagnosis of HIV in children important.
3. Discuss the sample collection for EID.
4. Discuss the testing algorithm for EID diagnosis.

MODULE

6

UNIVERSAL PRECAUTIONS, POST EXPOSURE PROPHYLAXIS, SAFE HEALTH CARE WASTE MANAGEMENT AND SAFETY AT THE HIV TESTING SITE

Purpose

To provide basic information on universal precautions, post exposure prophylaxis, laboratory safety and safe health care waste management at HIV testing site.

Learning objectives

At the end of this module, participants will be able to:

- Describe the universal precautions for laboratory safety
- Explain the personal health and safety practices
- Explain the ways to maintain clean and organized workspace
- Explain the ways to safely manage the health care waste generated in testing site

Content outline

- Importance of laboratory safety
- Universal precautions
- Proper segregation, disinfection and disposal of laboratory waste
- Disinfection of work areas
- Safety documentation
- Safe health care waste management

Time: 60 minutes

6.1 Importance of laboratory safety

Performing laboratory tests have a potential health hazard to the laboratory personnel if precautions are not followed. Coming in contact with human blood or blood products is potentially hazardous. Safety involves taking precautions to protect us and the client against infection. All specimens should be treated as potentially hazardous or infectious.

Besides laboratory personnel and client, we need to protect other people from infection. In addition, it is important to protect the integrity of test products. If a new or unused test is contaminated by a drop of blood from a previous client, the test may not yield accurate result when used on the next client. It is also important to protect the environment from hazardous material avoiding the transfer contaminated materials into areas outside of the testing area.

6.2 Universal or standard precautions

Every specimen should be treated as though it is infectious because harmful and potentially hazardous agents/organisms may be present in a client's blood. If a person comes into direct contact with the blood that person could be infected. We must follow safety practices in every step of the testing process.

6.3 Safety practices throughout the testing process

Before testing, specimens should be transported in a manner to prevent contamination of staff, patients, and environment. This includes using appropriate packing containers, and following national and international postal and transport regulations.

- During testing, follow the safety rules when performing finger-prick or blood drawing and actual testing of the client's blood.
- After testing, remember to clean up working area and properly dispose contaminated waste.

6.4 Work habits for personal safety

- Wash hands between testing each client – wash away any germs that might be present on the tester's hands – this will ensure that no infections are passed from the tester or previous client onto the next new client.
- Wear fresh gloves for each new client to protect the client and tester from cross-infection (that is, the transfer of infection from one person to another).
- Wear lab coat or apron to protect the tester from reagent spills, client's blood.
- Get rid of used sharp objects such as needles or lancets, Sharp objects can cut human skin. Any germs or pathogens present on the lancet can be passed from the lancet into that person's blood through the cut. Sharps object should be disposed off into special sharps boxes and a new box used when the box is two-thirds full.
- Never re-sheath needles (if needed use single hand re-sheath method)
- Never pipette by mouth. You run the risk of accidentally swallowing or coming into direct contact with harmful materials.
- Never eat, drink, or smoke in the test area. Harmful germs or pathogens can be an entry point to the mouth from touching contaminated objects followed by contact with your mouth.
- Never apply make-up in the laboratory.
- Keep food away from the testing area or a refrigerator that contains blood samples. Infectious agents/pathogens can be carried in food and transmitted to people. Never take food or drink into the laboratory or patient areas.
- Never use cell phones in the laboratory. Cell phone should not be taken into the laboratory and should be kept with personal belongings outside of the laboratory.
- Never take outside garments or personal bags into the laboratory. Store these outside of the laboratory in personal lockers or equivalent spaces.

6.5 Maintain clean and orderly work space

- Keep work areas uncluttered so there is less chance for accidents.
- Disinfect daily because a work area was disinfected the previous day, it does not mean it is still free from germs today.
- Limit access to the lab. It is important to prevent other people from risk of infection, as well as to protect the client's confidentiality. Limiting access also prevents distractions.
- Keep supplies locked to prevent unauthorized persons having access to potentially dangerous objects such as lancets.

6.6 Precautions to avoid needle stick injury

Needle stick injury can be dangerous because infected blood containing pathogens can be transferred to the person and cause infection. Needle stick injury may occur due to lack of concentration, inexperience, lack of concern for others, or improper disposal of sharps. To prevent needle stick injury, you should focus on where the needle is, as well as where your hand is and also where your client's hands are. Do not let yourself be distracted. Only people who have received appropriate training should perform the finger-stick procedure.

Always follow proper procedures to dispose of used needles and sharps. For example,

- Dispose needles or lancets immediately after use in the sharps disposal container.
- Do not leave used needles or lancets lying around

- Use needle destroyer to burn or cut the needles
- Never re-sheath needles

6.7 Disinfect work areas with bleach

In order to keep a clean and orderly work area, disinfect working surface on a daily basis. It is part of a general safety practice that needs to be followed. Remember, disinfection kills germs and pathogens, keeps work surface clean, prevents cross-contamination and reduces risks of infection.

- For spills (blood), use paper towel/paper soaked in a 5-10% hypochlorite solution
- For general disinfection purposes such as wiping down all surfaces at the end of the day and decontaminating used item (test tubes, kits, and slides) use 0.5 to 1% hypochlorite solution.

Note: Facilitator will provide instruction for preparation of working solution of hypochlorite of different concentration using formula; $V_1S_1 = V_2S_2$. It is important to follow Standard Operating Procedures (SOP) for laboratory safety measurements.

6.8 Health care waste

Health care waste is the unwanted materials generated during health care activities such as laboratory testing, dressing etc. Different types of wastes are generated in health facilities, some of which are potentially hazardous and infectious and some might be general non-infectious.

6.8.1 Types of waste generated in health facilities

- Infectious solid wastes: used test kits, pipette tips, infected dressing material, etc.
- Infectious sharps: needles, lancets, broken glass, etc.
- Infectious liquid wastes: serum, other contaminated liquid wastes, etc.
- Non-infectious wastes: paper, plastic covers of syringes, other uncontaminated materials, etc.

6.9 Basic elements for waste management

- Minimization of waste: Adequate use of accessories
- Segregation of waste: Non-infectious, Infectious, Sharp
- Collection in color coding (color coded bins)
- Storage and transport
- Treatment: Autoclave/Chemical disinfection/Burial
- Disposal

6.9.1. Minimization of waste

Adequate use of laboratory supplies helps to minimize the waste generation. Unnecessary use of supplies should be minimized.

6.9.2 Segregation of waste

The laboratory waste generated should be segregated based on their type and infectivity. Infectious, non-infectious, sharps, etc. should be segregated. If no separation of waste takes place, the whole mixed volume of waste needs to be considered as being infectious. Infectious waste costs ten times as much to treat compared to non-infectious waste and so it costs money if not properly segregated.

6.9.3 Collection of waste by color coding

The waste should be collected in different colored waste collecting bins based on their character and type. The bins should be labeled in local or understandable language with photos of colored bins attached.

- **Red** – Infectious solid waste for autoclave
 - o Leak-proof white container with red cap: infectious sharp
 - o Jar (glass/thick plastic) with 0.5% hypochlorite for decontamination: infectious tubes, slides, etc.
- **Blue** - Non-infectious paper/plastic materials.
- **Black** – Non-infectious solid waste (decomposable).

Sharps in special containers

All sharps containers should have:

- A lid, puncture-proof or thick walls, a large enough hole for lancets and needles, leak-proof sides and bottom, a label or color code indicating bio-hazard material.
- Do not break, bend, re-sheath or reuse lancets, syringes or needles. You could injure yourself if you try to bend needles or lancets.
- Never shake sharps containers to create space because this leads to formation of aerosols. Aerosols are tiny invisible droplets in the air that can also carry infectious agents/pathogens.
- Sharps containers must be placed near workspace, closed when not in use and sealed when two thirds is full.

6.9.4 Treatment

The best method for treatment of infectious solid waste is autoclaving of waste. In autoclave, waste is treated at 121 degree Celsius at 15 psi pressure for 25-30 minutes, where all form of life (spores and infectious agents) are destroyed and hence safe for disposal. Incineration is another option but this is not environment friendly; fumes produced during burning may cause pollution and have effects on living things including humans.

6.9.5 Disposal of waste

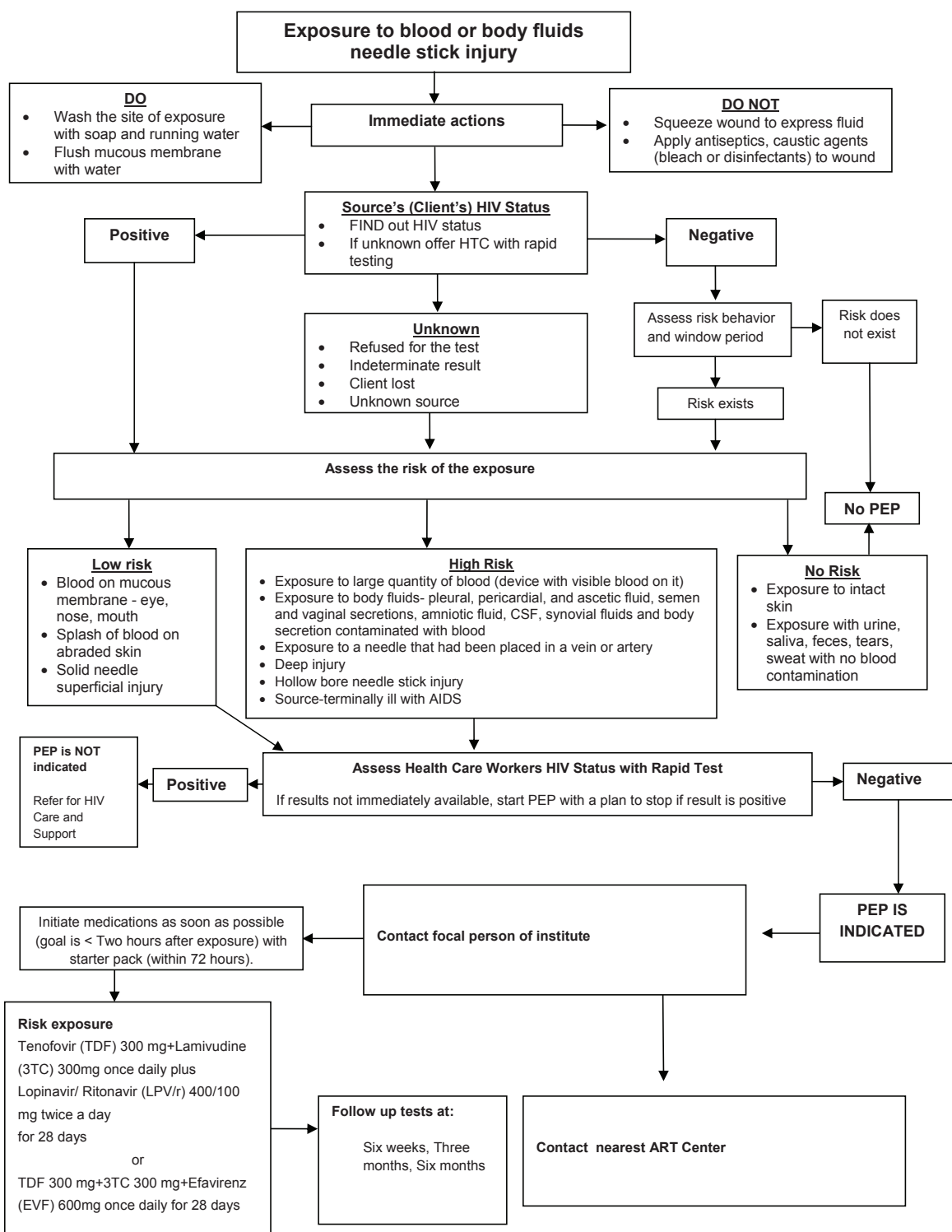
Use the following methods for disposal of wastes generated in laboratory sites:

- Disposal of sharps: When the sharps container is two/third full, it should be loosely closed and then autoclaved. Autoclaved sharps should be disposed off preferably by burying.
- Disposal of glasses: Collect daily autoclaved glass slides into gallon or jar and look to recycle after they have been autoclaved.
- Disposal of bio-degradable waste: Bio-degradable waste can be composted. Dispose into municipal waste stream if composting is not possible.
- Disposal of non-recyclable general waste: Dispose into municipal waste stream.
- Disposal of recyclable general waste: Separate recyclable general waste as per its recycling value and send for recycling
- Disposal of chemical and infected fluids: The chemical and infected fluid should be disposed after decontaminating. They can be separately flushed.

Module review

1. What is safety? Why is it important?
2. What is the universal precaution you must take when dealing with specimens?
3. What are some examples of safety practices related to personal habits and work space?
4. What are the types of waste generated in health facilities?
5. What are the major steps for waste management?
6. What is the colour coding for waste segregation?

**FIGURE 6.1: exposure to blood or body fluids:
management flow chart**



MODULE 7

BLOOD COLLECTION AND DBS PREPARATION FOR HIV EQAS AND EID

Purpose

To provide necessary knowledge and skills to perform venipuncture and finger prick, and prepare DBS samples from blood for HIV EQAS and EID.

Learning objectives

At the end of this module, participants will be able to:

- Describe the process to collect blood from a finger prick or/and venipuncture accurately and confidently
- Explain the process for preparation of DBS specimens from blood for HIV EQAS
- Explain the process for preparation of DBS specimens from blood for HIV EID

Content outline

- Performing venipuncture
- Performing finger prick
- Preparing DBS Specimens for HIV EQAS
- Preparing DBS Specimens for HIV EID

Time: 60+60+60 minutes

Safety precautions

It is important to follow universal safety precautions to protect client and yourself when performing venous blood collection or finger prick. Remember to always:

- Wash hands before and after testing each client.
- Put on gloves before collecting blood.
- If blood is spilled, mop it up and disinfect the area immediately.

Key message

- You must prepare your workstation and client prior to performing a venipuncture or finger prick.
- Always follow universal safety precautions to protect your client and yourself when performing finger prick.
- Follow SOP when performing a venipuncture or finger prick.
- An accurate HIV Rapid Test result is dependent in part on the quality of the sample collected

Note: Facilitator will provide the practical exercise on blood collection through venipuncture and finger prick.

Module review

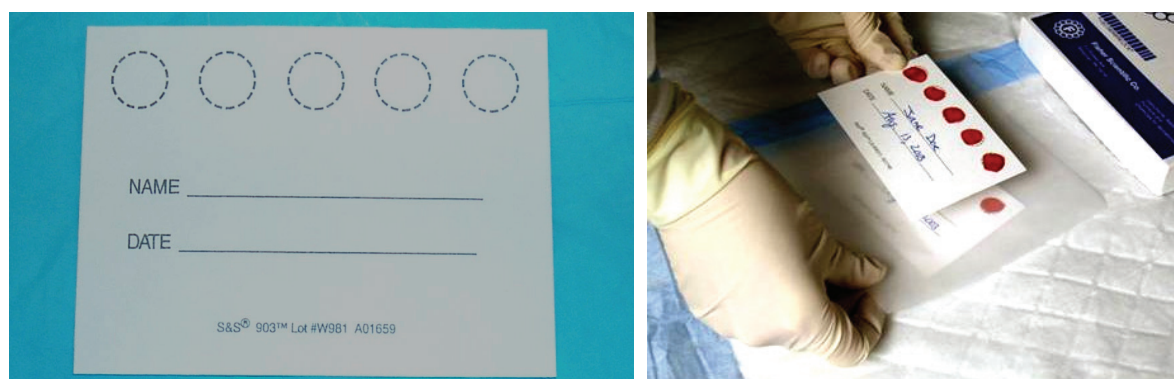
1. How do you put a client at ease while collecting blood?
2. What supplies do you need for a venipuncture?
3. What are the steps to performing venipuncture? What safety precautions should you follow?
3. Explain the process for preparing DVS sample for HIV EQAS and EID.

7.1 Introduction to Dried Blood Spot (DBS) paper

DBS papers are the specialized filter paper having specific absorbing and retaining properties for different bio-organic molecules such as proteins, nucleic acid and others. This is a very simple technique for collecting, shipping, and storing blood samples, for wide range of applications. DBS technique refers to the collection of whole blood directly from the client on a specialized filter paper with specific absorbing capacity.

The nature of DBS paper depends on the type of test to be done from the DBS elutes. The protein saver DBS cards are used for antibody/antigen detection which has the capacity to preserve the antibody/antigen whereas nucleic acid saver cards are used for cDNA detection for EID, viral load testing as a part of ARV monitoring.

Figure 7.1: Dried Blood Spot (DBS) paper



7.2 Advantages of DBS

DBS sampling can offer enormous advantages over liquid blood or plasma.

- Significant reduction in the volume of blood.
- Simplified process that does not need to centrifuge, sub-aliquot.
- Reduce freeze thaw cycle, safety in handling, risk reduction, shipping, and storage at room temperature.
- Improved compound stability for drugs and their metabolites, and considerable cost savings.
- The combined advantage of the above allows for a significant simplification of blood sample collection and handling for newborns, infants, and other special patient populations for various studies.

7.3 Application and use of DBS

7.3.1 Use of DBS for EQAS

DBS filter paper method has been chosen for HIV EQAS in Nepal. In resource limited settings, like in Nepal, it has been demonstrated to be effective by the validation studies conducted in the past.

7.3.2 Application of DBS for EID

The stability of DNA in DBS card has made it possible to apply PCR to detect proviral HIV DNA in newly born children. HIV DNA test is currently the preferred standard method for diagnosis of HIV infection in children less than 18 months of age. Use of DBS for DNA PCR has been shown to be a robust, reliable means of increasing access to infant HIV diagnosis since it is easier to obtain, store and transport for centralized testing.

EQAS re-testing and EID testing

Remember: Two types of specimens may be collected at the site for referral to a reference lab; HIV EQAS for re-testing and EID testing. The properties of the DBS paper used for EQAS and EID are different.

What are your responsibilities?

The responsibilities include:

- Collecting valid specimens
- Labeling and storing appropriately until transported for re-testing
- Ensuring records are properly maintained
- Avoiding transcription errors
- A test result is only as good as the specimen collected

7.4 Specimen transfer Log

An example of EQAS and EID specimen transfer log is provided for reference at the end of this module. Data recorded on transfer logs must be accurate.

Note: Facilitator will describe the sample selection for EQAS and EID

- Sample selection and DBS card preparation: EQAS
- HIV EQAS: All HIV positive and 10 % of HIV negative samples (Follow national EQAS protocol)
- EID: Babies born from HIV infected mothers or suspected or unknown history (Follow EID guideline)

7.5 Collection of blood and preparation of DBS

7.5.1 DBS card preparation

1. Use the permanent marker to label the DBS card with the sample identification number (date, where applicable).
2. Fill patient ID, date of collection, test results, and all the information in EQAS form every-time a DBS card is prepared and Patient ID, collection date, date of birth, breast feeding status in EID form for EID.
3. Take the blood specimen collected in Ethylene Diamine Tetra Aceticacid (EDTA) tube and mix it well to re-suspend RBC and carefully remove the top (avoid splashing blood). Venous bloods, heel, toe or finger prick for EID DBS sample collection.
4. Put one drop of blood (60 µl of blood) in each circle of the card (all together five circles) for HIV EQAS and one large drop of blood (120 µl of blood) in each circle of the card (all together four circles) for HIV EID. Place the drop into the center of the circle. Careful attention should be given to avoid blotting of the specimen.
5. Leave the cards on the lab bench/DBS drying rack for least three hours for complete drying. The card can be dried overnight if necessary but should be protected from insects, flies and rodents.

7.5.2 Steps for Dried Blood Spot (DBS) preparation: EQAS

1. Ready the supplies required for DBS

- Blood collection card (DBS paper)
- Sealable plastic bags
- Desiccant packs (silica-gel)
- Marker



2. Collection of DBS samples

- Use Universal Safety Precautions.
- Clearly label DBS card with ID .
- Follow finger prick procedure or vein puncture collection in EDTA vial.
- Dispense uniformly 60 µl of blood and saturate entire circle.

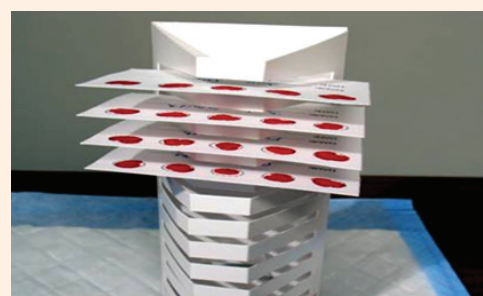


Always use universal safety precautions.

NOTE: Clearly label each card with appropriate identification number. It is unacceptable to submit a blood card for testing that has not been labeled properly.

3. Air dry DBS samples

- Avoid touching or smearing the blood spots.
- Allow the specimen to fully air dry horizontally (at least three hours) at room temperature.
- Keep away from direct sunlight or heat.
- Do not stack or allow DBS to touch other surfaces during the drying process.

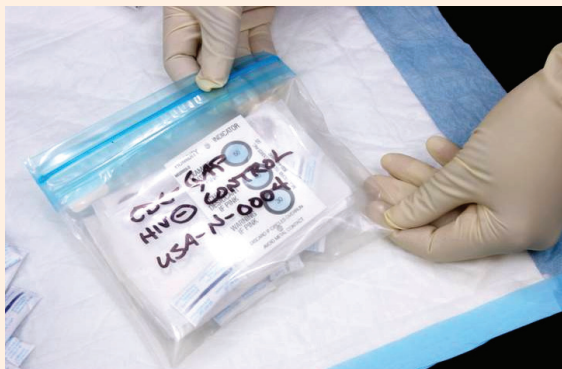
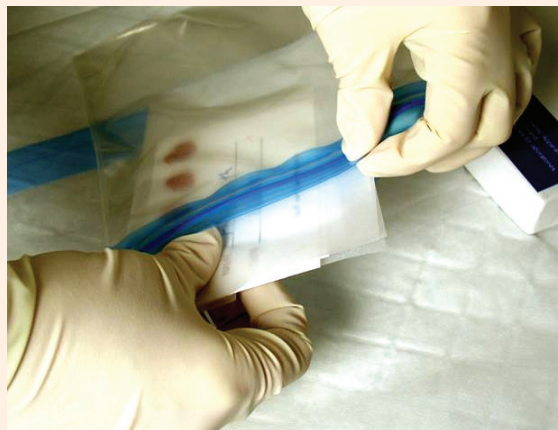


Valid DBS specimens:

- 60 µl of blood has been collected in each circle completely saturating or filling the circle.
- The filter paper card has been labeled with appropriate identification.
- Blood should not be soaked to the other side of the card.

**4. Packaging and storage of DBS**

- Insert into sealable plastic bag
- Add desiccant packets
- Label contents of bag and seal

Add desiccant packets**Insert into sealable plastic bag****Label contents of bag and seal****5. Packaging DBS samples for shipping**

- Keep packaged DBS (in sealable plastic bags) cool and dry until transported to reference laboratory.
- Insert bundled DBS into rip-resistant envelope.
- Include appropriate documentation.
- Insert both into brown envelop and seal for shipment.



Packaging

- Insert bundled DBS into rip-resistant envelope.
- Include appropriate documentation.
- Insert both into brown envelop and seal for shipment.

**7.5.3 Storage**

- Keep packaged DBS (in sealable plastic bags) cool and dry until transported to reference laboratory.
- Avoid leaving in vehicle, as sun and heat will deteriorate DBS.

7.5.4 Transportation of EQAS specimen (DBS cards) to reference laboratory

- At the end of every month all DBS cards need to be sent to National Public Health Laboratory (NPHL) (reference lab) Teku, Kathmandu or Regional Public Health Laboratory (RPHL).
- The shipment should be sent latest by seventh of next month (e.g. all DBS cards of November 1 to 30 must be dispatched by seventh of December).

7.6 EQAS specimen transfer sheet

Data recorded on transfer logs must be accurate. Care should be taken in transferring information from test records to EQA transfer log. Errors that are typically made include:

- Specimen ID on transfer log sheet does not match specimen ID filter paper
- Final result at testing site incorrectly transcribed from test records

Figure 7.2 EQAS specimen laboratory request form

Dried Blood Spot EQAS Form												
Country:			Test 1:		Lot#		Exp. Date:		For NPHL staff only			
Site Name:			Test 2:		Lot#		Exp. Date:		Receiving Date:			
Site ID:			Test 3:		Lot#		Exp. Date:		Examined by:			
Month			Total No. of specimen:						Date of examined:			
Mailing date			Completed By:									

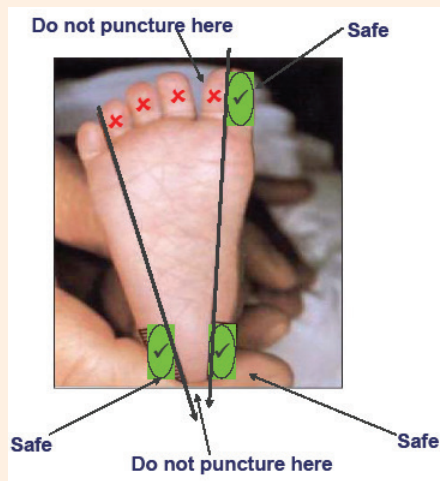
No.	Sample ID	Patient ID	Date collected	Age	Sex	Risk group	Test result				Quality of sample		
							Test 1	Test 2	Test 3	Final Test Result	Spot 1	Spot 2	Spot 3

7.7 DBS sample collection for EID: specimen collection, storage and transportation

7.7.1 DBS card preparation for HIV DNA PCR testing

1. Small infants (less than nine kg weight)
 - Puncture the heel
 - Do not puncture the fingers; there is risk of hitting the bone
2. Larger infants (more than eight month or nine kg weight)
 - Puncture the heel if callous is visible, the lateral aspect of the big toe is useful.
 - Do not stick the fingers or small toes; because there is risk of hitting the bone.
3. If veins of the infant can be felt, you may choose to collect blood specimen by vein puncture. Use small syringe (one to three ml) for collecting blood specimen by vein puncture.

Figure 7.3: Possible sites for pricking to collect blood sample for EID DBS card



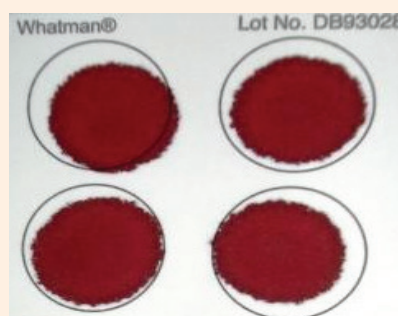
Procedure for DBS sample collection by heel pricking

- Wash hands, put on gloves
- Warm the area
- Position baby with foot down
- Clean the site with spirit swab and allow to dry for 30 seconds
- Press lancet into foot, prick skin





- Wipe away first drop
- Allow large drop to collect
- Touch blood drop to card
- Fill entire circle with drop
- Clean foot, no bandage is needed



Procedure for DBS sample collection by large toe pricking

1. Wash hands, put on gloves
2. Warm the area
3. Position baby with foot down
4. Clean the area, dry 30 seconds
5. Press lancet into foot, prick skin





1. Wipe away first drop
2. Collect large drop onto all four circles of DBS card
3. Clean foot, no bandage is needed



Procedure for using blood from vein puncture to make DBS

1. Wash hands and put on gloves.
2. Clean the puncture site with spirit swab and allow drying for 30 seconds.
3. Using small sterile disposable syringe (butterfly needle), collect one to three ml of the blood by vein puncture.
4. Remove the needle safely from the syringe.
5. Hold the syringe vertically above a circle on the DBS card.
6. Gently depress the piston of the syringe so that a large drop of blood is formed on the tip of the syringe. Touch the blood drop to the filter paper. Approximately 120µl of blood will fill a circle completely.

7.7.2 Packaging and storing DBS samples

1. When DBS card is completely dried.
NOTE: leave the DBS card overnight if not dried within three hours, put each DBS card in one small zip lock bag and put one silica gel in each bag.
2. Press air out of bag and seal.
3. Put the small zip lock bag with DBS card in a big zip lock bag.
4. Add some more silica gel packs in the large zip lock bag.
5. Put the large zip-lock bag in an envelope.
6. Store DBS papers at room temperature in cool and dry place.

7.7.3 Packaging DBS for shipping

1. Insert into an envelope.
2. Label outside clearly.

7.8 Special considerations for EID using dried blood spot technique

High level of precautions should be taken in each step for preventing contamination of DBS specimens to be used for DNA PCR testing. Contamination of DBS specimen may lead to a false result which further complicates the diagnosis of HIV in babies. Special precautions should be taken in the following steps of DBS collection for EID.

7.8.1 Preparation

1. Sterile alcohol gauze pad should be used for disinfection purpose before doing vein puncture, heel pricking or toe pricking procedures.
2. Sterile dry cotton or gauze pad should also be made available for wiping the first drop of blood obtained by vein puncture.
3. Powder free gloves should be made available for sample collection purpose.
4. If only powdered gloves are available, gloved hands should be properly washed with soap and water.
5. Small syringes (3ml syringe with butterfly needle) should be used on babies for collecting blood specimen by vein puncture.

7.8.2 Sample collection

1. Always use gloved hands for conducting vein puncture, heel pricking or toe pricking procedures.
2. Always wash gloved hands with soap and water if gloves used are powdered.
3. Warm site with soft cloth, moisten with warm water up to 41° C, for three to five minutes.
4. Always wipe away the first drop of blood obtained by heel or toe pricking with sterile gauze pad to avoid contamination.
5. Fill at least three circles of the DBS paper with whole blood. Filling four circles is recommended.
6. Never touch the circles of the DBS paper to the skin of the baby.
7. If blood specimen is collected from heel or toe, never dispense the blood specimens into test tubes or vials, it may clot soon.
8. Blood spots should be directly made from the blood drops formed on the tip of the syringe by depressing the plunger of the syringe gently.
9. Clotted blood cannot be used for DBS preparation.

7.8.3 Drying

1. Avoid contact of the blood drops with insects and dust while drying the blood spots.
2. Place one DBS card into each slot of the drying rack without allowing the cards to touch each other.
3. Never dry the blood spots under direct sunlight.
4. Blood spots should not be prepared using whole blood specimen collected by capillary tubes.
5. Blood spots should be dried at least for three hours before packaging.
6. Properly dried blood spots are dark red or brown in color.



7.8.4 Features of acceptable and unacceptable DBS samples

Acceptable:

- At least three pre-printed circles should be completely filled with blood.
- Laboratory bar-coded sticker should be affixed and the DBS card completely and accurately labeled. In absence of bar-code sticker, use permanent marker for labeling.

Unacceptable:

- Patient details on DBS card is not legible.
- Patient details on DBS card and laboratory request forms do not match with barcode sticker or labeling.
- Insufficient sample for processing.
- Blood spotted outside the pre-printed circle and DBS cards containing clotted/crusted blood.

7.8.5 Storage

1. DBS cards should be stored at room temperature until transportation to laboratory for further processing and testing.
2. DBS cards should be stored in a cool and dry place until transportation to laboratory.
3. Two or more than two DBS cards should not be piled up together.
4. DBS cards should be stored in zip lock bag with desiccants.
5. Desiccants should not be saturated before keeping them in zip-lock bag.
6. Air inside the zip lock bag should be pressed out before sealing it.

7.8.6 Transportation

1. Insert DBS bag into rip-resistant envelope
2. Include laboratory requisitions
3. Seal envelope
4. Label envelope clearly "Infant Specimens"

7.8.7. Recording and reporting

1. A correctly completed laboratory request form is necessary so that the laboratory can inform the clinic if there is a problem with the PCR sample and help deliver patient report to the correct facility. It also allows for monitoring of infant testing rates from the NPHL database.
2. The clinic PCR testing register, documents infants who have been tested and ensures that PCR test results are obtained from NPHL and communicated to parents or caregivers. Remember to document the NPHL site to know which HIV-infected infants have been referred for ARVs.
3. The specimen transfer checklist must be completed as a record of the PCR sample being transported to the laboratory for analysis.

7.9 HIV DNA PCR requisition form should include:

1. **Specimen Collected by:**
2. **Sample ID:** **Patient ID:**
3. **Date Collected:** record date DBS sample collected **Date of Birth:** enter birth (day, month, year)
4. **Age:** Specify in months. We can write ½ month. (i.e. many babies will be six weeks old. This can be written as 1.5 months)
5. **Sex:** "M" for Male / "F" for female
6. **Date of last breast feeding:** Record date that parent reports baby last had breast milk. If currently feeding, write current date. If exact date not known, write best estimate.
7. **Rapid Test Results:** Write positive or negative for rapid test.
8. **PMTCT status of mother and baby**
9. **PCR Results:** positive or negative

MODULE 8

PERFORMING HIV RAPID TESTS

Purpose

To provide participants with necessary knowledge and skills to accurately perform three HIV rapid tests and to determine HIV status.

Learning objectives

At the end of this module, participants will be able to:

- Explain how to perform HIV rapid tests according to Standard Operating Procedure (SOP)
- Explain accurately and interpret individual test results
- Explain accurately and determine HIV status

Content outline

This module requires live demonstration and hands-on practice

- Overview of testing procedures
- Work station setup
- Demonstration
- Practice session with known specimens and blinded specimens

Time: 120 minutes

8.1 Rapid HIV tests

Rapid HIV antibody tests provide same-day results and do not require additional reagents or equipment. Current rapid tests are based on three formats: particle agglutination, immunoconcentration and immunochromatography. Most HIV rapid tests contain antigens to HIV-1 and HIV-2 and detect antibodies to both. A positive test result is indicated by clumping, a spot, dot, or line, depending on the test format. The sensitivity and specificity of the latest generation of rapid tests are similar to those of Enzyme Immuno Assay (EIA).

Rapid tests are useful for resource limited small laboratories and for geographic areas with limited laboratory infrastructure. Many rapid tests do not require electricity, special equipment, refrigeration, or highly skilled staff, although a few require refrigeration for heat-sensitive reagents.

Advantages of Rapid Test:

- Increases access to prevention and interventions
- Supports increased number of testing sites
- Same-day diagnosis and counseling
- Robust and easy to use
- Most require no refrigeration
- None or one reagent
- Minimal or no equipment required
- Minimum technical skill
- Used with Serum, Plasma, Whole blood or Oral fluids

8.2 Three formats for rapid HIV test:

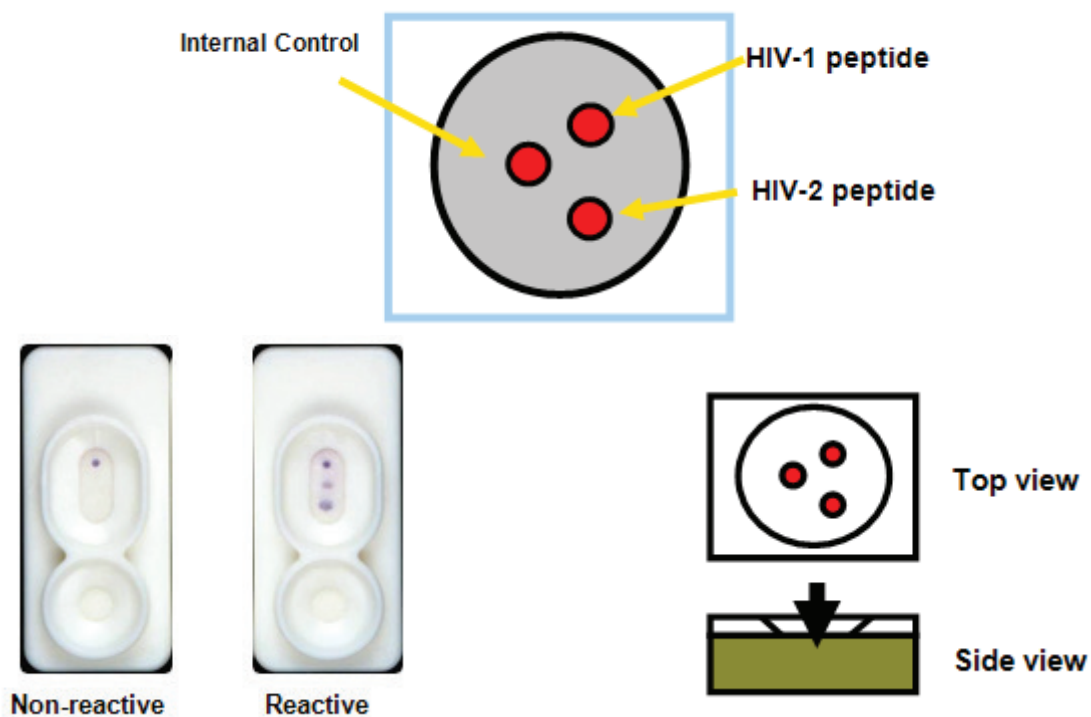
1. Immunoconcentration (eg, Multi-Spot, Genie II)
2. Immunochromatography (eg, Determine, Unigold)
3. Particle agglutination (eg, Capillus, Serodia)

8.2.1 How immunoconcentration works

HIV antibody links to bound HIV peptide antigens forming the color spot. Flow-through (or immunoconcentration) devices are usually cartridges, with HIV antigen attached to a membrane. The specimen and individual reagents are each added to the cartridge in a series of steps. Presence of HIV antibody is indicated by the development of a colored spot or line.

Some examples of flow-through devices are Multi-Spot and Genie II.

Figure 8.1: HIV rapid test device on immunoconcentration format



8.2.2. How immunochromatography works

Specimen is applied to a pad (filter) where it mixes with gold or selenium colloid-antigen conjugate. This mix migrates through the nitrocellulose strip to immobilized recombinant antigens and synthetic peptides at the patient window. If HIV antibodies are present then a red line will form in the test area of the strip.

Figure 8.2: HIV rapid test based on immunochromatography

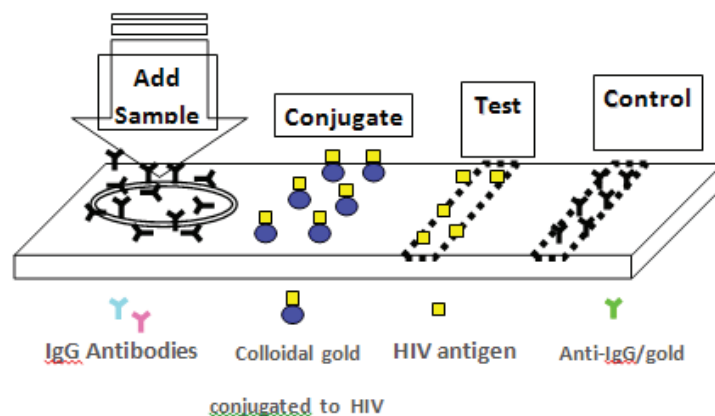
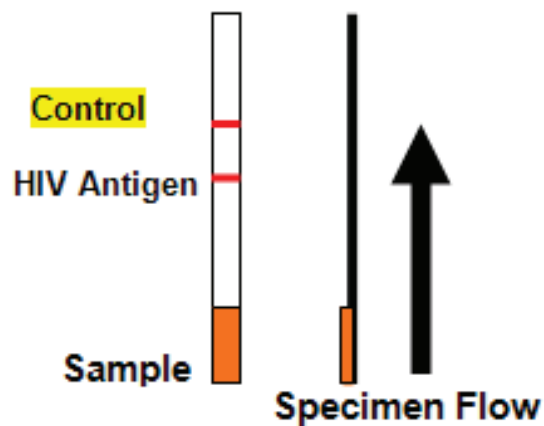
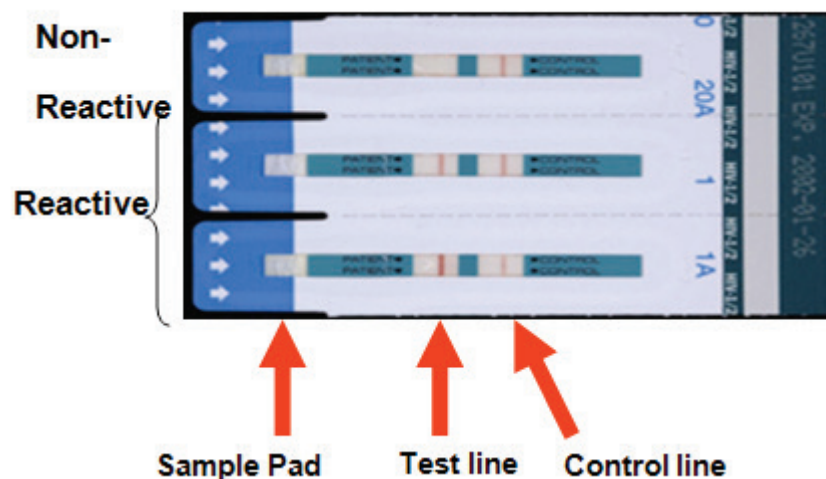


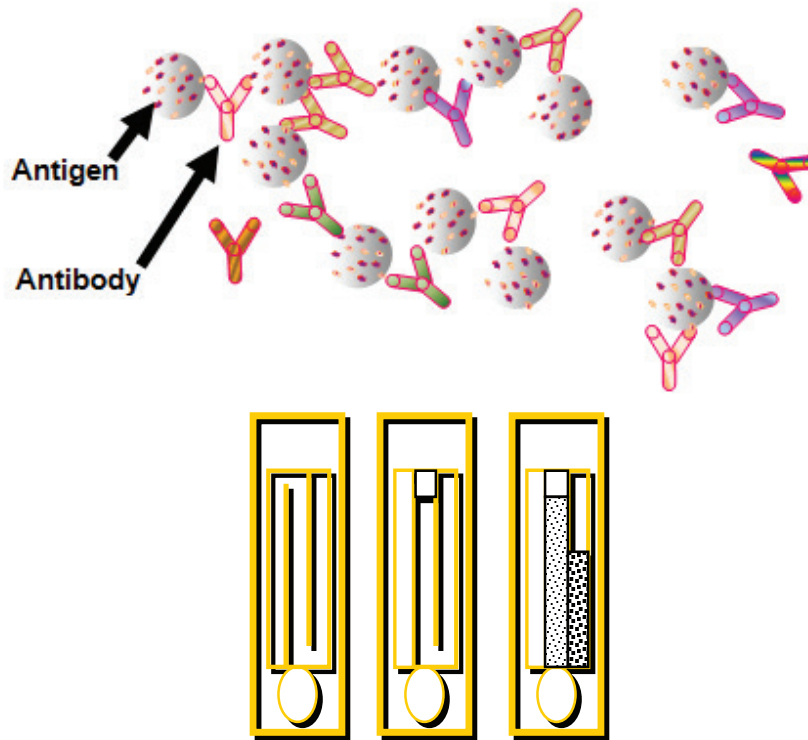
Figure 8.3: Test specimen flow in immunochromatography

Capillary flow (lateral flow) devices resemble dipsticks. All of the necessary reagents are usually incorporated into the test strip embedded in the device. Specimen (and sometimes buffer or a reagent) added to the strip flows across the reagents, and a colored line develops in the presence of antibodies. Most lateral flow devices also have an internal control that detects human IgG. This internal control indicates that specimen was added to the test strip. If no human IgG is detected, an internal control line does not develop indicating an invalid test.

Figure 8.4: Test specimen flow in immunochromatography

8.2.3 How particle agglutination works

Anti-HIV antibodies bind to the antigen-coated latex particles. Agglutination assays were among the first of the rapid tests developed. The round circles represent antigen-coated latex particles that bind antibodies to HIV. Agglutination or clumping occurs when the antibodies bind to the antigen-coated particles. Inexperienced persons or those who do not conduct the tests frequently may have problem with differentiating the coarseness or clumping of individual particles from true agglutination. They sometimes “over-interpret” agglutination, which result in a larger number of false-positives.

Figure 8.5: Test result based on Agglutination reaction**Points to note:**

- Always follow universal safety precautions when performing any laboratory procedure.
- Always follow national testing algorithm.
- Always follow approved SOP (national).

Note: Facilitator should share kit insert/ SOP for performing the rapid test with instructions.

Module review

1. Explain how to perform HIV rapid test

MODULE 9

QUALITY ASSURANCE AT HIV TESTING SITE

Purpose

To understand the basic elements of quality laboratory system and understand the importance of quality assurance in HIV testing.

Learning objectives

At the end of this module, participants will be able to:

- Explain the essential elements of a laboratory quality system and how they apply in HIV testing
- Explain the key factors that may compromise the quality of HIV testing
- Describe responsibilities in preventing and detecting errors before, during, and after testing

Content outline

This module requires live demonstration and hands-on practice

- Quality assurance
- System approach to quality
- Essential elements of a laboratory quality system
- Quality assurance procedures during HIV rapid testing
- How participants can contribute to quality before, during, and after testing
- Troubleshooting invalid results

Time: 45 minutes

9.1 Quality assurance

Quality assurance (QA) refers to planned, step-by-step activities that allows one know that testing is being carried out correctly, results are accurate, and mistakes are found and corrected to avoid adverse outcomes. Quality assurance is an ongoing set of activities that help to ensure that the test results provided are as accurate and reliable as possible for all persons being tested. These activities should be in place during the entire testing process, from the time a person agrees to be tested until after the test results are provided.

In other words, it is the planned and organized activities that help to ensure that certain requirements for quality will be met.

9.2 Systems approach to quality

The approach we take to ensure laboratory 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.

Let us explore the concept further by using the human body as an example. A headache may be caused by disorder in other parts of the human body system. You need to look at other parts to find out what is wrong with the head.

Similarly, to achieve total quality in the lab or testing site, you need to look at all the activities, direct or indirect, that may contribute to the quality.

9.3 Laboratory quality system

A laboratory 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.

By adopting the systems approach to laboratory quality, the laboratory quality system would encompass all activities that contribute to quality directly or indirectly.

9.4 Benefits of quality system

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.

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

9.5 Components of laboratory quality system

A laboratory quality system has 12 components. Read on to learn about each component.

9.5.1 Organization

Organization is the leadership or party responsible for establishing and managing the overall quality program. A quality system must start with the organization.

To ensure total laboratory quality, an organization needs to:

- Create quality policy and standards.
- Secure sufficient resources to maintain quality requirements.
- Clearly define roles and accountability.
- Cultivate a culture committed to quality.

This component is closely linked to other components such as personnel, equipment, process improvement, and customer service.

9.5.2 Personnel

Qualified and trained personnel are the most important component in the lab quality system because it is linked to all other components. To achieve total lab quality, we need to have the right people on the right jobs who are motivated and competent to perform. The label of laboratory personnel may vary depending on the type of facility and it should be based on the national laboratory policy. The person should be trained and oriented on the activity to be performed. There should be regular system for performance evaluation, and should be rewarded based on performance.

9.5.3 Equipment

Depending on the type of facility where HIV testing is done, nature of equipment varies. Generally, equipment used at the HIV rapid testing site may include refrigerator, centrifuge, rotator, microscope and micro pipettes. Laboratories that serve as referral laboratory for HIV rapid testing site must ensure that equipment used is appropriate for the task and kept in optimal working order. The equipment should be regularly calibrated and validated to ensure the correct function. This is achieved by:

Figure 9.1: Components of Laboratory Quality System



Source: WHO: Rapid HIV test training manual

- Selecting the right equipment. The purchasing contract should include installation and initial calibration; regular service; and training to operate the equipment.
- Setting up mechanism for regular preventive maintenance and routine calibration to ensure uninterrupted service and prolonged life span of the equipment.
- Ensuring readily available technical expertise for timely repair in case of equipment breakdown.
- Stocking up on parts that break frequently.
- Establishing troubleshooting procedures.
- Creating a maintenance log and regularly reviewing all documentation.
- Retiring equipment properly. This involves putting up signage, removing from premises, disinfecting before decommissioning and salvaging reusable parts.

This component is closely linked to other components such as personnel, purchasing and inventory, documents and records, and facilities and safety.

9.5.4 Purchasing and inventory

Purchasing is primarily handled by a central procurement and inventory process. Laboratory staff should be involved during the process of defining criteria for the materials and supplies needed.

Purchasing and inventory involves:

- Defining criteria for products and services to be purchased.
- Establishing a system to receive, inspect, accept, and store incoming materials.
- Maintaining proper inventory.
- Developing a system to connect materials to appropriate patients, activities, or records. This is important in the event of notices from manufacturers of potential problems with specific kit, lot number. You will know what lot number was used only if this information is recorded.

This component is closely linked to other components such as organization, process control, documents and records, and facilities and safety.

9.5.5 Process control

Process control refers to the activities and techniques performed to ensure:

- Testing procedures are correctly performed.
- The environment is suitable for reliable testing.
- The test kit works as expected to produce accurate and reliable results.

Process control concerns all aspects of the laboratory, not just the testing procedures. Examples include ensuring that:

- Test methods are appropriately evaluated.
- Testing sites have up-to-date SOPs.
- All staff follow SOPs exactly as written.
- Specimens are appropriately collected, handled/processed, stored, transported, and discarded.
- QC (quality control) is performed and monitored.

This component is closely linked to other components such as personnel, purchasing and inventory, assessment, and facilities and safety.

9.5.6 Documents and records

Documents and records may be paper-based or computer-based. Regardless of the format, a system must be established in order to:

- Create standards for forms.
- Manage document revision, approval, and distribution.
- Manage and retain patient test records and source of laboratory data.
- Maintain document storage, retrieval, and destruction.

This component is closely linked to other components such as purchasing and inventory, information management, assessment, and occurrence management.

9.5.7 Information management

Information management refers to these activities:

- Manage incoming and outgoing information.
- Establish standards for gathering information.
- Ensure the privacy and confidentiality of patient information.

These activities can often be facilitated by computers. If computers are used, personnel must be trained in relevant computer skills such as word processing, spreadsheet, and database.

This component is closely linked to components such as personnel, documents and records, and customer service.

9.5.8 Occurrence management

Occurrence management deals with laboratory problems and errors as they occur. Examples of occurrences include accidental spills or needle injuries. There must be a pre-defined approach and system for dealing with errors. For example, you should keep a record of all circumstances related to the error or problem, and corrective action taken or keep record of any communications with affected persons. This information is useful for those monitoring the testing, for any internal audits, and for use if further inquiries from patients or physicians occur.

This component is closely linked to other components such as process control, documents and records, and customer service.

9.5.9 Assessment

Assessment is the periodic examining and monitoring of laboratory operations compared to established requirements. It involves external and internal evaluation.

It is a good practice for testing sites to periodically conduct self-evaluation of their operations against quality requirements. Any gaps identified can be addressed immediately. There are two types of external evaluation or assessment.

- Testing sites may be routinely monitored in the form of supervisory visits.
- External assessments may be conducted by external agencies for accreditation purposes. This is usually done by an independent body to objectively assess compliance with established quality requirements of published standards.

This component is closely linked to other components such as organization, personnel, and process control.

9.5.10 Process improvement

Process improvement refers to activities designed to identify and eliminate the causes of poor quality, and to reduce waste and improve efficiency by eliminating non-value added activities.

This component is closely linked to other components such as organization, personnel, documents and records, process control, and customer service.

9.5.11 Customer service

Who are your customers? The patient/client, of course, is the ultimate customer. However, we must not forget the clinician, program staff, and epidemiologists. These people are our internal customers.

Everyone at the HIV rapid testing site has a responsibility for providing good customer service, from

the receptionist, counselor, and laboratory staff. Each test site should actively seek information on both internal and external satisfaction through customer surveys and interviews, and then use the data collected for process improvement. It is also a good practice to reward those staff providing good service.

This component is closely linked to other components such as personnel, documents and records, process control, and process improvement.

9.5.12 Facilities and safety

It is important to ensure that the facility, testing and storage areas are adequate in order to produce reliable test results, e.g., monitoring testing and storage area temperatures. It is also important to provide an adequate and safe work environment.

This component is closely linked to other components such as organization, personnel, purchasing and inventory, occurrence management, process control, and customer service.

9.6 Who is responsible for quality?

Quality is everyone's responsibility. For example,

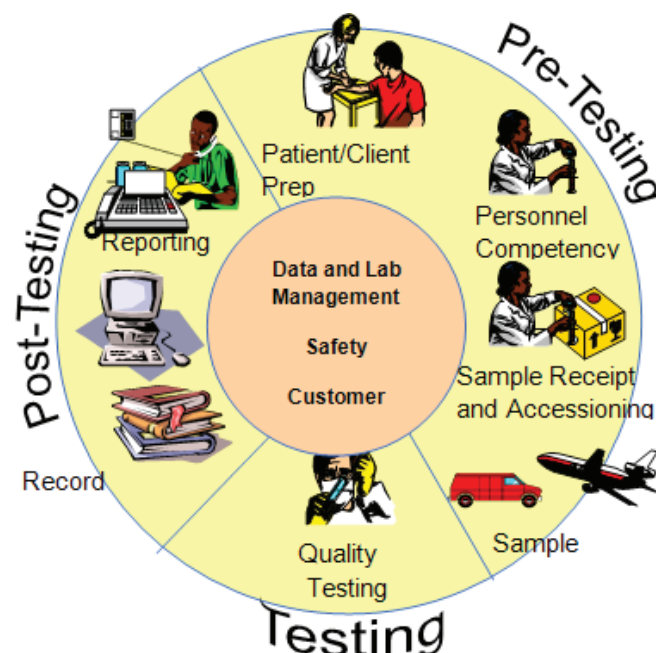
- Laboratory management and program staff establish quality assurance procedures.
- Test site personnel implement the quality assurance procedures.

How can you contribute to lab quality? You will find out more about the specific things you can do to help. But, first, let's explain how quality assurance differs from quality control.

9.7 Quality assurance vs. quality control

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

Figure 9.2: Quality assurance cycle



Source: WHO Rapid HIV Training Manual

9.8 Why do errors occur?

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

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

Table 9.1 Common errors during three phases of quality assurance cycle

	Before Testing	During Testing	After Testing
Common Errors	• Specimen mislabeled or unlabeled • Specimen stored inappropriately before testing • Specimen transported inappropriately • Test kits stored inappropriately • Incorrect specimen collected	• Country algorithm not followed • Incorrect timing of test • 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 • Report illegible • Report sent to the wrong location • Information system not maintained • Results reported when control results out of range • Result not properly reviewed before release
How to Prevent / Detect Errors	• Check storage and room temperature • Select an appropriate testing workspace • Check inventory and expiration dates • Review testing procedures • Record pertinent information, and label test device • Collect appropriate specimen	• Perform and review Quality Control (QC) • Follow safety precautions • Conduct test according to written procedures • Correctly interpret test results	• Re-check patient/client identifier • Write legibly • Clean up and dispose of contaminated waste • Package EQA specimens for re-testing, if needed

Module review

1. Explain the elements of Laboratory quality system.
2. Explain the common errors in pre analytical, analytical and post analytical phase.

MODULE 10

QUALITY CONTROL IN HIV TEST (IQC AND EQAS)

Purpose

To provide the concept on quality control, internal quality control and external quality assessment and implement in HIV testing site.

Learning objectives

At the end of this module, participants will be able to:

- Explain Laboratory Quality Control (QC)
- Describe Internal Quality Control (IQC)
- Explain the Difference between internal and external controls
- Describe External Quality Assessment and types
- Explain the Retesting method using Dried Blood Spots (DBS) for HIV External Quality Assessment Scheme (EQAS)

Content outline

- Laboratory quality control
- Internal quality control
- External quality assessment
- DBS in HIV EQAS

Time: 45 minutes

10.1 Laboratory quality control (QC)

Quality control is measures taken to monitor the quality of the test itself. Quality control ensures that the test is working correctly and the tester can report accurate test results with confidence. It is the operational techniques or tasks that are in place to find and correct problems that might occur.

Laboratory quality control is designed to detect and reduce deficiencies in a laboratory's internal analytical process prior to the release of patient results, in order to improve the quality and accuracy of the results reported.

Laboratory quality includes both internal quality control and external quality assessment.

10.2 Internal quality control (IQC)

Internal quality control includes the measures that are applied by laboratory staff with the laboratory to ensure the generation of correct result following correct procedures. In some instances, internal laboratory quality control is usually run at the beginning of each shift, after an instrument is serviced, when reagent lots are changed, after calibration, and whenever patient results seem inappropriate.

This may include temperature control, logistic control, use of internal controls, checking reagents, and checking expiry of kits, follow SOPs and universal precautions.

For example, IQC for HIV rapid testing can be done through:

- Testing of samples with known results to verify if the procedure is working properly
- Interpreting the presence or absence of control bands/lines within the device itself

If problems or errors occur, immediate corrective actions must be taken before dispatching results.

10.2.1 Sources of internal controls for test kits

There are two types of quality control for HIV rapid testing kits: internal and external.

1. Internal control:

- Control samples with known reactivity may be included with the test kit which are tested just like a patient/client specimen.
- Another type of internal control is an area or region within the individual testing device. This area or region is also termed as the procedural or in-built control. This type of control verifies the flow of either specimen and/or buffer through the test device resulting in an appearance of a line or dot in the control region. In other words, in some test devices, a line in the control area may appear even if a specimen is not added but as long as a reagent is added, unlike other test devices with an antibody control. In this instance, a control line will not appear if antibody is not detected.

Since it is not always known if the test device includes a true antibody control, it is important to test an external control sample.

2. External control:

- Control samples that do not come with the test kit. They are provided by an external source such as your regional reference laboratory or a commercial supplier.
- This type of control should also be tested in the same manner as you would test a patient or client specimen.

For both internal and external control samples, you already know whether the control is positive or negative. Once tested, you should receive the expected results. If not, this is one sign that there is a problem with your testing operation.

Internal and external quality control

Internal Control (In built control)

Included in testing device or as part of the kit



Control Band

External Control (External to kit)

Control samples are often received in tubes called cryovials. This photo illustrates control samples neatly stored in a Styrofoam container.



Examples of tests that include inbuilt internal control

Determine, Hema-Strip, OraQuick, and Uni-Gold all include an internal control in built within the kit.

Sources of internal quality control external to kit

Internal controls which are not in built or external to kit may either be obtained from commercial manufacturers, or from another laboratory that has prepared validated quality control samples in-house.

It is important to store controls appropriately. For controls obtained commercially, it is important to store according to the manufacturer's instructions. For in-house prepared controls, these should be refrigerated upon preparation.

For all controls, you must:

- Label vial with date when first used.
- Label vial with preparation date.
- Label vial with storage conditions.
- Label vial with some code to link to a batch name of a commercial control or a batch prepared in-house.
- Test before expiry date.
- Take care as to not contaminate the control materials.

10.2.2 Frequency of use: when should you test internal quality control samples?

At a minimum, test your external control samples:

- Once a week
- When a new shipment of control materials or test kits are received at the testing site
- In the beginning of a new lot number

Most kits do not require refrigeration, but some (such as Capillus, Tridot) some need. If these kits have been stored under non-refrigeration temperatures, then the lot must be tested using external controls to verify the integrity of the test kit. However it is highly unadvised to use test kits that have not been stored at the correct temperature even if controls appear to give the correct results.

10.2.3 Invalid results – what do you do?

If you get an invalid result, you must repeat the test. In addition, you should identify the cause of the problem, inform your supervisor, and take corrective actions.

Repeatedly invalid results may be due to problems either with the test product or test procedures. In this case, you should continue with an approved alternative testing algorithm.

10.2.4 Troubleshooting invalid results

It is important to always follow the SOP for each type of test used, as the following may differ from kit to kit:

- Sample volume – This may differ from kit to kit, and might differ depending on the sample type (e.g. whole blood vs. serum).
- Buffer volume – Some kits require different volumes of buffer.
- Incubation time – This time may also differ from kit to kit. Always follow the time required by the manufacturer and do not read the test after the stated maximum read time as given by the manufacturer.

Table 10.1 Potential cause and action for invalid results

Problem	Potential cause	Action
No control line or band present	Damaged test device or controls	Repeat the test using new device
	Proper procedure not followed	- Follow each step of testing according to SOP - Re-check buffer and/or specimen volumes - Wait for the specified time before reading the test
	Expired or improperly stored test kits or controls	- Check expiration date of kits or controls. Do not use beyond stated expiration date. - Check temperature records for storage and testing area. Do not use the test if it has not been stored under the correct conditions
	Test removed from foil pouch long before the test was performed	Only remove the device from the foil pouch when ready to do the test
Positive reaction with negative external control, i.e. false positive	Incubation time exceeded	Re-test negative control using a new device and read results within specified time limit
	Control contaminated	Use a fresh vial of negative control and perform a new test
Extremely faint control line	The control line can vary in intensity	No action required. Any visible line validates the results as long as the test was read within the time permitted by the manufacturer. After this maximum period faint lines will appear over time and must be disregarded.

10.3 Problems may occur throughout the testing process

Problems may lie anywhere in the testing process: pre-testing, testing, and post-testing. Most problems occur in the pre testing phase of testing. The integrity of the specimen may have been compromised during preparation, shipping or after receipt by improper storage or handling.

Problems such as with reagents, test methods, quality control, or competency of staff may occur during testing. Due to the number of specimens collected and transported by various test sites, care must be taken to ensure proper transcription of data throughout the testing process.

10.3.1 Take corrective actions

- Whenever problems are detected, corrective actions must be taken.
- Use problem-solving team.
- Investigate root causes and develop appropriate corrective actions.
- Implement corrective actions.
- Examine effectiveness and monitor over time.
- Record all actions and findings.

10.3.2 Maintaining quality control records

Maintaining QC records help with troubleshooting and provide proof of reliable test results. These should be recorded using standard worksheets. These should be recorded every time when tested with

QC materials. All invalid results should be recorded and timely informed to supervisor.

10.3.2 Quality control record

During a review of QC results, it is easier to have one log of all QC results, rather than going from page to page in a logbook. A format such as this provides an easy glance at consistent frequency in testing QC samples, and also a ready identification of problems.

Facilitator will demonstrate quality control record format.

10.3.4 Periodic review of records

You should review QC results periodically in order to detect any problems early. This review involves:

- Daily review of internal control results before accepting test results.
- Review of external control results by test performer.
- Weekly or monthly review of external quality control results by testing site supervisor.
- Periodic audits or assessments.

10.4 External Quality Assessment (EQA):

EQA is the objective assessment of a test site's operations and performance by an external agency or personnel. This process assesses and monitors the performance of a laboratory.

EQA allows comparison of performance and results among different test sites offering not only an opportunity for performance checks, but an opportunity to systematically identify problems with kits or operations. Additionally, EQA also provides objective evidence of testing quality, indicates areas that need improvement, and identifies training needs.

10.4.1 EQA: conducted at three levels

EQA should be conducted at all levels of testing service. These include national reference lab, regional or intermediate lab, and test site (point of service).

For EQA program to be effective, the Ministry of Health and Population must establish an organizational structure and assign responsibility to assure that on-site monitoring occurs in all locations. In most countries, the National Reference Laboratory (NRL) has overall oversight responsibility, NPHL for our country. However, to have better reach in meeting the needs of rural test sites or points of service, this may be best accomplished with oversight by regional public health labs.

10.4.2 Management responsibilities: an overview

Someone of authority must take responsibility for EQA. First of all, they must determine policies for EQA (who, what, when, how) and designate a staff member with the responsibility to establish and implement the EQA program.

Furthermore, it is the responsibility of management and lab staff to enroll in national EQA program, to receive/send samples for EQA and receive EQA results, and support corrective action measures. Management will determine how and when they are advised on the outcomes of the EQA program. The best way to ensure EQA reports are reviewed by management is by including them as an agenda item in management review meetings. Finally, the management must monitor and maintain records, investigate deficiencies, manage corrective action efforts, and communicate outcomes.

Testing personnel's responsibilities

Test providers' EQA responsibilities include:

- Participating in the EQA program.
- Taking corrective actions.
- Maintaining EQA records.

- Communicating outcomes to supervisors.
- Rotating EQA testing among all staff that perform the tests routinely.

10.5 External Quality Assessment (EQA) methods

There are three main methods for conducting External Quality Assessment:

1. Proficiency Testing (PT)
2. On-site evaluation
3. Re-checking or Re-testing/Inter-laboratory comparison

10.5.1 Proficiency testing

In proficiency testing (PT), a reference laboratory sends out panels of specimens to multiple test sites, which in turn perform tests on these panels and report results. The test should be done in the same manner as for regular samples. The reference laboratory sends the result to site with feedbacks and evaluation. Corrective actions are provided to site if needed. The reported results indicate quality of personnel performance and test site operations. Results are often compared across several testing sites.

10.5.2 On-site evaluation

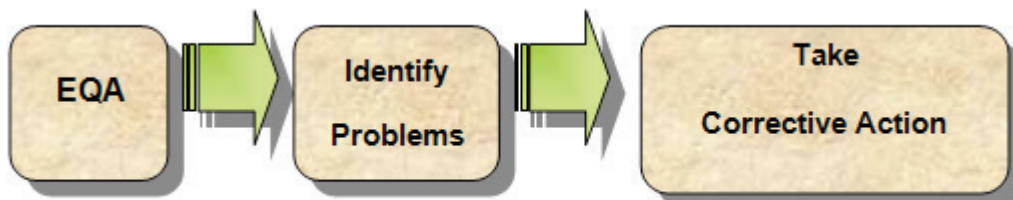
On-site evaluation is periodic site visits for systematic assessment of lab practices. These visits focus on how the lab monitors its operations and ensures testing quality. They provide information for internal process improvement. On-site evaluation is also referred to as audits, assessments, or supervisory visits.

These site visits enable us to learn “where we are” so we may measure gaps or deficiency. From the visits we can collect information for planning and implementation, monitoring, and continuous improvement. They are part of every lab quality system. These visits should be instructional rather than punitive. The main purpose of on-site visits is to observe the testing site under routine conditions in order to check that it is meeting quality requirements.

10.5.3 Re-testing/Inter laboratory comparison

Re-testing is the process by which a certain number of specimens are collected from the routine workload at the test site and sent to the reference laboratory for validation. The number of sample and collection system depends on the type of EQA program. For example, all positive samples and ten percent of negative samples are sent using DBS technique for HIV EQA program. It is used to identify errors in testing which leads to correction. The referral laboratory sends the summary report to the site with results and feedbacks. The corrective actions to be taken are also included in the summary report if discrepancy of result is observed between reference laboratory and site.

EQA should lead to corrective actions



A Corrective action is an action taken to correct a problem or non-conformance / deficiency within the quality management system. Examples of non-conformance include:

- Production of an incorrect result.
- Any step within a process which contributed to an incorrect result.

- When the documented quality system is not followed exactly as intended.
- When the quality system does not meet the requirements of quality standards.

10.6 External Quality Assessment Scheme (EQAS) for HIV

DBS filter paper method has been used for HIV EQAS in Nepal. In resource limited settings like in Nepal, it has demonstrated to be effective by the validation studies conducted.

10.6.1 Specimen requirements

All positive samples and ten percent of total negative samples should be selected. For negative sampling, the systemic random sampling should be done avoiding the positive sample number in accordance with the daily recording log book for HIV testing.

1. Suppose 100 samples per month is tested for HIV detection, blood samples from every tenth patients attending the HTC site will be collected for EQAS.
2. Beyond that all HIV positive tested samples should be collected
3. Mark every tenth row of HTC enrollment register for EQAS enrollment.
4. When the assigned (every tenth person coming for HTC person comes to HTC site take out one EQAS sample collection set.

NOTE: If the PMTCT service facilities along with HTC, in such case make a separate logbook (personal use for DBS sample only) for numbering of samples, then select the negative sample by random systemic sampling including all positive samples.

Example: Sample number selection for Service Delivery Point (SDP) with 50 samples testing per months

Example: suppose you have tested 50 samples, and the highlighted (red) are positive then:

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, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50.

Samples to be sent for EQAS:

- All positive: 3, 10, 18, 32, 35, 47
- Total: 6
- 10% negative = $4.4 \approx 4$
- Negative samples: 7, 19, 29, 41
- Total sample send = all positive + 10% negative = $6 + 4 = 10$

EQA Specimen Transfer Log (*Demonstration*)

Facilitator will explain how to fill the EQAS specimen transfer log form.

Module review

1. What is quality control ?
2. What is internal quality control ?
3. How often and when should internal controls be used ?
4. What would you do if your internal control tested invalid ?
5. What is external quality assessment and methods ?
6. Describe national HIV EQAS.

LOGISTIC MANAGEMENT AT HIV TESTING SITE

Purpose

To provide basic information on logistic management to manage stock level at HIV testing sites.

Learning objectives

At the end of this module, participants will be able to:

- Explain health logistics management, purpose of a logistics system and logistics cycle
- Explain the supplies required for HIV rapid testing and laboratory diagnosis of sexually transmitted infections
- Explain the test kits and accessories required for HIV rapid testing and laboratory diagnosis of sexually transmitted infections (STIs)

Content outline

- Health logistic management system
- Basic elements of logistics system
- Logistic management information system
- Storage, physical inventory count
- Accessories in HIV testing sites

Time: 60 minutes

11.1 Health logistics management system

Health logistics management includes the planning and management of all activities involved in sourcing and procurement and all logistics management activities. Importantly, it also includes coordination and collaboration with channel partners, which can be suppliers, intermediaries, third party service providers, and customers. In essence, health logistics management integrates supply and demand management within and across companies.

In other words, health logistics management considers activities as the operational component of supply chain management, including quantification, procurement, inventory management, transportation and fleet management, and data collection and reporting. Logistics management includes the logistic activities plus the coordination and collaboration of staff, levels, and functions.

11.2 Basic elements of health logistics system

During our lifetime, we will encounter hundreds of logistics systems—in restaurants, stores, warehouses, and many other places. This module describes logistics systems for health programs; however, if you understand a simple example of a logistics system, you will be able to understand almost any health logistics system.

A restaurant is one example of a simple logistics system:

- The kitchen is a storage facility; the food is held there until it is delivered to the customer.
- Waiters provide the transportation; they carry the food from the kitchen to the customer.
- The tables are the service delivery points, where customers sit to order and eat the food.

For customers, a restaurant is not a logistics system; it is a place to eat. However, we can directly relate to logistics.

We may expect that the:

- restaurant will be attractive and pleasing
- server will provide excellent customer service
- food you order will be available
- food will be served promptly
- correct order will be delivered to your table

The Six Rights of Logistics

- The RIGHT goods
- in the RIGHT quantities
- in the RIGHT condition

Delivered...

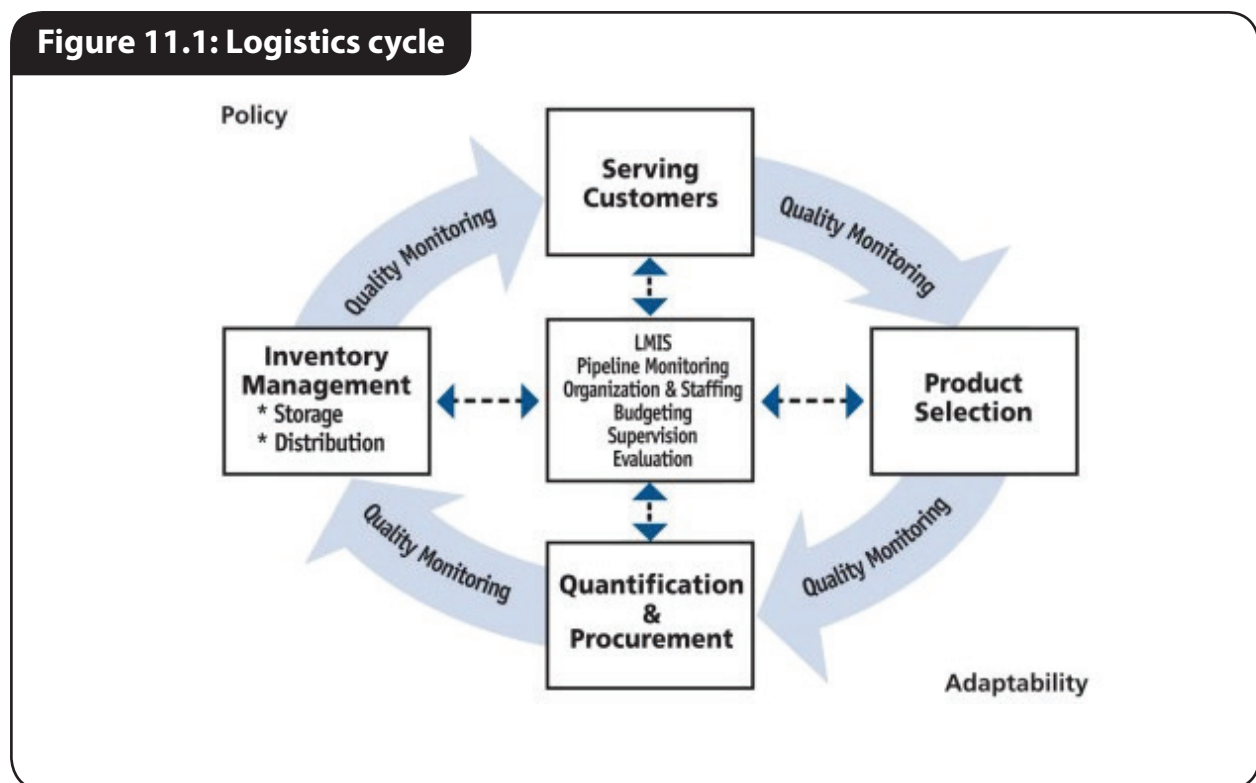
- to the RIGHT place
- at the RIGHT time
- for the RIGHT cost

11.2.1 Logistic cycle

Logistics management includes a number of activities that support the six rights. Logistic cycle is a continuous process from product selection to forecasting and procurement, inventory management, monitoring and evaluation of HIV and AIDS commodities. The logistics cycle comprises the following elements:

- The logistics management information system (LMIS), which is at the heart of the cycle
- Quality monitoring is a continuing activity throughout the cycle
- Policies and adaptability constitute the logistics environment.

Figure 11.1: Logistics cycle



11.3 Key logistics terms

The key logistics terms used throughout this module are defined below.

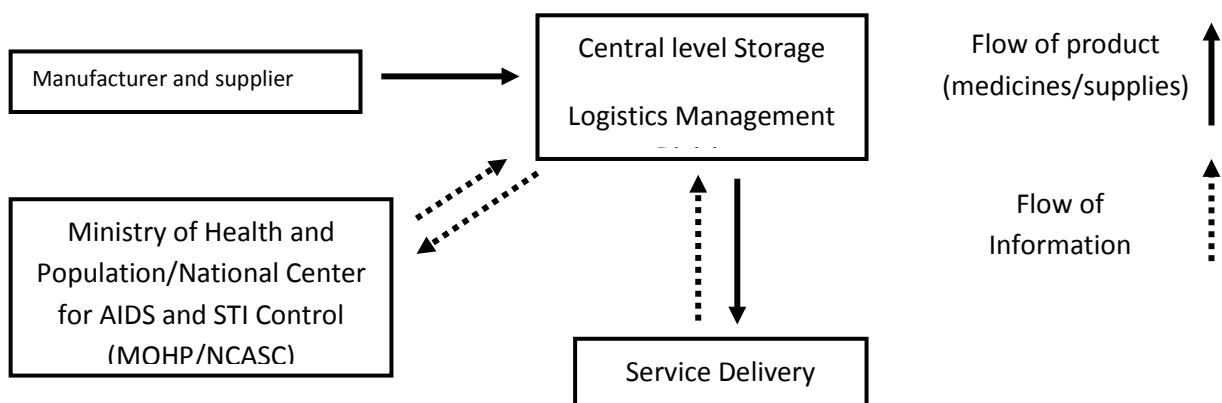
- **Pipeline:** The entire chain of physical storage facilities and transportation links through which supplies move from the manufacturer to the user, including port facilities, central warehouse, regional warehouses, district warehouses, all SDPs, and transport vehicles, including community-based distribution networks.
- **Lead time:** The time in between when new stock is ordered and when it is received and available for use. When logistics managers evaluate how well a logistics system is meeting the six rights, they measure the lead time and try to reduce it. Goods should be available to customers at the right time—before the customer asks for the product. Lead time can be calculated within the entire in-country system, from arrival in port to the end user, between specific levels of the system, or even the procurement lead time from when a product is ordered with the manufacturer until it arrives in port.
- **Pull System:** In pull system, the quantity to be re-supplied is calculated by the person placing the order and receives order accordingly.
- **Push System:** In push system, the quantity to be re-supplied is determined by the person who fulfills the order i.e. central level.
- **Consumption Data:** Consumption data provide information about the quantity of goods actually given to or used by customers.
- **Issue Data:** Issues data provide information about quantity of goods moved from one level of the system to another, or from one department to another within the same facility.

11.4 Logistics Management Information Systems (LMIS)

LMIS refers to an integrated system that collects processes and reports logistic data across all levels of the system. Most importantly, a LMIS enables logisticians to collect the data needed to make informed decisions that will ultimately improve customer service.

“No Report, No Commodities, No Program”

Flow of commodities and information



11.5 Three essential data for logistics management

The three essential data for logistics management are:

- Stock on hand – This data refers to quantities of usable stock in an inventory at any, or all system levels.
- Consumption data - The quantity of a particular item dispensed to users or used by service providers during a specific time period.
- Losses or Adjustment – Losses are quantities of unusable stock removed from the pipeline for any reason other than consumption by clients (example: losses, expiration, theft, damage, etc.) Adjustments include quantities transferred between facilities or levels.

Note: Facilitator will demonstrate steps to fill Combined report, Issue and requisition form for Test kits.

11.6 Storage

Products are stored at every facility in the pipeline; almost everyone working is responsible for product storage. Storage ensures the physical integrity and safety of products and their packaging, throughout the various storage facilities, until they are dispensed to clients. An important goal in storage of health products is the correct staging of health products to ensure that orders can be fulfilled and distributed.

11.7 Shelf life

Shelf life is the length of time from manufacturing date to the final date, that a product can be safely used, or the length of time that a product can be stored without affecting its usability, safety, purity, or potency.

11.8 FEFO

First-to-expire-first-out ensures that products with near expiry date are issued first.

11.9 Storage guideline

Storage guidelines for lab commodities are as follows:

- Store all drugs/supplies in a locked room to prevent theft. Take precaution during transportation.
- Keep the store room clean and unpolluted. Also prevent harmful insects from entering.
- Prevent HIV and AIDS commodities from direct sunlight and keep these commodities in a well-lit, dry and ventilated store room. Store at temperatures below 25°C.
- Commodities that need to be stored in cold storage should be stored at temperatures between 2°C to 8°C.
- Prevent damage from moisture and water.
- Keep fire extinguisher in an accessible place and train each staff regarding its use.
- Allow only authorized personnel to enter the store room.
- Store cartons at least ten cm (4") above the ground, 30 cm (1') from wall and from other cartons; and within a height of 2.5 m (8').
- Store cartons in an upward direction (↑) showing the label, expiry date and manufacturing date.
- Check the expiry date of received commodities and store as per FEFO to enhance stock management. Some drugs/supplies have shelf life of one year only and hence it is important to apply FEFO.
- Keep all drugs/supplies away from pesticides/insecticides, chemicals, fire, old files and office instruments. Also keep them away from bright light.
- Separate all damaged and expired drugs/supplies, test kits from useable commodities and destroy them as per the rules. Record the deduction in the stock book.

11.10 Visual inspection

Visual inspection is the process of examining products and their packaging to look for obvious problems with product quality.

When to conduct a visual inspection?

To ensure the quality of the product we must conduct a visual inspection. Follow below listed bullets to conduct visual inspections:

- Check expiry date of products
- Check physical status of commodities
- Follow storage guidelines
- Issue products from one level to another

11.11 Physical inventory count

A physical inventory count is on hand inspection for each commodity with the amount recorded on the stock card.

11.12 Supplies and materials checklist

Below is a list of materials and supplies required for rapid HIV testing and laboratory diagnosis of sexually transmitted infections.

Internal and External Quality Control

Gloves



Gloves are used for safety reasons – to protect both you and the patient or client. It is important that the proper size gloves are used. Wearing gloves that are too large may pose a safety hazard and make it cumbersome to work with. Keep in mind that long nails may puncture the glove, making them ineffective.

Gloves must be changed between patients, and disposed of in a container labeled as bio-hazardous waste. Never use gloves that have been previously used or are torn. Gloves come in Latex or polypropylene - consider latex allergies when selecting the type of glove to use.

Alcohol swabs



Alcohol is used to cleanse the area of skin above the vein from which venous blood is collected. Alternatively, use a bottle of rubbing alcohol and cotton wool.

Cotton gauze or cotton balls



Cotton balls are used to: wipe away the first drop of blood, and to stop bleeding after specimen is collected. They are for single use only. Contaminated cotton gauze or cotton balls should be disposed with other hazardous waste.

Disposable syringes

Sterile Disposable Syringes are used to collect venous blood specimens.

Pipette

Pipettes are used to collect a specified volume of blood specimen from the fingertip. There are two types of pipettes commonly used.

The *transfer pipette* is a disposable plastic item that is used only once. Be sure to dispose of this along with other contaminated waste.

The *automatic pipette* is used to collect a specified volume of blood and is most often used in laboratories. A disposable tip is attached to the end of the pipette for collecting the blood. After use, the tip is ejected or removed and is disposed of along with other contaminated waste.



**Transfer
Pipette**



**Automatic
Pipette**

	The loop is another tool used to collect a specified volume of blood. This is used with some kits.	
Timer	 	Shown here are two types of timers that can be used for waiting for the specified time to elapse before test results are read. You may also use a watch, or clock.
Standard Operating Procedures and forms	Each site will also need to follow standard operating procedures, and use standard forms for recording test results.	
Labeling pens and writing pens	 	A permanent marker as seen on the left is best used for labeling test devices. Ball point pens (seen on the right) are used to fill in forms. Never use pencils, especially for recording client results – results can be erased and changed.
Sharps disposal bins / disinfectant jar	 	

Examine test kits	Examine the test kits that are approved in your country. Pay attention to the components of each test kit. In addition, notice the following two components: • Desiccant packet – This is not used when performing the test. It only serves to keep the packet contents dry before use. It should be discarded when the test kit packet is opened. • Buffer solution – Required by some kits
Reagents for microscopic examinations	Reagents for Gram staining: • Crystal Violet • Gram's iodine • Decolorizer (50% acetone+50% ethanol) • Safranin Reagents for Potassium Hydroxide (KOH) mount: 10% KOH solution Reagents for wet mount : Normal saline
Organize your work area	Having an organized workspace is key to producing quality results. It is important to: • Keep working area neat, clean and organized. • Have necessary supplies placed within reach at the testing area before testing. Once the tester sits with the client, it will make the client more nervous if the tester has to keep getting up to collect more supplies. 

Module review

1. List and explain the components in the test kits approved for use in your country.
2. Physical count should be performed at least.....
 - a. While preparing bi-monthly report
 - b. Every year
 - c. Every day
 - d. Never
3. Which of these is not an essential data of LMIS?
 - a. Consumption data
 - b. Loss/adjustment
 - c. Beginning balance
 - d. Stock on hand
4. FEFO means.....
5. When should you forward the LMIS report to the center?
 - a. Quarterly
 - b. Every six months
 - c. Bi-monthly
 - d. Every month

MODULE 12

USE AND CARE OF EQUIPMENT AT HIV TESTING SITE

Purpose

To provide basic information on use and care of equipment used in HIV testing site.

Learning objectives

At the end of this module, participants will be able to:

- Explain the rationale and function of equipment needed at HIV testing sites
- Explain the proper use, maintain and monitor the equipment available
- Describe CD4 T lymphocyte count and viral load test machines

Content outline

- Rationale for using properly maintained equipment
- Use and care of equipment at HTC laboratory
 - o Microscope, refrigerator and freezer, pipette, centrifuge, RPR rotator

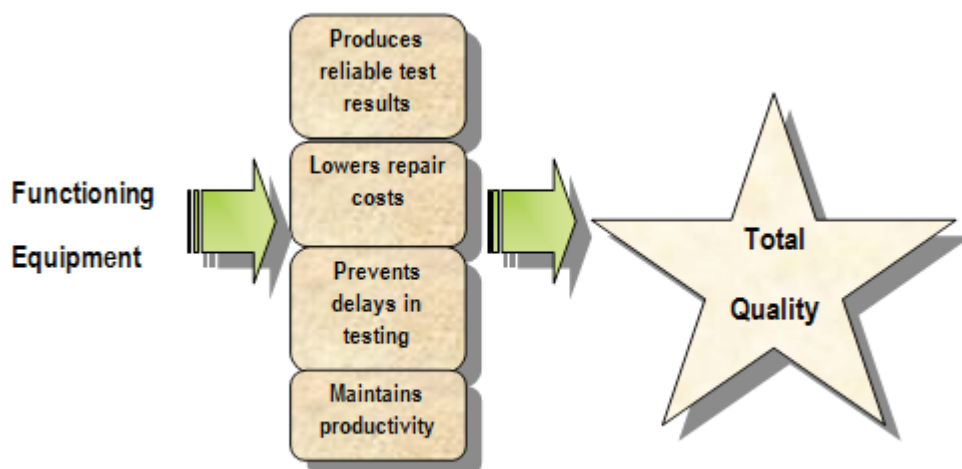
Time: 45+45 minutes

12.1 equipment

Appropriate, adequate and functional equipment are required for regular functioning of laboratory service in a reliable manner. As equipment is also one of the key components of the laboratory quality system, following things should be considered for each equipment used in the laboratory.

- Equipment selection
- Equipment acquisition
- Equipment installation and initial calibration/validation
- Maintenance, service and repair
- Troubleshooting
- Retiring equipment and disposal

12.2 Functioning equipment is vital to quality service



A reliable result provides basis for clinical diagnosis. Unreliable results may result in incorrect diagnosis and mistreatment of the patient. If equipment is properly maintained, it is less likely to breakdown before its next service and is less likely to perform inadequately due to lack of maintenance. All equipment used at the testing site must be properly maintained. Using equipment that has not been properly maintained may compromise the quality of test results.

12.3 Equipment at HTC laboratory

- Refrigerator: To store reagents, kits, and quality control materials
- Micropipette: To collect or transfer specimen to test device.
- Centrifuge: To separate red blood cells from whole blood to get serum/plasma
- Microscope: To examine Gram stained smears, Wet mount slides and KOH mount slides for Gram negative intracellular diplococci, Trichomonas vaginalis, Clue cells and fungal elements.
- RPR Rotator: To rotate the RPR cards containing a mixture of antigen coated carbon particles and patient serum on it within a circle for better mixing antigen and antibody.
- Treponema Pallidum Particle Agglutination (TPPA) Plate shaker: To shake the micro well plate for better mixing of the contents kept in the wells.
- CD4 T lymphocyte count machine: To enumerate the CD4 T lymphocyte as a part of immunological monitoring in people with HIV infection. This might not be available at HTC, this will be at sites with ART centers.
- Viral load Machines: To quantify the number of viral particles in HIV infected people and to assess the ARV efficacy. This is available at referral laboratory only, now at NPHL only.

12.4 Management responsibilities: ensure test site readiness

Lab management is responsible for making sure the test site is ready to receive and install a new piece of equipment. This includes:

- Assigning responsibilities for oversight of all lab equipment.
- Establishing inventory record – Each piece of equipment must have an inventory record. This record contains pertinent information such as manufacturer and name of equipment, maintenance and service record, and manufacturer contact information.
- Establishing maintenance program, including routine function checks, trouble-shooting, and maintenance log.
- Developing and implementing written protocols for operating procedures.
- Training the operators – Everyone using any piece of laboratory equipment must be properly trained. Training must include troubleshooting.

Responsibilities of laboratory staff

Once the equipment is installed, the following should be applied.

- Follow written operational procedures.
- Conduct routine maintenance, including function checks.
- Take corrective actions.
- Keep records.

Do not use malfunctioning equipment.

Function Checks: Verify that equipment is working properly. Function checks are activities performed periodically to ensure that equipment is working properly before use and appropriately maintained for peak performance. Function checks should be performed routinely such as daily, weekly, monthly, and after adjustment or repair. Examples of function checks include monitoring refrigerator temperatures, verifying pipette accuracy and checking centrifuge speed.

12.5 Refrigerator and freezer: use and care

What you need to do

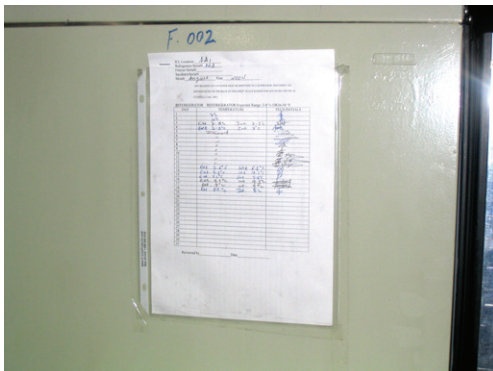
- Keep organized
- Periodically clean inside and outside
- Ensure door is completely sealed when closing

CAUTION! – DO NOT store food items or beverages in laboratory refrigerator or freezer

Refrigerator and freezer: temperature checks

Monitor your refrigerator and freezer daily and make sure they are within the acceptable temperature ranges:

- Refrigerator: 2°C to 8°C
- Freezer: -20°C, -40°C, or -80°C



This photo illustrates routine monitoring of temperatures of this refrigerator. It is a good practice to attach the form for recording temperatures directly on the front of the refrigerator for easy access. Inserting it into a protector page will guard against tearing of paper.

Refrigerator and freezer: Temperature log

12.6 Pipettes**12.6.1 Types of pipettes**

There are two types of pipettes: precision pipettes and graduated plastic bulb pipettes.



precision pipette



Graduated plastic bulb pipette

- Precision pipettes dispense precise and accurate volumes (e.g., 50 μl for Determine). They are not disposable, but use disposable, single-use, pipette tips.
- Graduated plastic bulb pipettes, on the other hand, dispense approximate volume, are easy to use, and are disposable.

Remember, never to re-use disposable items. Doing so will cause cross contamination.

12.6.2 Pipette: use and care

What you need to do:

- Select the appropriate pipette for the volume required (for example, if 50 micro liters (μl) of specimen is required, use a 100 μl pipette).
- Ensure that the pipette, tips, and specimen are at the same temperature.
- Firmly attach tip.
- Hold the pipette vertically when aspirating.
- Place tip just below the sample.
- Avoid air bubbles.
- Discard contaminated tips in appropriate container after completion of task.

Never lay the pipette on its side when liquid is in the tip – doing so will cause the specimen to flow into to the pipette shaft and damage the pipette.

Air bubbles in the tip can greatly reduce pipetting accuracy. If an air bubble is trapped within the tip during intake, do the following:

- Dispense the sample into the original vessel.
- Check the tip immersion depth.
- Pipette more slowly.
- If an air bubble appears a second time, discard the tip and use a new one.

Remember these safety practices:

- Carefully discard pipette tips in the appropriate container. Used pipette tips should not be found on the floor, as this poses a safety hazard.
- Never re-use a pipette tip, which can cause cross contamination and will compromise patient results. A fresh tip should be used for each sample.

12.6.3 Precision pipettes require performance checks

Precision pipettes should be checked periodically for performance. You will need the following supplies: pipette, pipette tips, analytical balance, weigh boats, and distilled or deionized water. The analytical balance should have a scale of 0.1 to 0.0001 mg.

Pipette: steps for checking reproducibility

Performance checks include reproducibility and calibration. The procedures for checking reproducibility are described below:

- Pipette a series of ten samples into a weigh boat on an analytical scale
 - Record weight of each sample to calculate calibration results
 - Verify that calculated results are within limits
1. If the results are not within limits, remove from service until appropriate adjustment can be made.
 2. Decontaminate pipette and scale after use

Table 12.1 Pipette troubleshooting and action

Problem	Potential Cause	Action
Leakage	Tip(s) incorrectly attached	Attach firmly
	Foreign articles between the tip and cone	Clean tip cones
	O-ring damaged	Change the O-ring
Inaccurate dispensing	Incorrect operation	Follow manufacturer's instructions carefully
	Tip incorrectly attached	Firmly attach tip

12.7 Centrifuge: use and care

What you need to do:

- Always operate with the lids closed – Operating a centrifuge without the lid closed poses an unnecessary safety hazard.
- Balance contents before turning on – For example, if there is only one sample to be centrifuged, a tube identical in size and volume must be placed in the rotor opposite the tube.
Note: The rotor is the part of the centrifuge that holds the tubes and rotates during operation.
- Check for vibration – There may be several reasons why a centrifuge vibrates. When vibration occurs, you'll need to:
 - o Stop operation of the centrifuge.
 - o Determine the cause of the noise or vibration.
 - o Correct immediately to prevent severe damage to the centrifuge or injury to the worker. Refer to the operation manual for possible causes aside from improper balancing.
- Do not open the lid until the rotor has come to a complete stop.
- Keep lids on tubes when spinning – Do not take the tops off the tubes before spinning. Doing so will cause splashing and creating of aerosols from potentially infectious material

12.7.1 Centrifuges: function checks

Separation activity is a function of both centrifugal force and timing. Proper balance, lubrication and rotor function are essential for proper centrifugation to occur.



12.7.2 Centrifuge: routine maintenance

When cleaning the centrifuge:

- Clean interior daily with soap and water, wipe with a disinfectant.
- Wipe spills using 10% bleach solution.
- After cleaning, run the centrifuge at varying revolutions per minute (RPM) to check the braking mechanism and ensure a smooth gradual stop.

When noticing unusual noises or vibrations:

- Stop operation of the centrifuge.
- Follow manufacturer's recommendation on activation and release of brakes.
- Correct immediately to prevent severe damage to the centrifuge or injury to the worker.

Inspect for evidence of wear, cracks in fitting, corrosion, uneven wear, or signs of fatigue by checking:

- Head, shaft head and coupling
- Rotor
- Brushes and bearings
- Power supply
- Motor and lubricant
- Gaskets, seals, mounts and lubricants
- Brushes need to be inspected every 3-6 months and replaced according to manufacturer specifications.

12.7.2 Centrifuge safety

Follow these safety rules when operating a centrifuge:

- Increase the speed slowly until optimal speed is reached.
- Disconnect the centrifuge from the electrical source before preventive maintenance, cleaning or inspection.
- Take caution when removing spills and broken specimen tubes after a .
- If tubes are broken, keep the door closed and allow to sit undisturbed for 30 minutes before attempting to clean.

- Use tweezers to remove broken glass.
- Simply turning the power off does not remove power to the centrifuge.

12.8 Microscope: use and care

Follow these directions when using the microscope:

- To carry the microscope, grasp the microscopes arm with one hand. Place your other hand under the base.
- Place the microscope on a table with the arm towards you.
- Turn the coarse adjustment knob to raise the body tube.
- Revolve the nosepiece until the low-power objective lens clicks into place.
- Adjust the diaphragm.
- Place a slide on the stage. Center the specimen over the opening on the stage. Use the stage clips to hold the slide in place.
- Look at the stage from the side. Carefully turn the coarse adjustment knob to lower the body tube until the low power objective almost touches the slide.
- Looking through the eyepiece, VERY SLOWLY turn the coarse adjustment knob until the specimen comes into focus.
- To switch to the high power objective lens, look at the microscope from the side. CAREFULLY revolve the nosepiece until the high-power objective lens clicks into place. Make sure the lens does not hit the slide.
- Looking through the eyepiece, turn the fine adjustment knob until the specimen comes into focus.

12.8.1 Process of using the oil immersion lens

- Focus at low power on a region of a smeared and stained specimen which is well-spread and stained (not too thin, nor too thick).
- Rotate nosepiece to 40x objective, locate desired portion of specimen in the center of the field. Refocus very carefully so that the specimen is focused as sharply as possible. (Do not alter focus for the following steps)
- Partially rotate nosepiece so that 40x and 100x objectives straddle the specimen.
- Place a small drop of oil on the slide in the center of the lighted area.
- (Take care not to dribble on the stage.)
- Rotate nosepiece so that the 100x oil immersion objective touches the oil and clicks into place.
- Focus only with fine focus. Hopefully, the specimen will come into focus easily. Do not change focus dramatically. If you still have trouble, move the slide slightly left and right, looking for movement in the visual field, and focus on the object which moved.
- With more than one specimen on a slide, do not alter focusing, rather, place a drop of oil on the second specimen, and slide the slide laterally until it is in place.
- Never go back to the 10x or 40x objectives after you have applied oil to the specimen since oil can ruin the lower power objectives. [The 4x objective can be used because it is high enough to be above the oil.]
- Clean up: When you have finished for the day, wipe the 100x oil immersion objective carefully with lens paper to remove all oil. Cleanse stage. Recap the immersion oil container securely, replace in drawer.

Note: Facilitator will explain and update on the CD4 T lymphocyte count machines and viral load test platforms in the country.

12.8 Keep a log for all maintenance activities

It is good practice to keep a log to document problems, corrective action, preventive maintenance, cleaning, and inspections. At the end of the module, you will find a sample, generic maintenance log for discussion.

12.9 Exercise: create a maintenance activity log

Use what you have learned in this module and create a maintenance checklist specific to your test site. Create a list of maintenance activities on a daily, weekly, monthly, and yearly basis.

Module review

1. Why is it important to keep equipment in optimal condition?
2. Describe proper use and care for: *a. Microscope b. Refrigerator c. Micropipettes*
d. Centrifuges e. RPR rotator
3. Describe your responsibilities for equipment at the test site.

MODULE 13

OVERVIEW OF SEXUALLY TRANSMITTED INFECTIONS AND OPPORTUNISTIC INFECTIONS

Purpose

To provide basic concepts on sexually transmitted infections (STIs) and opportunistic infections (OIs).

Learning objectives

By the end of the session, the participants will be able to:

- Describe common STIs
- Describe Transmission mode and common signs and symptoms of STIs
- Describe common OIs in HIV

Content outline

- Sexually Transmitted Infections
- Presentation of STIs: Syndromes
- Etiological agents of STIs
- STI transmission and risk behavior
- Common OIs in HIV infections

Time: 60 minutes

13.1 Sexually transmitted infections

13.1.1. Introduction

Sexually Transmitted Infections (STIs) are the infections transmitted from one infected individual to another primarily through sexual contact. It is one of the major public health burdens of society because of its effect on morbidity, its social impact, and relationship with HIV/AIDS. The importance given to STIs and its proper management has further increased due to its significant role in HIV prevention.

All STIs are divided into different syndromes, which include the major symptoms of STIs. STIs are treated based on the syndrome, called syndromic approach. In enhanced syndromic approach, laboratory diagnostic tests are also applied to detect etiological organism for STI infection. STI syndromes with symptoms and causative organism are given below.

Table 13.1 STI syndrome with major sign and symptoms with causative agent

STI syndrome	Major sign and symptoms	Causative agent
• Urethral Discharge Syndrome	• discomfort on passing urine (slight to severe) • discharge from the urethral opening (thin to thick, clear to pus)	• Neisseria gonorrhoeae • Chlamydia trachomatis • Mycoplasma genitalium • Ureaplasma urealyticum • Trichomonas vaginalis
• Scrotal Swelling Syndrome	• painful testis • dysuria (sometimes) • swelling and tenderness of testis and epididymis • discharge (occasionally)	• N. gonorrhoeae • C. trachomatis

STI syndrome	Major sign and symptoms	Causative agent
Inguinal Swelling (Bubo) Syndrome	- Painful swelling in the groin - Discharging sinus	<i>Chlamydia trachomatis</i> <i>Haemophilus ducreyi</i> (Chancroid)
Vaginal Discharge Syndrome	- Vulvo-vaginal irritation - Vaginal soreness and smell - Pain during intercourse - Discharge from the vaginal opening - Thin to thick - Clear to purulent - scanty to profuse	- Vaginal infection <i>Candida albicans</i> <i>Trichomonas vaginalis</i> - Bacterial vaginosis - Cervical infection <i>Neisseria gonorrhoeae</i> <i>Chlamydia trachomatis</i>
Lower Abdominal Pain Syndrome	- Pain in lower abdomen - episodic or continuous - Fever low or high grade - Vaginal discharge	<i>Neisseria gonorrhoeae</i> <i>Chlamydia trachomatis</i> - Anaerobic bacteria
Syndrome of neonatal conjunctivitis	Swelling and/or discharge from one or both eyes within 21 days	<i>N. gonorrhoeae</i> <i>C. trachomatis</i>

Source: National STI Guideline, 2009

Table 13.2 Different STIs and their etiological agents

STI	Causative agent
HIV infection	Human Immunodeficiency Virus
Gonorrhea	<i>Neisseria gonorrhoeae</i>
Syphilis	<i>Treponema pallidum</i>
Chancroid	<i>Haemophilus ducreyi</i>
Chlamydial infections	<i>Chlamydia trachomatis</i>
Moluscum contagiosum	Pox virus
Trichomoniasis	<i>Trichomonas vaginalis</i>
Vulvo-vaginal candidiasis	<i>Candida albicans</i>
Bacterial vaginosis	<i>Gardenerella vaginalis</i>
Granuloma inguinale	<i>Calymmatobacterium granulomatis</i>
Lymphogranuloma Venerum	<i>Chlamydia trachomatis</i>
Ano-genital warts	Human papilloma virus
Genital herpes	Herpes Simplex Virus
Hepatitis B and C	Hepatitis B and C Virus

13.2 Transmission of STIs

Routes of STI transmission:

- Transmission through sexual contact
- Mother to child transmission (vertical transmission)
- Other routes, occasionally contaminated fingers, blood and blood products, organ transplantation, contaminated needles, fomites like shared towels, sex aids such as dildos.

Major risk factor for transmitting the STI is sexual contact. Different behavioral factors increase the risk of transmitting STIs. They are as follows.

Sexual behaviors

- Having multiple sexual partners
- Practicing sexual contact without using condoms
- Practicing anal sex
- Keeping casual relationship

Health care related behaviors

- Less willingness to seek health care
- Lack of compliance with therapy
- Inadequate or no partner treatment
- Unhealthy practice of vaginal cleaning or douching

Other contributing factors

Age, gender, marital status, socioeconomic status and ethnicity, certain occupation, urban residence, alcohol and drug use, contraceptive methods used etc.

Note: Sex work is the highest but modifiable risk factor for STI and HIV transmission.

13.3 Complications of STIs

STI if left untreated can cause serious complications. These infections can lead to numerous serious, long-term, and sometimes deadly complications.

13.3.1 Complications of STI in males

STI can bring infertility and carcinoma of reproductive organ. STI Infection can occasionally result in partial or complete blockage of the sperm ducts, and disorders in sperm production, which contributes to male infertility. Infection with Human Papilloma Virus (HPV) is associated with the development of penile cancer.

13.3.2 Complications of STI in females

The major complications of STIs include pelvic inflammatory disease, adverse outcomes of pregnancy, ectopic pregnancy, infertility and cervical cancer.

Infection may become generalized and life threatening, and the resulting tissue damage and scarring may cause infertility, chronic pelvic pain and increased risk of ectopic pregnancy. STI such as chlamydia, gonorrhoea, syphilis, genital herpes etc. are responsible for the adverse outcomes of pregnancy. Infertility often follows after untreated pelvic inflammatory disease in women. Infection with HPV appears to be strongly associated with the development of cervical cancer.

13.3.3 Complications of STI in new born babies

Congenital syphilis, prematurity low birth weight and infection of gonorrhea, chlamydia are major complications in neonates. Congenital syphilis results from the transmission of *Treponema pallidum* infection from an infected pregnant woman to her foetus. There might be severe complications in congenital syphilis. *Neisseria gonorrhoea* infection may present with only conjunctivitis, which usually appears within the first four days of life and may progress to panophthalmitis unless treated. Chlamydia trachomatis can cause only conjunctivitis or have systemic infection like pneumonitis.

13.4 Opportunistic infections

Opportunistic infections are those infections which are caused by organisms that would not cause disease in an immuno-competent person (people with healthy immune system). A compromised immune system, however, presents an "opportunity" for the pathogen to infect. Immunosuppression, psychological stress, nutritional depletion are the major things that weakens the immune system and

provides opportunity for OIs. The common viral, bacterial, parasitic and mycotic opportunistic infections are listed below.

Table 13.3 viral, bacterial, parasitic and mycotic opportunistic infections

Group	Opportunistic infections
Bacterial	Streptococcal infection (mainly <i>S. pneumoniae</i>) Mycobacterial infections (tuberculosis) Mycobacterial avium complex Salmonellosis
Parasitic	Toxoplasmosis (<i>Toxoplasma gondii</i>) Cryptosporidiosis (stool, ZN stain modified) Isosporiasis Generalized strongyloidiasis
Mycotic	Pneumocystis jiroveci (pneumonia) Candidiasis Cryptococcosis neoformans
Viral	Cytomegalo virus (CMV) Herpes simplex Varicella-zoster Human papilloma virus, genital warts Molluscum contagiosum Oral Hairy Leukoplakia (OHL) Progressive Multifocal Leukoencephalopathy (PML)
Malignancies	Kaposi sarcoma, B-cell lymphoma or non-Hodgkin's lymphoma

The most common OIs seen in people living with HIV are *M. tuberculosis*, *Pneumocystis jiroveci*, *Candida albicans* and *Cryptococcus neoformans*. The common OIs diagnosed in Nepal includes Pneumonia, Pulmonary tuberculosis, Meningitis (mostly Cryptococcal), Oral candidiasis, Hepatitis B, C and "other" hepatitis, Skin: Herpes simplex, Herpes zoster, Molluscum contagiosum, Papular pruritic eruption, Candida dermatitis, Folliculitis. The less common OIs detected are Lymphoma (non-CNS), CNS Lymphoma, Possible PML, TB of the abdomen and TB osteomyelitis or joint infections.

Studies have revealed that association between level of CD4 T lymphocyte count and development of OIs. As CD4 T lymphocyte count is the hallmark of immune system, decrease in CD4 count below the normal range in HIV infection leads to different opportunistic infections as shown below.

Table 13.4 Relationship of CD4 T lymphocyte count with common opportunistic infections seen in PLHIV

CD4 T lymphocyte count	Opportunistic infection
400	Herpes Zoster, Tuberculosis
300	Oral Candidiasis
200	Pneumocystis carinii Pneumonia, Esophageal Candidiasis
100	Toxoplasmosis, Cryptococcosis Mycobacterium avium complex
50	Cryptosporidiosis, Progressive Multifocal Leukoencephalopathy

13.4.1 Tuberculosis: *Mycobacterium tuberculosis*

Tuberculosis is the most common opportunistic infection and a major cause of mortality among HIV infected people. It is the first manifestation of HIV infection and AIDS in more than 50% of cases in developing countries. Tuberculosis is an infectious disease that usually attacks the lungs, but can attack almost any part of the body. It is caused by *Mycobacterium tuberculosis* which are Gram-positive, non-motile, pleomorphic rods. Pulmonary TB can present at any CD4 T lymphocyte count and disseminated TB usually occurs at CD4 T lymphocyte <200.

13.4.2 *Mycobacterium Avium* Complex (MAC)

Mycobacterium Avium Complex (MAC) is caused by bacteria that can be found in soil, water, and many places in the environment. These bacteria can cause disease in people with HIV and CD4 counts less than 50. The bacteria can infect the lungs or the intestines, or in some cases, can become “disseminated”.

13.4.3 *Pneumocystis jiroveci*

Pneumocystis jiroveci is a common microorganism that exists in rats, guinea pigs, monkeys, dogs, sheep, humans, and other animals. Most people get infected with *Pneumocystis jiroveci* during childhood and develop no symptoms. In people with adequately functioning immune systems, *Pneumocystis jiroveci* remains a harmless, latent, lifelong infection. In people with impaired immune system, *Pneumocystis jiroveci* causes *Pneumocystis jiroveci* pneumonia (PjP) which can be very dangerous. PjP is the most frequently identified, serious OI in HIV. *Pneumocystis jiroveci* pneumonia only attacks people with a very weak immune system. It generally appears in people when their CD4 T lymphocyte count goes below 200 per cubic milliliter.

13.4.4. Candidiasis

Candidiasis is a fungal infection caused by *Candida albicans* that can affect the mouth, throat or vagina. Candida infections range from superficial to systemic and potentially life-threatening diseases. Thrush is a fungal infection, caused by candida, which usually infects the mouth, throat or vagina. Oral candidiasis is a rare condition in a healthy person, but is frequently the first indication of immune impairment in HIV infected patients. Recurrent episodes of oral candidiasis usually occur in patients with CD4 T lymphocyte <300. Over 60% of patients with CD4 T lymphocyte <100 develop oropharyngeal candidiasis each year.

13.4.5 *Cryptococcus neoformans*

Cryptococcus neoformans is an encapsulated fungal organism that can cause disease in apparently immunocompetent, as well as immunocompromised hosts. It is generally accepted that the organism enters the host by the respiratory route in the form of dehydrated haploid yeast or as basidiospores. After some time, the organism hematogenously spreads to extra-pulmonary tissues. The frequency of *Cryptococcus* infection in people with HIV/AIDS is about eight to ten percent. It is the most life-threatening fungal infection in patients with HIV/AIDS. Life-threatening infections caused by *Cryptococcus neoformans* have been increasing steadily over the past ten years because of the onset of AIDS and the expanded use of immunosuppressive drugs. It has a predilection for the brain, infected persons usually contract meningoencephalitis. If untreated, cryptococcal meningoencephalitis is 100% fatal.

13.4.6 Toxoplasmosis

Toxoplasmosis is caused by the parasite *Toxoplasma gondii* that can cause encephalitis and neurological disease in patients with low CD4 T lymphocyte counts. The parasite is carried by cats, birds, and other animals and is also found in soil contaminated by cat feces and in meat, particularly pork.

13.4.7 Cryptosporidiosis

Cryptosporidiosis is a diarrheal disease caused by the protozoa *Cryptosporidium*, and it can become chronic for people with low CD4 T lymphocyte counts. Symptoms include abdominal cramps and severe chronic diarrhea. Infection with this parasite can occur through swallowing water that has been contaminated with fecal material (in swimming pools, lakes, or public water supplies), eating, uncooked food (like oysters) that are infected; or by person-to-person transmission, including changing diapers or exposure to feces during sexual contact.

Module review

1. What are STIs?
2. What are the means by which STIs are transmitted?
3. What is Opportunistic Infection?
4. List the common OIs in HIV infected people in Nepal.

MODULE 14

OVERVIEW OF LABORATORY DIAGNOSIS OF SEXUALLY TRANSMITTED INFECTIONS AND OPPORTUNISTIC INFECTIONS

Purpose

To provide participants with basic knowledge on laboratory diagnosis of sexually transmitted infections (STIs) and opportunistic infections (OIs).

Learning objectives

At the end of this module, participants will be able to:

- Explain the spectrum of testing technologies for different STIs and OIs
- Explain the laboratory techniques in the diagnosis and management of STIs and OIs

Content outline

- Microscopy
- Culture
- Serology
- Antigen detection
- Nucleic acid detection

Time: 60 minutes

14.1 Laboratory testing technologies

Laboratory service provides the supportive role on screening and identification of infection in people who are at risk of STIs and OIs. Accurate diagnosis is crucial for timely medication and becomes highly important with HIV infection. Therefore, to support the syndromic approach to diagnosis, laboratories should perform the tests needed to facilitate clinical management of persons with and at risk for STIs and OIs.

There are different laboratory testing technologies to identify the etiology of STIs and OIs. In general, the following techniques are used to diagnose the STIs and OIs.

- Microscopy (Microscopic examination)
- Culture
- Serology
- Antigen detection,
- Nucleic acid detection

The sensitivity and specificity of these different approaches vary according to specimen type and organism assayed. Nucleic Acid Amplification Tests (NAATs) are the most sensitive methods, and culture the most specific. Antigen detection, nucleic acid hybridization, culture and microscopy are less sensitive but may be effective for certain types of patients and specimen types. Since not all diagnostic laboratories perform the same tests, clinical conditions and specimen types should be discussed before collecting the specimen. In some situations, serology is very useful (e.g., syphilis), but in others (e.g., C trachomatis) it is of no or limited use.

14.2 Microscopy (Microscopic examination):

Directly visualizing the organism on smear prepared under the microscope. Examples: Wet mount for *Trichomonas vaginalis*, *Candida* (budding cells), Bacterial vaginosis (BV), Gram staining for gonococcus, KOH for fungal infections, Acid fast staining for Tuberculosis.

Microscopic examination is done through various staining techniques depending on the type of organism suspected.

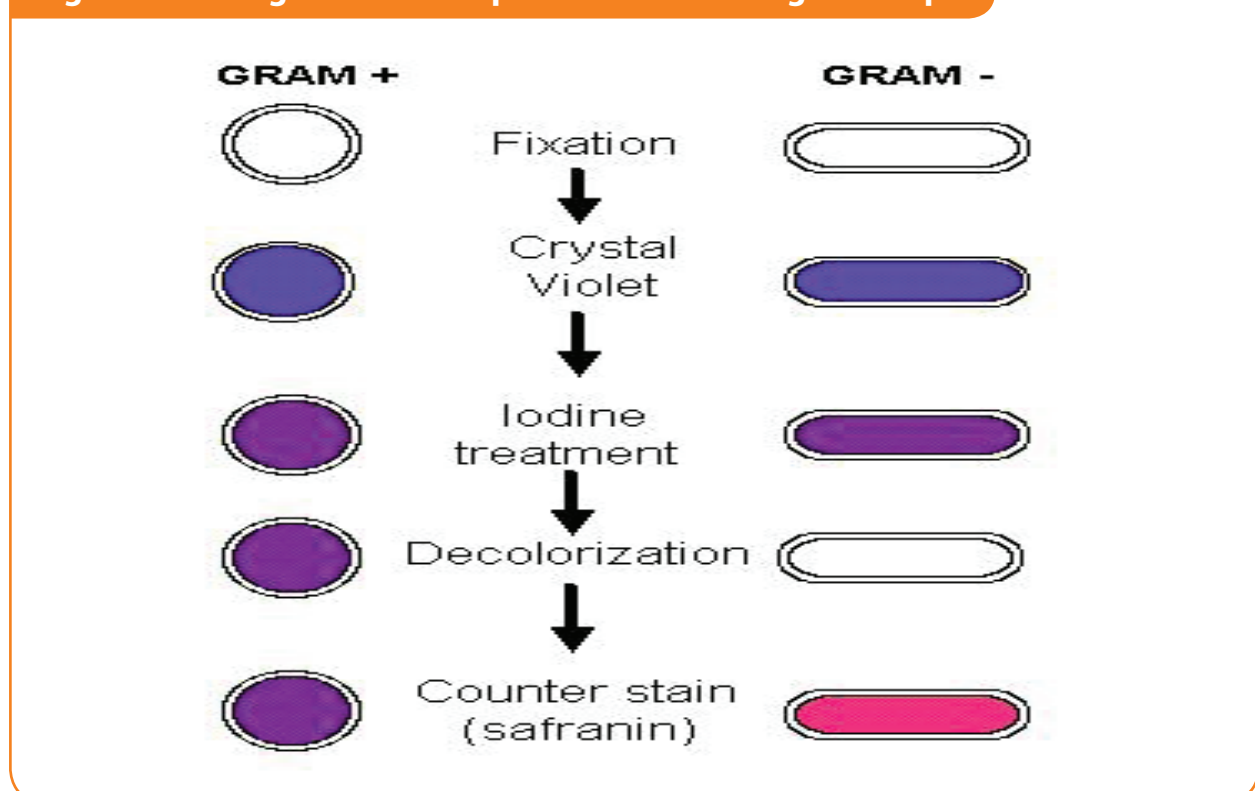
14.2.1 Gram staining

The Gram stain is particularly useful in the presumptive diagnosis of bacterial as well as fungal opportunistic infections. Properly Gram-stained preparations can quickly give considerable information that can be applied immediately to patient care. The Gram stain is useful in the diagnosis of gonorrhea, candidal vulvovaginitis, and bacterial vaginosis, and in the assessment of urethritis, cervicitis, and other infections characterized by mucosal discharge. Both the numbers of polymorphonuclear leukocytes (PMNs) and microbial flora present can be assessed.

Principle

The crystal violet stain is the primary stain, which stains everything in the smear violet blue. The Gram's iodine acts as a mordant that causes the crystal violet to penetrate and adhere to the gram-positive organisms. The acetone-alcohol mixture acts as the decolorizer that washes the stain away from everything in the smear except the gram-positive organisms. The safranin is the counter-stain that stains everything in the smear that has been decolorized: pus cells, mucus, gram-negative organisms.

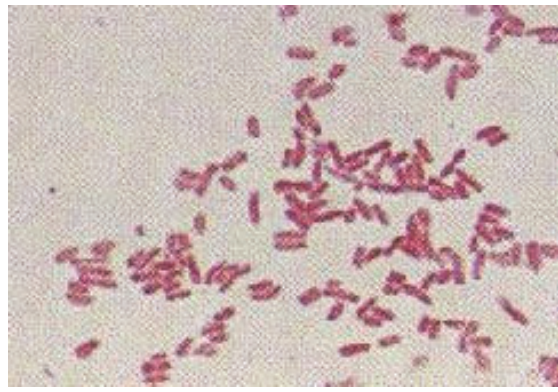
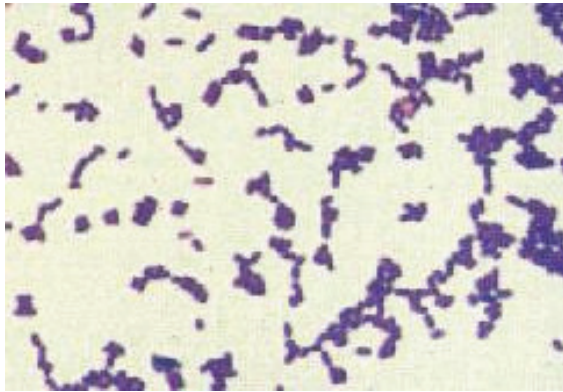
Figure 14.1 Diagrammatic Steps for Gram Staining Technique



Interpretation

- Organisms that retain the violet-iodine complex after washing in acetone or ethanol stain purple are termed Gram-positive.
- Those organisms that lose violet-iodine complex and stain red from the safranin counter stain are termed Gram-negative.

Figure 14.2: Gram Positive and Gram Negative smear observed under HPF Microscopy



14.2.2 KOH preparation

Principle

KOH is a strong alkaline solution used as a cleaning agent to determine fungal infection in specimen. It is used mainly to digest the keratin in the keratinized tissue present in the specimen, so that the fungal elements can be observed clearly. The KOH preparation is used to detect yeast. In addition, a characteristic amine odor may be observed in patients with bacterial vaginosis (Whiff test).

Interpretation

- *Candida albicans* can be detected in KOH preparations, microscopically, they are small, oval, measuring two to four μm in diameter. Budding yeast cells or pseudohyphae may be seen.

Figure 14.3: KOH staining under microscope



14.2.3 Ziehl- Neelsen (ZN) method /Acid fast staining and principle

Mycobacteria possess cell wall that contains mycolic acids, which are long chain, multiple cross-linked fatty acids. These long chain mycolic acids probably serve as complex basic dyes, contributing to the characteristics of acid-fastness that distinguishes mycobacterium from other bacteria.

Figure 14.4: Mycobacterium bacilli rods observed under microscope

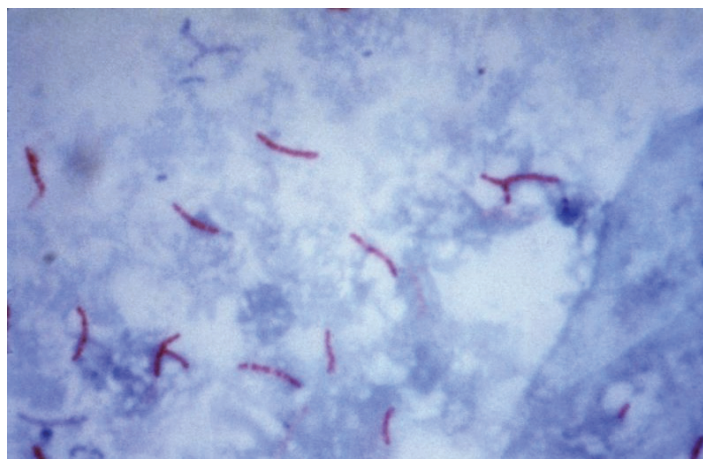


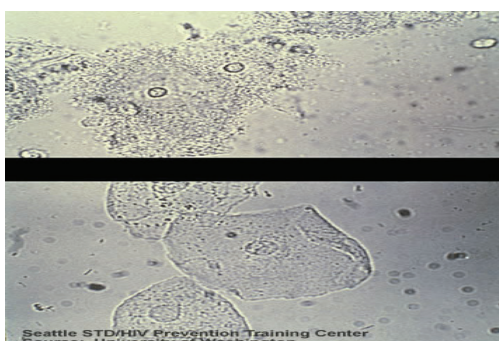
Table 14.1WHO grading: Acid fast bacilli

Examination	Result	Grading	No. of fields to be examined under HPF
≥ 10 AFB/ 100x	positive	3+	20
1-10/100x	positive	2+	50
10-99 AFB/100x	positive	1+	100
1-9 AFB/100	Scanty	Record exact number	100
0 AFB/100	Negative		300

14.2.4 Wet Mount

The saline wet preparation is easily prepared and is used for the rapid detection of *Trichomonas vaginalis* and "clue" cells associated with bacterial vaginosis. Through this technique, organisms can be observed in their original and live state so that it will be helpful to study the characters.

Figure 14.5: Clue cells under microscope



14.3 Culture

A culture (microbial) is a method of multiplying microbial organisms by letting them grow and multiply in predetermined culture under controlled laboratory conditions. Different types of bacterial culture media are available for culture. The nature and type of growth along with biochemical indicator results are applied to identify the type of organism and their character. It is one of the primary diagnostic methods of microbiology and used as a tool to determine the cause of infectious disease by letting the agent multiply in a predetermined medium.

Culture can be performed to detect *Neisseria gonorrhoea*, yeast, etc., but all types of laboratories do not have the facility to perform culture.

Figure 14.6: Bacterial culture (Bacterial colonies seen on media)



14.4 Antigen detection

Antigens are the specific protein or organic materials in cell, tissue or organ. Antigen detection tests can be performed for the diagnosis of STIs. Techniques like direct fluorescent antibody (DFA), ELISA, etc. can be used to detect the antigens. Antigen detection tests are helpful for the diagnosis of Genital herpes and *Chlamydia trachomatis* infection.

14.5 Nucleic acid detection

14.5.1 Nucleic acid amplification tests (NAATs)

A nucleic acid amplification test (NAAT) is a molecular technique used to detect the microorganism by identifying the genome (DNA or RNA) of microorganism. These tests were developed to shorten the window period, the time between when a patient has been infected and when they show up as positive by antibody tests.

These are used to identify small amounts of DNA or RNA in test samples. There are several different kinds of nucleic-acid amplification tests, but they are all based on the same principal. A nucleic-acid amplification test uses a series of repeated reactions to make numerous copies of the DNA or RNA that makes it easier to detect. This amplifies the signal of the nucleic acids in the test sample so that they are easier to identify. Nucleic acid amplification tests like PCR are used to detect gonorrhoea and *Chlamydia trachomatis* infection.

There are multiple methods that fall in this group, that includes Polymerase chain reaction, reverse transcriptase PCR (RT-PCR), Transcription mediated amplification, Branched DNA (quantiplex bDNA), and ligase chain reaction. Molecular tests are generally very sophisticated and require specialized laboratory systems and facilities so not all laboratories are able to perform these tests.

14.6 Serology

Serology, in practice, usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins/particles or to one's own proteins (autoimmune disease). There are several serology techniques that can be used depending on the antibodies being studied. These include ELISA, agglutination, precipitation, complement-fixation, and fluorescent antibodies.

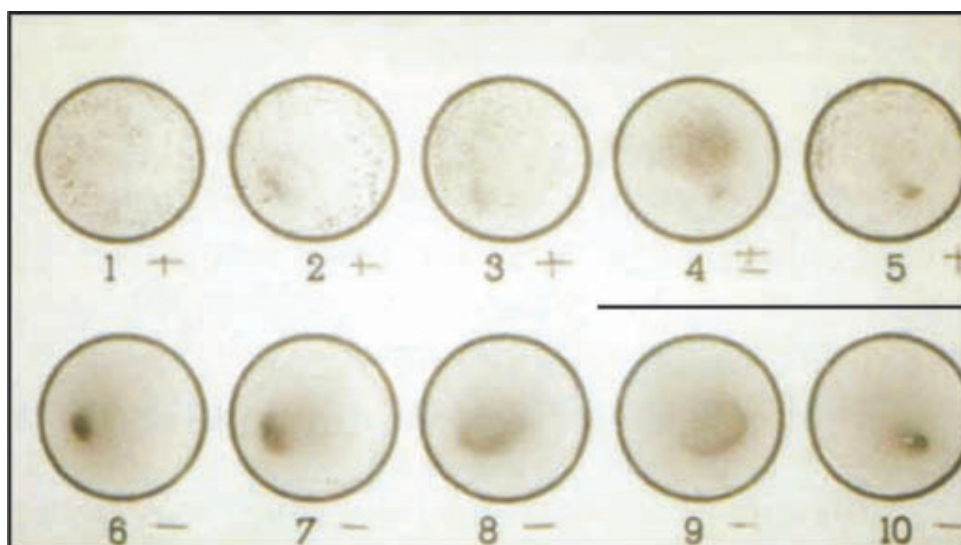
Detection of antibodies is very useful in the diagnosis of different STIs. Serological tests such as RPR and TPPA are important in the diagnosis of Syphilis.

14.6.1 Rapid Plasma Reagin (RPR)

The rapid plasma reagin (RPR) test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis. In the test, the RPR antigen is mixed with plasma on a plastic-coated card. The RPR test measures IgM and IgG antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material, and possibly cardiolipin released from the treponemes. The anti-lipoidal antibodies are antibodies that are produced not only as a consequence of syphilis and other treponemal diseases, but also in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs. If antibodies are present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card. If antibodies are not present, the test mixture is uniformly gray. Without some other evidence for the diagnosis of syphilis, a reactive nontreponemal test does not confirm *T. pallidum* infection. For accurate results, careful controls must be concurrently observed.

In addition to screening for syphilis, an RPR titer can be used to track the progress of the disease over time and its response to therapy.

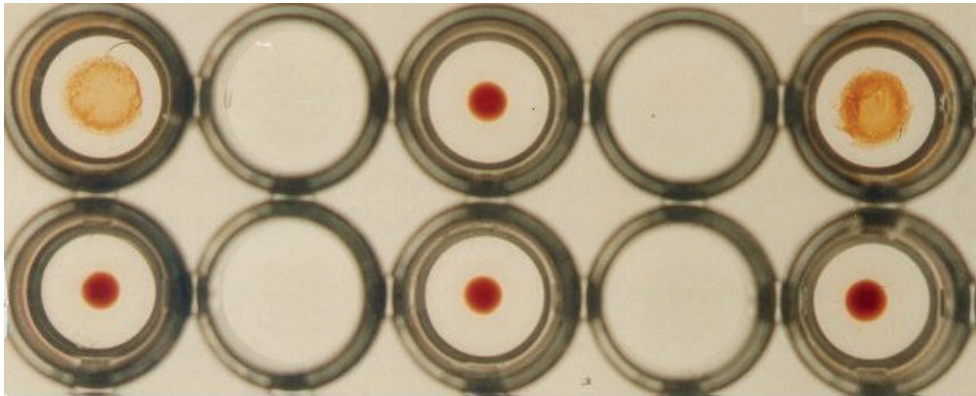
Figure 14.7: Card with Specimen



14.6.2 *Treponema pallidum* particle agglutination (TPPA)

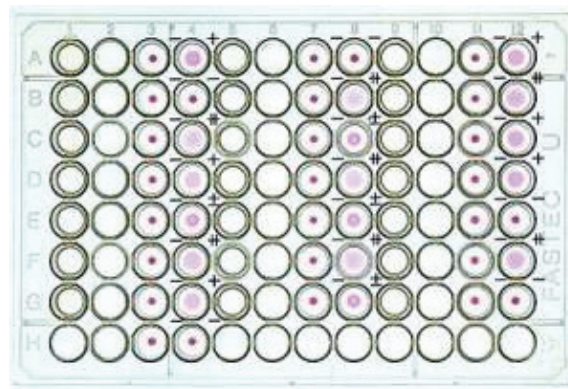
TPPA (or sometimes known as TPHA) is a treponemal serological test for the diagnosis of syphilis. The test is based on the principle that sensitized particles are agglutinated by the presence of antibodies to *Treponema pallidum* in human serum plasma. This is a confirmatory test for diagnosis of Syphilis.

Figure 14.8: TPPA results



The TPPA assay is an indirect agglutination assay used for detection of antibodies against the causative agent of syphilis, *Treponema pallidum* subspecies *pallidum*. In the test, gelatin particles are sensitized with *T. pallidum* antigen. Patient serum is mixed with the reagent containing the sensitized gelatin particles. The agglutinated particles spread out covering the bottom of well uniformly (or peripheral agglutination is observed) when the patient serum is positive for syphilis. In other words, the patient's serum contains antibodies to *T. pallidum*. A negative test shows clumping of gelatin particles in a heap of a button in the center of well with smooth round outer margin. It is used as a confirmatory test for syphilis infection. Now, rapid test kits on syphilis diagnosis are available in the market. These rapid test kits are based on detection of antibody against *T. pallidum* infection (Syphilis).

Figure 14.9: TPPA EIA plate with results



Module review

1. What is the principle and intended use of:
 - o Gram staining
 - o ZN Stain
 - o KOH Mount
 - o Wet Mount
 - o RPR
 - o TPPA
 - o NAAT

MODULE 15

PERFORMING LABORATORY TESTS FOR SEXUALLY TRANSMITTED INFECTIONS AND OPPORTUNISTIC INFECTIONS

Purpose

To provide you with necessary knowledge and skills to accurately perform different laboratory tests for diagnosis of STIs and OIs.

Learning objectives

At the end of this module participants will be able to:

- Explain how to perform different laboratory diagnosis of STIs and OIs following SOP
- Explain and accurately interpret individual test results

Content outline

- Grams staining techniques and interpretation
- Acidfast staining technique and interpretation
- Wet Mount techniques and interpretation
- KOH Mount techniques and interpretation
- RPR techniques and interpretation
- TPPA techniques and interpretation

Time: 60 minutes+60 minutes

Note: Facilitators will provide instructions and participants will perform following the laboratory test following the provided test protocol (SOPs).

MODULE 16

DOCUMENTS AND RECORDS

Purpose

To provide basic understanding on the importance of documents and records in testing site.

Learning objectives

At the end of this module, participants will be able to:

- Explain the difference between a document and a record
- Explain the rationale for following documents and keeping records
- Describe examples of documents and records kept at a test site
- Describe importance of Standard Operating Procedures (SOPs)

Content outline

- Documents and records
- Difference between document and record
- Types of documents and records in HIV testing sites
- Standard Operating Procedures

Time: 30 minutes

16.1 Documents and records

Documents and Records are essential component of the Quality System. As a matter of fact, it is the backbone of the quality system. Documents communicate the policies and procedures that should be followed at each test site. This is important for assuring consistency and accuracy at the test site.

Documents are written policies, process descriptions, and procedures used to communicate information. They provide written instructions for HOW TO do a specific task. Blank forms are also considered documents. Forms are used to capture data or information for performing a procedure.

Records are generated when written instructions are followed. In other words, after data, information, or results are recorded onto a form, it then becomes a record.

Documents and records may be paper or electronic.

A document is any piece of written information in any form, produced or received by an organization or person. It can include databases, website, email messages, word and excel files, letters, and memos. Some of these documents will be ephemeral or of very short-term value and should never end up in a records management system (such as invitations to lunch).

Some documents need to be kept as evidence of business transactions, routine activities or as a result of legal obligations, such as policy documents. These should be placed into an official filing system and at this point, they become official records. In other words, all records start off as documents, but not all documents will ultimately become records.

A document provides information in written, printed, or electronic form.

A record relates to an activity or transaction that has happened in the past; it is a record of history. A record can consist of one or more documents, which all relate to a single event in time.

Documents can be amended. The documents can be amended and they become a new version document. However, it must be ensured that once the new version is in use, the previous should be archived. There should be clear labeling of document such as prepared by, purpose, effective date in relevant area (header, footer) (Format is a document. Filled format becomes a record)

Records cannot be amended (if amended it becomes manipulation)

Note: sometimes corrections need to be made, and they are not necessarily manipulation or falsification of data. Typos, transpositions, putting the wrong value in the wrong column etc.

Not all documents are considered records, but all records can be considered as documents.

16.2 Examples of documents and records

Examples of documents include: country testing algorithm, safety manual, standard operation procedures (SOPs) for an approved HIV rapid test, manufacturer test kit inserts, temperature log (blank form), and quality control record (blank form).

Examples of records include: client test results, summary of findings from on-site evaluation visit, report of corrective actions, daily maintenance log (completed), stock cards and stock book (completed), and EQA specimen transfer log (completed).

Documents are the backbone of quality system

Verbal instructions often are not heard, misunderstood, quickly forgotten, and ignored. Policies, standards, processes, and procedures must be written down, approved, and communicated to all concerned. Documents should also be reviewed and revised as needed on a regular basis and no later than every two years or when a change is needed earlier.

16.3 Standard Operating Procedures (SOPs) are documents

SOPs are documents that describe how to perform various operations in a testing site. They provide step-by-step instructions and assure consistency, accuracy, and quality.

SOPs are one type of document. Using SOPs brings in reliable and consistent results in a uniform manner. SOPs must be followed. Staff following a SOP must be trained in the SOP and the competency should be documented.

SOPs are controlled documents

Controlled documents means documents must be approved for use in-country, have document control features, and be kept up-to-date. Key features of SOPs include:

- Cover page
- Descriptive title
- SOP number
- Version number
- Date when SOP becomes effective
- Signature of person responsible for writing the SOP
- Signature of person authorizing the SOP
- Number of pages

Each test site should have on hand current/approved SOPs. Typical SOPs kept at a test site include:

- Daily routine schedule
- Country policies and algorithm
- Safety manuals (for example, safety precautions, preparation of 10% (vol / vol) bleach solution, and post-HIV exposure prophylaxis management and treatment guidelines)
- Blood collection (for example, finger prick, venipuncture, and DBS)
- Test procedures

- Reporting procedures
- Corrective and preventive action
- IQC (if not included in the testing SOP)
- Storage and disposal of samples
- Archiving of laboratory records
- External Quality Assessment Scheme (EQAS) form and records (for example, submission of EQAS specimens to reference lab and for internal assessments)
- Reordering of supplies and kits
- Equipment use and maintenance
- Temperature monitoring of equipment and facilities
- Storage of reagents and test kits

Do not rely solely on manufacturer product inserts, follow SOPs

Manufacturer product inserts do not provide specific information for test sites. Examples include:

- Materials required, but not in kit.
- Specific safety requirements.
- Sequence of tests in country algorithm.
- External quality control requirements.
- Maintenance of equipment needed to perform the test, for example, pipette calibration

16.4 Proper record-keeping makes quality management possible

Record-keeping allows a test site to:

- Communicate accurately and effectively - Record keeping enables sites to timely report to program managers and site supervisors.
- Minimize error - All records must be written.
- Monitor quality system - Records allow for periodic review of testing operations. Only through the review of records can improvements be identified.
- Assist management in developing policy and plans and monitoring and evaluating programs.

16.5 Records at a test site

It is recommended that you keep these records at the test site:

- Specimen transfer logs
- HIV request / client test result
- Lab / Test register
- Temperature logs
- Equipment maintenance logs
- Inventory records

16.6 Tips for good record keeping

Here are some tips for good record-keeping:

- Understand the information to be collected. Before you record any information, make sure that you understand what is to be collected.
- Record the information every time. Record in the appropriate form each time you perform a procedure.
- Record all the information. Make sure you have provided all the information requested in a form.
- Record the information the same way every time. Be consistent in how you record information.

16.7 Client test records

Types of information captured on test records include:

- Client/Patient ID number
- Date of test
- Results from Test 1, Test 2, and Test 3

- Repeat results
- HIV status
- Kit Name and Lot number
- Person performing test

16.8 How long should you retain client records?

All records must be maintained and stored securely. The length of time needed to store test site records will depend on national policies, and the availability of secure storage space at the test site.

16.9 Logbooks are cumulative records of test site operations

These photos of logbooks are common. Storage of logbooks and records should be kept in a manner that will minimize deterioration. Although many sites use paper-based logbooks and records, they should be indexed to allow for easy access.

Figure 16.1: Photos of logbooks



16.10 Records should be permanent, secure, traceable

Facilities where records are kept should be secure to maintain patient/client confidentiality. Procedures and mechanisms should prevent unauthorized access.

Records should be permanent, secure, and traceable. Examples of keeping records permanent include: keep books bound, number pages, use permanent ink, and control storage. To keep records secure, you need to maintain confidentiality, limit access, and protect them from environmental hazards. To keep records traceable, make sure every record is signed and dated. If mistakenly written, the records should be corrected with initial signature on it. Never use the tipex marker or white wash for correction.

16.11 Information recorded will feed into monitoring and evaluation systems

Records must be kept permanent, secure, and traceable because they will be used for reporting and monitoring purposes. Monitoring is the routine tracking of program information. Accurate facility records offer essential information for providing quality health care and monitoring PMTCT programs. It is recommended that you analyze, on a monthly basis, the number of clients served and summarize the test results.

16.12 Examples of ledgers used and fill up process (demonstration and exercise)

Module review

1. What is the difference between a document and a record?
2. What are some examples of documents and records?
3. Name examples of information not found in a manufacturer product insert.
4. What are some key features of SOPs ?
5. How should records be maintained ?

MODULE 17

PROFESSIONAL ETHICS

Purpose

To provide concept on ethics and to apply ethical conduct in HIV testing.

Learning objectives

At the end of this module, participants will be able to:

- Describe ethical issues related to HIV testing
- Explain the importance of professional ethics
- Explain and apply ethical conduct in HIV testing
- Describe and take appropriate actions to maintain confidentiality and integrity in HIV testing

Content outline

- What is ethics?
- Why is ethics important?
- Who is responsible for ethics?
- How is ethics applied to HIV testing?
- Maintaining confidentiality
- Code of conduct

Time: 30 minutes

17.1 What is ethics?

Ethics is “A set of principles of right conduct”. This leads to perform the activity with integrity in the right manner. In other words if we do not apply a code of ethics the patient or someone else will suffer.

There are three widely recognized principles that apply to both clinical and research ethics: respect for persons, beneficence, and justice. Respect for persons entails respecting the decisions of autonomous persons and protecting persons who lack decision making capacity. It also imposes an obligation to treat persons with respect by maintaining confidence and keeping promises. Beneficence imposes a positive obligation to act in the best interests of patients or research participants. Justice requires that people should be treated fairly without any biases and discrimination. It is often understood that benefits and burdens be distributed fairly within society.

17.2 Why ethics is important?

Decisions about diagnosis, prognosis and treatment are frequently based on results and interpretations of laboratory tests. Irreversible harm may be caused by erroneous tests. The key ethical issues related to HIV/AIDS testing, treatment, and research includes confidentiality, informed consent, conflict of interest, and vulnerable populations.

17.3 Maintaining confidentiality

It is important to:

- Keep all client/patient information private including clinical checking of patients in a private area where they cannot be overheard or seen by other patients
- Secure all records / logbooks
- Restrict access to testing areas

Remember - prior to testing, clients should be informed about the purpose, advantages, and disadvantages of testing. This process ensures understanding of the counseling and testing process. Keeping information confidential means that it is kept a secret from everyone except those who have a direct involvement with their care. People often violate ethics not because they mean to, but because they are careless. Therefore, we must be extra vigilant about ethical conduct.

17.4 Who is responsible for ethics?

Everyone at a testing site plays a part. Anyone who plays a part in testing or has access to the test results must adhere to ethical conduct. This includes laboratory staff, nurse counselors, clerks, secretary, general hand, and driver.

Incidents may happen where specimens are damaged, results are falsified, or confidentiality is broken. Equally they can decide to ensure that specimens are delivered, recorded, stored and reported with high quality.

17.5 How do we apply ethics to HIV rapid testing?

Ethics is applied in the work that we do at the test site. This includes:

- Using only kits approved for use in the country
- Ensuring quality outputs: Follow SOPs as written. If a test procedure calls for 20 minutes incubation or wait time, DO NOT take shortcuts. Wait the full time before recording and reporting test results.
- Keeping supplies and kits in safekeeping. Unauthorized use of test kits outside of the testing site is prohibited.
- If you have questions, ASK.
- DO NOT falsify results.
- DO NOT tell anyone about the results unless they are authorized to know.
- Only perform the tests agreed to by the patient and nothing else.

Ethics is also applied in your behavior. Always conduct yourself in a professional manner. Examples of professionalism include:

- Dressing appropriately. If lab coat or apron is soiled/spoiled, change to a clean one
- Turning cell phones off. It is disruptive and not considerate of clients to talk on the phone or text/SMS during the course of testing.
- Not discussing with others, the results or interaction with clients. Maintaining patient confidentiality is a MUST.
- Not making any comments that judge the client. Treating them with the respect that you yourself expect to be treated with.

Behavior of management: Management sets the example or expectations of how staff should conduct themselves.

17.6 What is a code of ethics?

A Code of ethics is an expression of basic values - the principles and standards by which you should conduct yourself. A number of laboratory professional organizations have code of ethics, with common principles of conduct.

17.6.1 Code of ethics (IFBLS)

Excerpts from International Federation of Biomedical Laboratory Science (IFBLS):

- Maintain strict confidentiality of patient information and test results.
- Safeguard the dignity and privacy of patients.
- Be accountable for the quality and integrity of clinical laboratory services. You must take personal responsibility for everything you do and be able to answer for your conduct and moral obligations to choose to do right over wrong.

17.6.2 Code of ethics (ASCP)

Excerpts from American Society for Clinical Pathology (ASCP)

- Treat patients and colleagues with respect, care and thoughtfulness
- Perform duties in an accurate, precise, timely and responsible manner
- Safeguard patient information as confidential, within the limits of the law
- Prudently use laboratory resources

a. Scenario I

A pregnant woman comes for HIV testing. Your test site has just run out of the second test in the algorithm. You tell her that she will have to come back in two days. She becomes very emotional and explains that she has traveled a long distance after finally deciding to get tested and won't be back in the area for a long time.

Feeling sorry for her, you proceed to perform test one, and report a resulting positive test to the client.

What are the issues here?

What is the right thing to do?

What are the consequences if you do not do the right thing?

b. Scenario II

At the HIV rapid testing site, you discover that you just ran out of the buffer for Test 1 of the algorithm. Rather than denying testing to clients, you decide to go ahead and perform Test 1 using the buffer from kits of Test 2.

What are the issues here?

What is the right thing to do?

What are the consequences if you do not do the right thing?

c. Scenario III

Today is Monday. You discover that there are enough test devices to last through the entire week, but they will expire on Wednesday.

Since resources are tight and you don't want to waste any test kits (it is only a couple of days past expiration anyway), you decide to use the test devices until the end of the week.

What are the issues here?

What is the right thing to do?

What are the consequences if you don't do the right thing?

d. Scenario IV

Ram, the tester, is excited about getting home at the end of his work day, because a relative he has not seen in quite some time is scheduled to arrive. Right before he is ready to leave, he gets distracted by a phone call and forgets to lock up the lab register in the cabinet.

What are the issues here?

What is the right thing to do?

What are the consequences if you do not do the right thing?

How hard is it for you to do the right thing?

17.7 Consequences of a false positive or false negative result

A false positive HIV result can lead to considerable personal stress and, family and marital problems. A false negative result can lead to increased transmission.

Each result you report is connected to a patient/client. A lot is at stake – people's health, lives, mental health. The emotional costs of a wrong result can be huge. Also the health consequences can be enormous. Considerable personal stress also includes depression, fear and even suicidal tendencies.

You must strive to do the right things right.

Module review

- In your own words, what is ethics?
- Why is ethics important?
- Give examples of actions you can take to maintain client confidentiality
- Give an example of a code of ethics to which you are willing to personally commit.

ANNEX I: GROUND RULES

- **Punctuality:** All the participants and facilitators are requested to arrive on time.
 - >> Starting time:AM
 - >> End Time:PM
- **Participant's performance evaluation:**
 - >> Pre-test: On the first day.
 - >> Post-test: At the end (on the last day)
- **Attendance Record:**
 - >> Daily attendance record will be maintained. All the participants are expected to stay full time. (For DA provision: attendance record will be referred).
- **Laboratory safety:**
 - >> Since the participants will be handling infectious agents/materials, the participants should follow the universal precautions.

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