

National AIDS Control Organization

Government of India

Write-Ups of Modules for ICTC Laboratory Technician's Training

1.NACO LABORATORY TRAINING PROGRAMME

SLIDE 1: Introduction

- Interact with Laboratory Technicians
- Ask simple questions about HIV/AIDS to assess how much they already know.
- Try to find out what their beliefs about HIV are.
- Find out if they are scared or apprehensive about dealing with HIV positive samples & clients.
- Explain the basics-to assure them that they have no need to be apprehensive. Infection cannot be acquired if they practice standard work precautions.
- Ensure that they are immunized for HBV.

SLIDE 2: What do LTs want to achieve?

- Explain in brief taking help of slides as to what is expected from the LTs and how they can deliver it.
- Also what they should know to deliver quality services.

SLIDE 3: What do we want to achieve?

- Read out all the details given on the slide.

- Briefly describe the content of the training being imparted i.e. there will be theoretical lectures, demonstrations, practicals and interactive question and answers sessions on each of the topics mentioned on the slide.
- Also take LTs input on language to be spoken, any additional information required by LTs.
- Make a note of it to incorporate the same into the content subsequently.

2. VIROLOGY, PATHOGENESIS AND TREATMENT OF HIV INFECTION

SLIDE1- Sessions Objectives

- This module details the basic of HIV, how it is acquired, how it produces disease and what we can do to diagnosis, manage and prevent this deadly infection.
- This is a very important module this basic knowledge not only will help you do your job efficiently, it will also help you to educate your family members, friends and community about HIV & AIDS, remove myths & misconceptions and reduce stigmatization and victimization.

SLIDE2- HIV and AIDS

- Give a brief history of how & where HIV was discovered i.e. way back in 1981 in New York & San Francisco normal adult males started having Kaposi sarcoma & Pneumocystis jirovecii pneumonia.
- The common denominators in these adult otherwise healthy males was immunodeficiency, were homosexual and the agent was being spread to sexual contacts.
- The condition was named AIDS.
- Give expansion of HIV (Human immunodeficiency virus) and AIDS (Acquired immunodeficiency syndrome).
- Describe why the condition can't be cured & why so far no vaccine due to virus changing its coat (genetic mutation) & evading immune response.
- The virus infects and kills the cells which give us resistance to fight microbes i.e. CD4 cells.
- Power to fight infections is gradually reduced (give example of termite slowly chipping away the wood) till there is collapse of immune system infections & death if ART is not given in timely manner.

SLIDE3- Disease burden due to HIV

- HIV infection can occur in anyone who practices risk behaviours irrespective of caste, creed, social and education status.
- All over the world there are estimated to be 33 million infected individual & every day more infections are taking place.
- It is estimated, based on sentinel surveillance that .36% of Indians are infected with HIV we do not know who these people are and where these people are.
- This is because HIV on infection does not produce any symptoms suggestive of HIV and diagnosis can only be made by testing blood for HIV antibodies. Blood will be HIV antibody positive only after the lapse of window period.

SLIDE4- Structure of HIV

- The slide shows a picture of HIV.
- The mushroom like structure on the outside have the head/(GP120) with which virus will attach to CD 4 receptor on CD 4 T lymphocyte. It fits on cell like a lock & key system.
- Next the stem of mushroom which is GP 41 bring about the fusion of virus with the cell and viral DNA goes into the cytoplasm of cell. Once this happens virus has established itself for life in the individual.
- Mention the other antigens and enzymes mentioned in the slide and emphasize that virus has the complete software to replicate itself, it only needs a computer (living cell- CD4 carrying cells) to start its programme in the infected individual.
- Emphasize that antibodies are produced against all these proteins which form basis of HIV diagnosis.

SLIDE 5- Routes/Mechanisms of Transmission

- HIV is such a disease which will not occur as it is acquired through high risk behavior except for infants borne to HIV positive in mothers. These babies get infected from their mothers.
- Describe the various routes of transmission given in the slide.
- Emphasize most common route of transmission unsafe sexual practices.

SLIDE 6- Prevention of HIV Transmission

- This slide describes how to prevent and control transmission of HIV and hence new cases through each route of transmission mentioned in the previous slide by taking specific precautions specific for each route.
- Emphasize use of condom every time a sexual contact is made.
- Emphasize blood safety screened/tested blood.
- Inform about PPTCT i.e. Nevirapine administration to HIV Positive mother and breast feeding as per guidelines to prevent transmission to baby.
- Emphasize use of sterile needle & equipment. Needle exchange programme for IDU & harm reduction i.e. injectable to oral drugs for IDUs.

SLIDE 7- Susceptibility of HIV

- Fortunately HIV is a fragile virus. It dies rapidly on drying.
- Every method used in a microbiology for disinfection & sterilization kills HIV.
- However, always take standard work precautions while working in lab.

SLIDE 8-HIV Pathophysiology – Life Cycle

- HIV coming near CD4 cells due to GP 120 and CD4 being complementary.

SLIDE 9- Attachment

- Diagrammatic picture of HIV & receptor to show how the GP 120 (head of mushroom) fits onto the CD4 receptor depicted as round empty vessel like structure.

SLIDE 10-HIV Pathophysiology – Life Cycle

- This slide shows of HIV binding to CD4 receptor on CD4 T cell by its GP 120 protein (mushroom head) on the surface 72 such binding sites on HIV surface.

SLIDE 11-HIV Pathophysiology – Life Cycle

- The stem of mushroom GP41 along with co-receptors (CCR5 or CXCR4) shown in previous slide on cell surface fuse together, a sort of channel is formed between HIV and cell.

SLIDE 12-HIV Pathophysiology – Life Cycle

- The HIV RNA along with the enzymes are released into the cytoplasm of the cell.

SLIDE 13- HIV Pathophysiology – Life Cycle

- HIV RNA with the help of enzyme “Transcriptase” starts transcribing HIV RNA into complementary DNA (cDNA).

SLIDE 14- HIV Pathophysiology – Life Cycle

- Reverse transcription means that HIV RNA directs the synthesis of cDNA.
- Normally DNA directs transcription resulting into formation of corresponding RNA.
- Here give example of our genes.
- HIV is a retro – virus so reverse transcription.

SLIDE 15- HIV Pathophysiology – Life Cycle

- Once the double stranded HIV C DNA is formed, it translocates i.e. enters into the cell nucleus.

SLIDE 16- HIV Pathophysiology – Life Cycle

- The HIV DNA integrates into the cell chromosome under the effect of HIV enzyme integrase. Wherever open chromatin framework is available.
- Now HIV DNA (the virus software) has become a part of nucleus and takes over the function of formation of HIV RNA from DNA.

SLIDE17- HIV Pathophysiology – Life Cycle

- A big HIV RNA molecule is synthesized at the command of HIV DNA present in nucleus --- and is released into the cytoplasm.
- Under the effect of the 3rd HIV enzyme protease – the HIV accurate size proteins are formed from the big molecule protein formed by translation of a HIV RNA.
- HIV RNA in cytoplasm→cDNA→enters nucleus→HIV RNA→Big protein molecule→accurate size proteins→assemble at cell membrane→released by budding as HIV.
- This infected cell becomes a factory synthesizing thousands of virion on and on till cell is destroyed.

SLIDE18- HIV Pathophysiology – Life Cycle

- Proteins helping to cut accurate size proteins.
- Assembly of virus RNA and proteins at cell membrane
- Release into circulation/exterior by budding
- Acquires lipid coat from host cell membrane during budding

Normal Defense Of Body And Host ResponseTo HIV Infection (Natural History Of HIV Infection)

In order to understand how a perfectly healthy individual becomes immunodeficient progressively, slowly after acquiring HIV infection, we have to know the normal defense mechanisms of our body.

SLIDE 1-Various Blood Components

- Blood has plasma, serum with various proteins.
- Blood has many cells which include white blood cells (polymorphs, monocytes. Eosinophils and lymphocytes) and Red Blood Cells.

SLIDE 2-Normal Body Defenses and HIV

- Lymphocyte is a composite population of many sub population which have different receptors on their surface.
- Important cells involved in body defense are
 - B - cells
 - T- cells (CD4 T cells, CD8 T cells)
- Describe functions of these cells give examples.

SLIDE 3-Window Period

- After a person gets HIV infection she/he can't be diagnosed as HIV infected by HIV antibody tests for 3 weeks to 3 months (sometimes). This period is called window period.
- During this period HIV is multiplying in the body tissues, person can infect others through blood and unsafe sexual practices and we cannot detect infection in this individual by available antibody detection systems.
- This is because body takes sometime to form antibody against HIV proteins and it takes sometime for these antibodies to rise up to the levels at which they can be detected.

SLIDE 4-Natural History of HIV – 1 Infections Progressing to AIDS*

- This slide depicts the natural course of HIV disease from infection, passing through different stages, ultimately resulting in AIDS if ART is not administered at the right time, the kinetics of HIV and CD4 cells in the blood through these stages.
- First stage is when person gets infected with HIV and may or may not suffer from cold flu like symptoms. This is acute HIV infection stage HIV levels rise in blood, antibody start forming, CD4 cells fall sharply.
- Next stage sets in as the individual starts mounting an immune response (both humoral as well as cell mediated). So CD4 cells level rises to almost normal, virus level falls & person is totally healthy. This is clinical latency stage and can last for years. Person can spread infection to others, virus replication continues and CD4 cells are slowly and progressively destroyed.
- Finally the CD4 cell levels fall to 200 cells/cm or even lower and opportunistic infections(OIs) set in this is beginning of AIDS.
- If not treated this patient will die within 1-2 years.
- CD4 cells are low & virus level is high. Once HIV infection occurs what happens is like two equally matched sumo wrestlers (our body defense and HIV) fighting each other for supremacy. As long as the body defense system is strong the person stays healthy. Since the virus kills CD4 cells which protect us, slowly and progressively the defense system is destroyed and the body gives in to invaders the microbial infection. If ARVs (ART) is given then virus is killed and becomes weak and body defense is protected and the individual lives and almost near normal life with certain limitations due to presence of HIV in the blood, though at undetectable levels.

SLIDE 5-Association between opportunistic infections and CD4+-Lymphocyte count

- This slide shows how an HIV infected person become susceptible to more and more microbial and opportunistic infections as the CD4 cells responsible for our defense are gradually destroyed by HIV.

- Life threatening infections (OIs) occur when CD4 cell count < 50 T cells/cmm
- As TB is endemic in our country it can occur inspite of higher CD4 counts.
- Explain the slide-read out infections which commonly occurs at various different levels of CD4 T cells.

SLIDE 6-Rate of progression of HIV infection without ART

- HIV infection progresses at different rates in different individuals.
- This is because of various host factors (genetic, presence of other diseases (TB,STI & IDU) nutritional and immune status at the time of HIV infection) on one hand and HIV virulence on the other hand. HIV 2 is less virulent and less transmissible compared to HIV1.
- Based on multicentric data three types of HIV disease progression have been observed – classic, rapid & slow.
- Explain the three types from the slide.
- Give example of your own experience on progression on HIV.

SLIDE 7-Life Cycle and ARVs site of action

- This slide depicts the various steps in the replication cycle of HIV at which different groups of anti-retroviral drugs act.
- Describe the steps of replication like
- Fusion →where fusion inhibitors act & stop entry of HIV into the cell.
- Reverse transcription →on which RT enzyme inhibitors act and stop the reverse transcription. So that cDNA is not formed or faulty molecule is formed which is no good for virus.
- Integration →HIV cDNA has to be integrated into nucleus to start forming HIV RNA, enzymes & proteins. Integrase inhibitors stop/inhibit this step so virus is not formed.
- Protease inhibitors→stop the HIV enzyme protease from cutting the big HIV protein into accurate size HIV protein molecules.

SLIDE 8- Art Works: Progression to AIDS/Death

- You must be wondering why should we be giving three anti retroviral drugs to HIV infected individual. This is based on evidence depicted.
- HIV infected were given the first drug which was discovered and were O.K. for sometime and then deteriorated (monotherapy).
- The second ARV was discovered and both drugs were given together to counter and delay the development of drug resistance by HIV. These individual fared slightly better.
- Then more and more drugs were discovered and it was formed that when a combination of 3 drugs (acting at different sites of HIV replication) was given, best result was seen.
- A combination of 3 ARVs is given to hit the virus hard, achieve undetectable HIV level in blood, delay development of drug resistance.

3. DETECTION OF HIV INFECTION IN THE LABORATORY USING HIV ELISA AND RAPID TESTS

SLIDE 1: Learning Objectives

- This module will enable the lab supervisor and the LT to understand various issues related to HIV testing.
- It will help to understand the strategies of testing which are to be followed as per HIV testing guidelines.
- LT will also learn the process, of HIV testing.

SLIDE 2: Detection Of HIV Infection

- HIV testing is important as diagnosis of HIV infection is laboratory based and not clinical.
- There are no specific symptoms produced by HIV infection as such which indicate that a person is HIV infected.
- HIV infection, in addition, is asymptomatic for a long time. During this stage HIV antibody detection tests are positive and person is HIV infected.
- The only way to detect HIV infection is by various laboratory tests used either to detect HIV antibodies or HIV antigens (P 24 Ag) and HIV DNA or RNA.

SLIDE 3: Aims Of Laboratory Support

- This slide enumerates various benefits of HIV testing even through the cure for HIV does not exist so far.
- HIV testing is undertaken in different situations to fulfill different objectives e.g. for blood safety, for PPTCT by surveillance, for PEP management and for estimating disease burden in the community.

SLIDE 4: Approach to HIV Testing

- The individual who access the ICTC services are usually those practicing some risk behavior. This should be kept in mind. Though, there may be some who just want to know there HIV status.
- There is definite process which has to be followed at ICTC. The steps of the process are given in this slide.
- Describe each step and also explain the importance of pre-test counseling, confidentially and post-test counseling.
- Emphasize that all those found to be positive must be referred to the designated ART site for further care.
- In case the test result is found to be negative, the client must be record for follow-up and repeat testing to rule out window period.

SLIDE 5: General Principles

- HIV testing is not done in isolation. It is always done as a part of the care and support programme.
- The HIV test used should be technically the right ones i.e. they should detect antibodies to all types and sub-types of HIV I and II, should have the desired sensitivity and specificity, should detect infection in early seroconverters.
- The strategy and protocol of testing followed should be commensurate with the objective of testing.
- Strategy III is followed at ICTC for asymptomatic cases & strategy II B is used in symptomatic (symptoms suggestive of AIDS) cases.

- Quality assurance is practiced for HIV testing each and everyday as wrong results have grave implications. False positive at ICTC can play havoc with the life of the client and false negative at blood bank can infect individual.

SLIDE 6: Testing Procedure

- There are three types of HIV testing undertaken to fulfill various objectives.
- Unlinked, anonymous HIV testing is undertaken for sentinel surveillance. In this type of testing the result of the test cannot be linked to an individual and secondly even the LT or the doctor do not know the name of the individual testing positive. Here, two different test kits are used and testing is as per strategy II A for sentinel surveillance.
- Voluntary and confidential testing after informed written consent or provider initiated HIV testing(without consent) is undertaken at ICTC. Pre test and post test counseling is a must for testing at ICTC and confidentiality has to be maintained.
- Mandatory testing is not recommended in our country.

SLIDE 7: Three Types of Tests

- The HIV test used for detecting HIV antibodies are named according to the purpose for which they are being used.
- The first HIV test used for detecting antibodies is called the screening test. It can be a rapid or an ELISA, but has to be highly sensitive(NACO recommendation 99.8%) and a reactive screening test indicates that this serum has to be tested again with the second and third HIV test, which are based on different HIV antigens or different principle of HIV testing. These tests are called supplemental or confirmatory tests.
- The third category are tests which are used to detect HIV P24 antigens and/or HIV RNA or DNA. These tests are not recommended as screening tests. These are used for specific situations eg. HIV. DNA PCR is to be used for diagnosing HIV infections in HIV exposed infants less than 18 months old.

SLIDE 8: Laboratory Diagnosis

- The main stay of HIV diagnosis in India is detection of HIV antibodies.
- At ICTC three different HIV test kits are used to designate a sample as HIV positive. These are the first kit-screening test A1, if A1 gives a reactive result then supplemental second and third kit are used to test the sample. These are labeled A2 and A3 respectively.
- A sample to be labeled as positive for HIV antibodies has to test as reactive with A1, A2 and A3.
- A sample testing reactive with A1 and A2 or A1 and A3 and testing negative with A3 or A2 i.e. two tests out of three are reactive is labeled as indeterminate.
- Such a case has to be followed up and retested.
- A repeatedly indeterminate sample should be sent to NRL for WB and/or any other test (P24 or DNA PCR) which may help to resolve the indeterminate result.
- If A1 is non-reactive report as negative and if A1 is reactive and A2 and A3 are non-reactive report as negative.

SLIDE 9: Characteristics of HIV rapid tests

- Rapid test available commercially are based on four different immunologic principles.
- The four principles are given in the slide.
- Explain this:
- Results of rapid tests are ready in less than or upto by 30 minutes.
- Rapid tests are used at ICTC, so that result may be given on same day to the client.
- Rapid tests are procured as per the specifications given by NACO.

SLIDE 10: How Immunoconcentration Works

- One of the principles on which some of the rapid HIV tests are based is immunoconcentration.
- Device used is usually a cartridge and various reactants flow through the membrane (with HIV peptides) mounted on the device.

SLIDE 11: Tests Based On Immunoconcentration

- This picture shows an example of flow through device.
- One dot is control (Procedural or Ig), one is HIV peptides. The third is HIV 2 peptides.

SLIDE 12: How Immunochromatography Works

- Immunochromatography where reactants flow laterally-**capillary** action.
- Explain the chart as shown.

SLIDE 13: Test Based On Immunochromatography

- This slide shows an example of an ICT strip-
- This strip is determine test.
- There are many commercially available rapid based on ICT from different manufactures.

SLIDE 14: Reading Results: Determine

- This slide shows the results with two different sua (one non reactive and the other reactive)
- Explain the charts as shown.

SLIDE 15: How Particle Agglutination Works

- The slide shows antigen and antibodies forming a lattice by agglutination (clumping).

SLIDE 16: Test Based On Agglutination

- The slide shows the test cartridge for agglutination test from Capillus.
- There are other manufactures also who make HIV rapid tests based on principle of agglutination.

SLIDE 17: Reading Result: capillus

- This slide shows the same cartridges after the test has been performed.
- Slide shows strong reactive, weak reactive and negative test result cartridges.
- Explain the slide as shown.

SLIDE 18: There are only three possible outcomes for single HIV antibody tests

- In rapid kits after the test has been performed the reactive result will appear as a colored line, colored dot, agglutination, etc.
- For the rapid test result to be valid the controls should work and the control dot/line/band must always give a positive reading irrespective whether the test sample HIV positive or negative. If control does not work, test is invalid. Repeat.

SLIDE 19: ELISA Principle

- This picture depicts the reactants that take part in different steps of an indirect ELISA and how in a positive/reactive sample ultimately the color develops after adding the substrate.

- Explain the slide as shown.

SLIDE 20: ELISA Plate Showing Positive (colored) and negative (colorless) Result

- The slide shows an ELISA plate after performance of ELISA.
- Colored walls are reactive result.
- Colorless walls non reactive samples.

SLIDE 21: Validation of ELISA Result

- For performance of ELISA, a known external positive control(in addition to kit controls) is always used as a measure of IQC.
- Both sets of controls have to perform accurately within the range for the ELISA run to be valid.
- Kit controls must give result i.e. OD values within the range given in kit insert.
- Kit controls are used to calculate the cut off values.
- External QC samples must give the ODs equal to mean \pm 2 SD of the given value. So variation can be only \pm 2 SD of the mean value which is already known.
- Whether the external control samples OD is within range or not is known by plotting the obtained OD value on the Levy Jennings Chart.

SLIDE 22: HIV Testing strategy II B

- This slide shows strategy IIB of HIV testing.
- Three different HIV test kits are required to follow this strategy.
- It is used to diagnosis who are clinically symptomatic with symptoms pointing to AIDS.
- Serial testing is done on a sample found to be reactive with the screening assay.

- A clinically symptomatic as is diagnosed as HIV infected if his/her sample tests reactive with 2 different HIV test kits.(rapid or ELISA)

SLIDE 23: HIV Testing Strategy III

- This slide given the Algorithm followed for strategy III of HIV testing used of ICTC.
- Three different HIV tests kits are required to practice this strategy.
- Sample is tested serially one kit after another in case sample gives reactive result with screening tests.
- This strategy is practiced on symptomatic healthy individuals who opt to undergo HIV testing.
- A1, A2, A3 all have to give the reactive result for the sample to be declared as HIV positive.
- Two out of three tests giving reactive results mean the sample result is indeterminate.
- Such a case has to be retested (2-4 weeks) to resolve the indeterminate results.

SLIDE 24: HIV Testing Strategy III

- This slide is the description of flow chart of strategy III given in the previous slide.

SLIDE 25: Invalid result-What do you do?

- If any test results rapid or ELISA is found to be invalid result the repeat testing has to be done. Sometimes repeat testing may have to be done on a repeat sample- if the problem was with the sample.
- Must investigate and find out the cause of invalid result and take corrective action.
- Make record of all activities the invalid result and activities related to invalid results.
- Inform the supervisor.
- Do not report such a result to the client.

4.Prevention of blood borne and hospital acquired infections including HIV/AIDS standard work precautions.

SLIDE 1:Introduction

- How are exposed to blood and body fluids while they are working for patients(collecting blood, processing specimens doing other jobs in hospital) and there is a chance though remote that if proper SWP are not practiced then may get infection from the contaminated materials.
- So, it is important to learn and to know how to protect ourselves, not be panicky and provide the services to patient and fulfill the requirements of our profession efficiently.

SLIDE 2:The chain of infection

- Micro-organisms(bacteria, viruses, parasites or fungi) are the disease producing agents. HIV is the agent.
- Humans are the reservoirs for HIV.
- Blood and secretions.
- Person to person.
- Compromised skin and mucous membranes.
- All are susceptible to HIV.

SLIDE 3:Descending order of resistance to germicidal chemicals

- If we see how sturdy and resistant micro-organisms are to the disinfectant and germicidal chemicals we use in labs and hospitals we find that bacterial spores are the most resistant followed by mycobacteria, fungi and other bacteria.
- On this scale HIV is the most sensitive virus, even more than hepatitis B virus.
- So, we should not be scared of working at ICTCs and if we take precautions, chances of getting infected are almost nil.

SLIDE 4:Important infections associated with exposure to contaminated blood

- HIV is not the only virus transmitted through blood and body fluids.
- There are many micro-organisms which are present in blood of an infected individual and which can be acquired from blood, body fluid of these patients if SWP are not practiced.
- Some of these organisms include HBV, HCV etc.

SLIDE 5:Risk Procedures

- The supervisors should understand that there are various routine procedures in the hospital in wards, OPD and OTs etc, which can lead to transmission of blood borne pathogens if SWP are not practiced.

- These are given in the slide, read out all.
- As far as laboratory are concerned the collection of blood and processing of blood and processing of various specimens are potentially hazardous and SWP should be practiced.

SLIDE 6: Modes of exposure of blood borne pathogens in the laboratory

- HCW working in the laboratory is exposed to the risk of accidental exposure right from collection of blood sample, example needle stick injury, transport of sample(broken vial, spillage etc.) and processing of samples.
- Care should be taken to prevent infection during all procedures in the laboratory by practicing caution and SWP.
- We should not forget that the person who cleans the laboratory surfaces and disposes off the waste is as much at risk as the LT and officer in-charges.
- So, always disinfect surfaces and waste at the place where activity is going on so that cleaners are also safe and do not get infected.

SLIDE 7: Risk factors for occupational HBV & HIV infection in health care professionals.

- The risk of getting infection through accidental exposure is proportionate to the risk behavior of patient population, occupational area of HCW in the hospital.
- Risk is more in some disciplines of medicine like Obs-gynae, Dialysis units, laboratories and others.
- Whenever there is exposure to large amounts of blood and body fluids chances of infection are more compared to other specialities like psychiatry, general physician etc.

SLIDE 8: HIV and Environment

- Some experiments have been done with cultured virus to see for how much time it can survive outside the human body.
- This slide shows the results of those experiments.
- As you can see that most of the virus dies when kept at different temperatures and for different times.
- However, still whenever one comes across dried blood or body fluids even though environment most of HIV has died, still SWP must be followed.

SLIDE 9:Characterstics of blood borne viruses during the window period

- It is important to know that there is a period following infection with viruses(HIV, HBV, HCV) during which the antibodies to the virus cannot be detected. The virus is present in the blood and such individuals are infectious during window period.
- The important implication of this is that if a client is testing negative for HIV antibody he may be infected and must be retested after 2-4 weeks to ensure the HIV status of client.
- Window period for HIV, HCV and HBV are shown in the slide both when antibody test and nucleic acid detection tests are performed.

SLIDE 10:Standard work precautions apply to:

- HIV is present in almost all body fluids and blood and all the tissues.
- Only the concentration of HIV may vary in different fluids i.e. why most infections are transmitted through blood and not through other body fluids.

SLIDE 11:Standard work precautions usually do not apply:

- The secretions and excretions mostly do not have HIV, but since mostly these may be mixed with blood particularly HIV infected individual low concentration of HIV may be present in these also.

- So, we practice SWP for blood, all body fluids and blood mixed secretions and excretions.

SLIDE 12:Essential Dos and Don'ts of Biosafety

- Protect yourself at all times while performing activities like collecting blood, processing specimen etc. In fact any activity where contact with blood and body fluid is expected, barrier precautions must be practiced.
- There should not be any direct contact with blood and body fluids or clients.
- Correct precautions must be taken at all times.

SLIDE 13:Components of SWP

- Hand washing with soap and water is very important after each activity. It is simple and most important component of SWP.
- The other important component is disinfection and care of used sharps at the place of activity.
- This will minimize accidents and will render the items non-infectious.
- An extremely important component is segregation of various types of waste at the point of generation into various color coded bags as per the NACO guidelines: disinfection of infectious waste and sharps and final disposal of waste as per NACO guidelines.

SLIDE 14:Safety Precautions

- Follow the NACO guidelines for disinfection of waste i.e. use 1% freshly prepared sodium hypochlorite solution. This solution is made every morning for use during the day.
- All HCW should take HBV immunization doses and should have optimum level of antibodies against HBS Ag. Otherwise, additional doses of vaccine may have to be given.

- Wear PPE(personal protective equipment) as warranted by the activity you are going to perform. Gloves, apron, white coat for collection of blood and all barrier articles while conducting a delivery in the labor room etc.
- Know and practice right method of sterilization and disinfection.

SLIDE 15:Safety Precautions

- Be careful with sharps. Take special care not to handle sharps after use. This will protect you as well as others from accidental exposure to contaminated sharps like needles and lancets etc.
- Do not bend or cap the needle.

SLIDE 16:Limitations of SWPs

- Nothing in life has 100% guarantee. Same is the case with SWP. Needle can pierce the glove and skin. Sometimes, one is careful and practicing SWP as directed, still freak accidental injuries may happen. What to do then? Do not panic or put injured are in mouth.
- Do not squeeze the area.
- Wash under running tap water.
- Report accident to supervisor immediately.
- Try and take first dose of ARVs.
- Then manage the PEP as per NACO guidelines.

SLIDE 17:Occupational exposure to HIV is very low, however it may happen.

- This slide describes various types of accidental exposures which can happen in a health care setting. These may be piercing by needle, cut with a sharp, spill on skin, spill/contact of eye month etc.
- Two factors determine whether transmission of virus will happen or not. One is amount of virus in patients blood and second the severity of exposure.
- Carefully examine these two factors and decide how PEP will be managed.

SLIDE 18:Risk of Virus transmission in work place HIV vs. HBV

- This slide shows the rate of transmission of various blood borne viruses after accidental exposure.
- The figures are based on retrospective analysis of data.
- Every exposure should be taken as an emergency and managed as such.
- Very few about 57 HCW got infected with HIV(CDC report) on account of accidental exposure. One report of infection in a nurse in defense services reported by NICD.
- So exposure are many but infections are minimal.
- Keep in mind that the person who gets the infection is infected for life so always follow SWP diligently and never compromise or be lax as far as SWP are concerned.

SLIDE 19:Is PEP needed for all types of exposures?

- Every occupational exposure to contaminated blood and body fluids does not require PEP drugs.
- However, every exposure should be managed seriously and aim should be to take all precautionary and preventive measures to stop transmission of HIV to the HCW.

SLIDE 20:Management of accidental occupational exposure to blood and body fluids

- After an injury or accidental exposure take the actions as described in the slide. All actions to be described by the faculty taking the session.
- Address each point and make sure the LT understands what he or she has to do in case of an accidental exposure.

SLIDE 21: Conclusions

- So, if you know how to protect yourself and fellow, the risk of HIV transmission in work place becomes minimal.
- SWP are our responsibility as we have to take care of our life ourself.
- So be responsible and practice SWP.

5.BIOSAFETY: PRACTICAL CONSIDERATIONS(USE AS REFERENCE FOR PRACTICAL)

We will now undertake practically what we have learnt theoretically to ensure that each one of you knows how to practice SWP and protect yourself from getting any infection through blood and body fluids.

SLIDE 1: OBJECTIVES

- HCW providing services are exposed to various types of hazardous specimens and articles.
- It is very important for them to understand what these hazardous specimens and articles are and how to protect themselves.

- It is the duty of the HCW(LTs) to know all about the potential risks associated with their occupation, the precautions which need to be taken(eg. HBV vaccination, standard work precautions, safe disposal of waste etc.)
- The risk of transmission of HIV at work place for LTs is minimal but still the risk is there and precautions must be followed at all times for all clients in all specimens.

SLIDE 2: Policy for Safety in the Laboratory

- LTs and other HCW must not all the time complaint about poor resources for SWP but should themselves be resourceful and get materials like soap and water and practice SWP.
- Must ask for gloves.

SLIDE 3: Standard Work Precautions

- Standard precautions must be practiced at all times for all clients.
- Do not be careless if client happens to be a doctor, your friend or a VIP.
- SWP must be practiced at all times.

SLIDE 4: SWP includes

- SWP are common sense practices which we should have been practicing.
- Somehow we have become careless. We do not wash hands even if soap is not available, was under water, you can get soap from home as it is for your own life.
- Ensure that you protect yourself and your colleagues.
- Disinfect/cut/destroy needle syringes, all other infectious waste, so that individuals who are going to transport and dispose of the waste are not at risk.

SLIDE 5: Hand Washing

- Wash hands thoroughly after each procedure.
- Do not be lazy.
- Wash all areas of hands- as shown.

- Must wash hands systematically so that no area is left contaminated.

SLIDE 6: Effective hand washing

- Take care of these areas of the hands which are usually missed during washing due to creases etc.

SLIDE 7: Sharps Safety

- Never ever touch broken glass with bare hands.
- A blood spill must be covered with 10% sodium hypochlorite.
- Manage spills with gloved hands. Details will be described later.

SLIDE 8: Splashes to the eye

- In case a specimen or chemical falls into the eye, wash eyes thoroughly for 15 minutes depending upon what splashed into the eye.
- Show how to splash eyes for washing.

SLIDE 9: Commonly used disinfectants in the laboratory

- 1% sodium hypochlorite mean 1 gm chemical in 100 ml of water. See the %age of sodium hypochlorite on bottle and then teach how to make 1 % solution.
- In the same way other solutions will be made.
- Before making the solution do the calculation and get it checked by the supervisor for accuracy.

SLIDE 10: Recommended strength of Sodium Hypochlorite

- 10% solution is used for blood spill/large organic matter disposal due to possibility of heavy load of micro-organisms in these type of waste.
- 1% sodium hypochlorite made fresh everyday is an effective disinfectant.

SLIDE 11: Management of Spills

- This exercise-all steps have to be demonstrated in the laboratory.
- Spill can be of a solution-presume as blood and demonstrate.

SLIDE 12: Wastes Disposal

- Different types of bags with color codes and containers containing different types of wastes to be shown.

SLIDE 13: Disposal methods

- Show different types of containers and how the waste is transport(Discard jars).

SLIDE 14: PEP is not indicated

- Do role play.

SLIDE 15: Pre & Post test counseling, testing & follow up

- Do role play for management of an accidental occupational exposure.

6. PROCESS AND FLOW OF CLIENT AT ICTC

SLIDE 1: The client accessing ICTC may be:

- It is extremely important to ensure that the client who is accessing the services is made comfortable. This is best achieved by smooth flow of client from counselor to technician, being polite and understanding.
- The counselor and LT should keep in mind the background of clients and should be cordial and polite so that client is comfortable.
- LT and counselor should not pass any comment on client's behavior and should not be judgmental.

SLIDE 2: Process flow for HIV testing

- This slide shows the various steps through which the client accessing ICTC services passes.
- Sequence of these steps must be clearly understood so that all the important information is recorded in appropriate registers and the work of ICTC goes smoothly.
- This also helps an HIV Positive person to reach the designated ART site for further management.

SLIDE 3: Client Flow

- This slide shows how the work flow should go at the ICTCs as a flow chart. Same as was explained in the previous slide.

SLIDE 4: Flow of client continues.....

SLIDE 5: Pre-test Counseling

- In case sometimes the LT or laboratory in-charge has to counsel the client it should be ensured that counseling is done as per NACO guidelines.
- We must have the right kind of attitude (not judgmental and criticizing), current knowledge of HIV and services available.
- Aim should be to motivate the client to undergo HIV test and also have hope of getting services if found to be positive.
- Always motivate to practice safe behaviors.
- Give hope of ART care if found positive.

SLIDE 6: PPTCT

- At ANC clinics the emphasis is on group counseling of all pregnant women on the day and giving them the option either to get tested or not.
- Like at ICTCs, the flow of client after group counseling at PPTCT is sent for counseling, informed consent, blood collection, testing and reporting with post test counseling.

7.COLLECTION, STORAGE AND TRANSPORT OF BLOOD FOR HIV TESTING

SLIDE 1: At the end of this module you will be able to:-

- Collection of blood sample is an invasive and hazardous activity. This module describe how to accurately carry out this activity, minimize accidents, errors and the procedure to collect blood sample for various HIV related, associated tests.
- By understanding this module Lab Technician(LT) will perform the activity correctly.

SLIDE 2:Who should collect blood specimen

- LT/Nurse should be skilled/trained in procedure of collection of blood sample.
- LT/Nurse should accurately follow each step of phlebotomy, finger pricks, DBS-
So that optimum quantity of quality blood sample is obtained.

SLIDE 3: Materials required to collect the blood sample

- This slide describes various materials which should be available at the blood collection site.
- Describe the material from slide.
- The type of collection tube will vary according to the purpose for which the blood sample is going to be used.

SLIDE 4: Types of syringes used to collect blood sample

- This slide describes the various types of collection syringes/devices available commercially for collection of blood for various tests.
- Describe the material/devices on the slide.

SLIDE 5 :Pre –analytical variables

- This slide describes various pre-analytical activity to be undertaken for collection of blood sample.

SLIDE 6: Collecting Blood Sample

- This slide shows the blood sample being collected from vein following the standard work precautions.

SLIDE 7: Procedure for drawing blood

- Before collecting the actual blood sample-talk to the client, describe the procedure and assure.
- Prepare all materials required.
- Wear gloves
- Keep the discard jar/needle destroyer near the activity site(on table).

SLIDE 8: Procedure for drawing blood

- Describe the steps of blood collection procedure from the slide.
- This will be demonstrated in the practical.

SLIDE 9: Procedure for drawing blood

- Describe the steps from the slide.
- Demonstrate the steps in the practical class.

SLIDE 10: Labeling & Test Requisition

- Once the specimen has been collected, it has to be labeled in such a way that there will never be a mix up of this specimen with any other specimen(similar name etc.)
- Test requisition form also. Always label while patient is available and match details.

SLIDE 11: Check Patient Data

- Double check the labeling on the sample tube and data entered on the report form.
- Careful recording of client details, sample and other relevant information is an important step to ensure quality of testing.

SLIDE 12: Adult and Infant sample volumes

- The volume of sample to be collected will depend upon the test to be performed.
- An expert should collect sample from infants & young children.
- Ensure that sample is collected in the correct tube as per the requirement of the test to be performed.

SLIDE 13: Blood Sample stability for enumeration of CD4 T Cells

- It is important to know what is the duration for which the sample will be stable for testing. For CD4 testing stability of sample is usually limited, check the type of machine being used and keep the stability in mind. Samples for CD4 should be kept at room temperature(22-25 deg. C)

SLIDE 14: Specimen Handling & Transport

- It is very important to maintain (storage/transport) cold chain (4-8 deg, C) for blood samples for serological tests.
- For CD4 cell count store & Transport at room temperature(22-25 deg. C)
- Higher temperatures affect the viability of cells.

SLIDE 15: Specimen Handling & Transport

- This slide describes how the sample has to be packaged for transport to another laboratory.
- Describe each step as on slide.
- Demonstrate accurately in the practical.
- Give an exercise to LT to package a few samples.

SLIDE 16: Specimen Handling & Transport

- A few things which have to be kept in mind and practiced for transporting the samples are described in this slide.
- Must declare the samples are hazardous.
- Must put Bio-hazardous sign on the external most container also.
- Must put an arrow up showing how the container has to be handled and placed so that samples do not spill.
- Follow the national and international guide for transporting samples nationally and internationally respectively.

SLIDE 17: Specimen Requisition and Receipt

- All the specimens being transported must be recorded in a specimen identification log for traceability from sender-courier-recipient-testing etc.
- Each specimen must be accompanied duly with a duly completely filled requisition form.

SLIDE 18: Assessment of specimen quality/integrity

- The sample receiving laboratory must check the samples, accompanying records, temp. etc. and only then sign the specimen receipt document.
- Describe from the slide the various details to be checked by the LT at the sample receiving laboratory.

SLIDE 19: Assessment of Specimen quality/integritycont.

- In case the integrity of sample is compromised in any way-the sample must be rejected.
- Provide the feedback about problem samples to the sending laboratory to take corrective actions for future.
- Records of rejected samples and reasons for the same must be maintained.

SLIDE 20: Summary

- This slide re-emphasizes and summarizes the important messages of the module.
- It is important that LT and all others in the laboratory have and follow the correct procedures for collection, storage and transport of blood samples for HIV and related testing.
- SOPs should be prepared on: Collection of Blood samples from vein & by finger prick.
- SOPs to be prepared for: Transport of samples for different tests.
- SOPs to be prepared for: Storage of blood samples.

8. INTRODUCTION TO HIV TESTING

SLIDE 1 & 2: Learning Objectives

- A very important function the LT performs is collecting and testing of blood sample from the client either after referral from a physician(provider initiated testing) without consent or from counselor after informed consent, in this case.
- LT should be conversant with all the components and requirements of quality systems, the meaning of external quality control sample, why do we put it, what purpose it serves, etc. to render quality HIV testing services.
- The LT should know how to perform tests, how to read results and how to validate results.
- LT should also know the strategy of HIV testing to be followed at ICTCs(IIB and III)

SLIDE 3: Requirements for HIV testing laboratory

- In order to achieve the accurate results it is important that the LT knows exactly what he is supposed to do and how. This can only be done through training & retraining.
- A trained technician then ensures that all the requirements for HIV testing are in place.
- Describe each parameter from the slide including quality test kits, SOPs, standard equipment and biosafety measures etc.

SLIDE 4, 5 and 6: Procedure

- The HIV test kits used should be licensed and should be good quality i.e. should fulfill NACO specifications for the kits.
- Examine and make sure all the components are within expiry date.
- The SOP should be for the test being performed.
- Follow steps of the test as described.
- Every time you perform an HIV test whether it is ELISA or rapid, external quality control know EQAs samples (one negative and one positive) must also be tested just like the samples from the clients.
- For ELISA the kit controls are used to calculate the cut off values as per the manufacturer instructions and own external controls are put to validate the accuracy of results. Levy Jening's Chart is used for the purpose.

- For rapid test one set of external quality control samples as above are used at least once a day in addition to the kit controls.
- External quality control samples are tested preferably in the centre/middle of all client samples (9 if total 15 samples are to be tested put controls at 7th or 8th position.)
- While performing the test, be careful, do not get distracted see to it that there is no mixing or carryover of samples.
- Follow the sequence of spotting of samples as per the worksheet.
- Observe correct timings.
- Read the results as recommended by manufacturer in the kit insert.

SLIDE 7: Reading the result

- The result of the external control samples used must always be correct.
- If the external control sample results are not correct within range, this means some parameter of quality has been compromised.
- It is better to examine the methods and parameters of testing. Try to locate the error and correct it. Repeat testing should be done in such cases.

9. No WRITE UP for performance of Rapid tests with practicalas it is a performance practical.

10.Total Quality Management and Quality Assurance in Laboratories

SLIDE 1:Learning Objectives

- The LT has to understand the different factors operative in the laboratory which can affect the quality of results means wrong results may be produced. So, it is important to learn

about all the correct procedures right from collection of specimen-processing-report the results.

- Each procedure is a combination of a large no. of steps/process and each step may involve reagents, equipment, documentation, interpretation and reporting of results to the right client in the right way.

SLIDE 2:Quality Assurance(QA)

- This slide defines and explains the quality assurance programme which is to ensure that every time an activity test is performed the quality aspect is always kept in mind so that the results produced are accurate(keeping in mind the sensitivity and specificity aspects of the test.
- The quality processes encompass pre-analytical, analytical and post analytical activities.
- Describe the factors given in the slide.

SLIDE 3:Factors affecting Quality assurance

- Important factors which can influence the outcome of result are described in this slide.
- Give examples for each parameter from your experience.

SLIDE 4:Internal Quality Control(IQC) Process

- This slide describes what is IQC and why we practice. It is to ensure that the performance of test is accurate, that the sample being tested is of desired quality, that the reagents and equipment which are going to be used are of the desired quality and equipment is calibrated.
- However, the test results can never be 100% accurate, as every test has limitations. Also, in case of HIV if results are not informed with post test counseling-this way compromise quality.

SLIDE 5: Internal Quality Control(IQC)

- To practice IQC known negative and positive samples are run along with kit controls and the test samples.

- The known samples are samples which have been already tested and the results of these samples are already known.
- The results of these known controls must fall within the desired range.
- Explain the Levy & Jenings's chart for validating ELISA run here as an example. The OD of control known sample must fall within $\pm 2.5D$ of the known OD of control.

SLIDE 6:Control Serum Samples for use as IQC

- The standard known external positive control which is to be used for day to day QC while performing the tests needs to be prepared in bulk in the laboratory so that it can be used over a period of time.
- The source of serum for such a positive control can be obtained from blood banks i.e. the unit of blood testing positive for HIV only (-ve for HBV & HCV).
- Separate the serum and filter through 0.2 μ m bacterial filter(for sterility)
- Heat inactivation done to kill bacteria & viruses.
- The serum is processed further to prepare standard known positive control.
- The dilution of serum 10-20% above the cut off value by ELISA and positive by all the rapid tests is to be taken as positive control.
- This serum sample should give -ve result with all the known HIV test kits.

SLIDE 7: Control Serum Samples

- The positive control should be distributed as aliquots as 0.5 ml in vials(screw capped).
- Randomly pick 20 aliquots and test by ELISA to calculate mean and standard deviation and prepare levy jenings chart.
- These samples are also to be tested with all HIV test kits available and should give clean positive result.
- Such a positive external control is used to practice QC(IQC) day to day while performing the test.

SLIDE 8:IQC Programme method

- The external QC sample is included in every run of ELISA every day and tested one a day for rapid tests.
- The OD value of ELISA of this external positive QC sample should be within range i.e. mean +/- .25OD as calculated when it was prepared-such ELISA run is valid means all quality assurance parameters have been met.
- For rapid tests the control should give positive result when tested once every day while performing rapid HIV tests.

SLIDE 9: IQC Programme Method-for ELISA only

- This slide depicts the flow of events for IQC and describes each step of the flow chart in your language.

SLIDE 10:IQCcontd...

- Important elements of QC are described in this slide. Go through each step and describe the implication of each step. The bottom line is that if the known controls results are not accurate, it means something has gone wrong somewhere in the performance of tests and the LT has to examine the process, the reagents and performance to find where and how the error was committed and rectify it.
- However, in such a case the test has to be repeated.

SLIDE 11:Variables that can affect quality of results

- This slide gives various variables which can influence the quality of the test results. Knowing these, it becomes easy for the Lab supervisor or LT to investigate and find out the cause of error and rectify it.

SLIDE 12:Errors During Performance

- These are some of the common sense areas where if LT/supervisor is not careful error can happen and the results may be affected.

SLIDE 13: Important issues for consideration

- In order to produce accurate results the LT/supervisor has to take into account all the processes and procedures which have to be followed and practical right from selection of sample to reporting of result.
- These procedures are listed in this slide.
- Describe slide and give examples from your experience relevant to LT.

SLIDE 14: Criteria for Rejection of Specimen

- This is an important slide as it details the situations and reasons for rejecting a sample and asking for a repeat sample.
- Describe each criteria so that it sticks in the mind of LT.

SLIDE 15: Laboratory Management

- Management of Laboratory is extremely important to use space properly, to work comfortably and to ensure that there are not stock outs and records/data are maintained.
- Describe each item on the slide and emphasize the importance of each item.

SLIDE 16: Corrective action in case of poor performance

- Whenever it is detected that inaccurate result has been produced the laboratory supervisor/laboratory technician must review the procedure used, reagents used, SOP used, equipment used, IQC followed, interpretation and reporting of result.
- In fact each step of the procedure from collecting, logging of sample to testing and reporting is reviewed to detect the error and take corrective action.
- Record of all such events should be maintained in the quality register.

SLIDE 17:External Quality Assessment

- The slide describes the EQA and process of EQA.
- The role of EQA participating laboratory is to test samples received from EQA conducting laboratory as routine samples are tested within the same week and report back the result to the EQA conducting lab.
- The lab conducting EQA must maintain confidentiality of result of the participating lab and mentor the lab.
- Whenever there are some discordant or wrong result the EQA conducting lab must find the reason for this and do the trouble shooting.
- This can be done by just dialogue with the defaulting lab and examination of reagents kits and performance.
- Training may be conducted.

SLIDE 18:External Quality Assessment Programme

- The purpose of EQA is not to penalize the defaulting lab. It is to bring all the testing sites up to the minimal standards required for testing so that accurate results are produced by all the labs.
- EQA is conducted once/6 months or once/three months. So, it cant be used as a substitute for daily QC practice.
- Success in EQA increases the confidence and esteem of the laboratory.
- Describe conduct of EQA from your experience.

SLIDE 19:External Quality Assessment Programme..contd.

- EQA is also know as proficiency testing.
- Day to day quality control using own known +ve and –ve controls must be practiced by all the laboratories irrespective of whether the laboratory did well in EQA or not.

SLIDE 20:External Quality Assessment: Method

- One way to EQA is the external audit which can be done by a senior/reference lab. In this all the processes, procedures equipment, calibration, technical competence and performance are assessed by visit to the lab on site.

SLIDE 21: External Quality Assessment contd...

- Advantage of participating in EQA is that it builds esteem, helps to find out grey areas in laboratory, helps to improve performance helps to improve knowledge.
 - This also brings the laboratory into a network wherein consistent good performance can be achieved.
-

11. No WRITE UP for performance of ELISA as it is a performance practical.

12. Syphilis testing

SLIDE 1 & 2: Learning Objectives

- LT should be conversant with all the components and requirements of quality systems, the meaning of external quality control sample, why do we put it, what purpose it serves, etc. to render quality syphilis testing services.
- The LT should know how to perform tests, how to read results and how to validate results.
- LT should also know the rationale for performance of syphilis test with the HIV test in the ICTC for STI attendees, ANC attendees and HRG through a single prick, providing a single window of services.

SLIDE 3, 4: Serologic tests for syphilis

- *Treponema pallidum*, the etiological agent of syphilis, induces the production of at least two types of antibodies in human infection: anti-treponemal antibodies that can be detected by FTA- ABS antigen, and anti-nontreponemal antibodies (reagin) that can be detected by RPR antigen
- Serological tests to detect antibodies to *Treponema pallidum* are in-vitro qualitative or quantitative tests.
- Can be performed on plasma or serum. Most commonly serum is used.
- The kits to perform STS contain non-specific antigen (Cardiolipin).

SLIDE 5: Requirements for syphilis testing laboratory

- In order to achieve the accurate results it is important that the LT knows exactly what he is supposed to do and how. This can only be done through training & retraining.
- A trained technician then ensures that all the requirements for HIV testing are in place.
- Describe each parameter from the slide including quality test kits, SOPs, standard equipment and biosafety measures etc.

SLIDE 6 to 20: RPR kit and procedure for qualitative and quantitative test

- The RPR test kits used should be licensed and should be good quality i.e. should fulfill NACO specifications for the kits.
- Examine and make sure all the components are within expiry date.
- The SOP should be for the test being performed.
- Follow steps of the test as described.
- Read and interpret the results as described.

12 A. No WRITE UP for performance of Rapid tests with practical as it is a performance practical.

13. USE AND CARE OF EQUIPMENT AVAILABLE AT ICTCS

SLIDE 1: Learning Objectives

- The purpose of this module is to enable the laboratory personnel as to understand what their responsibilities are to ensure appropriate monitoring and use of equipment and its care.

SLIDE 2: Common equipment used at ICTC.

- This slide show the equipment commonly used at ICTCs.
- These include a refrigerator, a centrifuge and micropipette, droppers etc.
- Incubator may also be present at some of the sites.
- Needle destroyer /cutter also is present at ICTCs.

SLIDE 3: Functioning Equipment is vital to Quality Service.

- It is the duty of LT and the officer in-charge ICTC to ensure that all the equipment is of good quality is maintained properly and is functional.
- This will help to maintain quality of testing and prevent delays in testing on account of non functional equipment.
- Preventive action like procuring good quality equipments, putting equipment on AMC and managing equipment as per the instructions of the manufacturer and calibrating as per guidelines will also lower the cost incurred otherwise.

SLIDE 4: Management Responsibilities(Hospital)

- Maintain inventory of the equipment (name of equipment, manufacturer and supplier, when received, on AMC or not, when to calibrate etc.)

- The equipment should be used properly as instructed by the manufacturer.
- The details of AMC, calibration, records, details of breakdowns, etc. should all be maintain in the equipment inventory.

SLIDE 5: Responsibilities: Lab Supervisors

- It is important not to ever use malfunctioning equipment as it will affect the test result.
- As soon as a fault in equipment is detected corrective action should be taken and recorded.
- Maintain calibration record for equipment.
- Maintain temperature logs for all temperature sensitive equipments (refrigerator, water bath and incubator etc.)

SLIDE 6: Function checks to verify that equipment is working properly.

- Maintain records of function checks of each equipments as recommended.
- Record also when equipment was repaired and how it performed after repair.

SLIDE 7: Refrigerator and Freezer: Use & Care

- This slide describes the common sense actions which need to be taken to ensure refrigerator and freezer use functioning.
- Also the storage of samples and kits (how it is to be done)
- Do Not store kits in the door shelves, so that kits do not deteriorate.
- Properly catalogue and store samples.

SLIDE 8: Refrigerator & Freezer: Temperatuer Checks

- The temperature log for refrigerator has to be maintained.
- Record temperature inside the refrigerator at least once a day, preferably morning & evening.

SLIDE 9: Refrigerator & Freezer: Temperature Log

- This slide shows an example of the document-Temperature check chart for refrigerator.

SLIDE 10:Types of Pipettes

- With constant use the volume delivered by micropipette may be affected.
- To ensure micropipettes are functioning optimally these must be calibrated at least once every 3 months.
- Some kits provide disposable graduated fixed volume dropper pipettes.
- These are used for the test and then disposed off as per recommendations.

SLIDE 11 & 12 : Pipette: Use and Care

- When using micropipettes ensure that the corresponding, right volume micro-tips are used.
- Use pipettes properly.
- This will be explained in the practical class.
- Presence of air bubbles and holding the pipette in different ways affects the volume of serum being delivered and hence the results of the test.

SLIDE 13: Precision pipettes require performance checks

- In case the laboratory is confident, the calibration micropipettes can be done in house using electronic balance with calibrated weights(NABL accredited center)
- It is better to get the micropipettes calibrated by the experts(vendors or NABL accredited center)
- The slide shows different methods of calibration.

SLIDE 14: Pipette Troubleshooting

- This slide shows minor error which may be encountered while using micropipettes. These minor problems can be resolved in house-as described above.

- Explain the cause of error and how to resolve as given in the slide.

SLIDE 15: Centrifuge: Use and Care

- Centrifuge is used for separating serum from blood at ICTCs.
- Centrifuge should be properly calibrated(from accredited center using tachymeter)
- While using-balance the tubes, close the lid and ensure proper speed.
- There should be no vibrations when the centrifuge is in use as this will indicate that balance is not proper.

SLIDE 16: Centrifuge: Function Checks

- Function Checks of a centrifuge include seeing that it is balanced
- Rotors are functioning as per requirements.
- It is not noisy when in use.

SLIDE 17: Centrifuge: Routine Maintenance

- Centrifuge must be cleaned regularly(also from inside)
- It must be placed under AMC.
- In case of any doubt in performance or any breakdown call the expert (service engineer) for corrective action.

SLIDE 18: Centrifuge Safety

- This slide describes the safety steps to be observed while using the centrifuge.
- Disconnect the centrifuge while performing the checks from the mains.
- Do not spill etc. inside the centrifuge.
- In case the tube breaks inside the centrifuge due to improper balance manage as described. Use forceps to take the glass out.
- Disinfect after spills.

SLIDE 19: Keep a Log for All Maintenance Activities

- This slide show the generic maintenance form for various checks performed daily, weekly or monthly for various equipment being used in the laboratory.

SLIDE 20: Ensure proper Storage of Inventory

- While storing the items in store. Place them neatly on shelves.
- Design a system so that you can access the items easily(store items alphabetically or usewise, whatever system you feel will work for you.
- Store in such a way that early expiry items are taken out and used first.
- Store items, kits as per the directions of the manufacturer.
- Maintain appropriate storage temperatures eg. 2-8 degrees Celsius for HIV test kits.

SLIDE 21: Carry-home Messages

- Adequate stocks of items should be maintained at all times.
- Ensure there is never a situation of stock out resulting in stopping of work. Plan in advance.
- All items(kits, reagents, equipments etc.) present in the lab must be accounted for in the stock register.
- Manage stocks in such a way that there is minimum wastage.
- Always carefully examine and record details whenever new stock is received.

14.COMMON ERRORS AND TROUBLESHOOTING

SLIDE 1: Learning Objectives

- The purpose of this module is to be sensitized the LT to the common errors which happen in the laboratory, the factors responsible for these errors so that LT can take preventive action and avoid the errors.
- However, in spite of all the precautions taken an error can happen then the LT should know what corrective actions need to be taken.
- Record of all these happening has to be maintained.

SLIDE 2: Factors responsible for some common errors

- The slide describes the various factors responsible for errors in lab.
- The blood/serum sample is the starting point for the test. If the serum/sample is not as desired it will lead to wrong result. Make sure serum is as per the requirements
- The quality of sample, quality of equipment, reagents and quality of performance will influence the quality of result.
- Give some examples from your own experience e.g. a pipette.

SLIDE 3: Other errors

- The LT might have done everything right but sometimes due to carelessness, there may be error in copying the result from the print out/worksheet on to the report form.
- Name of the patient and the result may not correspond due to similar names and wrong transcription.
- Sometimes the early seroconverter may give positive result with one test kit and negative result with the other test kit. This is because antibodies to all antigens of HIV have not yet reached the detectable level.
- So such grey zone samples may test positive in one lab and negative in the another lab.

SLIDE 4: Method to identify common problems

- Whenever a wrong result is obtained in the lab an investigation/internal audit has to be carried out to find out the reason for wrong result.
- The quality of sample is examined, the kits used are examined for all parameters which assure quality of kits.
- Equipment quality is checked.
- Quality of performance.
- Competence to interpret & report results.
- In fact all the procedures, methods, SOPs and materials used from collection of samples to reporting of results are checked to locate error.

SLIDE 5: Specimen errors and preventive action

- This slide describes what kind of errors or problems can happen with the sample and actions to be taken to correct these errors.
- Describe the material on slide clearly.
- Give examples from your own experiences.

SLIDE 6: Clerical errors and preventive action

- This slide gives a summary of errors which can happen during recording various details and also while filling up the report forms with data from work sheet.
- Describe the error and how to rectify it slowly from the slide.

SLIDE 7: Test Kit dependent errors and preventive action

- Errors in result can also happen if the quality of kits and reagents used is compromised.
- Never use expired kits.
- Never, ever mix reagents from different kits.
- Check for expiry of each reagent/component of kit before during the test.
- Describe the slide slowly.

SLIDE 8: Performance errors and Preventive action

- It is very important to get the calculations made for dilution of sample or reagents checked by lab supervisor.
- Dilutional errors have been found to be very common.
- Do not mix SOPs.
- Uniform technique & SOP should be used for each sample.

SLIDE 9: Equipment based errors and preventive action

- Sometimes incorrect pipette tips are used due to stock out situation of the accurate volume tips. This will result in delivery of wrong volume of reagents and wrong results.
- Make sure temperature of incubator in lab environment are as desired for the test.
- High temperatures may accelerate enzyme reaction of ELISA & may give a few false positive results & vice-versa.
- Calibrate all equipment as per guideline in quality standards manual.

SLIDE 10: Environment dependent errors and preventive action

- It is important to maintain proper temperatures humidity in the lab where testing is performed and make sure the reagents never dry during performance of test. This will produce wrong test result.

SLIDE 11: Causes of non-reproducible results and preventive action.

- This slide describes the factors which can affect the reproducibility of the test results i.e. when the test is repeated, same result is not obtained.
- Explain each factor and what action to be taken to rectify/correct the error from the slide.

SLIDE 12: Records

- Each time an error happens, inaccurate result is produced which has to be recorded.
- What was done to find out the reason/cause for the error i.e. the investigation/internal audit has also to be recorded.
- Record what corrective and preventive actions were taken first to correct the error and second to prevent recurrence of the error.
- Never report wrong result to the client.
- In case wrong result has been reported, recall the client, explain counsel, and retest.

15. INVENTORY/STOCK MANAGEMENT

SLIDE 1:Definitions

- Inventory: List of items & Stock: Quantity of each item
- Every laboratory or testing site must maintain an inventory which is the listing of all the items(reagents, equipment)available in the laboratory.
- Stock register will have the details of each item of inventory like name, how much received, when received, expiry date, when issued and to whom and how much and stock balance available in store.
- Management of inventory and stock is extremely important to ensure the laboratory never runs out of any item(no stock outs) and at the same time there is no wastage of any item.

SLIDE 2:Learning Objectives

- Please ask questions and be very sure that how you will maintain inventory and stock at your site. This will help you to indent items at the right time and the right amounts, so that you will never be out of stock for the items used to run you laboratory.

- Maintenance of stock will help you to calculate the amounts of items required for your site, when to order these and how much to order.
- This is also an important requirement of quality management systems.

SLIDE 3:Inventory/stock management leads to high quality testing, minimize wastage

- Stock management helps to avoid/minimize the wastage, prevents stock out situations.

SLIDE 4:Inventory/stock management involves....

- This slide shows the different components or activities undertaken for management of stock.
- The faculty has to read out each point and give examples from their own laboratory. How and what they do in their own laboratory.

SLIDE 5:Perform a “Stock Count”

- Stock count is counting of each item in the stock by a designated person of the laboratory.
- All items available/present in the laboratory must be accounted for in the stock register.

SLIDE 6:Maintain proper inventory records

- NACO has prepared a format of stock register and all information regarding stocks, items available in the laboratory must be recorded on this format.
- A copy of format will be given to you during practical.

SLIDE 7:Stock Register-Example.

SLIDE 8:Reconciling Stock with Records.

- Ideally when you are checking stock the stock in balance should be equal to total number of an item/kit received minus the amount used which will be sum total of no. of tests performed and number of controls put. However, this does not usually tally as there will be times when the test has to be repeated due to one or the other reason (invalid test, equivocal results, expiry kits etc.). This should be duly recorded so that the amount of reagent/tests wasted can be calculated and added to the used tests column.
- We should make all the efforts to follow the quality assurance practices to minimize the wastage.

SLIDE 9:Determine when to Re-order.

- Suppose it takes 3 months for you to procure kits i.e. the process takes 3 months becomes the lead time and the minimum stock which you need at the time you place the order should be sufficient for 3 months.
- Ensure that at no time the work of laboratory is stopped due to shortage of kits or reagents.
- Total number of kits, reagents used in a given time period is known as maximum usage.

SLIDE 10:Calculating Minimum Stock Level

- This slide gives an example of how to calculate minimum stock level from the lead time and maximum usage values provided.

SLIDE 11:Determine how much to Re-order.

- Whenever placing order for materials, do not be greedy, calculate the size of the order keeping in mind maximum usage and lead time.
- Never order for more than that which can be used.
- This is to ensure that there is no or minimal wastage.
- At the same time ensure that you do not run out of suppliers.

SLIDE 12:Determine full stock level

- The slide gives an example of how calculations of stock required are made.
- The example given in the slide is to make LTs lab in-charges understand that how to calculate the stock order amount.
- Must explain them to make it understand.

SLIDE 13:Place Orders Properly

- In case your institute has some other way of calculating the size of order or stock required that system may be used.
- However ensure that there are no stock outs and no wastage(expiry of reagents, kits due to non-use).

SLIDE 14:Inspect Delivery of New Orders

- Whenever you receive kits, reagents examine carefully to ensure the integrity and quality, date of expiry, supplier name etc. Details should match the specifications in the purchase order.
- Record all the details in the inventory or stock register along with date received and signatures of the person who received the items.
- Store the items received appropriately.
- Update balance and other records in the stock register.

SLIDE 15:Examine Lot Number & Expiry Date

- For kits received carefully examine and record lot no. and expiry date.
- Make sure that expiry date is such that all the kits will be used before this date.

16.EXTERNAL QUALITY ASSESSMENT (EQA) PROFICIENCY TESTING

SLIDE 1: Learning Objectives

- This module is more for lab supervisors, also for LTS. It describes how to inspect and assess the quality of testing of a participating lab.
- In case the participating lab fails in EQA, What action need to be taken, how the feed back is provided and how the corrective actions are taken. The details are described in this module.

SLIDE 2: Content Overview

- EQA is defined as an external quality assessment exercise conducted by NRLs for the attached SRLs ICTCs & blood banks. It is done to improve quality of testing & not to find fault and penalize the defaulting sites.
- The results are kept confidential.
- There are 3 ways in which assessment can be done
 1. By sending out coded panel to participating labs and analyzing the result sent by them.
 2. By doing on site inspection and assessment and
 3. By retesting a percentage of positive & negative serum from participating labs to conform the results produced by them.(not a true component of EQA)

SLIDE 3: External Quality Assessment: Definition

- This slide describes the important components.

SLIDE 4: Why EQA?

- The purpose of EQA is to assess the performance of laboratories.
- Bring all labs to a standard level.
- Continuously and consistently encourage the labs to conform to quality standards

- Mentor the labs. Identify weakness & improve the same.
- Provide CME as per need.

SLIDE 5: EQA: Conducted at All Levels of Testing

- Networking of various laboratories is depicted in this laboratories.

SLIDE 6: Management Responsibilities: Overview

- EQA is an important component of NACP III.
- NACO has placed the testing laboratories of the country in 4 tiers.
- There is an Apex lab, NRLs (13) and SRLs (117) ICTCs (4500 appx) & blood banks labs.
- One tier of lab supervises, monitors & mentors the labs of next tier assigned to them.
- Apex lab collects, collates and analysis the data & feeds to NACO for required, relevant actions.

SLIDE 7: Testing Site (ICTC/PPTCT) Responsibilities:Overview

- This slide defines the jobs done by the ICTCs in the EQA Programe ICTC received panel, tests samples as the routine client samples, interprets the results and sends to SRL/NRL.
- Maintains all the records of EQA.
- Record and undertakes corrective actions recommended by SRL/NRL.

SLIDE 8: Quality Assessment Methods Used In National Program

- The chart depicts-the different methods of EQA.
- Describe the methods again.

SLIDE 9:What Is Proficiency Testing?

- This slide describes the mechanism and method of proficiency testing.
- Give example from your experience & describe each step.

SLIDE 10: What Is On-site Evaluation?

- On site physical inspection & assessment is another method of EQA.
- A checklist is prepared.
- A competent and experienced person conducts the inspection and assessments.
- Lacunae found are communicated to lab supervisor on site and corrective actions to be taken are informed.
- Report is submitted to NACO for action.

SLIDE 11: What Is On-site Evaluation?—Cont'd

- The process of on-site evaluation is like internal audit which tells about the existent level of performance and quality standards of the lab.

SLIDE 12: What Is Re-testing?

- Describe the slide and inform about the NACO guideline to be followed.

SLIDE 13: EQA/Assessments Should Lead To Corrective Actions

- One important result of EQA has to be that the defaulting labs take corrective actions and preventive actions to ensure that same error is not repeated.

SLIDE 14: Problems may occur throughout the testing process

- Wrong/inaccurate results on EQA panel obtained by the participating lab may be due to:
 1. Deterioration of sample during transport.
 2. Error in performance
 3. Bad quality kits.
 4. Also describe the slide.

SLIDE 15: Take Corrective Actions

- The NRL/SRL has to investigate and find out the reasons for wrong results reported by the participating lab by site visit.
- Help the participating lab to undertake corrective actions according the cause of error found.
- The NRL/SRL must continue to monitor and mentor so that errors are not repeated.
- The details of each activity must be reported for later reference.

SLIDE 16: Sample Of Corrective Action Form

- This is a sample to show the lab reporting the corrective actions taken.

SLIDE 17: How To Implement Quality Assurance As Per The National Program.

- A percentage of negative and positive some samples are tested again by the participating lab are re-checked by NRL/SRL to ensure that results reported by the participating lab are accurate.

SLIDE 18: Issues To Consider For Re-testing

- The purpose of cross checking certain percentage of tested samples some positive and some negative received from the participating labs (mostly ICTCs & blood band labs) is to find out how many false positive or false negative results are being produced.
- This is also a kind of measure of quality assessment; as the NRL or SRL is going to cross check the samples by retesting.
- All testing and interpretation of results is done as per the NACO HIV testing guidelines.
- NLLs & SRLs should ensure that the samples received from the participating assigned labs are tested immediately and feed back is provided in order that corrective action can be taken at earliest possible.
- NACO has recommended that 20% of all positive and 5%of all negative samples during first week of every quarter and be sent for cross checking.
- EQA samples should be labeled as such with code numbers tested & interpreted.
- Results should be recorded.

SLIDE 19: Re-testing (Cross-checking) Process

- This slide gives details of the specimens to be sent for cross checking and the process followed for this type of external assessment.
- Describe the steps given in the slide.
- Give examples from your own experience.

SLIDE 20: Testing sites' Responsibilities: Re-testing

- The NRL/SRL receiving samples from ICTC/ Blood Banks for cross-checking must undertake the testing as per guidelines and protocols as is done routinely for testing a specimen from the client.
- NRL/SRL must provide feedback.
- All records must be made as done routinely.
- The participating lab who has sent the samples for cross-checking must take corrective action if required and record the same.

SLIDE 21: Specimen Requirements

- Participating lab must send at least 0.5 volume of sample.
- Samples must be stored and transported at 4-8° C. For up to one week, if delay is anticipated store samples at -20° C (if not available then freezer compartment of refrigerator)

SLIDE 22: Specimen Management: Common Problems

- The factors which may lead to discordant result at NRL/SRL may be due to transcription errors at either participating or referral lab.
- Sample sent may not be sufficient to complete the testing.
- The participating lab should ensure that due precautions are taken to avoid transcription errors and volume of sample sent is adequate to complete testing.

SLIDE 23: Summary

- This slide is a summary of carry home messages.
- Ask these questions
- Let the participant's answers.
- Re-explain and re-emphasize important issues from the slide giving some examples from experience.

17.DOCUMENT CONTROL AND VARIOUS DOCUMENTS

SLIDE 1: Learning Objectives

- There are many different types of documents which are essential for following the various processes in the laboratory. Each process, job undertaken in the laboratory is as per the guidelines laid down standard method. These details are contained in documents
- Records are the results of work we do in the laboratory, entries we make in various documents.
- Example an empty temperature log format is a document and when it is filled with daily recorded temperature of a refrigerator, it becomes a record.

SLIDE 2: What are documents and records?

- Documents will be the policy, description of a method, SOPs, books, journals etc.
- Records are the information we enter/log in registers, forms, charts etc.

SLIDE 3: What are documents and records?

- In this slide, guidelines, SOP and Kit insert are the documents and client result is a record.
- Finding from an onsite evaluation visit is a record.

SLIDE 5: Documents are the backbone of the quality system

- Why we need to have so many documents? It is because if not written, the processes will not be understood properly, will not be followed properly. Some steps will be missed. **“To a supervisor what is not written is not done.”**
- Also in case somebody goes to court the documents and records help us.

SLIDE 6: Controlled Document: Definition

- Controlled documents are those which have limited access, limited distribution have title and are authorized by the appropriate authority eg. SOP of various procedures followed in the laboratory.
- All controlled documents are kept safely.
- Old controlled documents which are not in use are kept in files/archived.

SLIDE 7: SOPs are controlled documents

- Every SOP has to be made in the proper way. It will be described later.
- SOPs/documents must be updated from time to time.
- SOP should be signed by the authorized signatory(HOD).

SLIDE 8: What SOPs should you keep at test site?

- These are examples of SOPs which should be kept in the Laboratory.
- Ask the LTs which SOPs they would like to keep on the working bench.

SLIDE 9:SOPs must be followed

- It is important to perform the activity as per the SOP.
- This is important to get accurate results.
- Also important to manage and maintain quality in the laboratory.

SLIDE 10:Do not rely solely on Manufacturer product inserts

- Some kits may not include all materials required to perform test.
- When you make an SOP it should include all the materials and all the steps of the test.
- For steps for performance of test follow the steps given in the kit insert.

SLIDE 11: Record Keeping Recording the results of each and every activity undertaken in the laboratory is important example of calibration records, testing records, test results, results of quality practices etc.

- If these things are not recorded then it cannot be checked, quality systems cannot be improved and the site cannot be accredited.
- It is presumed that what is not recorded is actually not done.
- So, record all activities undertaken in the laboratory.

SLIDE 12: What Records should you keep at site? This slide gives the minimal essentials of records to be maintained by the laboratories.

SLIDE 13:Tips for good record keeping

- You must understand that whenever some information is received and some results are generated it all has to be recorded.
- If same information is recorded a number of times it must be recorded each time and not only once.
- The method of recording the information must be uniform.

SLIDE 14:Client test records

- Record the information, results etc. legibly, as far as possible there should be no overwriting.
- All the information recorded must be dated and signed.
- The laboratory in-charge should ensure and check that records are maintained.

SLIDE 15:How long should you retain client test records?

- How long the records to be kept
- All the records have to be kept for 5 years.
- The record should be stored so that there is no chance of these being damaged.

SLIDE 16:Records should be permanent, Secure and traceable.

- The records should be safe from all environmental hazards.
- The records should be accessible only to authorized individuals.
- Records should be dated and signed by authorized officer.

SLIDE 17:Key messages

- Records help to meet the program reporting requirements.(to SACS and NACO)
- Maintaining records is an essential requirement of quality management system.

