

INVITED ARTICLE

Current approaches to the laboratory diagnosis and monitoring of progression of HIV infection

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Introduction

Early diagnosis of HIV infection and effective treatment of infected individuals are the keys to the success of HIV control programme. In the last two decades, significant progress has been made in the diagnosis and treatment of HIV infected patients as a result of the development of new diagnostic techniques. This has facilitated the rapid identification of many new

four to six weeks of infection (figure 1), and within 24 weeks in virtually all infected individuals^{1,2}. In primary HIV infection, there is a period during which the level of viraemia is high, but antibody is absent³. This period of absent antibody in the presence of established HIV infection is called the "window period". Antibody tests are usually negative at this time; therefore, tests for virus or its components in

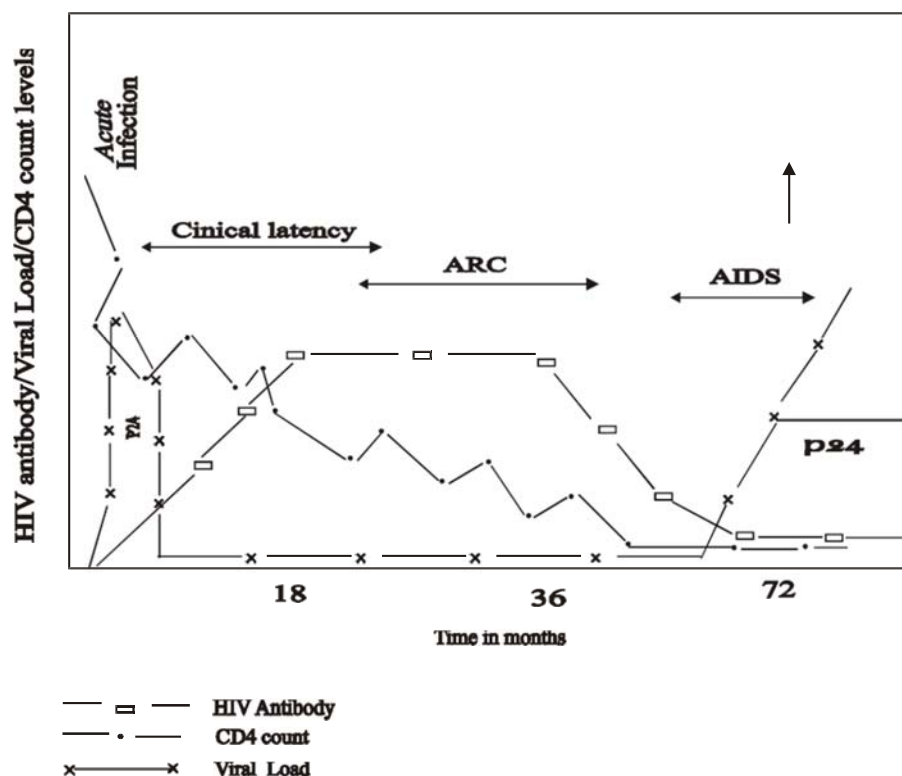


Figure 1 Time course and stages of HIV Infection

cases of AIDS and HIV infection, accurate monitoring of disease progression, and the efficacy of antiretroviral therapy. Laboratory diagnosis of HIV infection is based on the demonstration of the antibodies in the plasma or serum, and of the viruses in the blood. HIV antibodies are detectable within

blood are used for diagnosis. The virus can be demonstrated in the blood with nucleic acid-based tests (PCR for proviral Deoxyribonucleic Acid (DNA) and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for plasma viral Ribonucleic Acid (RNA), culture and p24 antigen assay.

ANTIBODY TESTS

The standard test for diagnosis of HIV infection is antibody detection in the blood. The objectives of HIV antibody testing are;

- i. To enable individuals know their HIV status and provide those with positive results access to treatment and care (antiviral and prophylaxis against opportunistic infections)
- ii. To prevent mother to child transmission by testing pregnant women and their newborns, and provide treatment and care to those with positive results.
- iii. To guide clinicians on medical management of patients.
- iv. To screen blood and organ donors to ensure public safety
- v. To carry out epidemiological surveillance and research.

Several different HIV antibody assays are now commercially available including Enzyme Linked Immunosorbent Assay (ELISA) and rapid tests. The latter are the most widely used in developing countries. Antibody tests for HIV diagnosis consists of screening and confirmatory tests⁵. Because of the serious consequences of false positive and false negative test results, it is imperative to use assays that are very sensitive and specific.

Screening Test

Rapid Tests : Rapid Tests are available in small test packs and therefore, are suitable for a laboratory testing of few samples. They are technically simple to perform, do not require any equipment but have a sensitivity and specificity comparable to ELISA. The commonly used rapid antibody tests in laboratories are based on the principles of dot immunoassay, or particle agglutination test^{6, 7,8}. Dot blot assays utilize recombinant or synthetic peptides spotted onto nitrocellulose paper or micro

particles. The antigen containing matrix is placed inside a plastic device containing absorbent pads to collect reactants. These assays are useful for single test applications such as in an emergency, during autopsy, or in labor rooms or in peripheral blood banks. Agglutination assays utilize antigen – coated carriers such as red blood cells, latex particles, gelatin particles and micro beads. The particles are used to support or carry HIV antigens by non – specific attachment. In this assay, HIV antibody in the patient's serum binds to the HIV antigens and a lattice network between antigen and antibody is formed that can be seen macroscopically. Agglutination tests are very sensitive, easy to perform and do not require sophisticated equipment and washing procedures.

ELISA: The ELISA procedure is carried out to screen for HIV IgG antibodies in plasma or serum. The principles of ELISA are classified as direct, indirect and competitive test. The competitive principle is not popular because of the low sensitivity. Antigens derived from HIV grown in human T – lymphocytes or recombinant proteins or synthetic peptides are used to coat beads or micro titer plates. To perform an indirect ELISA, the patient's serum is incubated with the antigens on the beads or in the micro titer plates. A conjugate, i.e. enzyme – labeled antibody specific for human immunoglobulin is then added. Detection of the enzyme – labeled antibody is carried out by the addition of a substrate that produces a colour reaction^{5,9}.

ELISA requires a plate washer, a reader, and ELISA test kit which contains the reagents for the test and the micro titre plate. It is a suitable test for laboratories where more than 30 samples are tested at each time. A good ELISA kit should have a specificity and sensitivity that exceeds 98% to 99%. A specimen that is reactive in ELISA is usually retested in an ELISA kit that uses an antigen made from another source. If the sample is reactive in the second ELISA, it is considered to be repetitively

reactive. Repetitively reactive samples are usually subjected to confirmatory tests such as western blot. False positive sometimes occur in western blot, especially in persons infected by other retroviruses, or in individuals with immunological disorders. False negative reactions are often seen ^{5, 10} during the 'window period' of primary HIV infection.

Confirmatory Test

Western blot: standard confirmatory test for HIV antibody assays. The test utilizes HIV antigens from purified viruses that have been electrophoretically separated and "blotted" (transferred onto a nitrocellulose paper). The paper is then cut into stripes, each containing all the separated HIV antigens. To carry out a western blot assay, a stripe is incubated with the patient's serum. Antibody in the serum binds to the HIV antigens on the strip, and is detected with the aid of a conjugate consisting of labeled anti – human immunoglobulin. Using the CDC criteria for interpretation of results, a Western blot is interpreted as positive, if two or more of the following bands are present on the strip: p24, gp41, gp 120/ 160. It is interpreted as negative, if no band is present, and indeterminate, if the result is not consistent with the criteria set for positive result ^{5,10}.

Western blot should only be used to resolve

discordant results and diagnosis of HIV – 2. A major drawback in the use of Western blot is its high cost and the high frequency of the occurrence of indeterminate results in specimens, especially those obtained during the early stages of HIV infection.

Indirect Immunofluorescent Antibody Assay (IFA): Indirect Immunofluorescent antibody assay employs HIV – infected cells (lymphocytes) fixed to the microscope slide. The patient serum is added and reacts (if antibody is present) with the intracellular HIV. After washing the slide, a conjugate consisting of anti – human immunoglobulin labeled with FITC is added, and the reaction is visualized under a fluorescent microscope. IFA has been used to

confirm diagnosis in sera producing indeterminate results in Western blot.

NUCLEIC ACID – BASED TESTS: These consist of DNA Polymerase Chain Reaction (PCR) and reverse transcriptase PCR (RT – PCR)

HIV DNA polymerase Chain Reaction (PCR) The DNA PCR involves the amplification of specific DNA sequences in the proviral DNA that has been integrated in the host cell ^{8,9}. This test is the preferred procedure for diagnosing HIV infection in infants less than 18 months of age. Because of the high sensitivity of the test, false positive results may occur as a result of contamination by minute quantities of extraneous DNA. A positive test result is indicative of HIV infection; however, it must be confirmed by a repeat test. Negative tests should similarly be confirmed.

RT – PCR: RT PCR is used to detect and quantify the amount of HIV RNA in plasma (11). The assay requires the conversion of viral RNA to DNA and amplification of specific sequences in the DNA produced by a process known as reverse transcriptase chain reaction (RT – PCR).

OTHER LABORATORY TESTS

Antigen Detection: Detection of p24 antigen is ¹³

an ELISA – based test . It is useful for diagnosis

during the 'window period' of early infection and in the newborn. It was the major assay used to measure viral replication directly before viral load assays became available. The disadvantage of this assay is that it has low sensitivity, therefore it may not detect p24 antigen in many positive individuals.

Virus isolation: HIV is usually isolated in peripheral blood mononuclear cells (PBMCs) ¹⁴. The procedure involves co – cultivating uninfected, mitogen – stimulated PBMCs with the PBMCs obtained from the test specimen. After several days, the supernatant culture fluid is assayed for reverse transcription activity, p24 antigen or HIV RNA. HIV isolation in PBMC is quite sensitive and is comparable to DNA PCR in

sensitivity. Positive results may be obtained within 1 – 2 weeks, but a negative result is not reported until after 30 days. Both negative and positive results should be confirmed with a second test. HIV culture is an acceptable technique for the diagnosis of HIV infection in infants and children under 18 month's age. Virus isolation is time – consuming; it generates a large number of infectious particles. Therefore, it should be carried out in laboratories with adequate facilities for biocontainment.

WORLD HEALTH ORGANISATION (WHO)/UNAIDS HIV TESTING ALGORITHMS

An algorithm describes the sequence of tests to be performed during laboratory testing of Specimens. Because of the high cost of Western Blot and the frequent occurrence of indeterminate results, especially when used to test specimens collected from patients in the early stages of HIV infection, the WHO and UNAIDS, the organ of the United Nations for the control of HIV/AIDS recommended algorithms for HIV antibody testing that, ordinarily, will not require confirmation of test results with Western Blot. This involves the use of two or three rapid test or ELISA kits that are based on different antigen sources. WHO/UNAIDS Testing Algorithm for Scaling Up Voluntary HIV Counseling and Testing (HCT) in Resource – Limited Settings. A serial or parallel double rapid testing protocol was recommended by WHO in order to scale up voluntary HCT and enhanced access to ART in resource – limited countries as illustrated. Serial testing algorithm refers to the use of 2 screening tests sequentially to test for HIV antibody. If the initial screening is negative is negative, no further testing is required. If the initial is positive, it is followed by one more test. The first test should be the most sensitive test and the second test should be very specific, and be based on antigen source different from that of the first test. Samples that produce discordant results in the two tests are subjected to further testing.

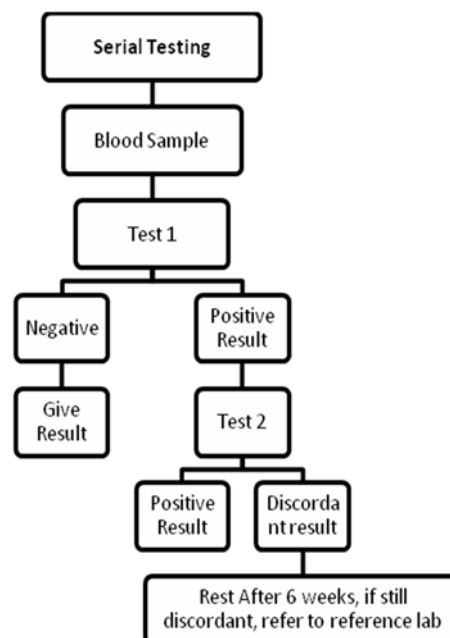


Figure 2A: WHO parallel testing algorithm

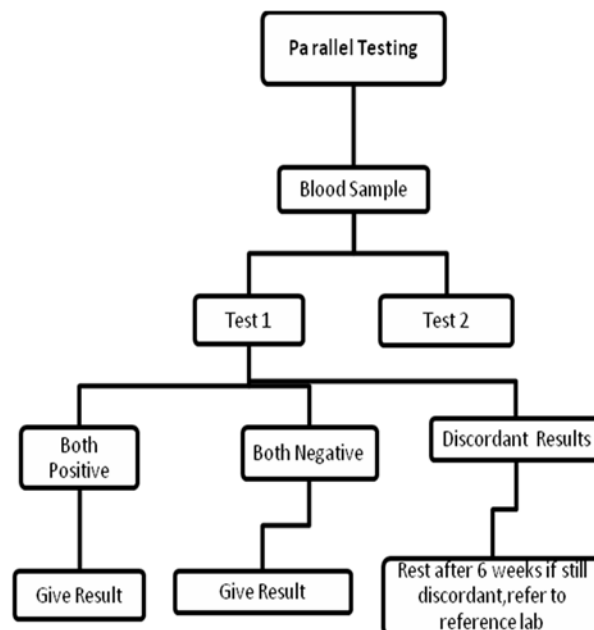


Figure 2B: WHO Serial testing algorithm

Parallel testing involves the use of two screening tests performed simultaneously. Samples reactive in both tests are regarded as positive; however, those with discordant results require further testing. Parallel testing is performed to minimize the chances of false negative results. It ensures sensitivity and

guards against technical errors. It is often used when a very sensitive test is not available for the initial screening, and when the concordance of two tests is to be evaluated.

WHO/UNAIDS Testing Algorithms Based on Different Objectives of HIV Antibody Testing and Prevalence of HIV Infection in the Population

The objectives of HIV antibody testing have been outlined on page 1, under the section on Antibody Tests. Three different HIV testing algorithms were recommended by WHO/UNAIDS based on these objectives and the prevalence of infection in the population. Test algorithm 1 makes use of only test kit, algorithms 2 and 3 utilize 2 and 3 test kits, respectively.

HIV Testing Algorithm I: This testing algorithm is used when the objectives of HIV antibody testing are screening of blood and blood products for transfusion, or surveillance in a population where the prevalence of HIV infection is > 10%, or diagnosis in population where the prevalence of HIV infection is > 30% and clinical signs and symptoms of HIV infection are present. In this algorithm, a sample is tested with one rapid test or ELISA kit, and if it is repetitively reactive, it is considered positive and no further testing is required. If it is non – reactive, it is regarded as negative.

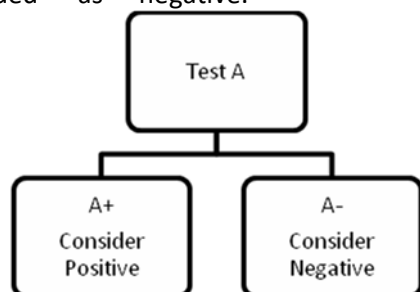


Figure 3:WHO/UNAIDS Testing Algorithm I

HIV testing Algorithm II: This HIV testing algorithm is used when the objectives of testing are surveillance in a setting where the prevalence of HIV infection is < 10%, for diagnosis where the prevalence of infection < 30%, and for identification of asymptomatic

individuals where the prevalence of infection is > 10%.

In this algorithm, samples are screened for HIV antibody with a rapid test or ELISA (Test A). Reactive samples are retested in a different assay (Test B). If a sample is reactive in both tests, it is regarded as positive for HIV antibody (Figure 4). A sample that is reactive in test A, but is non – reactive in test B should be retested in both assays. A sample with concordant reactive results is indeterminate. Any sample with concordant non – reactive result is negative.

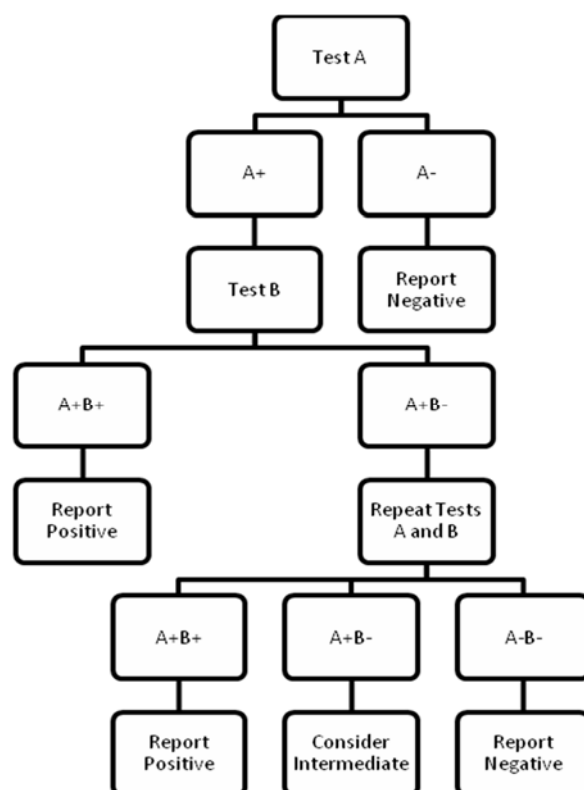


Figure 4 WHO/UNAID HIV Testing Algorithm (Strategy) II

HIV Testing Algorithm III: This algorithm (Figure 5), an extension of strategy II, is recommended for identification of asymptomatic individuals in setting where the prevalence of HIV infection is < 10%. All samples that are regarded as positive

after tests A and B and all indeterminate samples are subjected to Test C. If they are reactive in all the three tests, they are regarded as positive. A sample that is reactive in two of the tests is considered to be indeterminate, if the individual tested is from a high – risk population, but is regarded as negative if he is from a low risk population. Individuals whose samples yielded indeterminate results should be requested to have a repeat test done after 2 weeks with a fresh sample.

CLINICAL LABORATORY MONITORING OF HIV DISEASE PROGRESSION AND OF HIV/AIDS PATIENTS ON ANTIRETROVIRAL THERAPY (ART)

The progression of HIV infection to AIDS varies widely in different individuals^(15,16). It varies from non-progression to rapid progression⁽¹⁷⁾ with death occurring within a few years. Once an individual is identified as positive, it is important to assess the stage of infection, especially if therapy is being contemplated. It is also necessary to monitor disease progression in patients undergoing ART. The two test procedures most commonly used for monitoring are viral load, also known as plasma RNA load, and CD4⁺ lymphocyte count. In developed countries, a combination of HIV viral load assay and CD4 counts are used as markers of progression. However, in resource-limited settings, the use of CD4 counts alone has been recommended because of the high cost of viral load assays.

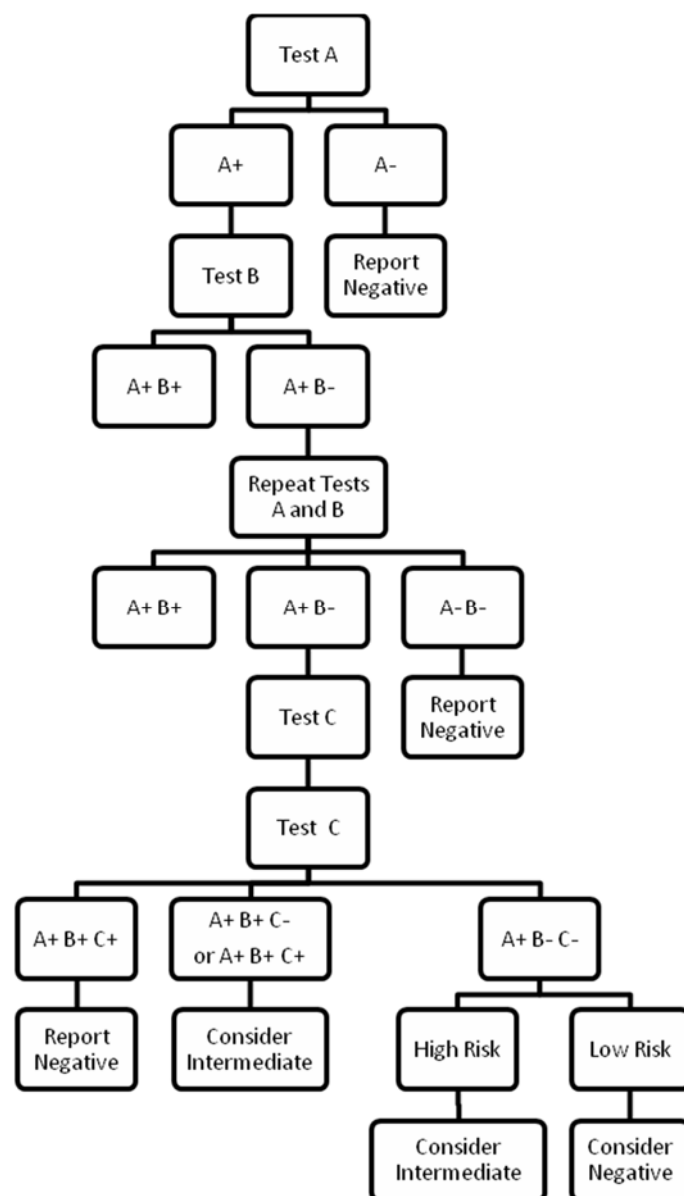


Figure 5. WHO/UNAIDS HIV Testing Algorithm (Strategy) III

CD4+ T lymphocyte Assay: T – Lymphocytes account for 70 – 90% of all lymphocytes (18). They are about 1600 per mm³, with CD4 accounting for approximately 1,000 and CD8 approximately 500. The decline in CD4 count in HIV infection is gradual, about 50 – 100 cells per year. It is believed that a CD4 cell count of less than 500mm³ is indicative of immunodeficiency. The lower the CD4 count, the more severe the

disease progression and the less favorable is the prognosis Enumeration of these cells is the most important parameter for assessing the immune system of HIV/AIDS patients (19,20). Accurate and reliable measurements of CD4 T-lymphocytes is very essential for monitoring the rate of progression to AIDS, and for initiating prophylaxis for opportunistic infections as well as monitoring the success or failure of ART. T – lymphocyte enumeration is performed by flow cytometry or by manual methods.

Flow cytometry is a laboratory procedure used for differentiating and counting cells and micro particles. It involves staining of lymphocytes in whole blood with monoclonal antibodies conjugated to fluorochromes (21, 22) followed by an analysis of the cells. Fluorescent antibody – stained cells in suspension pass through a nozzle in a narrow stream into a focused light beam, usually a laser. Light that is scattered and emitted by cells is then separated into constituent wavelengths by a series of optical filters and mirrors. This separated light is then translated into electrical signals that are subsequently detected and processed for cell distribution analysis.

Flow cytometers are the equipment used in this assay. They fall into two groups: classical flow cytometers and simplified flow cytometers. Examples of classical flow cytometers are FACS Calibre (Becton Dickinson) and FASCalibre. Examples of simplified flow cytometers are partec cyflow, FASCount and Guava Easy CD4.

Manual methods of enumerating CD4+ T-lymphocytes are microscopic methods using Dynabeads or Cytospheres (Coulter). Other manual assays include Opti – CIM (CIMA), Zymune (Zynaxis), TRAxCD4 (T cell Dx and Innogenetics), CD4 Count Chips (SemBio), the Capcelliaa (Sanofi), and the CD4 Biochip (Lab Now).

Viral Load Assay: Detection and qualification of HIV – 1 RNA in plasma is carried out with reverse transcriptase polymerase chain reaction (RT PCR). There are three stages of this test:

Conversion of viral RNA to DNA by reverse transcription, amplification of the DNA and detection of the amplified DNA with the aid of oligonucleotide probes.

HIV viral load measurement is a strong predictor of disease progression and death. For example, studies have shown that infected patients with viral levels of > 100,000 copies / ml at 6 months of infection are 10 – fold more likely to progress to AIDS in 5 years than those with viral load measurements of <100,000 copies per ml. 350, cells/mm, therapy should be considered when the viral load is up to 100,000 copies/ml. The procedure is also used to monitor the success or failure of ART and whether or not there is need to change the treatment regimen.

Viral load assay, in conjunction with CD4 enumeration, is an important tool used by clinicians and researchers to assess the virological status of a patient (23,24,25). This assay must be performed on HIV positive patients to determine the amount of HIV in their plasma and the need to initiate ART.

Three types of assay kits have been approved by Food and Drug Administration (FDA),USA and NAFDAC for the measurement of viral load. They are Roche Amplicorr HIV – 1 Monitor version 1.5 and Roche Amplicor HIV – 1 Monitor Ultrasensitive version 1.5, the NucliSens HIV – 1 QT, a nucleic acid sequence – based assay and VERSANT HIV – 1 RNA 3.0 a branched DNA assay.

There are two types of the Roche Amplicor HIV – 1 Monitor version 1.5: the standard and the Ultrasensitive. The range of assay sensitivity using the standard Roche Amplicor HIV – 1 Monitor is 400 – 750,000 copies per ml, and that of the Ultrasensitive assay is 50 – 75,000 copies per ml. The Standard Roche Amplicor HIV – 1 Monitor Assay should be used for new patients , and if viral load is greater than 75,000 copies per ml. Ultrasensitive assay should be used when the upper limit of viral load is less than 75,000 copies per ml, or when the lower

limit is anticipated to be less than 400 copies per ml.

NucliSens HIV – 1 RNA QT: Marketed by bioMeriux, the NucliSens HIV – 1 RNA QT, selectively and directly amplifies HIV – 1 RNA without PCR in a one – step sandwich hybridization procedure, resulting in the production of single – stranded RNA. This assay may use specimens that include tissue or body fluids such as signal secretions and blood spots.

Branched Chain DNA (bDNA): Branched chain DNA, also known as Versant, is marketed by Bayer Diagnostics. Initial steps in this assay require concentration of virions by centrifugation and release of the RNA by disruption of the virus with detergent and proteinase K. The test has good reproducibility, requires fewer hands – on time and amplifies HIV subtypes.

Viral Load Interpretation: Baseline Measurement of viral load should be obtained prior to commencement of therapy; another measurement of viral load should be obtained after initiation of therapy, and repeated every 24 weeks during therapy, if the level is < 400 RNA copies per ml. The test should also be repeated if there is a clinical event or a decline in CD4 count. Successful ART is defined as a decrease to < 400 RNA copies per ml and an increase of > 50 cells/ mm³ in the CD4⁺ T- lymphocyte count by week 24 of treatment failure is defined as a viral load that is 400 RNA copies per ml at 24 weeks or that increases by 0.5log₁₀ at any time.

MONITORING OF DRUG RESISTANCE IN HIV/AIDS PATIENTS ON ANTIRETROVIRAL TREATMENT (ART)

The scaling up of ART worldwide has made a dramatic impact on longevity and quality of life of many AIDS patients. However, a considerable number of patients receive suboptimal doses of the drugs used for treatment, or fail to adhere to the treatment regimen due to a variety of factors including drug toxicity, lack of money to

purchase the drugs, inconvenient appointments with the clinic, etc. This creates an in vivo selection pressure on the virus resulting in the emergence of drug resistant mutants ^(27, 28, 29). Knowledge of the nature of drug resistance of the indigenous HIV strains is absolutely essential so that clinicians may be properly guided in the use of antiretroviral drugs for the management of HIV / AIDS patients. Two methods are used for the assessment of drug resistance: Phenotypic Susceptibility Testing and Genotype assays.

Genotype testing detects specific mutation in the reverse transcript and protease genes. Phenotypic testing on the other hand, determines the relative amount of drug needed to suppress viral growth compared to a reference wild-type virus. Genotype tests are less technically demanding to perform; they are relatively less costly and have a shorter turnaround time. Phenotypic testing requires elaborated facilities for viral culture and may therefore be difficult to set up in laboratories without a virology unit.

Antiretroviral drug resistance testing is carried out to determine whether a patient has been exposed to a resistant virus so that the physician could be guided on the choice of drugs to use for treatment. It should also be performed promptly in case of virological failure i.e. when viral load levels does not fall despite ART or in cases of incomplete virus suppression and when patients have been off therapy for a considerable length of time.

THE LABORATORY AND HIV IN NIGERIA

The HIV / AIDS pandemic has made an enormous impact on the Nigerian population because of the morbidity, mortality, and socioeconomic problems it causes in the society. Despite the efforts to contain it, many new HIV infections continue to occur daily. In an effort to stem the spread of HIV infection in the country, the Federal government of Nigeria launched the AIDS prevention initiative in Nigeria (APIN) in which it proposed a scaling-

up of HIV counseling and testing (HCT). Prevention of mother to child transmission (PMTCT) antiretroviral treatment (ART), and care and support of people living with HIV and AIDS (PWLHA) and increase the health budget devoted to these activities. The success of this scale – up requires major contributions from the laboratories.

In supporting the Federal government's initiative on prevention of HIV / AIDS, the laboratory is responsible for carrying out diagnosis of HIV infections and clinical laboratory monitoring of disease progression and of patients on ART. It is important that the processes of testing clinical specimens be monitored through quality control and quality assurance to ensure that accurate and reliable test results are obtained. This will enable physicians to establish correct diagnosis quickly, thus facilitating good clinical management of the patients.

The launching of APIN and the provision of development assistance from the International donor agencies considerably improved the laboratory facilities for HIV diagnosis in the country. This led to an improved monitoring of the AIDS epidemic through sentinel surveys (29), an increase in the number of donated blood units screened for HIV in the blood banks, identification of more cases of HIV infection in voluntary HIV testing and counseling centres and the recruitment of more HIV infected patients into the ART programme. The progress made in the Prevention of Mother to Child Transmission (PMTCT) Programme also increased considerably.

The increase in the number of patients undergoing ART has led to the establishment of more treatment centres all over the country. This effect of this is a widespread use of antiretroviral drugs and an improvement of the longevity and quality of life of many Nigerian AIDS patients. Unfortunately, this has been accompanied by the development of resistance to some of the drugs (27, 28). Monitoring and prevention of the development of resistance to

antiretroviral drugs by the laboratories should be an important goal of the National HIV / AIDS control programme.

A lot still needs to be done by government before success can be achieved in the fight against HIV / AIDS. Recent need assessment of diagnostic laboratories carried out in parts of the country revealed that infrastructural facilities such as laboratory space, water and electricity supply, etc are grossly inadequate. Both major and minor equipment as well as consumables are in short supply. Also, there are not enough well trained personnel to perform certain laboratory procedures for HIV diagnosis. It is therefore necessary for the Federal and State government to better equip the laboratories and train laboratory personnel in adequate numbers to perform these laboratory procedures, especially those for diagnosis of infection and monitoring of patients undergoing antiretroviral therapy and emergence of drugs resistant HIV strains. This will contribute in no small measure to the effective management of AIDS patients by clinicians and the success of HIV / AIDS control programme of the Federal government.

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