ABSTRACT
The AIDS epidemic has already led to serious consequences for health care systems worldwide. The information attained by the laboratory tests is useful for prophylaxis, medical management, safety of blood, sentinel surveillance, to motivate behavioral modifications and to monitor trends of epidemic. This review highlights and updates various laboratory methods for diagnosing, monitoring and managing acquired immunodeficiency syndrome (AIDS) patients.

Keywords: HIV infection; HIV diagnostic test; Serology; Nucleic acid assay.

INTRODUCTION
The Human Immunodeficiency Virus (HIV) was unknown until the early 1980’s but since that time it has infected millions of persons worldwide. The result of HIV infection is relentless destruction of the immune system leading to onset of the Acquired Immunodeficiency Syndrome (AIDS). The AIDS epidemic has already resulted in the deaths of over half its victims. The debate about the HIV antibody test had been long, complex and anguish. No single diagnostic test in the history of modern medicine has had such a momentous impact on the lives of the individuals who rely on it. Since the beginning of the AIDS crisis, people have had very dramatic responses to the test lapsing into severe chronic depression and anxiety. Given that the test holds such power, its flaws and shortcomings are extremely significant. Unfortunately, it is only now that this immensely important subject is being investigated. The development of increasingly sophisticated virologic and immunologic techniques has further enhanced our ability to diagnose HIV-1 infection early and accurately. Despite the potential value of newer techniques, various modifications of the original solid phase serologic methods have remained the standard means by which most HIV-1 infections continue to be diagnosed in the United States and in many developed nations. The ability to accurately determine viral and infected cell burden is essential in understanding the natural history of HIV infection, predicting disease progression and assessing the efficacy of various therapeutic drug regimens and vaccines. The present work is thus aimed at reviewing the laboratory methods used in the diagnosis of HIV infected patients.

DISCUSSION
Once the HIV enters the human body via infected blood, semen, vaginal secretions etc, the virus immediately targets cells which display the viral receptors - CD4. These receptors are seen on lymphocytes (T helper cells and regulatory T cells) and other cells such as, monocytes, macrophages, and dendrite cells. The virus enters these cells, forms DNA, integrates into the host genome, multiplies and persists for many years. After a period of 3-12 weeks, the host mount an immune response against the virus which is detected as antibody in the blood. This stage is called seroconversion. Current routine laboratory diagnosis of HIV is mainly based on the detection
of these specific anti-HIV antibodies. The period following the entry of HIV into the body and the appearance of detectable levels of antibodies with the available tests is called the “window period”. During the window period, the patient is highly infectious but the antibody test is negative. This is also a worrying time for patients who have had an exposure and then want to be tested for HIV. Although many tests can be used to detect virus in general, specific tests has been described for detecting HIV-1 & 2.

Serological Assays

1. HIV -1 antibody screening assays

a) Enzyme Linked Immunoasorbant Assay (ELISA)

The first enzyme immunoassay test to detect antibodies against HIV was introduced in 1985. The enzyme-linked immunoabsorbant assay (ELISA), or enzyme immunoassay (EIA), was the first screening test commonly employed for HIV. It has a high sensitivity.

In an ELISA test, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens have been attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared “secondary antibody” — an antibody that binds to human antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and negative result.

b) Rapid tests

Rapid HIV tests can play an important role in HIV prevention activities and expand access to testing in both clinical and nonclinical settings. Rapid HIV formats include:

A. “Flow through cassettes” or “membrane immuno concentration devices”, capture and detect HIV antibody in a specimen flowing through a porous membrane. A visible dot or line forms on the membrane when HIV antibodies are present.

B. “Immunochromatographic strip (ICS) tests utilize a one-step method in which the patient’s blood specimen is combined with signal reagent and migrates through a special membrane. A positive reaction is seen as development of a line or the membrane.

2. HIV-1 antibody confirmatory antibody assays

Confirmatory tests must be highly specific to ensure that individuals who test reactive in screening assays are correctly identified as being HIV infected.

[A] Western blot

Like the ELISA procedure, the western blot is an antibody detection test. However, unlike the ELISA method, the viral proteins are separated first and immobilized. In subsequent steps, the binding of serum antibodies to specific HIV proteins is visualized. Specifically, cells that may be HIV-infected are opened and the proteins within are placed into a slab of gel, to which an electrical current is applied. Different proteins will move with different velocities in this field, depending on their size, while their electrical charge is leveled by a surfactant called sodium laurel sulfate. Some commercially prepared Western blot test kits contain the HIV proteins already on a cellulose acetate strip. Once the proteins are well-separated, they are transferred to a membrane and the procedure continues similar to an ELISA: the person's diluted serum is applied to the membrane and antibodies in the serum may attach to some of the HIV proteins. Antibodies that do not attach are washed away, and enzyme-linked antibodies with the capability to attach to the person's antibodies determine to which HIV proteins the person has antibodies.

There are no universal criteria for interpreting the western blot test: The number of viral bands that must be present may vary. If no viral bands are detected, the result is negative. If at least one viral band for each of the GAG, POL, and ENV gene product groups is present, the result is positive. The three-gene-product approach to western blot interpretation has not been adopted for public health or clinical practice. Tests in which less than the required numbers of viral bands are detected are reported as indeterminate: a person who has an indeterminate result should be retested, as later tests may be more conclusive. Almost all HIV-infected persons with indeterminate western blot results will develop a positive result when tested in one month; a persistently indeterminate result over a period of six months suggests the results are not due to HIV infection. In a generally healthy low-risk population, indeterminate results on western blot occur on the order of 1 in 5,000 patients. However for those individuals that have had high-risk exposure to individuals where HIV-2 is most prevalent, Western Africa, an inconclusive western blot test may prove infection with HIV-2. The HIV proteins used in western blotting can be produced by recombinant DNA in a technique called recombinant immunoblot assay (RIBA).
[B] Indirect immunofluorescence assay
Immunofluorescence assay (IF) is widely used for the rapid diagnosis of virus infections by the detection of virus antigen in clinical specimens, as well as the detection of virus specific IgG or IgA or IgM antibody. The technique makes use of a fluoresce in labelled antibody to stain specimens containing specific virus antigens, so that the stained cells fluoresces under UV illumination. There are mainly two types of IF assay such as direct and indirect IF assays.

Alternative Antibody Testing Technologies
Non-invasive methods provide alternatives to diagnostic blood tests and have high patient acceptance, increased safety and reduced costs. Currently saliva, gingival crevicular fluid, oral mucosal transudates and urine are considered to be the alternatives to blood.

(a) Oral fluids
Whole saliva, glandular duct saliva or mucosal transudates are the specimens that can be collected for tests to detect antibody to HIV in oral secretions. The fluid most frequently employed for salivary diagnostic purposes is expectorated whole saliva, and oral mucosal transudates. Several devices are commercially available for the collection of oral mucosal transudate specimens for the detection of HIV antibodies.

Screening for HIV Antibody in Oral Fluids
A number of screening assays have been employed for the detection of HIV antibodies in oral fluids.

These include both conventional enzyme immunoassays (EIA) and rapid tests designed for use with serum or plasma samples, as well as an IgG antibody capture radioimmunoassay (GACRIA) and enzyme-linked immunosorbent assay (GACELISA) optimized for the detection of HIV antibody in specimens that contain low concentrations of immunoglobulin.

Modifications for oral fluids
The oral-fluid Vironostika HIV-1 Microelisa system has been licensed for use with the associated OraSure collection device. This EIA is identical to the serum based Vironostika HIV-1 assay, except that the procedure has been modified by decreasing the sample dilution from 1 to 75 for serum to 1 to 2 for oral fluids. Rapid and simple “point-of-care” tests which use techniques involving membrane capture or particle agglutination have also been used for the detection of HIV antibody in oral fluids.

(b) Urine analysis
A high prevalence of antibodies to the glycoprotein gp 120 and gp 160 in urine samples among seropositive specimens led to the proposition of detection of antibodies specific for these glycoprotein. Several studies have utilized IgG antibody capture particle adherence tests and have concluded that performance of urine antibody screens is similar to that of serum tests.

(c) Antibodies against HIV in vaginal mucosa
A study has shown that “high risk sero negative” subjects had IgA in their genital mucosa, that it likely was produced locally, and that it probably served as a barrier to systemic HIV-1 infection. Belec et al 1994 studied 150 paired serum and vaginal secretions obtained from HIV-1 sero negative women. The results showed detection of IgG antibody among 2.5 % of the vaginal secretions. Antibodies in such specimens were broadly reactive with HIV-1 core and env antigens.

Viral Identification Assays
[1] Polymerase Chain Reaction [PCR] / Nucleic acid-based tests (NAT)
Polymerase chain reaction (PCR) has emerged as one of the most powerful tools for the amplification of genes and their RNA transcripts. Nucleic-acid-based tests amplify and detect one or more of several target sequences located in specific HIV genes, such as HIV-1 GAG, HIV-II GAG, HIV-env, or the HIV-pol.

Since these tests are relatively expensive, the blood is screened by first pooling some 8-24 samples and testing these together; if the pool tests positive, each sample is retested individually. Although this results in a dramatic decrease in cost, the dilution of the virus in the pooled samples decreases the effective sensitivity of the test, lengthening the window period by 4 days (assuming a 20-fold dilution, ~20hr virus doubling time, detection limit 50 copies/ml, making limit of detection 1,000 copies/ml). Since 2001, donated blood in the United States has been screened with nucleic-acid-based tests, shortening the window period between infection and detectability of disease to a median of 17 days (95% CI, 13-28 Days, assumes pooling of samples).

A different version of this test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the management of HIV-1-infected patients.

In the RT-PCR test, viral RNA is extracted from the patient's plasma and is treated with reverse transcriptase (RT) to convert the viral RNA into cDNA. The polymerase chain reaction (PCR) process is then applied, using two primers unique to the virus's genome. After PCR amplification is complete, the resulting DNA products are hybridized to specific oligonucleotides bound to the vessel wall, and is then made visible with a probe bound to an enzyme. The amount of virus in the
sample can be quantified with sufficient accuracy to detect threefold change. In the Quantiplex bDNA or branched DNA test, plasma is centrifuged to concentrate the virus, which is then opened to release its RNA. Special oligonucleotides that bind to viral RNA and to certain oligonucleotides bound to the wall of the vessel are added. In this way, viral RNA is fastened to the wall. Then new oligonucleotides that bind at several locations to this RNA are added and other oligonucleotides that bind at several locations to those oligonucleotides. This is done to amplify the signal. Finally, oligonucleotides that bind to the last set of oligonucleotides and that are bound to an enzyme are added; the enzyme action causes a color reaction, which allows quantification of the viral RNA in the original sample. Monitoring the effects of antiretroviral therapy by serial measurements of plasma HIV-1 RNA with this test has been validated for patients with viral loads greater than 25,000 copies per milliliter.

[2] Virus culture
The qualitative culture enables one to isolate HIV and may be used to confirm infection in an individual with equivocal serologic studies, or in infants born to seropositive mothers. The quantitative assay enables one to determine the number of infected cells or titre of infectious virus in a given sample by evaluating serial dilutions of infected peripheral blood mononuclear cells (PBMC) or plasma.

[3] P24 antigen capture assay
The p24 antigen test detects the presence of the p24 protein of HIV (also known as CA), the capsid protein of the virus. Monoclonal antibodies specific to the p24 protein are mixed with the person's blood. Any p24 protein in the person's blood will stick to the monoclonal antibody and an enzyme-linked antibody to the monoclonal antibodies to p24 causes a color change if p24 was present in the sample. This test is no longer used routinely in the US or the EU [25] to screen blood donations since the objective was to reduce the risk of false negatives in the window period. Nucleic acid testing (NAT) is more effective for this purpose, and p24 antigen testing is no longer indicated if a NAT test is performed. The p24 antigen test is not useful for general diagnostics, as it has very low sensitivity and only works during a certain time period after infection before the body produces antibodies to the p24 protein.

Monitoring tests

Lymphocyte analysis
The CD4 T-cell count is not an HIV test, but rather a procedure where the number of CD4 T-cells in the blood is determined. A CD4 count does not check for the presence of HIV. It is used to monitor immune system function in HIV-positive people. Declining CD4 T-cell counts are considered to be a marker of progression of HIV infection. A normal CD4 count can range from 500 cells/mm³ to 1000 cells/mm³. In HIV-positive people, AIDS is officially diagnosed when the count drops below 200 cells/µL or when certain opportunistic infections occur. This use of a CD4 count as an AIDS criterion was introduced in 1992; the value of 200 was chosen because it corresponded with a greatly increased likelihood of opportunistic infection. Lower CD4 counts in people with AIDS are indicators that prophylaxis against certain types of opportunistic infections should be instituted.

Low CD4 T-cell counts are associated with a variety of conditions, including many viral infections, bacterial infections, parasitic infections, sepsis, tuberculosis, coccidioidomycosis, burns, trauma, intravenous injections of foreign proteins, malnutrition, over-exercising, pregnancy, normal daily variation, psychological stress, and social isolation. This test is also used occasionally to estimate immune system function for people whose CD4 T cells are impaired for reasons other than HIV infection, which include several blood diseases, several genetic disorders, and the side effects of many chemotherapy drugs. In general, the lower the number of T cells the lower the immune system's function will be. Normal CD4 counts are between 500 and 1500 CD4+ T cells/µmicroliter, and the counts may fluctuate in healthy people, depending on recent infection status, nutrition, exercise, and other factors. Women tend to have somewhat lower counts than men.

Lymphocyte subsets are usually quantities in two related ways. The first of these is as a percentage of the total T lymphocyte (CD3 bearing) population. This value is generated directly from the fluorescence activated cell sorter (FACS). The second measurement (more commonly used) of the CD4 subset is the total CD4 cell count, as an absolute number derived by multiplying the FACS's percentage of CD4 cells by the total lymphocyte count. The CD4 T cell count is an important determinate of disease stage and prognosis in HIV infected individuals. Nonspecific markers such as CD4 cell count, is expressed either as an absolute value (normal adult range 600-1700 /mm³) or as a CD4:CD8 ratio (normal adult ratio 1.2: 3.5). According to most guidelines followed, a CD4 T cell count < 350/µL is an indication of initiating antiretroviral therapy, and a decline in the CD4 T cell count of > 25% is an indication for considering change in therapy. Once the CD4 T cell count is < 200 /µL, patients should be placed on a regimen for Pneumocystis carinii pneumonia.

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prophylaxis and once count is < 50 /µl, primary prophylaxis for MAC (Mycobacterium avium complex) infection is indicated. CD4 T cells (determined by flow cytometry) and total lymphocyte count (determined by the WBC count and differential percent) has been shown to correlate very well with the level of immunologic competence. Flow cytometry is an important method used to evaluate cell kinetics, in which large numbers of cells can be analyzed rapidly, providing distribution profile of several thousand cells at one time.26,28

HIV-2 tests
The antigens of HIV-2 are similar to those of HIV-1, but the molecular weights may vary slightly. The gag proteins of HIV-2 have designations of p56, p26, and p16. The designations of pol proteins are p68 and p34 and the envelope glycoprotein gp36 (or gp41), gp140, and gp105. As with HIV-1 screening tests, a variety of test formats are available to detect antibodies to HIV-2, including ELISA beads, ELISA micro titre, and rapid/simple assays. Commercially available HIV-1/2 "combination tests," which incorporate antigens from both viruses, can be used to screen sera in an attempt to identify either infection. HIV-2 confirmatory tests include the Western blot and the RIBA. In addition, EIA tests and some rapid tests that use chemically synthesized peptides corresponding to a unique immunogenic region within the respective transmembrane glycoprotein exhibit good correlation with the Western blot and the RIBA for identifying and differentiating HIV-1 and HIV-2 antibodies. Furthermore, these tests are valuable for differentiating samples that produce reactions to both viruses (dual reactors). For HIV-2 confirmation the WHO requires reactivity to at least 2 HIV-2 envelope antigens, whereas other organizations require reactivity to p26 (gag) and to gp34 or gp105 (Env). To conclude, if a specimen is tested by both HIV-1 and HIV-2 Western blot, the blot exhibiting the strongest reactivity to envelope antigens usually indicates which infection is present.29

Laboratory diagnosis of HIV in infants
Laboratory diagnosis of HIV infection in infants is complicated by the fact that all infants born to infected women are seropositive due to passively acquired maternal antibodies, irrespective of their infection status. Sensitive diagnostic tests designed to detect small amounts of HIV antibodies, give positive results in uninfected infants for 12 to 15 months, till they seroconvert. Therefore, a definitive diagnosis using traditional ELISA and Western Blot can be made only after 18 months of age when uninfected infants will lose all maternal antibodies and infected infants will develop their own HIV specific antibodies. For diagnosis of HIV in infants, reports are available that suggest detection of HIV can be done before 18 months using HIV RNA PCR.

CONCLUSION
HIV diagnostic tests continue to be developed and improved with the hope that widely available, simple and highly sensitive assays will continue to contribute to early clinical diagnosis of HIV infection. Early detection will ensure the safety of patients dependent on blood transfusions. But most importantly, it will facilitate early counselling and treatment of HIV-infected patients and possibly help to prevent the spread of this debilitating virus.

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