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Review Article

TRENDS IN THE DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS

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ABSTRACT

Ever since the first HIV case reported, there is a dramatic increase in the prevalence of HIV infection which urged the need for an effective diagnostic method. There are several improvements in diagnostic methods employing various technologies to screen for HIV infection using enzyme immunoassays and rapid diagnostic tests which simultaneously detect antigen and antibody with significantly reduced window period. Further, western blot or immunofluorescence methods are used to confirm the results. Nucleic acid amplification tests, though sensitive, are expensive and hence not employed for screening but instead used to monitor the antiretroviral drug response. In spite of appreciable advancements in the sensitivity and specificity of the detection techniques, the algorithms are still the method of choice for ensuring the accuracy of test results.

Keywords: Oraquick ADVANCE Rapid HIV1/2 antibody test, Enzyme immunoassays, HIV (HIV-1 and HIV-2), the enveloped retrovirus containing

INTRODUCTION

HIV (HIV-1 and HIV-2), the enveloped retrovirus containing single stranded RNA, is the causative agent for acquired immunodeficiency syndrome (AIDS) in humans, while HIV-1 being the cause for majority of HIV infections worldwide [1]. HIV -1 based on difference in its nucleotide sequence is subdivided into major – M; new – N; and outlier – O. Group – M is further divided into minimum of nine distinct subtypes A – J [2]. In Asia, three major HIV-1 subtypes (B, C and CRF 01AE) are dominant [1].

Worldwide about 19 countries with highest prevalence of reported infections are all African countries with more than 24.5 million. South Africa, with over 5 million infected, is reported to have the largest population living with the disease followed by Nigeria. India being the 3rd country with largest of HIV infected population with more than 2 million people reported due to its large overall population, showing a prevalence rate of 0.30 in comparison to a prevalence rate of 0.60 in US and 18.10 in South Africa [3].

Following HIV infection, it typically takes several weeks to a few months before antibodies against HIV antigens are detectable (Seroconversion). The lag period between infection and seroconversion (window period) however, may last upto 3 years in some individuals.

Ever since the first case was reported in 1981, 25 million people died and 33.4 million are currently living with HIV infection. It is critical to detect the serostatus of the individual as acute HIV infection is a highly infectious stage and accounts for disproportionate amount of HIV transmission. HIV infection is diagnosed either by the identification of antigen, antibody or viral RNA in the serum or plasma of the suspected individual. According to WHO and FDA, HIV screening is done preliminarily with antigen and antibody combined EIAs and it is further confirmed by western blot. The screening strategy employed is summarized in the following flow chart (Figure 1).

(Reproduced from Protocol of Public Health Laboratory Centre, Centre for Health Protection, Department of Health)

Type of Sample

Blood is the most common specimen for HIV testing where a small amount of blood is withdrawn from the suspected individuals for diagnosis. In some rapid tests, a saliva sample can also be collected using a sterile cotton swab for diagnosis.

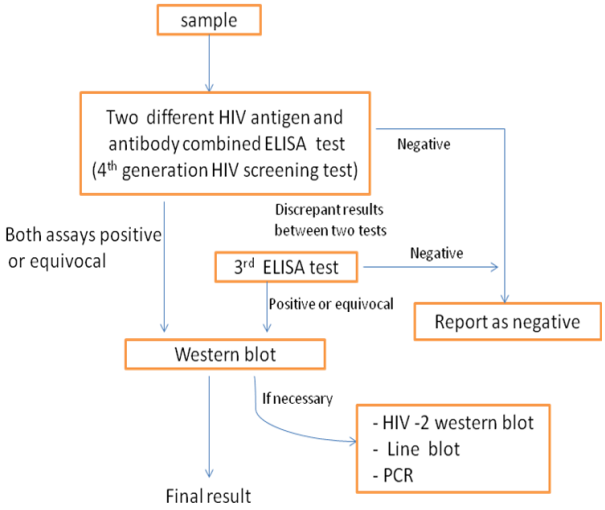

Fig. 1: Flow chart for screening strategy of HIV

Enzyme immunoassays

Enzyme immunoassays (EIAs) are the preliminary screening tests for the HIV infection. In the first generation EIAs, plates coated with viral lysate were used to bind IgG antibody of the infected individual. In the second generation immunoassays, although the same principle was used, purified antigen or recombinant viral proteins were employed in place of crude antigens. However, the window period ranging between 4 – 12 weeks was similar for both the first and second generation EIAs. In the third generation, detection of both IgM and IgG antibodies of the infected individual using sandwich EIAs came into existence. Since the third generation assays detects IgM antibodies, which are the primary antibodies soon after the immune response, the window period is less (3 – 4 weeks) as compared to the first two generations. In the fourth generation EIAs, in addition to the detection of antibodies similar to the third generation assay, detection of p24 antigen of the HIV was developed. Plates were coated both with antigens and the antibodies to detect the antibodies developed in the infected individuals and the P24 antigen of HIV respectively. Since the fourth generation assays permit the detection of viral antigen, before the immune system responds to the infection, these enzyme immunoassays allows

earlier detection of HIV than the previous generation assays [4, 5]. The fourth generation combined Ag/Ab assays can be used to screen in situations during blood or organ transfer where nucleic acid amplification tests are not feasible or not affordable [6].

Western blot

Western blot serves as a confirmatory test to the enzyme immunoassays [7]. The test employs a membrane transferred (blotted) with electrophoretically separated natural HIV proteins. Upon reaction with patient's serum, and subsequent washes, antibodies in the serum reacts with the proteins, which after incubation with an enzyme conjugated secondary antibody results in a series of dark colored lines on the membrane. Later recombinant or synthetic HIV proteins were mechanically impregnated onto the nitrocellulose membrane and were used as immunoblots the dark colored lines were identified by comparison with a positive control strip. The positive control strip contained one of the nine bands of HIV antigens (gp160, gp120, gp41, p66, p55, p51, p31, p24 and p17) varying with the manufacturers. A positive result is one in which the patient's serum reacts with two of the following proteins GP160 and P31 [8]. Further, antibody reactivity to two of three antigens (p24, gp41, and gp120/160) is necessary for a diagnosis of HIV infection by western blotting according to criteria commended by the Centers for Disease Control and Prevention (CDC). Recent improvements in the sensitivity of immunoassays (IA) coupled with increasing recognition of the importance of rapid point-of-care testing have led to proposals for adjusting the algorithm for serodiagnosis of HIV that can be performed using a dual or triple IA sequence that does not require Western blotting for confirmation [9].

Indirect Immunofluorescence

The indirect immunofluorescence test can also be used as confirmatory test [10].This test consists of reacting human serum or plasma containing cells infected with the human immunodeficiency virus type (HIV) which have been fixed on microscope slides. If the human serum contains HIV antibodies, they will react with the fixed antigen and form an antigen-antibody complex that can be visualized as by its fluorescence after the addition of human anti-immunoglobulin antibody conjugated with fluorescein isothiocyanate (FITC). Fluorescence is absent in a negative reaction. Positive and negative controls are used in all reactions. The fluorescence is read using a fluorescent microscope.

Dipstick assays

Dipsticks also known as lateral flow immunochromatographic assay is a rapid and qualitative diagnostic test based on antigen – antibody interactions used to detect anti HIV antibodies. The components include a sample pad, conjugation pad, nitrocellulose membrane and absorption pad (Figure 2). Sample contains mixture of anti HIV antibodies (both IgM and IgG) and human IgG antibodies. These antibodies react nonspecifically with the colloidal gold coated with protein A and moves towards the test line. Color appears at the test line upon cross binding of anti HIV antibodies with the impregnated HIV antigens. At the control line, the unbound human IgG antibodies react with the impregnated anti-IgG antibodies and light up as a color band. If sample is positive, a band appears on test line as well as on the control line. Only control band appears if sample is negative. The sensitivity and specificity of this assay range from 99.3% to 100 % and 99.7% to 99.9 % respectively, with 95% confidence interval (Table 1) [11]. However, the sensitivity may vary in recently infected individuals with regard to their ability to detect anti-HIV antibodies [12].

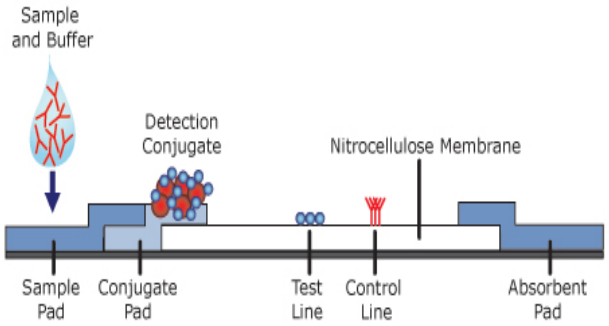

Fig. 2: Diagram of the lateral flow immunoassay (Reproduced from: web)

Table 1: Some FDA approved rapid diagnostic kits for HIV

Rapid HIV test	Specimen	Sensitivity	Specificity
Oraquick ADVANCE Rapid HIV1/2 antibody test	Oral fluid	99.3% (98.4-99.7)	99.8% (99.6-99.9)
	Whole Blood/Plasma	99.6% (98.5-99.9)	100% (99.7-100)
Uni-Gold Recombigen HIV	Whole blood	100% (99.5-100)	99.7% (99.0-100)
	Serum & Plasma	100% (99.5-100)	99.8% (99.3-100)
Reveal G3 Rapid HIV -1 antibody test	Serum	99.8% (99.2-100)	99.1% (98.8-99.4)
	Plasma	99.8% (99.0-100)	98.6% (98.4-98.8)
MultiSpot HIV-1/HIV-2 Rapid Test	Serum	100% (99.94-100)	99.93% (99.79-100)
	Plasma	100% (99.94-100)	99.91% (99.77-100)
Clearview HIV 1/2 STAT-PAK	Whole blood	99.7% (98.9-100)	99.9% (99.6-100)
	Serum & Plasma	99.7% (98.9-100)	99.9% (99.6-100)

Advantages

Antibody based tests which are inexpensive, take less Time To Positivity (TTP), require minimal laboratory settings are suitable for point of care (POC) diagnosis. These methods are used in blood screening for transfusions.

Limitations

Sensitivity may be less than 100% due to low level of antibody to specific antigen or no antigen variant strains of virus. Specificity may be poor due to cellular contaminants.

Nucleic acid amplification tests

There are several commercially available assays for HIV- RNA quantification (Table 2) which consists of reverse transcription-polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA) and branched-DNA assay (bDNA) [13, 14]. These are the three major methods available for monitoring antiretroviral therapy.

First method is the Reverse Transcription PCR method that converts viral RNA target to a single-stranded DNA template by using the enzyme reverse transcriptase. The DNA template is then amplified with a thermophilic DNA polymerase by the polymerase chain reaction using a dual labelled fluorescent probe [15] with fluorescent dye attached at 5' end and quencher dye at the 3' end preferentially gets hybridized to the target sequence, the fluorescent dye dissociating from the quencher during the extension stage by the 5' nuclease activity of the Taq DNA polymerase, an increase in the fluorescence intensity proportional to the amount of amplicon produced.

Second method is nucleic acid sequence based amplification (NASBA), an isothermal transcription-based amplification system specifically designed for the detection of RNA targets [16, 17]. The method employs enzymatic activities of avian reverse transcriptase, ribonuclease H and RNA polymerase simultaneously [18, 19]. The viral RNA amplification is measured by electrochemiluminescence (ECL). Further developments have led to the combination of NASBA and real-time detection using molecular beacons, hairpin shaped

molecules with an internally quenched fluorophore whose fluorescence is restored upon binding to a target nucleic acid [20, 21, 22].

Third method is a signal enhancement bDNA assay, which is based upon the hybridization of viral RNA (or nucleic acid target) with branched chain DNA (bDNA) probes [23]. Synthetic oligonucleotide probes attached to the solid surface capture the target nucleic acid. A complex multi branched DNA probe binds to the target that has been captured, and a solution of enzyme-labelled probes that bind these branches is added, thereby greatly increasing the signal for each target molecule. Detection is done by incubating the complex with chemiluminescent substrate and the light emissions are read with a luminometer. The signal is directly related to concentration of the target in the specimen and the target quantity is determined with an external standard curve [24].

Nucleic acid amplification methods are used to monitor HIV infected individuals before or during antiretroviral therapy to understand the response [25, 26]. Genotyping is used to monitor the development or presence of drug resistance in patients before or during therapy. It is also used to assist physicians in choosing the appropriate antiretroviral drug combinations for the patient [27, 28]. Nucleic acid amplification methods are used for diagnosing HIV infection in newborns from infected mothers. Antibody tests are not reliable in infants below 18 months of age, as an uninfected HIV exposed infant carry transplacental maternal antibodies [29, 30].

Table 2: FDA approved nucleic acid amplification kits

Trade name	Extraction	Target gene	Amplification and detection	Plasma volume(ml) used for extraction	Sensitivity
Abbott RealTime HIV-1 assay	Automated m2000sp RNA	polIN	Real- timePCR: noncompetitive fluorescent detection	0.8	39copies/ ml(68 IU/ml)
COBAS® TaqMan® HIV-1 Test	Automated Cobas AmpliPrep RNA	gag	Real-timePCR: fluorescent detectionwithTaqMan probes	1.0	40copies/ ml(68 IU/ml)
Nuclisens Easy Q HIV-1assay	Automated EasyMagtotal nucleicacid	gag	Real-time nucleic acid sequence-based PCR with fluorescent detection (molecular beacons with reporter and quencher molecule)	1.0	250IU/ml
VERSANl@ HIV-1 RNA 3.0 assay (bDNA)	Direct Plasma	pol	Chemiluminescence, bDNA; sandwich nucleic acid hybridization method; signal amplification	1.0	50copies/ml

IU – international units; ml – millilitre

Advantages

Realtime PCR is the only quantitative method which can detect viral load. Detection of HIV viral load is important for antireteroviral therapy and also for screening of new borns for HIV infection.

Limitations of nucleic acid amplification tests

Expensive and requirement of a sophisticated laboratory to perform the test.

CONCLUSIONS

Although there are many diagnostic methods for the detection of HIV infection, no single method can stand alone. The fourth generation immunoassays has reduced the window period as it can detect both antigen and antibody. However, the enzyme immunoassay suffers from false negatives due to varying window periods and difference in the sensitivity amongst the tests. Although nucleic acid amplification tests are highly sensitive having less window period for detection, these methods are expensive and suffer from amplicon contamination issues. Further developments with respect to the ease of use and cost for nucleic acid amplification tests can improve the HIV diagnosis significantly in the future.

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