International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 10 Issue 5 ǁ 2022 ǁ PP. 61-65

A Review On Evaluation Of Current Techniques Used In The Diagnosis Of Human Immunodificiency Virus.(HIV).

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Abstract
Out of estimated value of 1.2 million people infected with human immunodeficiency virus (HIV) in the United States, of which 20% are not aware of their HIV diagnosis status which fueled the transmission and incidence of the disease. Highly advanced methods of HIV testing techniques such as ELISA and Western blot could reduce these numbers of cases, as well as detecting those who have freshly acquired HIV infection and are at the most dangerous stage of the infection.

Acute HIV patients have been demonstrated an accelerated transmission of HIV in multiple epidemiologic and pathogenic studies. Approximately more than 50,000 HIV cases occur annually in the United States, and 50% have been attributed to persons with new infection.

The genuine HIV diagnostic testing protocols were established by the Centers for Disease Control and Prevention in 1989. Currently the proposed additions to the algorithm would incorporate improvement and enhancements made in HIV diagnostic testing, hence increasing sensitivity while reducing turnaround time and cost. This advancement has improved diagnosis of acute HIV, especially in HIV type 2. This in turn reduces the speed of the infection by providing adequate knowledge to health care providers for proper HIV treatment regimen.

KEYWORDS;
- ✓ *Human immunodeficiency virus,*
- ✓ *Enzyme linked immune sorbent assay*
- ✓ *Western Blot Techniques*

Date of Submission: 07-05-2022 Date of acceptance: 22-05-2022

I. INTRODUCTION

HIV is the abbreviated form of Human Immunodeficiency Virus. HIV is a virus that attacks the cells of the immune system (T-Lymphocytes), which is the body's natural defense system; it destroys the body's ability to fight against diseases and weakens the entire defense system. Without a strong immune system, the body has trouble fighting off diseases. Infection with HIV/AIDS is shown by the presence of antibodies in the blood. HIV infection is one of the leading causes of death. Human immunodeficiency virus is lenti virus which is a subgroup of retrovirus. The family of this virus is latency, recurrent viremia, infection of the nervous system like dementia, and weaken the immune system to makes it susceptible to infections or diseases. Human immunodeficiency virus is microscopic organisms that attack living organisms and depends on them to makes copies or multiply, when one's immune system is destroyed by HIV, AIDS set in. (Gallant, 1999).

It was first reported in 1981 in the USA at New York from homosexual men and soon after, other countries started reporting cases. It was first reported in India in 1986 at Chennai by sex workers and Ghana in 1986 and now a pandemic disease. It is a sexually, as well as, blood - borne disease. Currently, in USA, HIV is known to have evolved from chimpanzees, and transmit to humans.

Definition of HIV/AIDS

HIV is the abbreviated form of Human Immunodeficiency Virus. HIV is a virus that attacks the cells of the immune system (T-Lymphocytes), which is the body's natural defense system; it destroys the body's ability to fight against diseases and weakens the entire defense system. Without a strong immune system, the body has trouble fighting off diseases. AIDS stands for:

- **Acquired** which means: you were not born with it the disease compared to most immune deficient conditions. You can only be born with it if your mother had HIV and doesn't take precautions during pregnancy.
- **Immune deficiency** which means: the disease is characterized by weakened or ineffective immune system with no resistance to infection.
- **Syndrome** which means: AIDS is a combination of signs and symptoms which occur together due to the HIV infection as well as many other infections as a result of the immune deficiency

AIDS is therefore a group of opportunistic infections and serious illness that a person develops after being infected with HIV for a long time. HIV infects the cell (T Lymphocytes) of the immune system weakening the entire system. The strength of the body's immune system is determined by CD4 test. The lower the CD4 cells the less immune you are and the more cells count tell you have a higher immune system. A normal count is between 600 and 800 microlitre of blood (Offei, 2014).

TYPES OF HIV

There are two types of Human Immunodeficiency Virus.

- **HIV-1**
- **HIV-2**

HIV 1

HIV type1 was first discovered, and it's mostly spread worldwide. HIV type1 is the predominant form of HIV, and generally when people are referring to HIV without specifying the type of virus, they will be referring to type1. It is more pathogenic and easily transmitted. In Ghana it is 95% prevalent.

HIV 2

HIV type2 is less easily transmitted and the period between initial infection and illness is longer, that is, it develops more slowly. Type2 is relatively uncommon and is mostly concentrated in West Africa and rarely found elsewhere. HIV type1 and 2 are both transmitted by sexual contact, through blood and from mother - to - child.

According to the global reports of HIV/AIDS, Indian Council of Medical Research (ICMR) initiated surveillance for HIV infection in India in 1985-1986 and the first evidence of HIV infection in sex workers in Chennai, Madurai and Vellore was obtained in 1986-19871, progressively centers were established in the State Capitals of India. National AIDS Control Organization (NACO) set up under the Ministry of Health and Family Welfare instructed to implement initiatives like establishing HIV testing centers, strengthening blood-safety and controlling hospital infection took over surveillance activity in 1992. The epidemiologic data on HIV/AIDS in India has emerged primarily from the network of sentinel surveillance, ongoing testing in antenatal clinics and blood banks, research studies, reporting of AIDS cases and information generated from mortality statistics.

As per the recently released, India HIV Estimation 2019 report, Overall, the estimated adult (15–49 years) HIV prevalence trend has been declining in India since the epidemic's peak in the year 2000 and has been stabilizing in recent years. The estimate for this indicator was 0.22% (0.17–0.29%) in 2019. Below is the latest available data:

- 2.3 million people with HIV
- 0.22% adult HIV prevalence
- 69,000 new HIV infections
- 58,000 AIDS-related deaths
- 64% people with HIV on antiretroviral treatment

Since the first patient with acquired immunodeficiency syndrome (AIDS) was reported in 1981 its causative agent, human immunodeficiency virus (HIV), has led to 77 million HIV infections globally and remains a major public health issue.

II. Literature Review

Human immunodeficiency virus (HIV) is classified into 2 viral groups: HIV-1 and HIV-2. Both viruses are in the family of the retrovirus and in the genus of lenti viruses (Tang and Chan 2007). HIV-1 is globally the most common one, while HIV-2 is rare and mostly seen in the parts of Western and Central Africa. HIV-1 and HIV-2 are distinguished by their genome organization, but have the same basic structural genes, such as gag, pol, and env (Fanales-Belasio et al. 2010).

Even though, both viruses cause acquired immunodeficiency syndrome (AIDS), but HIV-2 is less virulent (Fanales-Belasio et al.2010). According to (Tang and Chan 2007) the strains of HIV-1 viruses are separated into major (M), new (N),and outlier (O) groups. The Strains in group M are responsible for the HIV/AIDS pandemic and are so different that they are sub-classified into subtypes A – K. These subtypes of HIV-2 are eight and amongst them only groups A and B cause transmissible disease. (Bhad et al. 2016).

People at a higher risk for contracting HIV, include those who: have been exposed to

HIV, show clinical features of HIV, those who are pregnant, or are involved in high risk behaviors, such as intravenous drug use and unprotected sex. Men who have sex with men and multiple sex partners are

also at a higher risk for HIV infection. Again, people infected with hepatitis B, hepatitis C, tuberculosis, or any sexually transmitted diseases are also at higher risk of HIV by Sharma et al. 2008). Clinical manifestations of HIV infections start with an influenza or mononucleosis like sickness that occurs within 1 - 4 weeks of the infection (Fanales-Belasio et al. 2010). The commonest symptoms are; fever, maculopapular rash, oral ulcers, lymphadenopathy, arthralgia's, pharyngitis, weight loss, malaise, and myalgias but not limited to only these ones.(Fanales-Belasio et al. 2010). The average period initial HIV infection is approximately 1-10 days (Fanales-Belasio et al. 2010).

Approximately, about 1.2 million people are infected with HIV in the United States and out of these patients 20% are not aware of their diagnosis according to Cornett and Kirn 2013. In this case, early detection with proper diagnostic tool and better treatment plan can decrease the incidence of the disease. Treatment of HIV is started when the patient CD4 is less than 500/ml or viral load is greater than 5,000 copies/ml(Laboratory diagnosis of human immunodeficiency viruses infection which is HIV slide test. HIV treatment is done with highly active antiretroviral therapy (HAART). This therapy regimen includes nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors. Different classes of HAART include fusion inhibitors and chemokine receptor 5 antagonists. The first line of action for the treatment of HIV is a combination of two NRTIs and one NNRTI.

Therapy recommendations can be change based on patient's response to initial therapy, which is monitored and evaluated via viral load and CD4 counts throughout the treatment regimen or Laboratory diagnosis of human immunodeficiency viruses infections(HIV Slide Set).

According to Bhad et al. 2016, it is of great significance for patients to be compliant with HAART or drug resistance will develop and patients will be left with less viable treatment options or methods.

Blood tests for HIV infection have been employed in most of Western Countries during mid of '80s. Since then, the quality and sensitivity of HIV screening tests has been advance. There are much clinical significance associated with HIV testing. For instance, since 1985 screening of the blood supply has resulted in the protection of numerous numbers of individuals from HIV infection. Again, HIV testing can be used for the diagnosis of infection to individuals who wish to know their HIV status. Lastly, HIV tests are also utilized for epidemiologic surveillance, providing health officials with information about the extent of the infection among communities, thereby allowing them to target populations for vaccines and treatment, to assess economic concerns, and also to provide counseling to prevent the infection of other individuals.

The worldly HIV/AIDS pandemic brought keen interest in the medical community, requiring vigilant diagnoses of the virus by continuously enhancing and ensuring the accuracy of the clinical, molecular, and/or laboratory diagnostics. There are a several variety of HIV tests available with the most sensitive and widely used being the ELISA. Patients with a positive ELISA for HIV are confirmed with a confirmatory test, known as the western blot, in order to improve specificity and reliability. Other techniques such as qualitative PCR techniques minimized the window period between infection and detectability, while NAATs magnify viral RNA, in order to detect viral genes rather than antibodies or antigens. A continuous advancement in diagnostic ability for HIV is very important, which support the development of advanced approaches to fast track, diagnoses and treatment of this virus.

Techniques

HIV diagnostic testing has come a long way since its creation in the early 1980s. Given many options available for selecting an HIV test for a specific clinical or research setting can be challenging or daunting. Conversely, an informed decision depends on an accurate assessment of the likelihood of acute infection in the test population and comprehends with the key aspects of the test techniques. The ability of individual tests to reliably detect HIV infection depends on the target(s) or sample being detected, the time they can be expected to be present after infection, and the concentration of stable target in test specimens, all of which are explained by the virologic and serologic events after infection.

III. Methodology

Electronic literature review search was done on Pub Med, Google Scholar, and Medline Plus. The articles were selected if the publication included keywords, includingbut not limited to, laboratory or molecular diagnostics of HIV. Articles were then reviewedand included based on importance to the topic.

Laboratory Diagnosis

HIV laboratory diagnostic procedure, which consists of a continuously reactive enzyme immunoassay for HIV antibodies and a positive HIV-1 western blot, has been the best and confirmatory standard for laboratory diagnostic tool for HIV-1 infection in the United States since the 1980s, (Branson 2014).

Also, numerous enhancements, advancements and developments in HIV laboratory testing have led to enzyme immunoassays, which have improved its sensitivity, to the extent that antibodies can be detected within

one to two weeks after infection, and the invention of a new variety of assays (Fearon 2005). In -vitro or laboratory tests that are used for HIV diagnosis can be grouped into the following: Enzyme Linked Immunosorbent Assay (ELISA); Western Blot
(WB); Polymerase Chain Reaction (PCR); and Nucleic Acid Amplification Test (NAAT).

ELISAs is mostly used as the current screening method due to high sensitivity (Chan

Kenny 2007). Though being highly sensitive, ELISAs have higher false positives results occurrence which is due to a lower specificity and false negatives due to small or scanty antibody levels in the specimen during the window period in Aids infection (Chan Kenny 2007). Although, the rate of false positives continue to fall with each sequential generation of assay(Chan Kenny 2007). The first and second generation assays, now known as IgG sensitive assays, are particularly or specifically for detecting IgG to a mid-window period of 45 days (Hurt 2017). According to Hurt 2017 the third generation assays, which is known as IgM / IgGantibody (Ab) laboratory test, reduced the window period to a median of 23 days after infection. There are various Ab assays including: ADVIA Centaur HIV 1/O/2 Enhanced (EHIV) Assay, Avioq HIV-1 Micro Elisa System, GS HIV-1/2 Plus O, Vitros

Anti-HIV 1+2, DPP HIV-1/2 Assay; and several rapid antibody tests such as HIV 1/2 STAT-PAK and OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (Branson 2014; Hurt 2017). The HIV 1/2 STAT-PAK and OraQuick ADVANCE Rapid HIV-1/2 Antibody Test identify anti-HIV IgG and IgM in oral fluid specimen, whole blood, plasma, or serum (Cornett and Kirn 2013). The Ora-Quick ADVANCE results are produce within 20 minutes and have a sensitivity of 93% and a specificity of 99%. According to Cornett and Kirn 2013, majority of these rapid tests can detect both HIV-1 and HIV-2 in about 30 minutes or less, which is specifically important in patient populations with poor follow-up or women present at labor. Furthermore, oral fluid-based rapid tests can decrease limitations to HIV diagnostic testing as they can be used as home-based,

Self-testing or in deprived areas (Cornett and Kirn 2013). Lastly, antigen/antibody (Ag/Ab) combination assays which was previously called fourth generation, binds IgM/IgG sensitive assays with separate, but concurrently p24 antigen detection to decrease the window period to 18 days after infection (Hurt 2017). Currently, five positive Ag/Ab combination laboratory tests are in existence, namely: Architect HIV Ag/Ab Combo Assay, BioPlex 2200 HIV Ag-Ab, GS HIV Combo Ag/Ab EIA, ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay, and rapid test Determine HIV-1/2 Ag/Ab Combo (Branson 2014).

Continuous reactive ELISAs test are performed first according to gold standard HIV laboratory protocols which is then followed by western blot in order to improve specificity and confirm sero-positivity when antibodies against both the env(which is in the envelope protein) and the gag proteins are detected (Chan Kenny 2007).

Recombinant antigens advancement has begin to improve the sensitivity of western blots (Chan Kenny 2007).

In this technique (PCR assays), HIV RNA or provirus DNA is magnified enzymatically in-vitro by chemical reaction to enhance early detection of the viral infection.(Sharma et al. 2008). Also, quantitative PCR techniques reduced the window period between infection and detectability to about 12 days. This include: reverse transcriptase-PCR (RT-PCR), nucleic acid sequence based amplification (NASBA) and branched-DNA (b-DNA) (Sharma et al. 2008). Whiles qualitative DNA PCR assays that detect provirus DNA in peripheral blood, mononuclear cells are prescribed for early diagnosis of HIV-1 in infants; if they are positive within the first 48 hours of life, an in-utero infection is indicated (Martin et al. 2017; Sharma et al. 2008). If DNA PCR is initially negative within the first 48 hours of life and then positive within one month, it's indicates an intrapartum infection.(Sharma et al. 2008). In neonatal diagnosis of HIV, PCR should be performed at 48 hours, 1 week, 3 months, and 6 months, with serological confirmation at 18 months (Sharma et al. 2008). There are two HIV-1 DNA assays for the early diagnosis of infants namely; the COBAS1 AmpliPrep/COBAS1 TaqMan1 HIV-1 Qualitative Test and the real-time HIV-1 Qualitative Test (Martin et al. 2017). These assays are costly which needs expensive equipment in order to be conducted at highly sensitive level.(Marin et al. 2017). Advanced technologies designed for use at or near the point-of-care with less costly equipment, such as the Cepheid Xpert1 HIV-1 Qual assay, the AlereTM q HIV-1/2 Detect, and the Xpert1 HIV-1 Qual assay, which can use either whole blood or dried blood samples, have begun to be developed (Marin et al. 2017).
Finally, nucleic acid amplification testing amplifies viral RNA, in order to detect viral genes rather than antibodies or antigens (Sharma et al. 2008). Nowadays, Parma HIV-1 Quant
Assay is the only FDA approved NAAT assay which is used for the quantitation of HIV-1 RNA in plasma (Corner and Kirn 2013). NAAT assays, which are highly sensitive, have relatively huge number of false negatives, are expensive, require a blood draw, and are time consuming to perform (Corner and Kirn 2013).

IV. CONCLUSION

In conclusion, it is very vital that the pivotal process of laboratory testing, leading to clinical diagnosis, is being continuously improved and enhanced to ensure quality and accurate diagnosis and a comprehensive treatment plan. In estimation, about 1.2 million people who have been infected with HIV in the United States. An estimate number of about 20 % are unaware that they are infected (Corner and Kirn 2013). Hence less expensive lab tests such as Elisa should be made accessible to people globally for early detection and treatment of the infection. Out of the estimation 50,000 new cases of HIV infections occurs annually in the United States, approximately 50 % of these cases are attributed to coming into contact with newly infected persons.(Corner and Kirn 2013).

Recently, studies have shown that a person who is diagnosed with HIV and treated before the disease progresses can live as long than those who does not have the diagnosis of HIV infection (Chan Kenny 2007). Hence, it is very important to continue the routine diagnostic techniques as about 70% of hospital, admission, diagnosis, treatment and discharge depends on accurate lab test. HIV laboratory techniques are used to detect, quantify, diagnose and to eliminate the burden of the illness caused by HIV infection. Since the CDC published the diagnostic algorithm in 1989, the techniques have been continuously reviewed to allow various new techniques that affirm sensitivity and specificity rapidly and more cost effective.

Currently there are a variety of advanced tools available in the detection of HIV infection in a patient. However, the most commonly used ones are Elisa and western blot method which is confirmatory test to Elisa test.

Also it is very important to understand the procedure and significance of each diagnostic tool and the time or phase to use each technique. The initial HIV screening assay should be the most sensitive available test; for example, in routine HIV testing, an assay that detects the HIV p24 protein and the antibodies against HIV should be first used (Chan Kenny 2007). In case this is reactive, confirmatory western blot method is performed to confirm the result. Specimen that is negative on the confirmatory test should be tested using a nucleic acid test (Chan Kenny 2007). In children less than 18 months of age, diagnosing HIV infection requires the direct detection of the virus or its component in the child's serum (Sharma et al. 2008). In all cases, if tests are negative and suspicion is high RNA RT-PCR could be repeated within 2-4 weeks while serology should be rechecked in 3 months.

No technique or procedure can accurately satisfy every possible scenario; therefore, it is vital for physicians and other healthcare providers to understand the significance and limitation of each diagnostic technique and how to implement or execute them for different patients. It is clear that the key to eradicate the burden of HIV infection depends on early detection of the infectious virus for proper treatment (Cornett and Kirn 2013).

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