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Comparison: Imported and Indigenous HIV Fourth-generation immunodiagnostic kits

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Abstract:

Early detection of any disease is always a life-saving methodology for human beings likewise the detection of p24 antigen is always better than the detection of HIV antibodies. In the current era, p24 antigen is added additionally in HIV antibodies detection assays and called HIV fourth-generation immunodiagnostic kit. The different HIV fourth-generation kit having different capacities to pick up the low concentration of analyte as well as represent the detection values in different units. A total of 30 assays were used in this study and it was found that the detection concentration assigned by the manufacturers in the case of imported kits was 0.48 IU/mL to 2 IU/mL. However, the detection range of the indigenous kits was 25 pg/mL to 1000 IU/mL. This study gives an idea/knowledge for the startups concerned with high hope and broad scope for make in India concept in the IVDs market.

Keywords:

Detection concentration, HIV, p24 antigen

Introduction

As per updated report (July 2022) of the World Health Organization (WHO), from the beginning of the epidemic, 84.2 million (64.0–113.0 million) people have been infected with HIV, and about 40.1 million (33.6–48.6 million) people have died of HIV. Globally, 38.4 million (33.9–43.8 million) people were living with HIV at the end of 2021. India HIV Estimation 2019 report showed that 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. In the same year, HIV prevalence among adult males (15–49 years) was estimated at 0.24% (0.18%–0.32%) and among adult females at 0.20% (0.15%–0.26%). The data is still terrifying and there is a need for timely detection of HIV infection to reduce the risk of transmission. *In vitro* serological tests have been playing a pivotal role in the diagnosis and management of an array of such diseases. HIV fourth-generation test kit is one of the serological test devices available in the commercial market and is used as very important tool for the detection of early infection HIV present in the blood of asymptomatic individuals globally. Since HIV is one of the most common and dangerous transfusion-transmissible infections, it is always better to use good quality HIV fourth-generation kits for screening of blood for donation purposes as it can detect early infection, thus reducing the risk of transmission to a very low level.[1,2] Screening for HIV by using a good quality HIV fourth-generation kit to reduce the

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risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that blood transfusion is as safe as possible. Fourth-generation assays simultaneously function as both a third-generation immunoassay (for the detection of immunoglobulin [Ig] G and IgM antibodies) and a capture immunoassay for the direct detection of p24 antigen (the most abundant protein of HIV virions).[3] The fourth-generation HIV immunoassay used synthetic peptides and recombinant protein antigens to detect IgG and IgM for direct format but in indirect format, p24 detecting substance is also added to detect p24 antigen. The fourth-generation immunoassay maximizes the sensitivity for the detection of HIV antibodies. Several studies have proven the sensitivity of fourth-generation immunoassay in early detection of HIV infection.[4-11] However, the specificity of fourth-generation immunoassay varies from 97.6% to 99.8%.[10,12,13] Only 46% of the HIV-infected people in the world are aware of their status.[14] The detection of HIV during the acute stage of infection is critical for many aspects of HIV public health and clinical diagnostics.[15] Mostly, the infectious disease specialist can diagnose HIV infection during the acute stage.[16] Acute HIV infection refers to the presence of the infection without any development of antibody response; recent infection refers to when the immune response is still developing and only sensitive diagnostic techniques can detect it.[17] As a result, early detection encompasses both acute and early phase infection, as well as the period between the onset of a stable viral set point and immune response. Acutely infected individuals are the key drivers for the highest rates of secondary HIV transmission.[18]

HIV RNA is the only viral biomarker detectable during the initial stages of acute infection, so only NAAT can be employed for virological detection.[18,19] Although NAAT have long been used to quantify viral load during patient monitoring in resource-rich settings, they are not generally approved for use as qualitative diagnostic techniques in most countries.[19] Nevertheless, the 24 KD, a gag gene product called p24, is the key internal structural protein of HIV-1 that is present at high levels in the early stages of infection and can be detected as early as 16 days after infection before the establishment of an immunological response[18] through serological test. In recent era, various serological diagnostic assays are available in the commercial market to detect early infection of HIV/AIDS. The fourth-generation tests, i.e., the combination of p24 antigen and HIV-Ab reduced the detection time up to 2 weeks.[19-22]

In the present study rapid diagnostic tests (RDT), enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) were used. RDTs are diagnostic assays that are designed to be used at the point of care, where they are fast, economical, and user-friendly. It is a paper-based platform for detecting and quantifying analytes in diverse mixtures, with the sample being loaded on a test device and the results are visible within 5–30 min. Whereas, ELISA are analytical methods that show antigen-antibody reactions through color change obtained by using an enzyme-linked conjugate and enzyme substrate that serve to identify the presence and concentration of molecules in biological fluids.[23] CLIA in the other hand advent in blood donor screening which offers automation, higher sensitivity, and reduces human errors.

Methods

The laboratory procured WHO International Standard HIV-1 p24 antigen (code 90/636) from the National Institute for Biological Standards and Control, Hertfordshire, UK, in lyophilized form. It was reconstituted as per instructions for use, the concentration was then 1000 International Units per mL and then prepared aliquoted, and stored at – 20°C for this study. Whenever required, take out the aliquot (s) from – 20°C and further dilute as per claimed value by manufacturers of selected kit (s) with negative plasma that is free from HIV-Ab, HCV-Ab, hepatitis B surface antigen, and syphilis.

A total of 30 different assays of fourth-generation HIV serology, i.e., RDT, ELISA, and CLIA-based *in vitro* immunodiagnostic kits were selected for this study. Out of 30, 17 were indigenous (Kit-I to Kit-XVII) and 13 were imported (Kit-A to Kit-M). As per the study design, the WHO International Standard HIV-1 p24 antigen (code 90/636) was used as a sample on all 30 different kits and diluted as per the claim of particular kit.

Results

The performance of the tests and interpretation of results was done as per the manufacturer's information for use/Kit Inserts. The selected kits have a very large difference in the detection concentration of p24 antigen. In reference to imported kits, a RAPID assay which is even not required a sophisticated laboratory setting is claimed to detect 0.48 IU/mL p24 antigen, whereas, indigenously developed RAPID assay claims 1000 IU/mL. It was found that all the imported kits were concordant with the claimed values of p24 antigen [Table 1]. However, 41.17% of the indigenous HIV fourth-generation immunodiagnostic kits were not complied with the claim concentration of P24 Antigen [Table 2].

From the results, it is clear that around 41% of the indigenous HIV fourth-generation immunodiagnostic kits are found not of standard quality and all the imported kits are of standard quality. In the case of rapid diagnostic kits, it is also found that the band intensity

Table 1: Imported fourth-generation human immunodeficiency virus serological assay

Imported kits				
Name of the kit	Method	Analytical sensitivity claimed by manufacturer (IU/mL)	Result	Remarks
Kit-A	RAPID	0.48	Detected	Complies
Kit-B	CLIA	0.48	Detected	Complies
Kit-C	CLIA	0.5	Detected	Complies
Kit-D	CLIA	0.59	Detected	Complies
Kit-E	ELISA	0.85	Detected	Complies
Kit-F	CLIA	1	Detected	Complies
Kit-G	CLIA	1.26	Detected	Complies
Kit-H	CLIA	1.26	Detected	Complies
Kit-I	CLIA	<2	Detected	Complies
Kit-J	RAPID	2	Detected	Complies
Kit-K	CLIA	2	Detected	Complies
Kit-L	CLIA	2	Detected	Complies
Kit-M	CLIA	2	Detected	Complies

CLIA=Chemiluminescent immunoassay, ELISA=Enzyme-linked immunosorbent assay

Table 2: Indigenously manufactured fourth-generation human immunodeficiency virus serological assay

Indigenous kits				
Name of the kit	Method	Analytical sensitivity claimed by manufacturer	Result	Remarks
Kit-I	RAPID	25 pg/mL	Not detected	Does not comply
Kit-II	RAPID	25 pg/mL	Detected	Complies
Kit-III	RAPID	1 ng/mL	Not detected	Does not comply
Kit-IV	RAPID	1 ng/mL	Not detected	Does not comply
Kit-V	RAPID	2 IU/mL	Not detected	Does not comply
Kit-VI	RAPID	2 IU/mL	Not detected	Does not comply
Kit-VII	ELISA	2 IU/mL	Detected	Complies
Kit-VIII	ELISA	5 IU/mL	Detected	Complies
Kit-IX	CLIA	8 IU/mL	Detected	Complies
Kit-X	ELISA	8.4 IU/mL	Detected	Complies
Kit-XI	ELISA	10 IU/mL	Not detected	Does not comply
Kit-XII	ELISA	10 IU/mL	Not detected	Does not comply
Kit-XIII	RAPID	1000 IU/mL	Detected	Complies
Kit-XIV	RAPID	1000 IU/mL	Detected	Complies
Kit-XV	ELISA	Not mentioned	Detected	Complies
Kit-XVI	ELISA	Not mentioned	Detected	Complies
Kit-XVII	ELISA	Not mentioned	Detected	Complies

CLIA=Chemiluminescent immunoassay, ELISA=Enzyme-linked immunosorbent assay

of indigenous kits is very low as compared to imported kits. Sometimes the bands are so faint that it becomes difficult to discriminate between negative and positive samples. Since *in vitro* diagnostic kits are used not only for screening, diagnosing, and treating patients but also for rehabilitating patients and tracking health indicators to prevent different diseases, therefore, improving the quality of *in vitro* medical devices at every manufacturing step is the need of the hour to facilitate the move to a healthier India.

Discussion

The *in vitro* immunodiagnostic kit either with a combination of antibodies and antigen of HIV or only HIV antigen reduces the window period to about 2 weeks which helps early detection of infection and accordingly clinicians can start antiretroviral therapy in the beginning of the disease. Early detection means kit to be picked up least concentration of antigen and/or antibodies. This study was planned to check kit assigned detection concentration of p24 antigen and it was found that the detection range of the kits was very wide, i.e., 0.48 IU/mL to 2 IU/mL and 25 pg/mL to 1000 IU/mL. It was also observed in the study that the unit was also different, i.e., pg/mL, ng/mL, and IU/mL. Whereas the IU/mL unit is internationally accepted even WHO also uses the same. This paper gives an idea and knowledge for the start-ups concerned with high hopes, and broad scope for the "Make in India" concept in IVDs market.

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Conflicts of interest

There are no conflicts of interest.

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