Original Research Article

Serodiagnosis of HIV by Rapid Test and ELISA Test Assay in a Tertiary Care Centre in Northern India

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A B S T R A C T

Keywords	
HIV, ELISA, Rapid diagnostic test (RDT)	AIDS has been a very fatal and challenging disease worldwide. It is very important to diagnose the HIV symptoms timely at early stage with accuracy for its prevention and proper retroviral treatment. Nowadays, the Primary HIV detection procedure has been changed to rapid diagnostic test for fast detection of AIDS. Indeed, the Rapid diagnostic tests (RDTs) need confirmation in some crucial cases. The purpose of our study was to determine the positivity of HIV virus in patients attending the OPD department at Dr. RMLIMS, Lucknow based on the given algorithm by SD-Bioline. Samples of 200 patients (180 male, 20 female) were taken and the sera of the samples were separated using centrifuge, all the samples were tested for HIV virus using SD HIV test kit (immune chromatographic) for rapid screening and then further confirmed by Elisa test kit (Gen Screen TM Ultra) in both the test, all the samples were HIV-negative except one case. Overall the positivity of HIV virus in OPD patient is low because it is a tertiary care Centre.

Introduction

Acquired immune deficiency syndrome (AIDS) is one of the catastrophic and challenging sexually transmitted disease (STD) worldwide and human immunodeficiency virus (HIV) is the causing agent. HIV is a blood borne and fatal pathogen which is responsible for transfusion transmissible infections in human beings (Sushmita *et al.,* 2013). Globally, approximately 35.3 million individuals are suffered from HIV (UNAIDS report, 2013). Cases of HIV infection have been increased rapidly in the developing countries (Kassu *et al.,* 2004). In India, guessed 2-3 million people have been considered positive with HIV and this infection has been proven a link with other sexually transmitted diseases (STDs) (Kumarasamy *et al.,* 2008).

It has been revealed that the HIV positivity rate is not affected by age, occupation, sex, locality, literacy status and migration amongst the participant of the STDs clinic in

northern India (Hussain *et al.,* 2005). Since, the prevalence of HIV has a very lethal impact on human population worldwide so, it is really crucial to diagnose the HIV-1/2 infection timely for proper retroviral treatment. Parallel and Serial, two HIV diagnostic methods are commonly used. In parallel algorithm, simultaneously testing of the samples is carried out by two kinds of tests whereas in serial testing, firstly samples are tested by first test, which results are further confirmed by second test (Wright *et al.,* 2004). Voluntary counselling and testing (VCTs) centers in India refer algorithm/strategy III to diagnose HIV from the proposed guidelines of NACO (National AIDS control organization), India (NACO guidelines, 2007). Many serodiagnostic techniques are used to diagnose HIV like ELISA with the accuracy up to 99% but due to lack of skilled labour and enough spare time, Rapid kits have replaced those time consuming techniques (Francis *et al.,* 1988; Bhanu *et al.,* 2014). Acceptance of constructed antigens in some accelerated rapid kits have elevated the specificity (Khansari *et al.,* 1993), which are used to diagnose HIV-1/2 infection in very less time.

However, result can be drawn in 5-15 minutes by using rapid diagnostic test (RDTs) but for the assurance of HIV presence, it must be re-confirmed by ELISA and western blot techniques because sometimes specificity of the test matters.

Materials and Methods

The laboratory procedure for HIV-testing are performed on patient's blood serum 'or' plasma.

Collection of samples

Totally two hundred (180 males and 20 females) samples were collected by performing venipuncture. 3–5 ml of blood was taken aseptically by using sterilized/disposable syringe. The samples were carefully transferred from the syringe squirting into a sterile plastic vial.

Separation of sera from the blood

The vials/vacutainers were centrifuged at 3000 rpm for 10 minutes to separate the serum.

Storage of serum specimens

The serum samples were placed in leak proof plastic containers in the deep freezer at -22^0C.

All the samples were labelled adequately with patient's name and record no.

Detection of HIV specific antibodies

Two detection tests were applied for HIV in the sera of 200 patients. Rapid diagnostic test (RDT) along with ELISA was performed. Rapid assay was applied with the helpSD Bioline HIV-½ 3.0 (SD Bio-Standard diagnostics Private Limited, Gurgaun, Haryana) as an immune chromatographic test for the quantitative detection of antibodies of all isotype (Ig G, Ig M, Ig A) specific to HIV-1/2 types in the serum. In rapid diagnostic test (RDT), a fresh kit was used for each patient. 5-10 ml. serum was poured into the well of the kit using capillary pipette followed by adding a 4 (120 ml.) of assay diluent into the same well and test was interpreted in 5~20 minutes. The ELISA was performed for the confirmation of rapid kit test. ELISA was applied using The Genscreen ^{TM} Ultra HIV Ag-Ab (Bio-Rad Laboratories India Pvt. Ltd., Gurgaon Haryana, India), an enzyme immunoassay based on sandwich principle for the identification of HIV antigen and antibodies of HIV-1/2 virus in the serum.

The evaluation of rapid test and ELISA assay was conducted as per the given algorithm.

Interpretation of the test and assay validation

Interpretation of the rapid screening test was carried out by visual assay like dot-blot test, on the basis of the given algorithm for the specificity, sensitivity and the estimation of positive and negative valuation of HIV test.

Calculation and estimation of ELISA for the positivity or negativity of HIV-1/2 was decided by comparison of the absorbance values for each sample with the calculated cut-off value. Thermo scientific Multiscan EX (Thermo fisher scientific, Pune, India) was used as ELISA reader to observe the optical density (O.D) at 450^0 or 620-700nm for the calculation of cut-off value. Samples with absorbance values below than the absolute value were advised to be negative and absorbance values equal to higher than the absolute value (cut off) were advised to be positive by The Genscreen ^{TM} Ultra HIV Ag-Ab assay. Optical density (O.D) was calculated by following formula:-

1- The mean absorbance of the negative control (ODR3)

$$ODR3 = [OD(C^*) + OD(D) + OD (E)]/3$$

2- The cut off value-
The cut off value was given by the formula
$-CO = OD R3 + .200$

Results and Discussion

Sera of 200 (180 males and 20 females) patients were tested for screening of HIV, all the samples were tested with help of the rapid kit (SD BODLINE HIV ½ 3.0). Except one, all the samples were found HIV negative (Non-reactive). The overview is given in the table 2.

After this rapid tests, in which all the samples were negative except one, 40 samples out of 200 along with positive one were taken randomly as a subsets for confirmatory testing of HIV negative/positive samples. Elisa was employed for 40 samples with the help of Genscreen the Ultra Kit. The optical density of 39 samples were observed less than cut-off value (0.260), confirmed as HIV negative and optical density of only sample was found 3.092 nm, more than cut-off value, so it was confirmed as HIV positive. Assay summary is given in the table 3.

The diagnostic tests evaluation is cheap, simple to complete, and impose the less discomfort to the patient, since there a small specimen size is necessary (Truong Xuan *et al.,* 2000).

As per the research, both rapid kits and ELISA are used immunologically to diagnose the infection of HIV. Ambiguous False positive results have been observed with RDTs according to other studies (A.F. Aghokeng *et al.,* 2009). Interpretation of rapid test would be acceptable with lower rate of false negatives and high positive predictive value (Khan *et al.,* 2010). Positivity and negativity of a test sample depends on the sensitivity of the kits. Sensitivity reflects the adeptness of an analysis to accurately allocate those who accept the disease, or accurate positives, a part of those who analysis positive (Kleinbaum *et al.,* 1982; Weiss, 1998).

However, research suggested that confirmation of HIV by western blot is very crucial technique but the ELISA has its own importance for the confirmation of rapid kits assay.In this study SD BODLINE HIV ½

3.0 rapid diagnostic kit was used for the screening test and the result was interpreted with respect to the given protocol with kit.

Table.1 Interpretation of the rapid test on the basis of given algorithm for SD Bioline HIV-½ 3.0
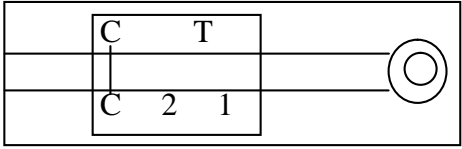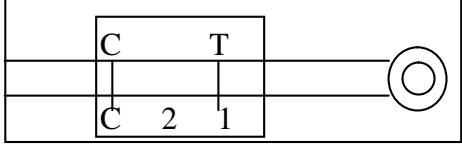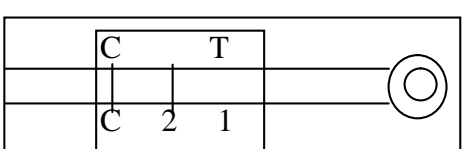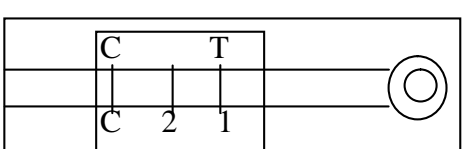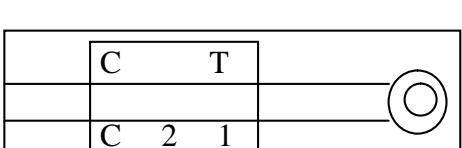
Negative Test Result	
	Appearance of only control line **'C'** within the result window.
Positive Test Result for HIV-1	
	The presence of two lines as control line **'C'** and test line **1(1)** within the result window indicates a positive result for HIV-1
HIV-2 Positive	
	The presence of two lines as control line **'C'** and test line **2(2)** within the result window indicates a positive result for HIV-2
HIV-1/2 Positive	
	The presence of 3 lines as control line **'C'** and test line **1(1)** and test lines 2(2) within the result window indicates 3 positive result for HIV-1 &/or HIV-2
Invalid test result	
	No presence of control line (C) within the result window indicates as invalid result

Table.2 Overview of rapid screening test result. (As per SD BODLINE HIV ½ 3.0 kit)

Test applied	Total number of samples(sera)	Number of Negative samples(sera)	Number of positive samples (serum)
SD BODLINE HIV ½ 3.0	200	199	01

HIV- human immunodeficiency virus.

Table.3 Summary of ELISA result by The Genscreen TM Ultra HIV Ag-Ab

Components of ELISA	Optical Density (O.D)	Cut-off Value	Assay Result
Positive Ag control	2.167	-	-
Positive Ab control	2.160	-	-
Negative Control	0.055	-	-
39 sera	> 0.260	0.260	NEGATIVE
1 serum	< 0.260	0.260	POSITIVE

ELISA- enzyme linked immunosorbent assay

ELISA result was interpreted on the basis of observance of the optical densities, calculated with cut-off value. Assay validation was done according to the given procedure-

The absorbance of each negative control (R3) should be less than 0.170. If one negative control does not respect this norm, disregard and recalculate the mean using the two remaining values only one value may be dominated by this way the mean of the absorbance of the negative control should be less than 0.150 the absorbance of HIV Ab positive control (R4) should be greater than 0.9:ODR4>70.9 the absorbance of HIV Ag positive control (R5) should be greater than 0.9:ODR5> 70.9 (Bio-rad laboratories, 2012).

Since our aim was to find out the spectrum of HIV Positive patients in RAM Monohar Lohia Institute of Medical Sciences Gomti Nagar, Lucknow, since Ram Manohar Lohia Institute of Medical sciences is tertiary care centre only referred patients' diagnosis was done for the screening of viral marker preparative. Thus the spectrum of patient at this institute does not correlate with that of community.

The assays were performed between the age group of 17-64 years. Most of the patients attended the department of neurosurgery, oncology, oncosurgery and urology. Mostly patients were male and they were diagnosed other illnesses too e.g., Hepatitis B, HCV and routine test like blood test.

In comparison to tertiary care centre, the population load is higher in ICTC (Integrated Counseling and Testing Centre) and PPTC (Prevention of parent to child transmission centre). ICTC is an important

component of HIV/AIDS care, prevention and control program. This serves as an entry point for counseling, testing, antiretroviral treatment, treatment for opportunistic infections, psychosocial support. For such types of services, direct population come here.

PPTC-Mother (Parent) child transmission of HIV is a major problem worldwide. Most of the children (90%) who are HIV positive were born to HIV infected mother. At this centre, pregnant ladies came for counseling and testing administration of antiretroviral drug to reduce the viral load, appropriate obstetric & prenatal care for mother counseling about breast feeding and administration of antiretroviral drugs to the HIV exposed new born. That's why the population load is high in primary & secondary care centre.

PPTC of HIV is a very important component of NACPII & NACPIII which is aimed to minimize transmission of HIV through this route.

Acknowledgement

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