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Comparative evaluation of Rapid and ELISA kits for detection of HIV infection

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We report results of seven Rapid and six ELISA test kits using a well-characterized sera panel comprising 50 anti-HIV positive, 10 cross reactive and 40 normal samples. ELISA kits detected the HIV infected samples with 100 % accuracy. Amongst the Rapid tests, CombAids-RS, HIV 1&2 BiSpot Immunocomb II and NEVA HIV kits detected the infected sera accurately. Other rapid kits had high specificity (100 %) but low sensitivity (84-98 %). The results presented here reiterate the need for independent body evaluation of Rapid and ELISA kits, with standardized and well-characterized large sera panel(s) for the accuracy of HIV testing.

Key words: Immuno-chromatography, immuno-filtration, agglutination, erythrocyte, ELISA, HIV.

INTRODUCTION

Transmission of the human immunodeficiency virus (HIV) remains a major concern for the public health departments of developed and developing countries alike. There are a recorded 36 million infected and more than 15000 infections are added daily, mainly in the developing countries[1]. There are no vaccines available yet for HIV and the knowledge of in vitro correlates of protection is far from satisfactory[2]. An effective vaccine development for human use is not yet in sight. Current efforts to contain the spread of HIV infection rely on good behavioral practices and effective blood screening. It is difficult to change the behavioral patterns, such as sexual practices, intra venous drug abuse etc. In this scenario, it becomes necessary to precisely detect the presence of HIV infection in the blood donated for transfusion and for health care professionals such as surgeons, dentists, gynecologists and paramedical staff as they are exposed to human blood and other fluids. The presence of antibodies in blood and products there of, indicates viral infection. Circulating antibodies, mainly against *env* and *gag* proteins of HIV are of immense diagnostic value[3]. Over the years, based on the detection of antibodies against the above-mentioned proteins, many different diagnostic formats such as enzyme immunoassay, agglutination and immunochromatography have been developed and are commercially available for detection of antibodies against HIV[4]. The initial tests used viral antigens derived from the whole viral lysates. Later, recombinant and synthetic antigens replaced the proteins derived from whole virus lysate as the latter was considered potentially infectious. The improved assays also had the capability to detect immunoglobulin M, the early antibodies to HIV, in addition to immunoglobulin G, thus resulting in a significant reduction in the seroconversion window period.

We have developed two different formats of HIV diagnostic tests namely NEVA HIV and ELIK HIV 1&2. The NEVA HIV is based on the principle of erythrocyte agglutination and utilizes bi-functional recombinant fusion proteins. The proteins consist of Fab molecules derived from the anti-erythrocyte surface proteins that are fused with antigenic epitopes from HIV-1 and HIV-2. The *E. coli* clones coding for the LC and Fd proteins, which on the C-terminus are fused with our chosen stretches of amino acids from the gp41, p24 of HIV-1 and gp36 of HIV-2 were obtained by us through a technology transfer contract between Cadila and University of Delhi South Campus; details about the recombinant proteins have been published elsewhere[5-7]. The LC and Fd fusion proteins are isolated, combined and purified to obtain the Fab molecules. The Fab molecules target the erythrocyte surface proteins and cause agglutination in presence of anti-HIV antibodies. The results of the reaction are visible to the naked eye. The other test is an ELISA based kit. The solid phase is coated with the synthetic (gp41 of HIV-1 and gp36 of HIV-2) and recombinant (p24 of HIV-1) antigens. The synthetic peptides are purchased from various commercial sources while the recombinant p24 protein is been constructed by us at our lab.

There are a number of rapid and ELISA kits available in the market for detection of HIV antibodies in the patient samples. Most of these tests are claimed to be highly accurate. However, in the actual market scenario, different kits perform differently. This leads to discrepancies in results particularly in the case of false-negative samples, which might lead to inadvertent spread of HIV infection amongst the recipients of infected blood/blood products. False positive samples also create avoidable stresses on the people due to the social stigma attached to the disease. Hence there is an urgent need to establish standardized testing procedures and protocols to evaluate various kits available in the market with a view to ascertain and identify the most suitable ones. In the present study we have evaluated NEVA HIV and ELIK HIV 1&2 kits and compared the performance with other commercially available kits using a well-characterized serum panel. The results suggest that most of the rapid kits and all ELISA kits give comparable results.

MATERIALS AND METHODS

Sera panel. A panel of well-characterized serum/plasma samples (n=50) obtained from patients infected with HIV-1 (n=45) and HIV-2 (n=5) was used for these studies. In addition, 10 cross-reactive samples were also included in the panel from patients suffering from tuberculosis, malaria, rheumatoid arthritis, syphilis, hepatitis B and hepatitis C virus. Samples (n=40) from healthy individuals were also included. The positive as well as negative samples were characterized using WHO algorithm for HIV detection[8, 9]. All the samples were tested under code.

Rapid assay kits. Seven kits based on the principles of flow-through, immunochromatographic and Dot immunoassay and erythrocyte agglutination were sourced for the study (Table-1). These were **CombAids-RS** (Span Diagnostics Ltd., India), **HIV TRI-DOT** (BIOTECH INC., India), **HIV 1&2 BiSpot Immunocomb II** (Orgenics, Israel), **SD BIOLINE HIV-1/2 3.0** (Standard Diagnostics, Inc. Korea), **Retrocheck HIV WB** (QUALPRO Diagnostics, India), **HIVue** (ACE Diagnostics and Biotech Ltd., India), and **NEVA HIV** (Cadila Pharmaceuticals Ltd., India). All tests were performed strictly as per the instructions of the manufacturers, as supplied with kits.
ELISA test kits. Five kits based on the principle of ELISA namely, **HIVASE 1+2** (General Biologicals Corp., Taiwan), **Anti-HIV TETRA ELISA** (Biotest, Germany), **Microlisa HIV** (J. Mitra & Co. Ltd., India), **HIV-1/HIV-2 Ab-Capture ELISA** (Ortho-Clinical Diagnostics, Inc., USA) and **HIV EIA** (Ani Labsystems Ltd., Finland) were procured from the market (Table-1). The sixth ELISA based kit was our ELIK HIV 12 (Cadila Pharmaceuticals Limited, Ahmedabad, India). All kits were used strictly as per the manufacturers' instructions supplied with the respective kits. All the kits are commercially available. Genelabs HIV 1/2 2.0 western blot kit was used to analyze and confirm the positive samples.

RESULTS

As mentioned earlier we have analyzed 7 Rapid and 6 ELISA kits on a sera panel comprising of 100 samples (45 HIV-1 positive, 5 HIV-2 positive, and 40 seronegative and 10 cross-reactive members). The tests were carried out under code by highly competent persons with experience of working with all types of formats such as erythrocyte agglutination, immunofiltration, immunochromatography and ELISA. The results are summarized in Table-2. The various ELISA kits performed equally well with the serum panel used in this study. The sensitivity and specificity of all the kits was found to be 100 %. The over all background with the normal human samples was less (OD = 0.02 to 0.07). The results of testing with the rapid format kits however varied amongst the seven kits tested with our sera panel. All the kits were 100 % specific (Table 2). Three kits namely CombAids-RS, HIV 1&2 BiSpot Immunocomb II and NEVA HIV detected all the infected samples with 100 % sensitivity. Other kits tested in this study showed lower sensitivity. For example, the Retrocheck HIV WB kit was only 84 % sensitivity. Similarly, other kits also when tested with the same samples showed false negative results. The immuno-flowthrough kit, HIV

Table-1 Description of HIV Diagnostic Kits used in the Study

Kit	Principle	Manufacturer	Antigens used	Discrimination between HIV-1&2	Hands on time	Sample type
CombAids-RS	Dot Immunoassay	Span Diagnostics Ltd.	Not specified	No	40-50 min	Serum or Plasma
HIV TRI-DOT	Flow Through	J Mitra & Co. Ltd.	HIV-1 (C terminal gp120, gp41), HIV-2 (gp36)	Yes	10-15 min	Serum or Plasma
HIV 1&2 BiSpot ImmunoComb II	Dot Immunoassay	Orgenics	HIV-1 (gp41 & gp120), HIV-2 (gp36)	Yes	35-45	Serum or Plasma
SD BIOLINE HIV-1/2 3.0	Immunochromatography	Standard Diagnostics, Inc.	HIV-1 (gp41 & p24), HIV-2 (gp36)	Yes	5-10 min	Whole blood, Serum or Plasma
Retrocheck HIV	Immunochromatography	Qualpro Diagnostics	HIV-1 (gp41 & p24), HIV-2 (gp36)	No	5-10 min	Whole blood, Serum or Plasma
HIVue	Immunochromatography	Ace Diagnostics & Biotech Ltd.	Not specified	Yes	5-10 min	Whole blood, Serum or Plasma
NEVA HIV	Whole blood agglutination	Cadila Pharmaceuticals Ltd.	HIV-1 (gp41 & p24), HIV-2 (gp36)	No	5-7 min	Whole blood, Serum or Plasma
HIVASE 1+2	ELISA	General Biologicals Corp.	HIV-1 (gp120/gp41), HIV-2 (gp105/gp36)	No	85-95 min	Serum or Plasma
Anti-HIV TETRA ELISA	ELISA	Biotest	HIV-1 (gp41, p24), HIV-2 (gp36)	No	180-200 min	Serum or Plasma
Microlisa HIV	ELISA	J. Mitra & Co. Ltd.	HIV-1 (gp41, C terminal gp120), HIV-2 (gp36)	No	100-110 min	Serum or Plasma
HIV-1/HIV-2 Ab-Capture ELISA System	ELISA	Ortho-Clinical Diagnostics, Inc.	Not specified	No	160-170 min	Serum or Plasma
HIV EIA	ELISA	Ani Labsystems Ltd.	HIV-1 (gp41, gp120, p24), HIV-2 (gp36)	No	85-95 min	Serum or Plasma
ELIK HIV 1&2	ELISA	Cadila Pharmaceuticals Ltd.	HIV-1 (gp41, p24), HIV-2 (gp36)	No	50-60 min	Serum or Plasma

Table-2 Comparative Evaluation of the rapid and ELISA test kits for detection of HIV-1/2*

Rapid Kit	HIV Reactive	False Negative	HIV Non-reactive	Sensitivity %	Specificity %	Faintly Reactive	Faint Results %
CombAids-RS	50	0	50	100	100	10	20
HIV TRI-DOT	49	1	50	98	100	7	14
HIV 1&2 BiSpot Immunocomb II	50	0	50	100	100	0	0
SD BIOLINE HIV-1/2 3.0	47	3	50	94	100	4	8
Retrocheck HIV WB	41	9	50	84	100	10	20
HIVue	45	5	50	90	100	0	0
NEVA HIV	50	0	50	100	100	10	20
ELISA Kit	**HIV Reactive**	**False Negative**	**HIV Non-reactive**	**False Positive**	**Sensitivity %**	**Specificity %**	**Cut off value**
HIVASE 1+2	50	0	50	0	100	100	0.12
Anti-HIV TETRA ELISA	50	0	50	0	100	100	0.281
MicroELISA HIV	50	0	50	0	100	100	0.308
HIV-1/HIV-2 Ab-Capture ELISA	50	0	50	0	100	100	0.136
HIV EIA	50	0	50	0	100	100	0.514
ELIK HIV 1&2	50	0	50	0	100	100	0.271

*All the tests were carried out under code by experienced persons and with high competence in testing the various formats of rapid and ELISA kits. The faintly reactive samples were retested to confirm the results.

Tri-Dot showed 98 % sensitivity. Besides Retrocheck HIV WB, other kits based on the principle of immuno-chromatography, namely SD Bioline HIV-1/2 3.0 and HIVue also showed false negative results and had poor sensitivity of 94 and 90 %, respectively.

Besides low sensitivity, the rapid kits also had problem of readability and in the interpretation of results. CombAids-RS and NEVA HIV kits did not give any false negative results but 20 % samples reacted poorly in both the kits. With Retrocheck HIV WB, apart from low sensitivity (84 %), approximately 20 % samples gave very faint lines on the immuno-chromatography membrane. HIV Tri-Dot and SD Bioline showed faint reactions with 14 % and 8 % samples, respectively.

A total of 12 samples in our panel showed poor reaction with more than one rapid kit used for this study. Moreover, eight samples gave poor reaction with at least one kit in this study (Table-3). Results of the Western blot analysis on some of these samples are presented in Table 3. Presence of different HIV protein bands observed in the Western blot analysis was confirmed in the samples, which were either missed or had poor reaction in the different rapid tests. Results of the Western blot analysis show presence of antibodies against various antigenic proteins in majority of these samples.

All the sera used by us in the study are available for independent study or cross verification of our results.

Table 3. Western blot analysis.

Sample Id	Antigen Bands present in WB	Name of the kits that missed the sample	Name of the kits that had poor reaction with the sample
4	p17, p24, p31, gp41, p55, p66, gp120, gp160	CombAids, HIV Tri-Dot, SD Bioline HIV-1/2 3.0, NEVA HIV	Retrocheck HIV
7	p17, p24, p31, gp41, p55, p66, gp120, gp160		Retrocheck HIV
8	p24, gp120, gp160	CombAids, HIV Tri-Dot, HIVue, NEVA HIV	SD Bioloine HIV-1/2 3.0, Retrocheck HIV
9	p24, gp41, gp120, gp160	CombAids, NEVA HIV	HIV Tri-Dot, SD Bioline HIV-1/2 3.0, Retrocheck HIV
10	p17, p24, gp41, p55, p66, gp120, gp160	CombAids, Retrocheck HIV, NEVA HIV	
11	p17, p24, p31, gp41, p55, p66, gp120, gp160	CombAids, HIV Tri-Dot	SD Bioloine HIV-1/2 3.0, Retrocheck HIV, HIVue
12	p24, gp41, p55, p66, gp120, gp160	CombAids, HIV Tri-Dot, SD Bioline HIV-1/2 3.0, NEVA HIV	
14	p24, p31, p55, p66, gp120, gp160	CombAids, NEVA HIV	
17	p17, p24, p31, gp41, p55, p66, gp120, gp160	Retrocheck HIV	
20	p17, p24, p31, gp41, p55, p66, gp120, gp160	NEVA HIV	
21	p24, p31, gp41, p55, p66, gp120, gp160		Retrocheck HIV
22	p17, p24, p31, gp41, p55, p66, gp120, gp160	Retrocheck HIV	
23	p24, p31, p55, p66, gp120, gp160	CombAids, HIV Tri-Dot, HIVue, NEVA HIV	Retrocheck HIV
27	N.D.	Retrocheck HIV	
28	p24, gp160	HIV Tri-Dot, SD Bioline HIV-1/2 3.0,	Retrocheck HIV
29	p24, gp41, p55, p66, gp120, gp160	HIV Tri-Dot, Retrocheck HIV	
30	N.D.	Retrocheck HIV	
35	N.D.	CombAids, HIV Tri-Dot, SD Bioline HIV-1/2 3.0, Retrocheck HIV, NEVA HIV	
38	N.D.	HIVue	
40	p17, p24, p31, gp41, p55, p66, gp120, gp160	CombAids, NEVA HIV	
41	p17, p24, p31, gp41, p55, p66, gp120, gp160	Retrocheck HIV	
43	p24, gp41, p55, p66, gp120, gp160	Retrocheck HIV	

DISCUSSION

The HIV infections are spreading at fast pace throughout the country as well as other developing nations[1]. Various Governments are taking the necessary steps towards stopping the new infections especially from the transfusion of infected blood to patients, by making it necessary for the blood banks to test all the donated blood units. Indian Government also has framed such rules and guidelines for blood banks and healthcare professionals[10]. Moreover, with the increased awareness amongst the healthcare professionals, the surgeons, dentists and gynecologists are conscious of the professional hazards associated with managing patients infected with HIV[11, 12].

Most of the commercially available diagnostic kits for detection of HIV are reported to have high sensitivity and specificity for detection of anti-HIV antibodies. We carried out a comparative evaluation of the various rapid and ELISA kits available in the Indian market on a very well characterized sera panel. The sera were obtained from patients reporting to the clinic with various ailments in the State of Gujarat, India. The results reported in this study indicate that all of the ELISA kits tested were able to detect the HIV

infected and normal human sera samples with 100 % sensitivity and 100 % specificity. All the ELISA kits were of third generation i.e. they were capable of detecting the IgG and IgM antibodies to *env* and *core* antigens of HIV-1 and/or HIV-2. All the kits required between 90 to 200 min for completion of the assay, except for the ELIK HIV 1&2 with a hands on time of less than 60 min.

In contrast to ELISA kits, the rapid tests showed greater variability in terms of performance, sensitivity and specificity. All rapid tests had excellent specificity (100 %) and no invalid results. However, only 3 (the dot immunoassay based CombAids-RS, HIV 1&2 BiSpot Immunocomb II, and the whole-blood agglutination based NEVA HIV) of the 7 tested kits were able to detect the positive samples with 100 % accuracy. The other 4 (HIVue, HIV Tri-Dot, SD BioLine HIV-1/2 3.0, and Retrocheck HIV) demonstrated sensitivity in the range of 84 to 98 %. Apart from the low sensitivity, in some of the kits, the reaction produced by the positive samples was poor. For example, in CombAids-RS and Retrocheck HIV kits, as many as 20 % of the positive results were poorly visible. NEVA HIV too showed poor agglutination with 20 % of the positive samples. HIV Tri-Dot kit also suffered with similar problem with very faint reactions in 14 % of the samples tested. The samples which gave poor reaction or were missed were subjected to reanalysis by Western blot kit to check the status. All such samples showed presence of antibodies against all protein antigens of the HIV. This phenomenon requires further analysis to understand the inability of fragments of the antigenic epitopes used in majority of the rapid kits to detect all the positive samples. Such samples can give rise to higher than recommended inter-reader variability in a multi-centric evaluation scenario. Moreover, such kits can lead to higher than normal false negative results in the clinical and pathology laboratory settings[13].

Five rapid test kits required 5-15 min for completion with very less hands on time for the user. The CombAids-RS and Immunocomb II required 35 to 50 min of hands on time for completion of the test including the reagent preparation and washing steps. Three of the seven tested kits were of third generation i.e. they were capable of detecting the anti-HIV-1p24 antibodies. Of these NEVA HIV had the highest sensitivity and specificity. Antibodies against the core antigen p24 are the first to appear in the patient infected with HIV. The kits, which can detect the anti-p24 antibodies, are especially useful as they can detect HIV infection early and can reduce the window period substantially.

The data presented in this study indicate that three of the seven rapid kits detected the negative and positive sera samples as accurately as the ELISA kits. ELISA methods took substantially longer time for completion, about 90-200 min as compared to 5-15 min for rapid tests. It has been observed in an independent study over a long period of time that more than 40 % of the people tested never return to receive their results[14]. The long time taken to complete the conventional tests has been cited as the main reason for this. The main advantage of using rapid tests is the availability of results in a very short time. This advantage may get diminished if the rapid test kits perform poorer than the ELISA kits. Such disadvantages may be overcome if more robust rapid tests with high accuracy in sensitivity and specificity are available. Such rapid test kits would go a long way in assisting the health care workers, surgeons, dentists, gynecologists etc. in understanding the status of their clients. Highly reliable rapid kits would also be useful in handling the emergency situations such as in the casualty and labor wards[15-18]. Besides, in situations where a person is bled for blood donation, a decision can be taken a priori within a couple of minutes about the suitability of the blood if the test results are available very rapidly. In the present study, performance of the NEVA HIV kit was found to be the best for rational use in terms of the total time taken to carry out the test as well as in terms of its sensitivity and specificity results.

This study has demonstrated that different kits available in the market give quite different results with identical samples. The results suggest that some of the rapid tests can be used in place of the more complex and time consuming ELISA based kits in small hospitals, pathology laboratories, surveillance and counseling centers as well as for the point-of-care tests.

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