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Original Article

Usefulness of enzyme immunoassay (EIA) for screening of anti HIV antibodies in urinary specimens: A comparative analysis

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ABSTRACT

Background: Standard HIV testing is done using serum or plasma. FDA approved ELISA to screen urine for IgG antibodies to HIV-1 in 1996. It is a simple, noninvasive test and is appropriate for developing countries where health care personnel may not be professionally trained or where clean needles for drawing blood may not always be available.
Methods: 436 individuals with high-risk behavior and strong clinical suspicion of HIV infection were screened for IgG antibodies to HIV-1 in urine by ELISA. Urine HIV testing was performed by enzyme immunoassay, at the ongoing Voluntary Confidential Counseling and Testing Center (VCCTC) at a large tertiary care microbiology lab. The individuals enrolled for the study had high-risk exposure to the virus and majorities were from a state with a high incidence of HIV infection. In all individuals, both serum and urine were tested for IgG antibodies to HIV-1.
Results: Overall, 135 individuals (30.96%) were HIV-positive, of whom 96 (71%) had never previously tested positive; 87% of those who tested positive received their results, and most were referred for medical care. Sensitivity, specificity and predictive values of HIV-1 urine ELISA test kit were determined. Sensitivity was found to be 89.6%; 95% CI [82.9–94.0], specificity 97.3%; 95% CI [94.6–98.8], positive predictive value 93.8%; 95% CI [87.8–97.1] and negative predictive value 95.4%; 95% CI [92.3–97.4].
Conclusion: Efficiency, sensitivity, and specificity of the urine-based screening for HIV-1 test kits were excellent as compared to the reference test.
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Introduction

Many HIV-infected individuals are unaware of their status, since the characteristic symptoms of AIDS usually do not develop until years after HIV infection. Early knowledge of HIV infection may allow infected individuals to seek early treatment, which has been shown to delay onset of AIDS and to help change high-risk behavior. Serologic studies for the

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presence of antibodies to HIV-1 are the standard method of screening for HIV infection. A time interval (window period) exists between infection with HIV and the development of detectable antibodies to HIV (seroconversion). 95 percent of infected persons seroconvert within six months. Arsenals of laboratory methods are available to screen blood, diagnose infection, and monitor disease progression in individuals infected by HIV. Tests to detect antibody to HIV can be classified as: (a) screening assays that are designed to detect all infected individuals, and (b) confirmatory (supplemental) assays, which are designed to identify individuals who are not infected but who have reactive screening test results. Accordingly, screening tests possess a high degree of sensitivity, whereas confirmatory assays have a high specificity. These classes of assays, performed in tandem, produce results that are highly accurate, reliable, and appropriate. To date, HIV testing can be performed on any of three body fluids: blood, oral fluid or urine.

The HIV Antibody Blood Test is a sequence of several different tests. The first Enzyme Linked Immuno Sorbent Assay (ELISA) for antibodies to Human Immunodeficiency Virus (HIV) were manufactured by coating purified HIV lysate on to the surface of micro-titration plates or beads (First Generation Assays). Later on, ELISAs have been developed which use antigens of either HIV recombinant polypeptide or synthetic peptide (second or third generation immunoassays).[1–3]

The use of the third generation immunoassay for the detection of HIV has reduced the interval between infection and antibody detection. These assays detect antibody to HIV earlier than the first and second generation assays including Western Blot (WB) from serum and urine.[4–6] Even with HIV antibody screening, assays that have excellent sensitivity and specificity, false-positive results cannot be ruled out, especially when used in a population with low prevalence of HIV antibodies.[1,7,8] Simple immunodot assays for HIV have been developed that do not require much equipment and that yield results after a few minutes.[1–3,9] The HIV oral fluid test (Orasure) was approved in 1994 and involves collecting secretions between the cheek and gum and then evaluating them for HIV antibodies.[10] Orasure is essentially as accurate as blood tests, and because it doesn't involve a needle stick, is favored by many individuals. The urine-based HIV Test was approved in 1996. The test uses urine samples to detect IgG antibodies to HIV-1 by ELISA.[11]

Urine-based HIV IgG ELISA is relatively simple, noninvasive, inexpensive test and the sample can be stored at room temperature for extended periods of time. The use of urine for testing is appropriate for physician's offices, health clinics, and in developing countries where health care personnel may not be professionally trained or where clean needles for drawing blood may not always be available. A positive screening test must be followed by a blood test to confirm the results. The present study attempts to evaluate the ELISA test kit to screen urine for IgG antibodies to HIV-1 for its accuracy.

Material and methods

The study was conducted in a city with high prevalence of HIV infection. The study subjects who presented voluntarily for the HIV testing and those referred to the OPD on clinical suspicion from a large tertiary care hospital to the VCTC were included in the study. All the subjects were HIV-1 status naïve. Informed consent was obtained and a pre test counseling was imparted to each individual. Blood sample in sterile vacutainers with clot activator and urine sample in sterile 15 ml tube (Falcon, from BD Biosciences) were collected under supervision. Post test counseling was done once the results were obtained.

Urine samples were tested for antibodies to HIV using a commercially available assay as per the manufacturer's instructions (Calypte Biomedical Corporation, Alameda CA., USA, HIV-1 Urine EIA Cat. No. 700000, 480 tests kit). Briefly, the Calypte HIV-1 Urine EIA is an enzyme immunoassay which utilizes a recombinant envelope protein of HIV-1 to detect the presence of antibodies to HIV-1 in human urine against the recombinant gp 160-envelope protein adsorbed onto the wells of a microwell plate. Appropriate positive and negative controls were included for each test run. The specimen was determined to be either reactive or non-reactive by comparing its absorbance value to a cut off value, which was calculated by adding the mean absorbance value of the negative calibrators to a value of 0.180.

03 HIV Kits were used concurrently for detection of serum IgG to HIV (Combaids HIV 1/2 Immunodot test kit, Span Diagnostics Ltd; TRI-DOT BIOTECH INC, J. Mitra & Co and MICROLISA – J. Mitra & Co). Sensitivity, specificity and predictive values with 95% Confidence intervals (CI) of the urine IgG HIV-1 test kit were determined using standard statistical tools.

Results

Of those 436 persons screened, 346 (79.3%) were males and 90 (20.6%) were females. The mean age of the subject was 38.5 years (range 3–77 years). 129 (29.58%) tested reactive for anti HIV antibodies and 307 non reactor by Calypte HIV-1 Urine EIA assay. Concurrently, blood samples of all the subjects were tested for HIV-1 antibodies by three different ELISAs (based on different principles and different antigens) as gold standard and 301 (69.04%) individuals tested true negative. Of the 129 samples that tested positive by urine ELISA, 121 (93.8%) turned out to be positive by all the three serum ELISA test kits. Therefore, 08 (6.2%) samples tested false positive by the urine ELISA kit. On the other hand, 307 samples that tested negative by the urine HIV ELISA testing kit, 293 (95.4%) were true negatives when compared to the serum ELISA tests and 14 (4.5%) reported false negative (Table 1).

Sensitivity, specificity and predictive values with 95% Confidence intervals (CI) of the urine IgG HIV-1 test kit being evaluated were determined. Sensitivity was found to be 89.6% with 95% CI [82.9–94.0], specificity 97.3% with 95% CI [94.6–98.8], positive predictive value was 93.8% with 95% CI [87.8–97.1] and negative predictive value was 95.4% with 95% CI [92.3–97.4] (Table 2).

Discussion

For the laboratory diagnosis of HIV, the mere presence of specific antibodies signals that infection has occurred.

Table 1 – Accuracy of the Test kit used for Urine EIA.

Urine EIA[a] result	Serum antibody test results by three EIA[a] test kits based on different antigen/principle		Total (*n*)
	True positive (*n*)	True negative (*n*)	
Test positive	121	8	129
Test negative	14	293	307
Total (*n*)	135	301	436

[a] Enzyme immunoassay.

Antigens used in HIV diagnostic tests must be appropriately specific, and are usually purified antigens from viral lysates, or antigens produced through recombinant or synthetic peptide technology. The use of such antigens allows HIV screening tests to possess both sensitivity (to detect infection) and specificity (to detect non-infection). ELISA are the most commonly used tests to screen for HIV infection because of their relatively simple methodology, inherent high sensitivity, and suitability for testing large numbers of samples, particularly in blood testing centers. Regardless of the screening method, a sample producing a reactive result must be screened again in duplicate, with at least two of the three results being reactive (repeatedly reactive) before verifying infection with confirmatory assays. New, laboratory-based, strategies have been devised that can distinguish recently infected individuals from those with established infection. They are based on the concepts of antibody titer or antibody avidity, and modifications to the procedures of conventional ELISA or rapid assays have been performed to allow discrimination of antibody titer or antibody avidity. These modified assays have been called "detuned assays" or "sensitive/less sensitive assay (S/LS)".[12]

Specific antibody to HIV is produced shortly after infection, but the exact time depends on several factors, including host and viral characteristics. Importantly, antibody may be present at low levels during early infection but not at the detection limit of some assays. Using the early generation tests, antibody could be detected in most individuals by 6–12 weeks after infection. Newer generation assays, including the third-generation antigen sandwich assays, can detect antibody at about 3–4 weeks after infection.[13] Most antibody tests currently on the market have near perfect and equivalent degrees of sensitivity for detecting most individuals who are infected with HIV (epidemiologic sensitivity), but they vary in their ability to detect low levels of antibody (analytical sensitivity), such as that occurring before complete seroconversion.[13]

Table 2 – Predictive value & the Confidence interval (CI) of the test kit used for Urine EIA.

	Percentage	95% Confidence interval
Sensitivity	89.6	82.9–94.0
Specificity	97.3	94.6–98.8
Positive predictive value	93.8	87.8–97.1
Negative predictive value	95.4	92.3–97.4

Simple test requires greater than 30 min but has procedures that can be performed easily without instrumentation. Within this class of tests are agglutination assays in which antigen-coated particles (red blood cells [RBC], latex particles, or gelatin particles) are allowed to react with serum antibodies to form visible clumping (agglutination).

Rapid assays for detecting specific HIV antibody were developed in the late 1980s, and are defined as tests that can be performed in less than 30 min. These tests gained popularity in the early 1990s, and as technology became refined, proved to be as accurate as the ELISA when performed carefully by experienced personnel. Technical errors are common with these assays, however, because users become careless with these simple procedures. For instance, pipettes are not always held in a vertical position as recommended, resulting in an incorrect delivery of reagent volumes. One class of rapid tests is the "dot blot" or "immunoblot". They produce a well-circumscribed color dot on the solid phase surface if the test is positive. Most of these rapid assays now incorporate a built-in control that indicates that the test was performed correctly. This control is an anti-human immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added appropriately.

The detection of p24 antigen by ELISA is a simple and cost effective technique to demonstrate viral capsid (core) p24 protein in blood during acute infection due to the initial burst of virus replication after infection. In order to maximize the detection of all infected individuals, including those in early infection, antibody, antigen and viral RNA tests should be used. However, viral RNA tests are expensive, time consuming, and are not available in many laboratories. Laboratories that possess ELISA capability can increase the ability to detect most infections by testing for both HIV antibody and p24 antigen.

Noninvasively collected specimens, such as oral fluids (saliva), have been used for HIV testing as an alternative to blood samples (Orasure).[10] These fluids, containing crevicular fluid from capillaries beneath the tooth-gum margin, are transudates of blood; therefore, they include the same fluid (plasma) that is used for testing with serum-based tests. The concentration of antibodies in oral fluids is about 1/400 of that in plasma, however, because of the dilutional effect of fluids from the salivary glands (true saliva)[14] necessitating extremely sensitive tests that are able to detect small quantities of antibody. The testing technology to detect these low quantities is now available and oral fluid tests; both ELISA and rapid tests are accurate.[15]

Intact IgG antibodies are found in urine, but their exact origin is unknown. The collection of urine is simple, noninvasive, and inexpensive, and the sample can be stored at room temperature for extended periods of time. The use of urine for testing is appropriate for physician's offices, health clinics, and in developing countries where health care personnel may not be professionally trained or where clean needles for drawing blood may not always be available. The major disadvantage is that there is not an approved confirmatory assay, necessitating the collection of blood when results are reactive. In 1996, FDA approved an ELISA for use to screen urine for antibodies to HIV-1.[11] There are a number of factors that influence rapid tests differently than ELISA-type tests. For

example, since urine is much less viscous and contains less protein than serum, flow rates through these rapid devices are dramatically increased. Consequently, this leaves less time for antigen–antibody reactions to occur. Also, the variability in the pH of urine appears to affect reaction time (since antigen–antibody reactions are pH dependent); the pH of urine varies considerably from individual to individual.

The validity of diagnostic test results depends on the quality of a number of measures used before, during, and after the test is performed.[16] To ensure the quality of test results, a program consisting of quality assurance, quality control, and quality assessment is necessary. The determination of HIV-1 antibody in urine by IgG ELISA assay for HIV-1 seropositive patients was found to have a sensitivity of 89.6% with 95% CI [82.9–94.0], specificity of 97.3% with 95% CI [94.6–98.8], positive predictive value of the test was 93.8% with 95% CI [87.8–97.1] and negative predictive value of the test was 95.4% with 95% CI [92.3–97.4]. The data obtained is excellent as compared to the reference serological test for the detection of HIV-1.

Conclusion

Recent studies indicate that sensitivity and specificity of noninvasive methods of detection of anti HIV antibodies in saliva and urine are comparable to the conventional methods using serum or plasma. Though saliva has now been considered better as a screening modality, the determination of HIV-1 antibody in urine by IgG ELISA assay can serve as screening test in the clinician OPD for detection of HIV-1 but confirmatory results is required to be given in conjunction with serum IgG assay.

Conflicts of interest

All authors have none to declare.

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