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Performance Characteristics of Urine HIV Screening Methods against Blood-Based Methods for Surgeons Guide

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Abstract

Testing for Human Immunodeficiency Virus (HIV), widely distributed in sub-Saharan Africa since it is mainly invasive but, could be non-invasive and quick also, reducing waiting time especially when required for presurgical procedures. This study determined the HIV status of patients using Urine screening test method and to compare its performance to blood-based testing methods. The routine pre and post-test counselling for HIV screening were done for all provider-initiated HIV testing using blood-based screening methods. Due to the cost and unavailability of enough urine testing kits, only patients who tested positive for HIV with blood-based methods and were scheduled for surgery or a surgical procedure were enrolled in the study. Informed consent was obtained. Paired urine and blood samples were collected at the same visit into clean universal bottles and analyzed immediately. A colloidal gold enhanced rapid immuno-chromatographic assay (Alliance Biomedical) kit for the rapid qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) I and II in urine were used in comparison to the standard HIV testing of ante-cubital venous blood collected in EDTA vacutainer and analyzed using Determine (T) HIV 1 and 2 *in vitro* qualitative immunoassay strip, UNI GOLD rapid test kit and the Chembio HIV 1/2 STAT PAK assay strip. A total of 7568 patients were tested for routine provider-initiated HIV testing, 521 tested HIV positive. There were 105 (20.15%) males and 416 (79.85%) females, age ranged from 15 years to >80 years. Most of the surgeries performed were Caesarian section 93 (37%), Hernia 55 (22%), Lumps 48 (19%), Acute appendicitis 33 (13%), Uterine fibroids 10 (4%), Ruptured ectopic pregnancy 2 (1%) and others (Intestinal obstruction, Post-

operative adhesions, Ingrown toe nails, Breast abscess, Hemorrhoids, Anal fissures etc.) 10 (4%). DETERMINE RAPID HIV TEST METHOD USING BLOOD: A total of 521 HIV positive samples were tested, 502 (96.35%) tested HIV positive and 19 (3.65%) tested HIV negative. These 19 HIV negatives were re-tested with Stak Pak: 19 (100%) tested HIV positive. UNI GOLD HIV TEST METHOD USING BLOOD: A total of 521 HIV positive samples were tested, 521 (100%) tested HIV positive. URINE TESTING METHOD: A total of 251 (48.18%) of the 521 HIV positive patients were scheduled to undergo a surgical procedure. These were re-tested using the Urine testing method, 235 (93.63%) tested HIV positive while 16 (6.37%) tested negative. The blood sample of the 16 who tested negative using the Urine testing method was subjected to confirmatory test using Stat Pak and all 16 (100%) tested HIV positive. The specificity for Unigold and Determine blood testing was 100%. All three tests had a Positive Predictive Value (PPV) of 100% while the Negative Predictive Values (NPV) were 100% and 99.73% for Unigold and Determine respectively. The use of Urine HIV testing method compared well to the blood HIV testing methods and could be a better non-invasive sample method for screening of HIV/AIDS in the population especially among surgeons' pre-surgical procedures.

Keywords

HIV, AIDS, Sensitivity, Specificity, HIV Rapid Testing

1. Introduction

The Human Immunodeficiency Virus (HIV), causative agent of Acquired Immune Deficiency Syndrome (AIDS) was discovered in 1983 [1]. UNAID/WHO 2007 epidemic update showed a worldwide distribution of people living with HIV to be 33.2 million with more adults (30.8 million) infected relative to children less than 15 years of age (2.5 million) [2]. Most cases are in sub-Saharan Africa (22.5 million) [2]. Based on this update, 2.1 million deaths resulted from AIDS-related illnesses with children making up 330,000 of this number [2]. Studies on surgical outcomes among HIV infected surgical patient showed that the infection attributed significantly to morbidity and mortality. HIV testing is a very important routine preparation for patients undergoing surgical procedures especially in endemic areas.

Blood based HIV testing is the gold standard for HIV diagnosis as determined by CDC and WHO. Other body fluids like the Oral fluids have been used for rapid HIV screening with expectant high sensitivity [1] [3]. There is however the window period of three months or more before seroconversion after getting infected. To overcome this bottleneck, researchers explored the use of other body fluids and urine was reported to be valuable. HIV infection can be detected in the urine within fifteen minutes of exposure to the virus [1]. The first Urinary HIV screening test was approved in the United States in August 1996 [2]. Defin-

itive testing methods used for confirmation include western blots and Polymerase Chain Reaction (PCR), while Enzyme-Linked Immunosorbent Assay (ELISA), direct fluorescent antibody assay and others are used for HIV types I and II [4].

The performance characteristics of urinary method measured against other standard methods of HIV diagnosis were to ascertain its suitability for use as a screening test in resource-poor environment. There is usually an interval of 3 weeks to 6 months from exposure to the production of measurable antibody against the virus before detectable HIV infection [5]. Patients are infective at this stage, although the infection may not be detected with the standard an antibody test [6]. Some patients may have delayed seroconversion or extended window period beyond 12 months if they have previously used anti-retroviral drugs [6] [7] [8]. These antibodies are usually found in all body fluids but most commonly used for diagnosis are the serum and plasma. Other body fluids such as urine and saliva have been found to be good alternatives to the former [9]-[15]. Urine has an added advantage of not being invasive, requires no special training for sample collection, no special sample containers and there is ease of collection from children [16].

The majority of antibodies detected in urine are immunoglobulin A (IgA) produced from urethral mucosa and a small amount of immunoglobulin G (IgG) following extravasations from the serum through the mucosa [17]. We compared the performance characteristics of urine HIV screening methods against blood-based methods as a quick pre-operative evaluation guide for surgeons in patients that require a surgical procedure at Faith Alive Foundation Hospital. Faith Alive Foundation which is a faith based NGO with comprehensive holistic approach to medical and social services began management of HIV/AIDS even with ARVs in 1997 and since then as early as 2004 was and still remains a beneficiary of PEPFAR with about 6000 clients currently on care, treatment and support.

2. Materials and Methods

The health facility where this study was conducted has a HIV treatment center with 5903 HIV infected patients on treatment, and 5234 in care and offers free HIV testing. The routine pre and post-test counseling for HIV screening was done for all provider-initiated HIV testing using blood-based screening methods. Due to cost and unavailability of enough urine testing kits at this time, only patients who tested positive for HIV with blood-based methods and were scheduled for surgery or a surgical procedure were enrolled into the study. However, most of participants preferred urine testing method making it a great potential for future use especially with the stigma associated with being openly seen in a HIV hospital-based HIV testing centers like Faith Alive Foundation. More importantly each participant was taught how to do urine testing which is reproducible. Ethical clearance was obtained from the ethics and research committee of the hospital for the study period from January 2014 to June 2016.

After obtaining informed consent from participants who were ART naïve they were instructed to rest for about 10 minutes prior to blood collection. Antiseptic preparation of the ante-cubital vein was done with methylated spirit after tourniquet was applied about 6 cm above the cubital fossa to identify a suitable vein. Five (5 mls) of free-flowing venous blood was collected into Ethylene Diamine Tetra acetic Acid (EDTA) vacutainer tube and analyzed immediately using Determine (T) HIV 1 and 2 *in vitro* qualitative immunoassay strip, UNIGOLD rapid test kit and the Chembio HIV 1/2 STAT PAK assay strip.

A colloidal gold enhanced rapid immuno-chromatographic assay (Alliance Biomedical) kit for the rapid qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) I and II in urine was used in to test HIV in the participants urine. The performance of the urine test strip was compared to the standard HIV testing venous blood using Determine (T) HIV 1 and 2 *in vitro* qualitative immunoassay strip, UNIGOLD rapid test kit and the Chembio HIV 1/2 STAT PAK assay strip.

Five milliliters (5 mL) of urine sample was collected into a clean universal bottle between 8 am and 10 am for every participant and analyzed immediately for HIV using a colloidal gold enhanced rapid immuno-chromatographic assay (Alliance Biomedical) kit for the rapid qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) I and II in urine was used. The principle of the urine test is based on antigen-antibody reaction. The HIV antigen-colloidal gold conjugate embedded in the sample pad reacts with the HIV antibody present in the urine sample to form a conjugate HIV antibody complex. The conjugate HIV antibody complex is captured by a second antibody immobilized on the membrane as it migrates along the strip to form a colored test band in the test region. A negative sample does not produce a test band due to the absence of a conjugate/HIV antibody complex. Antigens used in the conjugate test are recombinant proteins that correspond to highly immuno-reactive regions of HIV I and II. A colored band in the control region appears at the end of the test regardless of the test result and serves as a control. The whole process took approximately 15 minutes per test.

Determine (T) HIV 1 and 2 *in vitro* qualitative immunoassay strip was used. The principle of operation is based on the detection of HIV I and II in human serum, plasma or whole blood. The sample is added to the sample pad as mixture migrates through the conjugate pad; it reconstitutes and mixes with the selenium colloid-antigen conjugate. The mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV 1 and/or HIV 2 are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen and if absent, the antigen-selenium colloid flows past the patient window and no redline is formed.

The Chembio HIV 1/2 STAT PAK assay principle employs a combination of specific antibody binding protein which is conjugate to colloidal gold dye par-

ticles and HIV 1/2 antigens which are bound to the membrane solid phase. The sample was applied to the sample well and buffered to facilitate the lateral flow of the released products and promote the binding of antibodies to the antigen. Antibody present bounded the gold conjugate antibody binding protein to form a dye conjugated-immune complex which migrated to the nitrocellulose membrane and is captured by the antigen immobilized in the test area to produce a pink/purple-colored line. A control line at the control area also formed containing Immunoglobulin G.

The Unigold test kit is a rapid immunoassay principled on immunochromatographic sandwich technique. There's a nitrocellulose strip containing the test region which immobilizes the immunodominant region recombinant proteins of HIV (envelop protein HIV-1 and HIV-2, glycoprotein gp 41, gp 120 for HIV-1 and glycoprotein gp 36 for HIV-2). These proteins link to a colloidal gold impregnated below the test region of the kit. A control region is marked by a narrow sensitive band of nitrocellulose membrane. The test can be performed on plasma, serum or whole blood. A positive test results from the reaction between antibody of any immunoglobulin class specific to recombinant HIV-1 or HIV-2 and the colloidal gold linked antigen.

3. Results

A total of 7568 patients were tested for routine provider-initiated HIV testing, 521 tested HIV positive. There were 105 (20.15%) males and 416 (79.85%) females, age ranged from 15years to >80 years. See Table 1. Most of the surgeries performed were Caesarian section 93 (37%), Hernia 55 (22%), Lumps (48) 19%, Acute appendicitis 33 (13%), Uterine fibroids 10 (4%), Ruptured ectopic pregnancy 2 (1%) and others (Intestinal obstruction, Postoperative adhesions, Ingrown toe nails, Breast abscess, Hemorrhoids, Anal fissures) 10 (4%).

DETERMINE RAPID HIV TEST METHOD USING BLOOD: Total of 521 HIV positive samples were tested, 502 (96.35%) tested HIV positive and 19 (3.65%) tested HIV negative. These 19 HIV negatives were re-tested with Stak Pak: 19 (100%) tested HIV positive. See Table 2.

UNIGOLD HIV TEST METHOD USING BLOOD: Total of 521 HIV positive samples were tested, 521 (100%) tested HIV positive. See Table 3.

URINE TESTING METHOD: Total of 251 (48.18%) of the 521 HIV positive patients were scheduled to undergo a surgical procedure. These were re-tested using the Urine testing method, 235 (93.63%) tested HIV positive while 16 (6.37%) tested negative. See Table 4. The blood sample of the 16 who tested negative using Urine testing method were subjected to confirmatory test using Stat Pak and all 16 (100%) tested HIV positive.

The specificity for Unigold and Determine blood testing were 100%. All three tests had a Positive Predictive Value (PPV) of 100% while the Negative Predictive Values (NPV) was 100% and 99.73% for Unigold and Determine respectively. See Table 5.

Table 1. Treatment naïve participants in the study.

AGE GROUP	15 - 24	25 - 34	35 - 44	45 - 54	55 - 64	65 - 79	80+	TOTAL
MALE HIV+	9 (1.73%)	30 (5.76%)	24 (4.61%)	24 (4.61%)	12 (2.30%)	3 (0.58%)	3 (0.58%)	105
FEMALE HIV+	54 (10.36%)	158 (30.32%)	105 (20.15%)	84 (16.12%)	12 (2.30%)	0	3 (0.58%)	416
TOTAL	63	188	129	108	24	3	6	521

Table 2. Results obtained for Determine blood test assay.

Test outcome	HIV present	HIV absent	Total
Positive	502	0	521
Negative	19	7047	7066
Total	521	7047	7568

Test sensitivity = 96.35%; Test Specificity = 100%; Net sensitivity = 96.35%; Discordant result = 3.65%.

Table 3. Results obtained for Unigold blood test assay.

Test outcome	HIV present	HIV absent	Total
Positive	521	0	521
Negative	0	7047	7047
Total	521	7047	7568

Test sensitivity = 100%; Test Specificity = 100%; Net sensitivity = 100%; Discordant result = 0%.

Table 4. Results obtained for Urinetest assay.

Test outcome	HIV present	HIV absent	Total
Positive	235	0	235
Negative	16	0	16
Total	251	0	251

Test Sensitivity = 93.63%; Test specificity = 100%; Net Sensitivity = 93.63%; Discordant result = 6.37%.

Table 5. Comparison between Urine testing and Unigold and Determine blood test.

Test type	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
Unigold kit	100%	100%
Determine kit	100%	99.73%
Urine enzyme linked immunoassay	100%	Nill

4. Discussion

The performance of the test kits in detecting HIV antibody in both blood and urine was good. Sensitivity; the ability of a test to identify correctly participants with a HIV infection was 100%, 96.35% and 93.63% for Unigold, Determine and Urine sample testing respectively. Although Unigold had a higher sensitivity compared to Determine test strip and Colloidal gold enhanced rapid immuno-chromatographic assay (Alliance Biomedical) kit for Urine HIV testing, the

sensitivity of the two test kits shows that any of these tests can be used to screen patients especially because the specificity (ability of the test to identify correctly participants who do not have HIV/AIDS) was 100%.

This agrees with a study done by Connell *et al.* where they reported a 100% and 99.4% sensitivity comparing two different test kits for accuracy in detecting HIV antibody in the urine [10]. Urine HIV testing was also used for self-HIV testing among men who have sex with men (MSM) in China, where they reported a high sensitivity [18]. This study retreat the importance of urine as an alternative body fluid for HIV screening especially in vulnerable population that are difficult to reach.

Unigold had a Negative Predictive Value (NPV) of 100% while Determine was a little lower. The Net Sensitivity comparing the Urine HIV testing with the Unigold blood testing was 93.63% which compares well as a screening tool. A comparative analysis study done in India to evaluate the usefulness of enzyme immunoassay (EIA) for anti-HIV antibodies in urine found that the efficiency, sensitivity and specificity of the urine-based test strips compare excellently with blood-based HIV screening [19].

In this era of emerging and re-emerging infectious diseases, researches to look into the use of other non-invasive body fluids cannot be over emphasized. Urine can be easily collected by the patients themselves reducing the risk of blood borne diseases.

Stigma is still a problem among HIV infected patients and has remained an obstacle to testing among high risk individuals. The urine method is simple, non-invasive, and test strips can be stored in normal refrigerator for up to three 3 hours. It can be conveniently self-administered without risk to others and without stigmatization. The patients can then schedule appointment with the doctor based on a known diagnosis at a private time.

5. Limitations

The limitation of the use of urine testing is the fact that urine is prone to dilution and the timing of sample collection must be factored in its assay. Also, urine sample constituents begin to change with poor storage which may play a role in result obtained. However, urine sample can be a good alternative to invasive blood testing and can rapidly be done for unconscious patients and patients already catheterized prior surgery, delivery and other medical states.

6. Conclusion

The use of Urine HIV testing compared well to the blood HIV testing and could be a good non-invasive method for screening of HIV/AIDS in the population. Also, with emerging blood borne diseases, this may be the way forward.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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