

Offer of rapid testing and alternative biological samples as practical tools to implement HIV screening programs

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SUMMARY

Implementation of HIV testing has the objective to increase screening, identify and counsel persons with infection, link them to clinical services and reduce transmission. Rapid tests and/or alternative biological samples (like oral fluid) give the option for a better general consent in approaching screening, immediate referral of HIV positives to medical treatment and partner notification. We tested the performance characteristics of an oral fluid-based rapid HIV test (Rapidtest HIV[®] lateral flow-Healthchem diag. LLC) in comparison with routinely utilized methods in a selected population of known positive (N=121) or negative (N=754) subjects. The sensitivity of the rapid test was 99.1% (one false negative sample) and the specificity 98.8%. Five negatives showed a faint reactivity, 3 of these were reactive also in the reference test, one with a p24 only reaction in Western blot. If these 3 samples were excluded from the analysis the specificity increases to 99.2%. Results from our study confirm that, although a continuous improvement of the test performance is still needed to minimize false negative and positive results, rapid test and alternative biological samples may contribute to HIV prevention strategies by reaching a larger population particularly when and where regular screening procedures are difficult to obtain.

KEY WORDS: Dentin/enamel adhesives, MDPB, MDP, Antibacterial activity, Direct contact test, Agar diffusion test

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INTRODUCTION

Worldwide, human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) remain leading causes of illness and death (Uniyal & UNAIDS, 2008). The overall number of people living with HIV has increased as a result of new infections and a reduced mortality rate due to the beneficial effects of more widely available antiretroviral therapy.

Sub-Saharan Africa remains most heavily affected by HIV, accounting for 67% of all people living with HIV and for 72% of AIDS deaths in 2007. The global epidemic is stabilizing but at an unacceptably high level. Globally, there were an estimated 33 million (30-36 million) people living with HIV in 2007. The annual number of new HIV infections declined from 3.0 million (2.6-3.5 million) in 2001 to 2.7 million (2.2-3.2 million) in 2007 (Cohen *et al.*, 2008). The rate of new HIV infections has fallen in several countries, although globally these favorable trends are at least partially offset by increases in new infections in other countries.

An estimated 370.000 (330-410.000) children under the age of 15 became infected with HIV in 2007. The annual number of new HIV infections

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among children worldwide has declined since 2002, as initiatives to prevent mother-to-child transmission (pMTCT) have expanded.

As reported recently in the Morbidity and Mortality Weekly Report (Branson *et al.*, 2006a), the objectives of CDC recommendations for HIV testing are to increase HIV screening of patients, including pregnant women, in health-care settings, foster earlier detection of HIV infection, to identify and counsel persons with unrecognized HIV infection and refer them to clinical and preventive services, and as to further reduce perinatal transmission of HIV (Cohen *et al.*, 2008).

These revised CDC recommendations advocate routine voluntary HIV screening as a normal part of medical practice, similar to screening for other treatable conditions. In theory, new sexual HIV infections could be reduced by >30% per year if all infected persons could know their HIV status. CDC has also stated that the patient's right to refuse testing be preserved in order to facilitate a good working relationship between patient and doctor. HIV screening is recommended for patients in all health-care settings after the patient is notified that testing will be performed unless the patient declines (opt-out screening) (Bartlett *et al.*, 2008); persons at high risk for HIV infection should be screened for HIV at least annually; separate written consent for HIV testing should not be required; general consent for medical care should be considered sufficient and imply consent for HIV testing. Prevention counseling should not be required with HIV diagnostic testing or as part of HIV screening programs in health-care settings. HIV screening should be included in the routine panel of prenatal screening tests for all pregnant women (Branson *et al.*, 2006b).

In many developing world countries the equipment and staff to run these sophisticated tests are limited, and as a result infected blood is still being used for transfusions and many people remain undiagnosed. Therefore, there is a great need for a simple, manual, visual and robust test that can be used to quickly screen blood or alternative biological samples for HIV antibodies (Armstrong & Taeye, 2007) but due to foreseen difficulties in making the classic screening for HIV in blood products (ELISA) and due to the requirement of venipuncture as well as its cost, routine in developing countries is difficult to en-

visage thereby much hope is placed on rapid tests in these settings (Centers for Disease Control and Prevention (CDC), 2007a, Centers for Disease Control and Prevention (CDC), 2007b).

In the past few years new saliva-based tests for HIV antibodies have been developed (Debattista *et al.*, 2007, Delaney *et al.*, 2006, Hamers *et al.*, 2008, Hamers *et al.*, 2008). The Health-Chem Diagnostics RAPID HIV[®] SCREEN- LATERAL FLOW is a membrane-based solid phase assay, already commercially available in the United States after having been evaluated by the "Program for Appropriate Technology in Health" (WHO Collaborating Center on AIDS), is one such example of a rapid saliva-based test. These studies were conducted in different geographic regions, over a two year period: USA, India, Sub-Saharan Africa, Southeast Asia, Mexico (Delaney *et al.*, 2006).

The sensitivity and the specificity of RAPIDTEST HIV[®] SCREEN Lateral Flow test were found to be 100% relative to Western Blot and EIA assay. Notwithstanding this type of test is still subject to many controversies and concerns expressed by the European health authorities.

Saliva is a watery, viscous fluid composed of secretions of the parotid, submandibular, sublingual and other minor salivary glands. Saliva a serous transudate containing a complex mixture of organic and inorganic materials that fall into the following categories: electrolytes, enzymes, low molecular weight compounds, hormones, proteins (antibodies), vitamins. Thus saliva contains compounds that are representative of those contained in serum and plasma. This specimen is appropriate for use without further treatment such as centrifugation and filtration.

A recent study of the Division of infectious diseases at the McGill University Health Centre-Canada suggested the saliva test is a much better tool in preventing mother-to-child transmission than the blood test particularly for women entering labor with unknown serostatus and should become a standard procedure in India as well as in South Africa and China, where pediatric AIDS is a threat (Pai *et al.*, 2008). They demonstrated that even in a rural, resource-constrained setting, if you employ counselors and administer an HIV test, you could reduce transmissions. Testing pregnant women for HIV at the time of labor and delivery is the last opportunity for prevention of

mother-to-child HIV transmission (pMTCT) measures, particularly in settings where women do not receive adequate antenatal care.

A further advantage of the saliva test is that it does not have to be carried out in specialized laboratory facilities.

The RAPID TEST HIV[®] Lateral flow test is intended only as an initial screening test and reactive samples must be confirmed by a supplemental conventional assay such as the ELISA and Western Blot test. Rapid HIV tests can play an important role in HIV prevention activities and expand access to testing in both clinical and non-clinical settings. They can help overcome some of the barriers to early diagnosis and improve referral of care for infected persons.

Our study objectives were to evaluate the sensitivity and specificity and hence the applicability of the method in epidemiological population studies or as an adjunct in the control of the spread of HIV infection in low resource settings and, secondly, to determine the appealing of the rapid and non-invasive testing for a general population. This paper will review the operating and performance characteristics, quality assurance (QA) and laboratory requirements for commercially available rapid HIV tests, and their counseling implications.

METHODS

Ethical committee

Our study was conducted between April 2006 and December 2008 at the Department of Infectious Diseases at the San Raffaele Hospital in Milan, Italy. The Ethics Committee of the San Raffaele Hospital in Milan approved the study.

An informed consent form was prepared and explained the details of the study, the HIV testing procedure and interventions available for the patients. This information was also orally communicated (pre-test counseling) to patients by the personnel performing the test.

Criteria for recruitment

Subjects were informed that they could refuse testing at any time. Anyone could be included in the protocol, as long as they were over the age of 18 years and with known HIV serostatus (less than three months testing for HIV negatives). To

all those with unknown HIV serostatus, a regular screening (III generation ELISA and eventual Western blot confirmation of positive or dubious results) was offered for free.

Post-test counseling was provided to all HIV reactive subjects by the Infectious Diseases Department physicians involved in the study.

Samples

To assess the sensitivity and specificity of the test a total of 875 samples were evaluated. One hundred and twenty-one (121) known HIV positive and 754 HIV negative subjects were invited to participate in the experimental study, respectively between the out and in patients attending the Infectious Diseases Department of San Raffaele Hospital in Milan and health care workers and healthy volunteers.

Oral fluid sampling

Sample collection is obtained by a specifically designed device (Sani-Sal[®] saliva collection device) composed by a foamy material able to absorb mucopolysaccharides present in saliva, as well as food particles, cells, cell fragments and microorganisms while not absorbing analytes of interest such as drugs, hormones and proteins (antibodies), which are released together with the fluid component of saliva.

After sample collection by placing the collection device between cheek and gum (2-4 min), the device is assembled into a flexible plastic tube holder. Squeezing the flexible tube extracts the sample, which may be added directly into the test slide hole.

HIV testing

RAPIDTEST HIV[®] Lateral flow (HealthChem Diag, LLC): the test is a membrane-based solid phase assay that contains antigens that capture the antibodies from a plasma, serum or saliva sample on a membrane.

The antigens used consist of specific recombinant peptides corresponding to highly immunoreactive core (N=3) and surface (N=5) regions of HIV-1 and HIV-2. The antigens are absorbed on a nitrocellulose membrane and can capture any HIV antibody present in the sample. An internal reaction control is present on the membrane to assess the test validity regardless of the test result. This control band is the result

of colloidal gold conjugate binding to an anti-HIV antibody immobilized on the membrane. A pink control band in the control region appears at the end of the test and indicates that the colloidal gold conjugate is functional. The absence of this band renders the test invalid.

A positive sample thus produces two distinct red lines (test line plus control line) on the membrane while a negative sample produces only one line (control). Saliva samples are collected by the specific collection device (SaniSal®) and recovered from the device both by squeezing the tube or centrifuging it.

The assay starts with the addition in the sample well of one saliva drop (about 25 µl) followed by two Sample Diluent drops which immediately start to flow through the membrane. The HIV colloidal gold-antigen conjugate embedded in the sample pad reacts with the HIV antibody present in the saliva forming the conjugate/antibody complex which is captured by an antibody-binding protein A immobilized on a membrane

Reference tests

All the study participants with unknown serostatus (N=262) were submitted to HIV screening in blood samples by the AxSYM HIV Ag/Ab Combo Assay (ABBOTT diag. S.r.L). All the reactive and discordant results were confirmed by the HIV BLOT 2.2 (Alfa Wassermann diagnostics) following the manufacturer's instructions.

RESULTS

Test validation

A few problems due to insufficient wetting of the sponge and insufficient or null sample recovered from the device were encountered at the beginning of the study, particularly in HIV positive sub-

jects, who can suffer from drug-induced xerostomy. Keeping the collection device in the oral cavity a few minutes longer (at least 5 min) until the foam was fully expanded overcame this problem. Of the 875 samples collected (at the Department of Infectious Diseases in Milan) 15 (6 HIV positive and 9 HIV negative subjects) were excluded from the analysis because no band was visible at the control reaction site. The total number of valuable samples then include 860 samples of which 115 positive and 745 negative.

Samples results

Of the 745 HIV negative patients, 737 equal to 98.92% were negative at the saliva test, while eight (1.07%) showed a faint reactivity at the reaction line. In three out of eight of these low reactive samples, the corresponding serum showed a border-line reaction at the reference screening test, and one of these sera also showed a p24 only reactivity at Western blot confirmatory test. In one HIV negative saliva sample (0.13%) a light but clearly visible reaction was observed, thus was scored as false positive.

Of the 115 HIV positive subjects tested, 108 (93.9%) scored clearly positive, 6 (5.21%) showed a light reactivity both at the reaction and control line, while one (0.86%) was negative at the reaction band, thus was scored as false negative (Tab. 1).

All the discordant or light reactions were repeated at least three times, with the same saliva sample, maintaining an identical result.

The sensitivity and specificity of the test was calculated considering as positive all the dubious or faint reactions. The sensitivity thus was 99.1% and the specificity was 98.8%. If the 3 samples that resulted borderline also at the screening test are excluded from the evaluation, the specificity increases to 99.2%.

TABLE 1 - Summary of results.

HIV Status	N°	Rapidtest HIV® Lateral flow			AxSYM HIV Ag/Ab Combo	
		Invalid	Neg. (%)*	Reac. (%)*	Neg (%)	React (%)
Negative	754	9	737 (98.9)	8 (1.07)	751 (99.6)	3 (0.4)
Positive	121	6	1 (0.8)	114 (99.1)	0	121 (100)

*Percentages are calculated on evaluable samples.

The positive predictive value (PPV) is 92.7 (IC 95% 88.1-97.3) if all samples are considered and 95.0 (IC 95% 91.1-98.9) if the 3 borderline samples are excluded.

The negative predictive value (NPV) is 99.9 (IC 95% 99.6-100.1).

DISCUSSION

The US Centers for Disease Control and Prevention has revised its recommendations for screening for human immunodeficiency virus (HIV) (Armstrong and Taeye, 2007); (Branson *et al.*, 2006a) and now recommends HIV screening for all patients aged between 13 and 64 years in all health care settings, including hospital emergency departments, urgent care clinics, inpatient services, sexually transmitted disease clinics, tuberculosis clinics, and primary care services. The objectives of these recommendations are to increase HIV screening of patients, including pregnant women, in health care settings, foster earlier detection of HIV infection, identify and counsel persons with unrecognized HIV infection and link them to clinical and prevention services, and further reduce perinatal transmission of HIV in the United States. These revised recommendations update the previous ones for HIV testing in health care settings and for screening of pregnant women (Centers for Disease Control and Prevention (CDC), 2007c).

To increase the proportion of persons aware of their HIV serostatus, CDC launched the Advancing HIV Prevention Initiative with the objective to implement new models for diagnosing HIV infections outside medical settings. From 2004 to 2006, CDC funded a demonstration project to provide rapid HIV testing and referral to medical care, targeted to racial/ethnic minority populations and others at high risk in outreach and other community settings. Out of 23,900 subjects who received a rapid HIV test a total of 267 (1%) persons had a confirmed HIV-positive test result demonstrating that rapid HIV testing in outreach and other community settings can identify large numbers of persons at high risk who are unaware that they are infected with HIV.

A further option to increase the general consent in approaching screening activities could be the use of biological samples alternative to blood, as

for example oral fluid, which are more appreciated by persons reluctant to undergo venipuncture or with poor venous access like drug addicts, with a fast response rate and lower costs.

In this study we obtained similar results to those obtained in other similar evaluations (Debattista *et al.*, 2007, Delaney *et al.*, 2006).

The RAPIDTEST HIV[®] Lateral flow, despite the relatively low number of samples tested, showed sensitivity and specificity rates which fall in the expected range for a screening test also when compared to those of regular EIA testing.

In our experience some samples may show low level reactivity both in HIV positive cases, leading to dubious interpretation, and HIV negative (false positive) samples, and this phenomenon seems lot-dependent or due to interfering molecules in the sample. Being a screening oriented test, a reasonable number of results suggesting the need for a confirmatory step is however expected.

The single false negative result that we obtained in our series, although alarming, should be seen in the more general context of the global benefit that a population-based screening reaching a greater amount of population offers.

It should be taken into consideration the fact that a large number of HIV-infected subjects are unaware of their serostatus, with costly consequences in terms of late diagnosis (often at the onset of opportunistic diseases) and secondary transmission increase, because they either did not have access to screening or because they did not return for their test results. The possibility of rapid and more accessible testing methods gives the option of immediate counseling and referral to medical treatment as well as partner notification, with an optimal result in terms of control of infection spread and health cost containment. Globally, the number of children younger than 15 years living with HIV increased from 1.6 million (1.4-2.1) in 2001 to 2.0 million (1.9-2.3) in 2007. Almost 90% live in Sub-Saharan Africa. Since 2003, the rate of annual AIDS deaths among children has also begun to fall due to treatment scale-up and pMTCT.

Globally, half of the 33 million HIV-infected people are women and this percentage has remained stable for several years. The vast majority (60%) of new HIV infections occur in women of reproductive age and in infants and children. With only 9% of pregnant women receiving antiretroviral

therapy (ART), an urgent scaling up of HIV prevention efforts is needed to avert a pediatric HIV epidemic. Controlling HIV infection in women and children is crucial for changing the trajectory of the global HIV epidemic.

Reports from 147 countries on national progress in implementing the 2001 Declaration of Commitment on HIV/AIDS provide the most comprehensive global assessment ever undertaken of the HIV response.

In 2003, approximately 25% of the one million HIV positive persons living in the USA turned out to be unaware of their infection. However, that percentage might have been greater among persons at high risk for HIV infection, including racial/ethnic minority populations.

By the end of 2006 four rapid tests on blood had been approved for use by the Food and Drug Administration (FDA). The Rapid HIV test makes it possible for the patient to obtain pre-test and post-test counseling, their test results, and any medical referrals they may need, all in one visit. Moreover, CDC stresses the importance of reducing barriers to diagnose HIV infection early and increase access to treatment and prevention services (Branson *et al.*, 2006a).

Efforts for the continuous improvement of the rapid test performance are desirable together with the promotion and expansion of HIV screening programs.

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