



EVALUATION OF A RAPID TEST FOR HIV ANTIBODIES IN SALIVA AND BLOOD

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Objective. To test whole blood and saliva for HIV antibodies (anti-HIV) using a rapid test strip capillary flow immunoassay, and to correlate the test strip results with blood specimen results obtained from routine diagnostic anti-HIV assays.

Design. A prospective pilot study of selected HIV-positive and HIV-negative individuals, children and medico-legal cases from Gauteng, South Africa.

Methods. Whole blood specimens taken from every individual and medico-legal case (total study population 153) and saliva specimens taken from 76 selected cases were tested for anti-HIV using the respective Hema-Strip HIV-1/2, Sero-Strip HIV-1/2 and Saliva-Strip HIV-1/2 (Saliva Diagnostic Systems Inc.) rapid test strip methodology. All results were correlated with the currently recommended anti-HIV assays.

Results. The whole blood test strip results correlated 100% with the traditional diagnostic results. Only two saliva test strip results tested false-negative, both from marasmic and severely dehydrated babies, while the other results were in concordance. All test strip results on postmortem blood and saliva were fully concordant with the diagnostic assay results.

Conclusion. The anti-HIV test strip methodology for whole blood and saliva specimens is rapid, reliable and easy to perform and interpret. Saliva specimens can be readily collected from any individual, and there is a reduction in hazard risk. Anti-HIV saliva testing using the test strip methodology is recommended for South Africa, particularly in high-risk situations such as the paediatric and forensic medicine settings. A larger field study obtaining specimens from different regions in South Africa is advised.

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Worldwide, there is a critical need to test rapidly and easily for infectious diseases, especially in the global effort against the human immunodeficiency virus (HIV).^{1,2} Appropriate ways to conduct epidemiological surveys and to diagnose HIV need to be found in countries where traditional laboratory infrastructure may not be optimal and where clinical laboratory services are unreliable.^{3,4}

South Africa is currently experiencing a fast-growing HIV and AIDS epidemic; an estimated 3 million inhabitants were infected in 1997 and an estimated 1 500 new infections occur daily.^{5,6} The implications of this epidemic include the rising costs and logistics associated with laboratory analysis of traditional diagnostic methods for diagnosing HIV, which often involve the taking, handling, transport and storage of invasively obtained specimens as well as the availability of equipment, electricity and refrigeration. Specimen analysis in the field surroundings as well as using non-skilled personnel to perform surveys and diagnosis is an attractive option for many developing countries, including South Africa.

A number of studies on the detection of anti-HIV in saliva have shown its many advantages over the traditionally accepted blood specimen as it is non-invasive, more acceptable and easier for the patient, convenient, economical and less hazardous.⁷⁻⁹ However, conflicting reports on the sensitivity of saliva-based anti-HIV assays, probably related to the method of specimen collection and test methodology used to detect the low levels of antibodies, have given rise to caution when advocating use of saliva as a diagnostic fluid.^{8,9}

Many different types of successful rapid tests to detect anti-HIV are available worldwide.^{10,11} A study reporting a test strip methodology for detecting anti-HIV in plasma and serum not requiring refrigeration, further reagents or sophisticated laboratory equipment, appears to be an attractive option for South Africa.¹² The performance of the test strips was found to be comparable to that of the traditional diagnostic anti-HIV assays.¹² The manufacturers of this rapid test strip assay have also designed a similar saliva-based anti-HIV test which incorporates a specially designed saliva capture device.^{12,13}

In this pilot study we tested blood and saliva specimens from selected HIV-positive and negative individuals from the Gauteng region of South Africa for anti-HIV using the rapid test strip methodologies. The test strip results were compared with the blood specimen results tested on the routine diagnostic anti-HIV assays.

MATERIALS AND METHODS

Study population and specimens

A study population of 153 individuals was selected, of whom 69 were HIV-positive and 84 HIV-negative. The attributes of the individuals are depicted in Table I and the study population

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Table I. Attributes of study subjects

Age (yrs)		
Mean	29.3	
SD	14.9	
Minimum	0.4	
Maximum	78	
N	152*	
Gender (N)		
Female	82	(53.6%)
Male	71	(46.4%)
Ethnicity (N)		
Black	105	(68.8%)
Coloured	1	(0.7%)
White	47	(30.7%)

* Age for 1 individual not known.

Table II. Study population by underlying illness/condition

Illness/condition	Number	Ex postmortem
Healthy	24	
Pregnant	13	
HIV-positive	69	
Trauma	34	34
Needle-stick exposure	3	
Tuberculosis	3	1
Emphysema	1	1
Heart failure	1	
Pneumonia	1	
Respiratory distress	1	
Unknown	3	3
Total	153	39

defined by the underlying illness or condition is illustrated in Table II.

Sixty-six of the HIV-positive patients were selected from the hospital setting. Twenty-three HIV-negative specimens were selected from individuals in the community and 23 from hospital patients. Forty-one postmortem specimens were taken by a forensic pathologist at the Medicolegal Laboratory in Pretoria. All the medico-legal cases had been stored at 4°C for at least 48 hours and two bodies had been refrigerated for longer than 5 days. Ten saliva and blood specimens from 10 bodies and 31 blood specimens from 31 other bodies were collected. Three of the forensic cases were HIV-positive. Fourteen paediatric patients were also included in the study population and saliva and blood were collected from 10 children and blood alone from 4 children. Every blood specimen was tested using the routine diagnostic anti-HIV serological tests. All specimens were tested immediately upon receipt at the laboratory and thereafter stored at -20°C for later follow-up.

The patient identity of each specimen remained strictly anonymous and only results requested and tested using the routine diagnostic anti-HIV serological tests were made known to the patient. Participation in this study was voluntary, informed consent was signed, and ethical approval was obtained from the Pretoria Academic Hospital's Ethical Committee. All specimens obtained from the forensic pathologist were coded and tested blindly in order to ensure that the identity of each case remained unknown.

Anti-HIV tests

Routine diagnostic anti-HIV serological tests

The blood specimens were screened for anti-HIV using an enzyme immunoassay (EIA) which detected both HIV-1 and HIV-2 (Murex EIA HIV-1/2, Murex Diagnostics Ltd, Dartford, UK). Positive results were confirmed on two additional anti-HIV EIA test kits (Genelavia Mixt R, Sanofi Diagnostics Pasteur, France, and the Wellcozyme HIV-1 Recombinant EIA, Murex Diagnostics Ltd).¹⁴

Rapid test strip methodology

The Hema-Strip HIV-1/2 for whole blood specimens, the Sero-Strip HIV-1/2 for serum or plasma specimens and the Saliva-Strip HIV-1/2 for saliva specimens were tested for anti-HIV. The test strips were supplied by the Saliva Diagnostic Systems company (Saliva Diagnostic Systems Inc., Vancouver). The test strips are available in kits and all contain the respective test strips and a buffer solution.

The Hema-Strip kit contains a lancet and absorbent pad for specimen collection and the test strip is already placed within a sampler. The sampler, which has a small tip, is placed into the blood drop on the finger or heel of the patient and kept there until full. This tip is then firmly pressed through the foil cover of the buffer vial and timing is commenced for 15 minutes. After 15 minutes, results are interpreted by reading the lines that appear on the test strip. A control line should appear at the top of the strip, which indicates test validity. A test line should appear closer to the bottom of the strip, which indicates presence of the HIV antibodies.

A specimen transfer loop is added to the Sero-Strip kit. This plastic loop is dipped into the serum or plasma and stirred into a test tube containing the buffer solution. Thereafter a test strip is added to the test tube and results are interpreted according to the presence of the control and test lines after 15 minutes.

The Saliva-Strip kit also contains a saliva collector, a fluid filter and a plastic disposable pipette. The saliva collector consists of a plastic stalk with an indicator and an absorbent pad which can easily be torn loose. The collector pad is placed under the tongue of the subject, whose mouth is closed for the 1 - 6 minutes of collection time. When the indicator on the stalk turns from white to blue, the device can be removed. This absorbent pad is easily detached from the stalk and placed



inside the buffer tube. The buffer tube should be transported or taken to the place of testing in an upright position to prevent the dye of the indicator from discolouring the fluids. Should this happen, the test would be deemed invalid and a second specimen should be requested. Saliva is mixed thoroughly with the buffer fluid by pressing out all fluid in the pad using the fluid filter. The contents are then pipetted and transferred to a test tube and the test strip is added. After 20 minutes, the results are interpreted according to the presence of the control and test lines. Clear plastic or glass test tubes are required but not provided in the kit.

For each test strip, a negative result is indicated by a non-reactive test line and a reactive control line, a positive result by a reactive test line and a reactive control line, and an invalid test by a non-reactive control line.

The immunochromatographic test strip method is a capillary flow assay, and buffer-diluted specimen moves up the strip by this capillary action. The control line consisting of protein A becomes visible when the conjugate is immobilised at this line due to complex formation with immunoglobulin G (IgG) present in the specimen. The conjugate will only stop at the test line area on the strip if the specimen contains HIV-specific IgG which reacts with the impregnated target antigens.¹² The target antigens are synthetic peptides, gp41 and gp120 for HIV-1 and gp36 for HIV-2.¹²

All the tests, rapid and diagnostic, were performed in the routine diagnostic laboratory of the Department of Medical Virology under strictly controlled conditions, and were carried out strictly according to the manufacturer's instructions and specifications. All the control and test lines on each individual test strip were read and interpreted by the same person throughout the pilot study. This person graded the test line intensity as 4+ indicating a strongly positive reaction, and 2+ as a positive yet fainter reaction than the 4+ band intensity.

RESULTS

All the test strip results were compared with blood specimen results obtained using the routine diagnostic anti-HIV serological tests, and are illustrated in Table III.

Hema-Strip and Sero-Strip results were in full concordance with the blood results from the routine diagnostic serological tests. Five serum specimens were bilirubinaemic, 2 were lipaemic and 7 sera were severely haemolysed, but none of these conditions interfered with the performance of the Hema- and Sero-Strips. All postmortem blood specimens, including those from cases refrigerated for longer than 5 days, gave valid results on the test strips concurring with the diagnostic serological results.

Results obtained with the Saliva-Strips, except two, correlated with those from the diagnostic serological tests. One

Table III. Comparison of strip test results and routine test results (reference)

Category				Number*
Sero-Strip	Hema-Strip	Saliva-Strip	Routine	
4+	4+	4+	+	3
neg	neg	neg	neg	20
Total				23
4+	4+		+	37
4+	2+		+	5
2+	2+		+	1
neg	neg		neg	57
Total				100
		4+	+	23
		2+	+	4
		1+	+	6
		neg	+	2
		neg	neg	41
Total				76

* Each comparison includes cases also counted in other comparisons.

saliva specimen from a 4-month-old marasmic and severely dehydrated baby tested false-negative on the Saliva-Strip assay. Another saliva specimen from another severely dehydrated and severely ill baby, undernourished and diagnosed with bilateral otitis media, also tested false-negative on the Saliva-Strip. These 2 saliva specimens were collected according to the assay specifications and the indicator on the saliva collection devices turned blue for both specimens. All saliva specimens from postmortem material were valid on the test strips and correlated. Four medico-legal cases had extensive head and neck trauma and the saliva was collected from the pharyngeal regions and crevices of the remaining oral cavity. The indicators on all the saliva collection devices turned blue during collection from the postmortem material and the maximum collection time was only 3 minutes. Although rigor mortis was present in each body, the collection device could be inserted easily and the indicator readily observed for the colour change.

Fourteen children, with ages ranging from 5 weeks to 4 years (average age 16 months), were included in the study cohort. Ten children were known HIV-infected patients and 2 children, aged 19 months and 2 years respectively, born to HIV-positive mothers, remained uninfected. Two babies were very ill, one was severely undernourished, and the remainder were clinically well.

The 16 test strip assays, 4 Sero-Strips and 12 Saliva-Strips, repeated according to the manufacturer's instructions, gave the same results and were still in concordance with the diagnostic assays.



DISCUSSION

The Hema-Strip and Sero-Strip anti-HIV results correlated 100% with the traditional routine diagnostic assays. Blood was obtained from 4 patients deemed to be in the clinical terminal phase of HIV disease (so-called AIDS stage) and the antibody levels were still detected. Even unsatisfactory conditions of the specimen such as lipaemia, haemolysis and bilirubinaemia did not affect the assay performances. Postmortem blood, although received in most instances from refrigerated cases, was obtained from 5 bodies that arrived at the medicolegal laboratory for autopsy at least 48 hours - 5 days after death. These variations in temperature for extended periods did not interfere with the validity or the detection ability of the test strips. It would therefore appear from this small cohort of postmortem blood specimens that the Hema-Strip assay could be applied in the forensic medical setting. Although still invasive and still requiring the use of a lancet, smaller volumes of blood could be used and the collection method would be less hazardous. Two false-negative Saliva-Strip results were obtained from 2 babies, clinically considered to have advanced HIV disease, and extremely ill at the time of saliva collection. The indicator dye changed colour in spite of the dehydration status of the babies, and the levels of anti-HIV were probably below the detection level of the Saliva-Strips. The Saliva-Strip assay performed very well under the less optimal postmortem saliva specimen conditions. Saliva could be collected from all regions of the oral and pharyngeal cavities of the bodies, even though rigor mortis and refrigeration conditions were present and in spite of the lack of active saliva production. It therefore appears that saliva collection for anti-HIV could play a significant role in the medico-legal investigation of death and in hazard reduction and elimination of potential exposure of personnel to this bloodborne viral pathogen.

Saliva specimens were taken from 10 children, half of whom were older than 1 year. Staff members who assisted with this saliva collection found the procedure to be very easy and the older children to be co-operative. Young babies were inclined to chew or spit out the collection device. However, this was readily overcome by gentle persuasion and the correct handling and positioning of these babies. Parents of 3 children, present at the time of saliva collection, found this method of specimen collection much easier to cope with and readily cooperated with the staff. Saliva specimen collection in the paediatric setting appears to be a very attractive option, as it is easy and decreases the staff members' time spent taking specimens.

The test strip methodology for all three assays assessed was rapid, easy to perform and interpret and could be readily applied by non-skilled personnel. No refrigeration and minimal usage of laboratory equipment and materials were required for performing these three assays. The Hema-Strip assay, however, did involve obtaining a blood drop invasively

using a sharp instrument; this required staff training, emphasis on care and a method for disposal of the sharps. The Sero-Strip assay required no additional materials and can be performed in the field setting using the kit as provided.¹² However, a blood specimen needs to be drawn and serum or plasma is required. Centrifugation of the blood specimen will rapidly provide the serum specimen, but involves equipment usage. Alternatively, the specimens can be left overnight in the upright position and the supernatant serum can then be applied. The Saliva-Strip assay, although non-invasive and ideal for the field setting, does not provide glass or plastic test tubes, therefore this added cost would need to be taken into account. The circumstances of the field setting or laboratory infrastructure as well as the financial implications would need to be assessed thoroughly before a choice of rapid test strip anti-HIV assay can be made for field settings in South Africa.

The test strip methodology performed well in selected South African patients in this preliminary study. The data clearly indicate the need for a larger field study involving different regions in South Africa. A study of a larger number of forensic cases and postmortem material is also strongly recommended to assess thoroughly the value of anti-HIV Saliva-Strip testing under conditions peculiar to South Africa.

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References

1. Anonymous. Acquired immunodeficiency syndrome (AIDS): interim proposal for a WHO staging system for HIV infection and disease. *WHO Wkly Epidemiol Rec* 1990; **65**: 221-228.
2. Arya SC. Performance of a rapid on-site human immunodeficiency virus antibody assay in a public health setting. *J Clin Microbiol* 1996; **34**: 1600.
3. Anonymous. Recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec* 1992; **20**: 145-149.
4. Johnson AM, Parry JV, Best SJ, et al. HIV surveillance by testing saliva. *AIDS* 1988; **2**: 369-371.
5. Galloway M. Seventh national HIV survey results released. *AIDS Bulletin* 1997; **6**: 1-2.
6. Galloway MR. Results of the 8th national HIV survey of women attending antenatal clinics of the public health service in South Africa in 1997. *AIDS Bulletin* 1998; **7**: 16-17.
7. Luo N, Kasolo F, Ngwenya B, et al. Use of saliva as an alternative to serum for HIV screening in Africa. *S Afr Med J* 1995; **85**: 156-157.
8. Parry JV, Perry KR, Mortimer PP. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. *Lancet* 1987; **ii**: 72-75.
9. Gallo D, George JR, Fitchen JH, et al. Evaluation of a system using oral mucosal transudate for HIV-1 antibody screening and confirmatory testing. *JAMA* 1997; **277**: 254-258.
10. Parry JV. Simple and reliable salivary tests for HIV and hepatitis A and hepatitis B diagnosis and surveillance. *Ann N Y Acad Sci* 1993; **694**: 216-233.
11. CDC. Update: HIV counselling and testing using rapid tests — United States. *MMWR* 1998; **47**: 211-215.
12. Burgess-Cassler A, Angulo GB, Wade SE, et al. A field test for the detection of antibodies to human immunodeficiency virus types 1 and 2 in serum or plasma. *Clin Diagn Lab Immunol* 1996; **3**: 480-482.
13. Scramm W, Burgess-Cassler A, Barriga AG, et al. Rapid test for antibodies to HIV-1/2 in saliva. Presented at the XI International Conference on AIDS, Vancouver, British Columbia, 7-12 July 1996.
14. Fleming AF, Martin DJ. National strategy for serological diagnosis of HIV infection. *S Afr Med J* 1993; **83**: 685-687.

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