Use of saliva as an alternative to serum for HIV screening in Africa

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Saliva has been recommended as a safe and effective alternative to serum for enzyme-linked immunosorbent assay (ELISA) for HIV antibodies in surveillance programmes in developing countries. We evaluated the use of saliva specimens for detection of HIV antibodies using three different commercially available ELISAs. Saliva specimens from 107 patients selected at random from HIV high-risk (38), medium-risk (27) and low-risk (42) areas of the hospital were screened with the Wellcozyme HIV1+2 GACELISA VK61 (recommended for use with saliva), Wellcozyme HIV1+2 VK54/55 and Wellcozyme HIV-1 recombinant VK56/57. Of the 107 patients, 50 were positive and 57 negative for antibodies to HIV on confirmatory Western blot testing. For detection of antibodies to HIV in saliva, the Wellcozyme HIV1+2 GACELISA VK61 had a sensitivity and a specificity of 98%, the Wellcozyme HIV-1 recombinant VK56/57 a sensitivity and specificity of 96%, and the Wellcozyme HIV1+2 VK54/55 a sensitivity of 94% and a specificity of 95%. For detection of antibodies to HIV in serum, the Wellcozyme HIV-1 recombinant VK56/57 had a sensitivity and a specificity of 100%, the Wellcozyme HIV1+2 GACELISA VK61 a sensitivity and a specificity of 98%, and the Wellcozyme HIV1+2 VK54/55 a sensitivity and a specificity of 96%. This study illustrates that saliva can be used as an alternative to serum for screening for anti-HIV antibodies in African patients.

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Examination of serum by enzyme-linked immunosorbent assay (ELISA) is currently used for large-scale screening for HIV in developing countries. Various groups have used a number of commercial assays for detection of HIV antibodies in serum, and a wide range in sensitivity and specificity has been reported.[1] Western blot confirmation is used only when the two ELISAs prove indeterminate. The cost of widespread testing for HIV using serum in any developing country is prohibitive. Furthermore, obtaining a serum sample involves an invasive procedure and thus some degree of risk to paramedical personnel. Antibodies to HIV have been detected in saliva.[2] Studies from Europe, USA and Myanmar (Burma) have suggested that saliva is an effective alternative to serum for HIV antibody testing with ELISA for epidemiological surveillance.[3-9] Saliva has several advantages: it is easy to collect, samples are safer to handle, and there is no risk of needle-stick injuries. To ascertain the validity of the use of saliva in African patients, we compared three widely available commercial ELISAs for detection of HIV antibodies in saliva and serum obtained from patients at University Teaching Hospital in Lusaka.

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Patients and methods

Study population

Saliva and serum samples were collected from patients who were randomly selected from different recruitment areas of University Teaching Hospital (UTH) Lusaka. UTH is the only general hospital in Lusaka and the major site of ambulatory care for a large proportion of the city's population. Patients ill enough to require admission or specialist treatment are also referred to UTH from the peripheral clinics around Lusaka. Informed consent was obtained and, as suggested by the National AIDS Committee, pre-test counselling was offered. Ethical approval for the study was given by the research and ethics committee of UTH. Of the 130 patients interviewed, 107 agreed to take part in the study; 38 were recruited from the medical admissions ward (designated a 'HIV-high risk' area), where current HIV seroprevalence rates were over 40%, 27 were recruited from the obstetrics and gynaecology department (designated a 'HIV-medium risk' area), where HIV seroprevalence rates were between 20 and 30%, and 42 were recruited from the outpatient department (designated a 'HIV-low risk' area), where HIV seroprevalence rates were under 15%.

HIV-1 testing

Saliva samples (3 ml) were collected; the patient was asked to drool into a sterile plastic container. Antibodies to HIV in saliva and serum were tested using the manufacturer's instructions as follows:

1. Serum was tested using the Wellcozyme HIV-1 Recombinant VK56/57 (Murex Diagnostics Ltd, Dartford, UK). This ELISA is recommended and widely used for detection of antibody to HIV-1 in human serum or plasma.
2. Saliva from the same patients was tested using the Wellcozyme HIV1+2 GACELISA VK61 (Wellcome Diagnostics, Dartford, UK). This ELISA is an enhanced immunoassay for the detection of antibodies to HIV-1 and HIV-2 in saliva, urine or eluted dried blood. Using an immunoglobulin G antibody capture technique, the test detects antibodies to the envelope proteins as well as antibodies to the cross-reacting core proteins. The test is based on purified antigens to human immunoglobulin class G antibodies which are immobilised onto microwells. The conjugate is a mixture of highly purified immunodominant antigens labelled with alkaline phosphatase.

3. Serum was also tested using the Wellcozyme HIV1+2 VK54/55 (Wellcome Diagnostics Ltd, Dartford, UK). This test is recommended for detection of antibodies to HIV-1 and HIV-2 in human serum.

4. For confirmatory purposes, Western blot testing (DuPont de Nemours) was carried out on all serum samples and results were compared with those from saliva tested with the Wellcozyme HIV1+2 GACELISA.

Data recording and analysis

Data were analysed using the EPI-INFO software programmes. The sensitivities and specificities of the tests for HIV antibodies using saliva and serum were determined.

Results

Of the 107 patients, 50 were positive and 57 negative for antibodies to HIV by Western blot testing, which we used as the reference value for labelling the sample positive or negative. The sensitivity and specificity of the use of the three different commercial test kits for HIV antibody detection are shown in Table I (saliva) and Table II (sera).

Table I. Detection of HIV antibodies in saliva from 50 seropositive and 57 seronegative patients — sensitivity and specificity of three commercial test kits

	HIV result		Specificity	Sensitivity
	Negative	Positive	(%)	(%)
Wellcozyme HIV-1 recombinant VK56/57	59	48	96	96
Wellcozyme HIV1+2 GACELISA VK61	58	49	98	98
Wellcozyme HIV1+2 VK54/55	60	47	95	94

Table II. Detection of HIV antibodies in serum from 50 seropositive and 57 seronegative patients — sensitivity and specificity of three commercial test kits

	HIV result		Specificity	Sensitivity
	Negative	Positive	(%)	(%)
Wellcozyme HIV-1 recombinant VK56/57	57	50	100	100
Wellcozyme HIV1+2 GACELISA VK61	56	51	98	98
Wellcozyme HIV1+2 VK54/55	53	52	96	96

Detection of antibodies in saliva

For detection of antibodies to HIV in saliva, the Wellcozyme HIV1+2 GACELISA VK61 had a sensitivity and a specificity of 98%, the Wellcozyme HIV-1 recombinant VK56/57 a sensitivity and a specificity of 96%, and the Wellcozyme HIV1+2 VK54/55 gave a sensitivity of 94% and a specificity of 95%. The Wellcozyme GACELISA VK61 was the most sensitive and specific of the three tests for antibody detection in saliva, with only 1 false-positive and 1 false- negative result.

Detection of antibodies in serum

For detection of antibodies to HIV in serum, the Wellcozyme HIV-1 recombinant VK56/57 had a sensitivity and a specificity of 100%, the Wellcozyme HIV1+2 GACELISA VK61 a sensitivity and a specificity of 98%, and the Wellcozyme HIV1+2 VK54/55 a sensitivity and a specificity of 96%.

Discussion

HIV surveillance programmes monitor the prevalence of HIV seropositivity for purposes of control and planning of health budgets. Many African countries are using screening tests on serum for this purpose. This study illustrates that saliva can be used instead of serum for large-scale screening for HIV in Africa; it is a safer and more practical alternative, especially in poorer countries.[2-9] Saliva has many advantages over serum for use in epidemiological surveillance.[10] Collection is easier, safer and cheaper, several people can be sampled at a time, and the procedure is patient-friendly.

Several commercial test kits can be used for HIV testing of saliva, although their performance may vary considerably.[9] A review by Behets *et al.*[11] of several studies using saliva for detection of HIV antibodies reported sensitivities between 91% and 100% and specificities between 98% and 100%. Of the three ELISA-based tests we used, the Wellcozyme HIV-1 recombinant VK56/57 was the most sensitive and specific (100%) for HIV testing of serum. However, its sensitivity and specificity dropped to 96% and 95% respectively when it was applied to saliva from the same patients. The Wellcozyme HIV1+2 GACELISA VK61 test gave a consistent sensitivity and specificity of 98% when applied to both sera and saliva. These results indicate that when these tests are done on saliva samples for diagnostic purposes it is important to perform the appropriate confirmatory tests after initial screening. Furthermore, our experience in the use of saliva (N. Luo and F. Kasolo — personal observations) supports findings of other studies[9] that when ELISAs are done on the same dilutions of sera and saliva from HIV- positive patients, optical density readings for saliva are lower. It is therefore important to follow the manufacturer's instructions when performing these tests on saliva. Our study also shows that only tests recommended by the manufacturer for use with saliva should be used. We recommend that saliva be used for detection of antibodies to HIV in rapid epidemiological surveys in Africa as a cheaper and safer alternative to serum.

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