Detection of antibodies to HIV in saliva

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ABSTRACT

Background. Saliva has been recommended as an alternative non-invasive specimen for detection of antibodies to human immunodeficiency virus (HIV) because of the inherent disadvantages of using serum for such testing.

Methods. In a double-blind study, paired serum and saliva specimens were collected from 100 known HIV antibody seropositive and 100 seronegative individuals. The serum was tested in the conventional way while saliva was tested after modifying the routinely used serum enzyme-linked immunosorbent assay so as to detect antibodies to HIV from saliva.

Results. The sensitivity of saliva for HIV antibody detection using the modified test protocol was found to be 95% by GENELAVIA MIXT ELISA and 97% by DETECT-HIV ELISA, while the specificity for both was 100%. Identical results were obtained even after 7 months of storage of the saliva at 4 °C without any preservatives.

Conclusion. Saliva is a safe and cost-effective alternative to serum for HIV antibody detection for most surveillance purposes but not for diagnostic purposes.


INTRODUCTION

Infection with the human immunodeficiency viruses (HIV) is usually diagnosed by screening the serum or plasma for antibodies to HIV-1-2 (anti-HIV-2) using commercially available enzyme-linked immunosorbent assays (ELISAs). The confirmation is usually done by the Western Blot method. Most of these ELISAs are highly sensitive and specific (99.8%-100%) and using certain alternative testing algorithms, the need for Western Blot can be reduced or totally eliminated.

However, the use of serum as a specimen for testing has disadvantages. A trained phlebotomist is required to collect blood specimens using disposable gloves, syringes and needles and following appropriate universal precautions. In children, adequate amounts of blood may be difficult to obtain because of collapsed veins. There is an extremely small but definite risk of needle-stick injury. Serum separation from whole blood requires time, centrifugation, sterile test-tubes and carries the risk of splashing or cuts due to breakage of tubes in the centrifuge. It is risky to work with blood specimens due to the possible presence of several other pathogens such as hepatitis viruses and there is a problem of proper waste disposal. Further, patient compliance for blood sampling is known to be low in both adults and children. There is also the fear of lack of confidentiality or stigma associated with the disease.

Most of these disadvantages can be reduced by using a non-invasive specimen such as saliva. The collection of saliva is easier and safer, as it eliminates the risk of needle-stick injury. It is known that human salivary gland secretions contain substances which inhibit infection of lymphocytes by HIV-1 and other infectious viruses are also reported to be rare in the saliva. The problem of waste disposal is also less. The cost of testing decreases due to the non-requirement of syringes, needles, cotton swabs, spirit and centrifuge. Patient compliance for giving saliva instead of blood has been reported to be high. Frerichs et al., after conducting field trial studies in Myanmar, have recommended saliva as a safe and effective alternative to serum for anti-HIV testing with ELISA in surveillance programmes in developing countries.

Several studies have been conducted using saliva for anti-HIV screening for epidemiological and surveillance purposes, both in urban populations and in developing countries. The majority of studies from India suggest that the commonest strain of HIV is different from that in the West.

We, therefore, assessed whether saliva could be used as a reliable non-invasive specimen for detecting antibodies to HIV-1 and -2, compared to the sensitivity and specificity of conventional ELISAs using paired serum and saliva specimens, and modified the test protocol of the conventional ELISA so as to detect anti-HIV-1 and -2 from the saliva with a high sensitivity and specificity.

PATIENTS AND METHODS

This study was carried out at the Zonal AIDS Surveillance Centre, Department of Microbiology, B.Y.L. Nair Charitable Hospital, Mumbai. One hundred consecutive known HIV antibody seropositive patients attending various outpatient departments of B.Y.L. Nair Hospital for their clinical follow up were requested to donate a small amount of whole saliva along with a fresh blood sample for this study after appropriate pre-test counselling including assurances of complete confidentiality. All patients were informed that this was a research study and hence the results of the saliva test would neither be given to them nor considered for determining their antibody status. Prior to testing, informed consent was obtained from all patients. Patients who refused to give blood but only saliva and vice versa were excluded from this study.

Similarly, paired whole blood and saliva specimens were collected from 100 low-risk seronegative healthy volunteers after appropriate test counselling and informed consent.

Whole blood was collected aseptically from each participant...
early in the morning on an empty stomach, into a sterile glass test-tube using disposable gloves, syringes and hypodermic needles, following universal precautions. The blood was allowed to clot at room temperature for 2 hours, after which the tube was centrifuged and the clear supernatant serum stored in sterile screw-capped plastic disposable vials. Whole saliva was then directly collected in a sterile plastic vial from each participant early in the morning on an empty stomach by asking the person to dribble and spit into the sterile vial which was then immediately screw-capped. The outside of the vial was wiped with glutaraldehyde and transported to the laboratory. Approximately 5–7 ml of whole blood and 1–2 ml of saliva were collected from each participant.

As all testing was done on an anonymous unlinked basis, the paired serum and saliva samples were coded so that only the serum–saliva pair could be identified but could not be traced to any particular individual. Therefore, individual test results could not be given to the attending physicians.

Samples were stored at 4 °C in case of any delay in testing. However, no sample was stored for more than 72 hours.

The serum samples were tested for antibodies to HIV-1-2 by two commercially available anti-HIV-1-2 sandwich ELISAs (GENELAVIA MIXT (Sanofi Diagnostics, Pasteur, France) and DETECT-HIV (BioChem Immunosystems Inc., Canada)).

The saliva specimens were processed in two different ways. Firstly, the procedure recommended by the manufacturer for testing serum specimens was followed, but the serum specimens were replaced by saliva specimens (conventional method). Secondly, in order to standardize the kits for using saliva as a specimen, various modifications in the test protocol were carried out. These included testing different volumes of sample, diluent, conjugate and substrate, varying times and temperatures of incubation, and sequential lowering of optical densities so as to obtain an optimal sensitivity and specificity. Eliminating the diluent step and adding an equal quantity of saliva was found to be optimal and hence was used as a modified test protocol. This modified test protocol is similar to that described by King et al. In both kits, instead of the recommended diluent (80 μl with 20 μl of the specimen), 100 μl of saliva was added. This effectively concentrated the saliva specimens on the microwells in the plate itself.

The remaining procedure of the ELISA was the same. The results of all the tests were read on a Titertek ELISA reader at a wavelength of 492 nm for GENELAVIA MIXT and 450 nm for DETECT-HIV ELISA. The samples were not run in batches, but as and when they became available along with other samples routinely tested for the day, so that there was no bias in testing. All serum and saliva specimens using the conventional and the modified test protocols were tested only once. It was possible to run both the conventional and the modified protocol ELISA using serum and saliva on the same test plate.

The cut-off control for serum was as recommended by the manufacturer. In the case of saliva, using either of the procedures, the cut-off control was taken as 30% lower than that for the serum cut-off, as recommended by Major et al.

RESULTS

Sera of all the 100 known HIV antibody seropositive patients were found to be reactive in both the ELISAs. Sera of all the 100 low-risk HIV antibody seronegative volunteers were found to be non-reactive in both the ELISAs, giving a sensitivity and specificity of 100%.

Using saliva in the conventional ELISA, 90 of the 100 known HIV antibody seropositive individuals were reactive using GENELAVIA MIXT ELISA and 94 of 100 with DETECT-HIV ELISA, and all 100 of 100 antibody seronegative volunteers were found to be non-reactive by both the ELISAs.

Using the modified protocol for ELISA, 95 of 100 HIV antibody seropositive individuals were found to be reactive by GENELAVIA MIXT ELISA and 97 of 100 by DETECT-HIV ELISA, where all 100 of 100 HIV antibody seronegative individuals were non-reactive by both the assays. Therefore, the sensitivity of the modified ELISA for saliva using GENELAVIA MIXT ELISA was 95% and DETECT-HIV ELISA was 97%.

Twenty-four serum samples of 100 known anti-HIV seropositive persons were also tested by anti-HIV-1 Western blot (Cambridge Biotech HIV-1 Western Blot) according to the procedure described by the manufacturer. Those samples found to be reactive in the Western Blot showing all the bands were taken as gold standards. Only four saliva samples which were strongly positive in the modified assay could be tested by anti-HIV-1 Western Blot. All the four showed reactivities to gp41 and gp120 only in the Western Blot. Further, these bands were distinct but faint.

DISCUSSION

We modified the test protocols of two commercial ELISAs primarily designed for serum or plasma to give a good sensitivity (97%) and specificity (100%) for their use with saliva. The salivary glands have a local immunological system, including the production of secretory IgA, which constitutes 87% of the immunoglobulins in pure saliva. However, the predominant immunoglobulin in whole saliva is IgG. As saliva was expected to contain a lesser amount of IgG compared to serum, it was concentrated in the modified test protocol. Further, Shoeman et al. found that the substitution of an (anti-IgA, anti-IgM) enzyme conjugate for the conventional anti-IgG enzyme conjugate allowed the detection of antibodies to HIV in 93% of seropositive patients.

Most studies that have used oral fluids as a non-invasive specimen have modified the routinely used anti-HIV ELISAs designed primarily for use with serum or plasma. The earlier studies had indicated low sensitivities, but procedural modifications resulted in increased sensitivities (95%–100%) and specificities (98%–100%) of such assays for oral fluids. Frerichs et al. summarized the results involving 38 different ELISAs. They found that more than half of these yielded sensitivities between 98% and 100% and 38 of the 38 yields specificities between 99.5% and 100%.

There have been concerns about the degradation of immunoglobulins in saliva by proteolytic activity, if whole saliva is used without stabilizers. However, we found that storing saliva without any stabilizers at 4 °C for up to 72 hours before carrying out the assay did not seem to affect the test results. Further, when the above experiment was repeated by us on all the same samples preserved at 4 °C after a period of 7 months, we obtained identical results. This indicates that there is no loss in the level of immunoglobulins in saliva if stored properly at 4 °C. Cheingsong-Popov et al. have also reported that oral mucosal transudate specimens can be kept for many weeks at room temperature without losing antibody reactivity.

Concerns have been voiced about the transmission of Mycobacterium tuberculosis and other infectious agents through saliva. We have not looked at this aspect. We have used whole saliva collected by dribbling without the use of any stimulators so as to decrease the overall cost of testing. We obtained good results without the use of any collection devices. Other researchers have also reported good results by using unstimulated uncentrifuged saliva.
whole saliva. World Health Organization mentions that a single ELISA may be adequate for surveillance purposes. The surveillance tests measure the observed rather than the true prevalence of HIV. Therefore, if the specificity of the test is high, then the observed values will be very close to the true values.

We found a 100% specificity using saliva specimens. Frerichs et al. have shown that oral fluids for anti-HIV screening are an ideal alternative to serum for surveillance purposes, because of the high specificity. As both ELISAs gave a very high specificity (100%), both would give results very close to true values and, therefore, are suitable for surveillance.

Surveillance studies are designed to monitor trends and to make estimates of infection rates so that projected needs and prevention programmes can be designed, for which the target population sample size should preferably be as large as possible. Testing for HIV using saliva would be extremely beneficial, since fewer people will refuse to give a saliva specimen, thus reducing any bias introduced by high refusal rates.

The optimal sensitivity obtained by modification of the test protocol and using a cut-off value 30% lower than the serum value as recommended by Major et al. is 97%, which is lower than that reported by other researchers. This could be due to technical reasons. Use of saliva collection devices and stabilizers may increase the sensitivity in ELISA using whole saliva. van den Akker et al. found the mean sensitivity and specificity to be 96% and 99.96%, respectively, with collection devices.

Approximately 30% of the known HIV antibody seropositive patients and 2% of HIV antibody seronegative volunteers were also addicted to chewing paan (betel leaves) with the result that the saliva sample was stained slightly pink. This did not in any way appear to interfere with our assay, although the possibility of interference in levels of immunoglobulins in the saliva cannot be ruled out. Although we have not worked out the cost difference in using serum and saliva, it logically follows that testing saliva would be cheaper because it eliminates the use of disposable syringes, needles, tourniquet, cotton swabs, Pasteur pipettes and a centrifuge.

We, therefore, feel that saliva could be a good non-invasive specimen for anti-HIV testing, as it is safer and more cost-effective than serum for HIV antibody testing. Like serum, saliva may be safely stored at 4 °C to give accurate results. Thus, saliva specimens may be used for surveillance purposes, but due to its sensitivity being lower than that of serum by 3%, is not entirely suitable for accurate diagnostic purposes.

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