

DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS IN UNSTIMULATED WHOLE SALIVA WITH IMMUNOCHROMATOGRAPHIC TEST KIT USED FOR SERUM**CHANDRASEKAR LAKSHMI KRITHIKA^{1*}, SATHASIVASUBRAMANIAN SANKARAPANDIYAN²,
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ABSTRACT

Objectives: To determine the sensitivity and specificity for human immunodeficiency virus (HIV) antibodies in saliva using an immunochromatographic test kit used for serum for detection of antibodies specific to HIV-1/HIV-2 without any procedural optimization.

Methods: A study was carried out in 40 HIV patients, and 40 HIV free volunteers. 2 ml of unstimulated saliva was collected, using drooling method into sterile pre-weighed plastic containers for 10 minutes. The samples were labeled with numbers and laboratory personnel were completely blind to the specimens and analyzed with a kit used to determine antibodies in serum/blood. Analysis was performed using immunochromatographic test kit-SD BIOLINE HIV-1/2 - rapid test procedure (WHO approved) - third generation, for the qualitative determination of antibodies of all isotypes specific to HIV-1 and HIV-2. No procedural optimization was done. Appropriate statistical analysis was performed.

Results: Out of the 80 salivary samples, 38 were true positive, 40 were true negative and 2 were false negative for HIV. Sensitivity was 95%, specificity was 100%, positive predictive value of 100%, negative predictive value of 95% and disease prevalence of 50% were obtained.

Conclusion: Saliva samples collected without any specialized devices, and any test modification can be used instead of serum in rapid test assays for screening of HIV 1 and 2 antibodies and serve as a diagnostic tool.

Keywords: Saliva, Human immunodeficiency virus infection, Human immunodeficiency virus 1 and 2 antibodies, Rapid test.

INTRODUCTION

Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS) is a major global pandemic. India with a population of one billion was estimated to have 23.9 lakh people living with HIV/AIDS, with an adult prevalence of 0.31% in 2009 [1]. India serves as a home for 49% of Asia's total HIV infected population [2]. The National AIDS Control Organization of the government of India describes HIV infection, as most serious public health problem facing the nation and simplified testing methods are required to identify asymptomatic HIV carriers, to prevent disease transmission.

Saliva is a complex biologic fluid, and its use as a biomarker is relatively a recent trend. Obtaining saliva is easy, self-collection after instruction is possible and there is no need for trained staff and it does not carry the risk of needle stick injuries. Moreover, saliva samples can reflect real time levels of biomarkers [3]. Oral fluids for HIV screening are ideal alternatives to serum, for surveillance purposes as they have 100% specificity [4]. There are many rapid test kits available which make use of salivary samples to determine the levels of salivary antibodies to HIV antigens. In this study, a rapid test kit, which determines the presence of antibodies in plasma/whole blood, was used to estimate the sensitivity and specificity for HIV antibodies in saliva. Though the concentration of immunoglobulin G (IgG) antibodies to HIV antigens is about 1/1000 of that serum [5], the study was carried without the use of any specialized saliva collecting devices and procedural optimization for saliva.

METHODS**Subjects**

A total of 40 HIV infected individuals seen at the outpatient clinic of YRG care, Voluntary Health Services, Chennai, Tamil Nadu, and 40 controls

without the risk of HIV infection were enrolled in the study. Their age ranged from 20 to 50 years. Their details were recorded in a specific case sheet performa. Informed consent was obtained from subjects for participation in the study and approval from the Institutional Review Board, VHS-YRG care was obtained.

Sample collection

Participants were asked to have their breakfast and to abstain from eating, drinking and oral hygiene for 2 hrs before sample collection. Oral examination and sample collection was done after determination of CD4 lymphocyte count in peripheral blood by flow cytometry for HIV positive volunteers. The collection of unstimulated saliva by drooling method was initiated immediately after initial swallow action. The participants were instructed to refrain from any oral hygiene habits and to eat 2 hrs before collection. The subjects were instructed to rinse with distilled water and drain their saliva into sterile plastic containers of known weight, for 10 minutes. No specialized collecting devices were used. Samples were stored at 4°C until analysis. All the samples were coded, and number labeled that provided no information linking the specimens to HIV infected individuals and healthy volunteers and so the laboratory personnel were completely blind to the samples.

Analysis

SD BIOLINE HIV-1/2 - rapid test procedure (WHO approved kit) was performed for the qualitative determination of antibodies of all isotypes (IgG, IgA, IgM) specific to HIV-1 and HIV-2 in saliva of 30 HIV seropositive individuals. The test band is a strip of paper coated with an immobilized antibody specific for an antigen that is characteristic of HIV disease. Samples conjugated with the antibody allow chromogenic detection on the paper strip visualized in <20 minutes.

Test procedure

The test device was removed from the foil pouch, placed on a flat dry surface. A volume of 10 µl of whole saliva specimen is dispensed into sample wells. Four drops of assay diluents were added into the sample wells. As the test device begins to work, a purple color moves across the result window in the center of the test device. The test results were interpreted within 5-20 minutes.

Data analysis

Statistical analysis of data was performed to determine the sensitivity, specificity, positive and negative predictive values (NNPs) and disease prevalence (DP).

RESULTS

Presence of only control line C within the result window is indicative of a negative result (Fig. 1). Presence of two lines as control line C and test line within the result window indicates a positive result for HIV (Figs. 2 and 3).

Out of the 80 saliva samples, 38 samples were true positive for HIV, 2 were false negative, and 40 were true negative with no false positive results. Among the 38 HIV positive samples, 34 were positive for HIV-1 and 4 for HIV-2 (Fig. 4). The sensitivity was 95%, specificity 100%, positive predictive value of 100% and NNP of 95% and DP of 50% of the sample were obtained (Table 1).

DISCUSSION

HIV infection is diagnosed by screening the serum or plasma for antibodies for HIV-1 and HIV-2 antibodies, using ELISA and confirmed

with Western Blot method [6]. Using serum sample for HIV screening poses a lot of difficulties like the need for a trained phlebotomist, following appropriate universal precautions, risk of needle stick injuries [7] and patient compliance. Diagnosis based on salivary analysis has gained increasing interest as sampling is simple, safe, and non-invasive method. HIV is poorly transmitted via oral fluids [8]. Oral fluids contain small amounts of Igs and are more concentrated in crevicular fluid and mucosal transudate and are more suitable for screening of viral infections and require specialized collecting devices but unstimulated whole saliva collection is the most feasible method as there is good patient compliance, self collection can be made and more representative of the oral environment [9]. Specialized collecting devices with stabilizers and antibacterial agents are to be used to preserve these Igs. Saliva HIV antibody tests with specialized devices for proper specimen collection have been introduced [10].

Rapid testing for HIV has been introduced to enable inexpensive and convenient methods of screening. Hamill *et al.* in 2007 has reported that rapid tests modified to use oral fluid samples obviate the need for either vine puncture or finger prick blood analysis [11]. Oral fluid HIV tests offer additional advantages due to their non-invasive nature, can be performed anywhere, do not require specialist phlebotomy training or equipment, and reduce biohazard risk [12]. Peeling and Mabey in 2010 have suggested that rapid tests are reliable and affordable, requiring no equipment and minimal training [13]. Both adults at risk and adolescent population have a high level of acceptance for rapid oral tests [14,15]. Specific benefits of these tests include immediate communication of test results [16,17]. Rapid HIV tests produce results of comparable sensitivity and specificity to the ELISA test with high standards of sensitivity and specificity outcomes of 100% [18-21].

Procedural optimization like testing different volumes of sample, diluent or eliminating diluent, varying times of incubation, can be performed to obtain an optimal sensitivity and specificity. An equal quantity of saliva as that of serum, without any test modification and no saliva stabilizing agents and specialized collecting devices, were used

Table 1: Se, Sp, PPV, NNP and DP of the immunochromatographic test results for detection of HIV antibodies

Method	Se%	Sp%	PPV%	NPV%	DP%
Immunochromatographic test	95	100	100	95	50

Se: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NNP: Negative predictive value, DP: Disease prevalence, HIV: Human immunodeficiency virus

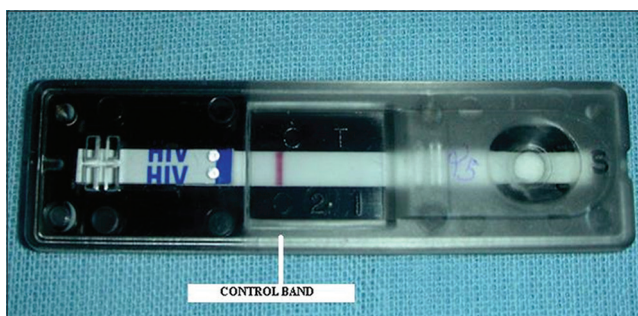


Fig. 1: A negative test result for human immunodeficiency virus antibodies in saliva

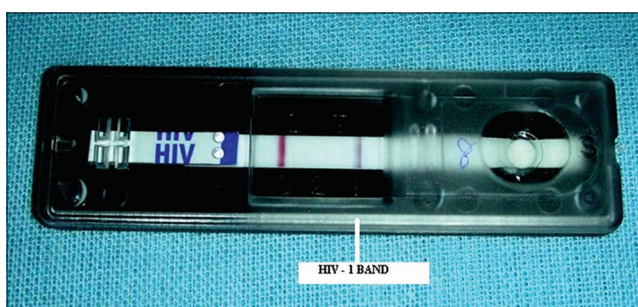


Fig. 2: A positive test result for human immunodeficiency virus-1 antibodies in saliva

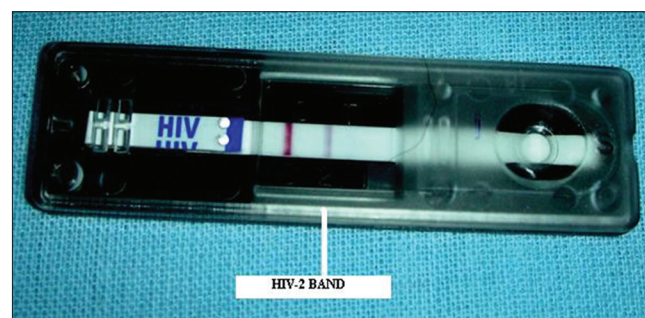


Fig. 3: A positive test result for human immunodeficiency virus-2 antibodies in saliva

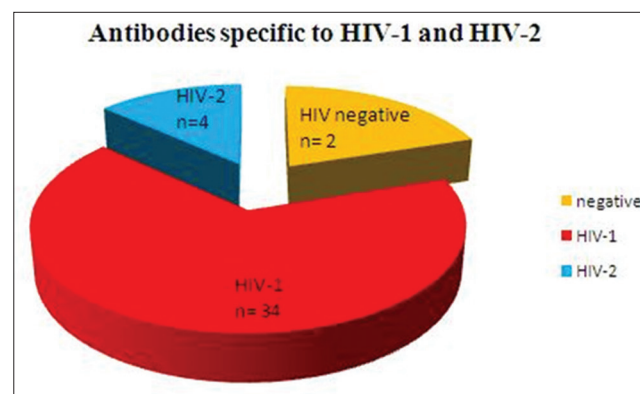


Fig. 4: Pie chart showing the detection of antibodies specific to human immunodeficiency virus (HIV)-1 and HIV-2

to perform our study. False negative results can be due to procedural errors and limitations of oral fluid assay like inability to detect in early stages of HIV and reduced viral load apply to rapid assays [22].

CONCLUSION

Many studies have been carried out on various aspects of test performance especially with saliva and oral mucosal transudate, but very few studies have been carried out to evaluate HIV antibodies in saliva with a kit that is used for serum. Our study indicates that without any procedural optimization and the use of specialized collecting devices, saliva could be used as an alternative to serum for detection of antibodies to HIV infection with a rapid assay kit used for serum.

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