

CLINICAL STUDY

Validation study of a conventional enzyme immunoassay to detect HIV antibodies in oral fluid

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ABSTRACT

OBJECTIVES: The aim of our study was to validate the Genscreen HIV ½ version 2 (BIO-RAD) for detecting HIV antibodies in oral fluid specimens (OF).

BACKGROUND: The advantage of assays to detect HIV infection in OF lies in the on-site easy access and noninvasive sample collection.

METHODS: Paired serum and OF were collected from 496 subjects (263 HIV-positive and 233 HIV-negative) using the Oraquick test kit (Oracle Diagnostics, Inc). The quality of OF was verified by measuring total IgGs using the Human IgG ELISA Quantitation Kit (Bethyl Lab. Inc). All reactive OF samples were retested by Western blot HIV1/2 BLOT 2.2 (MP Biomedical, Singapore, China).

RESULTS: Of 263 OF samples from participants with blood-based HIV-positive results, 259 were positive by Genscreen HIV ½ version 2 (98.48% sensitivity, 95% CI; 96.2–99.6). The 233 individuals who had a non-reactive HIV blood test were found negative on testing their OF by Genscreen HIV ½ version 2 (100% specificity, 95% CI; 98.4–100). NPV and PPV of the assay were 98.31% (95% CI; 95.74–99.34) and 100%, (95% CI; 98.53–100.00), respectively.

CONCLUSION: Genscreen HIV ½ version 2 (Bio-Rad) is a prospective method for HIV surveillance studies in hard-to-reach populations with high risk behavior using non-invasive OF collection (*Tab. 1, Fig. 1, Ref. 16*). Text in PDF www.elis.sk.

KEY WORDS: HIV testing, oral fluid, Genscreen HIV ½ version 2.

Introduction

Many persons with HIV do not get tested until late in their infection (1). It is estimated that approximately 30–50 % of HIV seropositive cases in European settings are diagnosed with Acquired Immune Deficiency Syndrome (AIDS) within a calendar

year of their diagnosis or have the CD4-cell count lower than 350/mm³ at presentation (2, 3). In addition, a significant proportion of tested individuals fail to return to get their results (4).

Oral fluid (OF)-based HIV tests could promote early diagnosis and increase the number of people who become aware of their status. The major advantages of replacing serum/plasma with OF include easy access to testing and noninvasive collection while maintaining high-level performance (5, 6, 7). OF tests do not require the presence of experienced healthcare personnel. Nevertheless, the staff should be trained on how to perform their assigned tasks (5, 7). In addition, OF-based testing is a key tool for successful implementation of Second Generation Surveillance approaches (8). The sampling methodologies for this purpose require collection of specimens from hard-to-reach populations at locations such as streets, bars, discos, and saunas where drawing blood is problematic and maybe unsafe. A CE marked OF-based assay could help collect and process easily, quickly and safely many samples in these settings.

HIV tests on OF had high levels of sensitivity and specificity across various populations and settings (9, 10, 11, 12). A recent meta-analysis of the accuracy of a rapid HIV-antibody-based point-of-care test (Oraquick advance rapid HIV-1/2) calculated pooled estimates of sensitivity and specificity at 98.03 % and 99.74 %, respectively (13).

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Given the great public health interest in OF-based HIV tests, we decided to evaluate the performance of Genscreen HIV ½ version 2 (Bio-Rad) on OF samples. This conventional assay is commercially available for use in serum or plasma. Preliminary results of this validation have been presented (14) as part of the SIALON I study that was conducted on men who have sex with men (MSM).

Material and methods

Paired serum and oral fluid specimens were collected from 496 subjects in 6 countries that participated in the European Union (EU)-funded project SIALON I (14). All study subjects gave informed consent to participate in the study. The HIV-positive group (n = 263) included Western Blot (WB)-confirmed patients (based on blood specimens) who were attending healthcare facilities and were invited to take part in the study (regardless of whether being on antiretroviral treatment or not). Controls (n = 233) were randomly selected health professionals and volunteers who looked for HIV testing and were HIV-negatives according to Enzyme Immunoassay (EIA; based on blood specimens).

The collection and transportation of serum were done according to standard procedures. The Oracol test kit (Malvern Medicals, Worcester, UK) was used for OF collection. Oral fluid samples were kept in a fridge and sent to the laboratory within 72 hours. On receipt, the testing laboratory extracted OF from each swab adding 2.0 ml of transport medium followed by vortex for 20–30 seconds. The transport medium was allowed to equilibrate with OF for at least 1 hour at 4 °C, after which the swab was removed from the plastic tube. Following centrifugation, the OF was collected and filtered through a Celtron 30, 0.2-µm filter (Schleicher & Schuell MicroScience, Dassel, Germany). The oral fluid was then stored in aliquots and at –20 °C.

The quality of OF samples was evaluated and verified by measuring total IgGs using the Human IgG ELISA Quantitation Kit (Bethyl Laboratories, Inc). Oral fluid samples with IgG < 3.5 mg/l were excluded from the study.

In this work, we evaluated Genscreen HIV ½ version 2 that is produced by Bio-Rad and has received CE marking (conformation with European standards) for testing HIV antibodies in serum and plasma (HIV-1:100 % sensitivity and 99.8 % specificity; HIV-2: 100 % sensitivity). The validation protocol we followed was developed according to the EU general principles set out in EU Commission Decision as of May 7th, 2002 on common technical specifications for *in vitro* diagnostic medical devices and the international guidelines for using HIV testing technologies in surveillance (WHO & UNAIDS, 2009).

All reactive OF samples were retested by Western blot HIV1/2 BLOT 2.2 (MP Biomedical, Singapore, China) according to standard procedures described in the instructions package for serum/plasma testing.

Results

Of 263 oral fluid specimens from participants with blood-based EIA-reactive and WB-confirmed results, 259 were as well

Tab. 1. Results of evaluating Genscreen HIV ½ version 2 (Bio-Rad) on oral fluid vs. serum samples.

Oral fluid/serum	HIV negative	HIV positive	Total	%	
HIV negative	233	4	237	98.31	NPV
HIV positive	0	259	259	100.00	PPV
Total	233	263	496		
%	100.00	98.48			
	specificity	sensitivity			

positive by Genscreen HIV ½ version 2. This corresponds to an overall sensitivity of 98.48 % (95% CI; 96.2 – 99.6). The 233 OF samples of the blood-based EIA-negative individuals were correctly identified as negative cases by Genscreen HIV ½ version (100 % specificity, 95% CI; 98.4–100). The Negative Predictive Value (NPV) and the Positive Predictive Value (PPV) of the assay were 98.31 % (95% CI; 95.74–99.34) and 100 % (95% CI; 98.53–100.00), respectively (Tab. 1). Assuming an HIV prevalence of 5 % and 15 %, the NPVs were 98.5 % and 99.73 %, respectively.

Three of the four oral fluid samples that were falsely negative by Genscreen HIV ½ version 2 were positive in Western blotting (Fig. 1) while 1 oral fluid sample showed one reactive band for HIV-1 (gp160), by using a five-fold amount of regular loading compared to serum testing (100 microlitres instead of 20).

Discussion

HIV testing on oral fluid has fewer requirements in terms of local staff training, it reduces the risk of injury from sharp instruments while it increases clients’ comfort and acceptability (7). Given the multiple advantages of OF-based sampling and testing, we evaluated the performance of the conventional Genscreen HIV ½ version 2 (Bio-Rad) assay by testing paired oral fluid and serum samples collected from 496 subjects in 6 EU countries that had

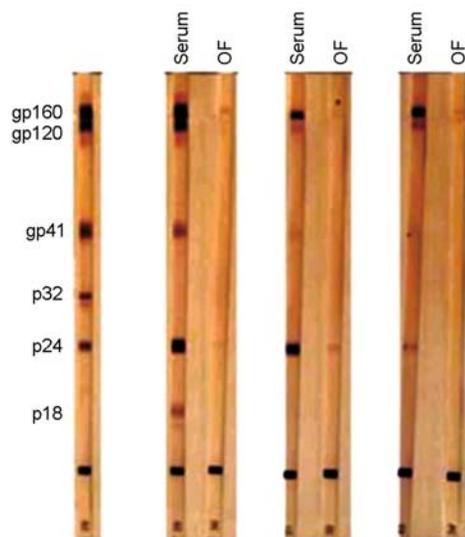


Fig. 1. Positive Western Blots of 3 false EIA-negative oral fluid samples.

participated in the SIALON I project (14). The assay performed very well at a sensitivity of 98.48 % and specificity of 100 %. Our results are comparable with the previous research that also showed high-level performance on oral fluids of both Genscreen HIV ½ vs 2 (Bio-Rad) (10) and fourth generation Genscreen assay, the Genscreen Ultra HIV Ag-Ab (5).

In our study, 3 of 4 OF samples that were false negatives by Genscreen HIV ½ version 2 assay were found positive in Western Blot testing. The other falsely negative oral fluid sample had a reactive band for HIV-1 (gp160) when we used a five-fold amount of regular loading compared to serum testing. The false OF-based negativity could be due to low levels of functional IgGs as a result of IgG degradation, bacterial contamination, or erroneous storage. Although the OF-based tests are simple and reliable, mistakes can occur at any point of the process. To reduce errors, a quality assurance program should be in place.

Recent research has also questioned the accuracy of oral fluid-based tests (in terms of sensitivity), especially when used soon after infection (15, 16). A meta-analysis in particular found a 2 % lower sensitivity of an OF-based test compared to blood-based analyses (13). In our study, however, we lacked the information for assessing the performance of the Genscreen HIV ½ version 2 (Bio-Rad) on samples from recently infected individuals, and especially whether the 4 false negatives were derived from patients who had acquired HIV recently. OF-based tests might need further evaluation in the setting of acute and recent HIV infection.

Conclusions

The conventional Genscreen HIV ½ version 2 (Bio-Rad) assay shows high-level performance for the diagnosis of HIV infection on oral fluids. It could thus be a suitable tool for HIV testing of oral fluids accessible to people in both clinical and non-clinical settings. Additionally, it could also prospectively contribute to enhancing the uptake of testing and implementing Second Generation HIV Surveillance in hard-to-reach populations with high-risk behavior.

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