Evaluation of accuracy of oraquick® rapid test in detecting HIV antibodies in saliva of Nigerians

***Nwoga MC, **Odukoya O, ***Savage KO, **Effiom OA**

*Oral Pathology Unit, Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, University of Nigeria;
**Department of Oral Pathology & Oral Biology, Faculty of Dental Sciences, University of Lagos;
***Department of Preventive Dentistry, Faculty of Dental Sciences, University of Lagos, Nigeria

Correspondence: Nwoga MC
Email: marknwoga@yahoo.co.uk; mark.nwoga@unn.edu.ng

Abstract

Objective: The accuracy of OraQuick® rapid test in detecting HIV 1 & 2 antibodies in saliva is evaluated against the blood EIA benchmark tests with confirmatory testing, against which OraQuick® accuracy is determined.
Method: Paired samples of saliva and blood from 281 Nigerians were tested for HIV antibodies, and compared for sensitivity and specificity. Subjects included in the study were those who had a complete test, which included saliva test with OraQuick® rapid test, serologic test using conventional Enzyme Immunoassay (EIA), and confirmatory serological test.
Result: From the 281 subjects who completed the oral fluid-based OraQuick® tests & EIA with confirmatory tests, 192 were seropositive and 89 seronegative for HIV 1 & 2 antibodies. The sensitivity (95% CI) of OraQuick® was 98.96% (97.98% to 99.96%) and specificity was 100% thereby comparing favorably with serum EIA with benchmark sensitivity of 100% and specificity (95% CI) of 96.63% (95.61 to 97.56).
Conclusion: Saliva based OraQuick® rapid assays for detecting HIV antibodies using oral fluids give accurate sensitivity and specificity results comparable to EIA results with serum samples. They are more acceptable, easier to use and not invasive.

Key words: Oraquick, saliva, HIV detection

Introduction

Most common diagnostic tests for detection of HIV antibodies use serum, plasma and blood as most reliable sample specimens, nevertheless, saliva shows promise as a viable alternative specimen to serum[1]. Other specimens not considered viable alternatives to blood included breast milk, sweat, seminal fluid, vaginal secretions, urine and lacrimal fluid due to inconsistent and unreliable results[2].
Some studies have assessed the feasibility of using saliva as an alternative to serum in detecting antibodies to HIV-1 and HIV-2[2-9]. In these studies, the test kit used was a self-contained device for collection, processing and analysis of the oral fluid specimen. These self-contained kits have immuno- chromatography test strips for analysis[6]. Most oral fluid collection devices yield about 0.5 to 1.5ml of Oral Mucosal Transudate (OMT) to saturate the devices[10].
Studies have demonstrated that most patients were more willing to be tested for HIV infection using saliva specimen rather than blood[8,11-17]. Children have also shown better compliance with oral fluid specimen compared to blood specimen collection for testing[18].
The wide use of oral fluid for HIV screening would have a far-reaching impact on the health system in selection and screening of blood donors, mass HIV screening, HIV research, ante-natal diagnosis and prophylaxis against vertical transmission. Other useful advantages of oral fluid HIV screening would include the rapidity of testing, absence of laboratory requirements, ease of specimen collection and handling, as well as being affordable for low income economies.
We were not aware of any previous study in Nigerians as at the time of this study, where efficacy of OraQuick® test kit was applied to detect HIV 1 and HIV 2 antibodies in saliva. We hereby evaluate the accuracy of OraQuick® as an oral fluid test device for testing and diagnosis of HIV infection.

Materials and method

Study population: A total of 319 subjects who attended the HIV, Dermatology, Medical outpatient clinics at the Lagos University Teaching Hospital, Nigeria, during the period from April 2002 to January 2003 were recruited for this study. All the subjects were selected via random sampling from those attending the clinics during the period of study, for routine medical check-ups or for the management of pre-existing medical conditions. Informed consent was sought and received for all the procedures on the patients, including for confirmatory tests where necessary.
This study received ethical approval from the Research and Ethics Committee of Lagos University Teaching Hospital, Lagos, Nigeria .

Each subject was informed of his or her HIV status through channels previously arranged in the respective clinics by their clinicians.All the subjects available for the study were dentate patients. The bio-data of each subject, was obtained from the subject and hospital records.

Basis of classifying participants as HIV infected or uninfected For the purposes of this study, the criteria for diagnosis of HIV infection or HIV positive serostatusis based on reactive EIA serum screening result or reactive oral fluid based OraQuick® screening result, followed by a reactive serum-based HIV antibody confirmatory test. In addition, a subject with non reactive serum-based HIV confirmatory test is considered to be free from HIV infection, even in the presence of a reactive EIA test or reactive OraQuick® screening result. Indeterminate results call for a repeat testing with new oral fluid or serum samples or repeat testing after a 3month window period after explaining to the patient.

Blood sample collections and testing: Blood samples from all patients were collected by the hospital laboratory staff through venepuncture, for HIV antibody screening, using an enzyme immuno-assay method: the GENSCREEN® PLUS HIV Ag-Ab, (BIO-RAD, Marnes-La-Coquette, France) and confirmatory testing using: ImmunoComb® II HIV 1 & 2 CombiFirm Kit.

OraQuick® HIV-1 and 2 test procedure (Oral Fluid)

Test items and saliva sample collection: OraQuick® test kit(Orasure Technologies Incorporation, Bethlehem, Pennsylvania, USA) consist of a test device, developer vial, a loop and a desiccant.

The saliva sample was collected using a porous flat pad attached to a handle, which is enclosed in the test device sachet. The sample was mixed saliva collected from the buccal and labial sulci, and external gum area of the oral cavity of each subject, through a brushing-like movement of the porous pad along the buccal and labial sulci. Alternatively, the OraQuick® test device was rubbed and swabbed across the outer gum line of both the upper and lower sulci, (between the gum and the cheek). The device was then removed from the oral cavity and placed into a vial containing a pre-measured amount of developer solution from the manufacturer.

Interpretation of OraQuick® tests

If a single line appeared on the test strip in the area labeled 'C', the result was non-reactive, suggesting the absence of anti-HIV antibodies in the specimen. If two lines appeared on the test strip in areas marked 'T' and 'C' respectively, the result was considered reactive, suggesting the presence of anti-HIV antibodies in the specimen. The result was interpreted as invalid, and such test was repeated with a new test device If there was no line in the area labeled 'C' and with or without any line on the test strip in area marked 'T'. Sensitivity and specificity tests: The sensitivity of the oral fluid test was measured using oral fluid samples from HIV-seropositive subjects while the specificity was measured using oral fluid specimens from HIV seronegative subjects.

Excluded incomplete tests

A subject was adjudged to have a complete evaluation if the subject has results from Oraquick® test, HIV serology test using the Enzyme Immunoassay method, and HIV serological confirmatory test. Subjects who do not have the three screening tests were adjudged to have incomplete evaluation and were excluded from the final study.

Result

From a total of 319 subjects evaluated in the study, thirty-eight subjects who had incomplete tests, were excluded. However, 281 subjects consisting of 162 females and 119 males(female: male ratio of 1.4:1) completed all three evaluations using OraQuick®, EIA and Confirmatory tests. The 281 subjects with complete evaluation studies, consisted of 192 HIV reactive subjects and 89 HIV non-reactive subjects. Out of the 162 females involved in the study, 120 females were reactive to the confirmatory HIV tests while 42 were non reactive. Among the 119 males in the study, 72 were reactive to the confirmatory HIV tests, while 47 were non reactive. The overall age range of was from 1.5 years to 75 years, with a mean age of 34.8 years **(Table 1).**

Table 1. Distribution of gender and HIV status in subjects

Sex	HIV +ve N (%)	HIV -ve N (%)	Total
Females	120 (74.0)	42 (26.0)	162 (100.0)
Males	72 (61.0)	47 (39.0)	119 (100.0)
Total	**192**	**89**	**281 (100.0)**

Result of serological test with EIA and Confirmatory tests: 195 subjects had a reactive serological test for HIV antibodies of either HIV-1 or HIV-2 or both, using the EIA, while a total of 86 subjects were non-reactive. Sensitivity of the benchmark EIA serological test was determined as 100%. However, confirmatory testing of the 195 EIA reactive samples, were reactive in 192 subjects and 3 samples were non-reactive (3 false positive). Specificity(95% CI)of EIA tests was thus determined as 96.63% (95.61 to 97.56) **(Table 2).**

Result of OraQuick® HIV-1 & 2 oral fluid tests: From saliva samples tested with OraQuick®in 281 subjects, 190 subjects were reactive for HIV antibodies irrespective of the type-specific HIV-1 or HIV-2 infections, while 89 were non-reactive. Two subjects with reactive serum EIA tests, produced saliva samples that were non-reactive with OraQuick® HIV-1 & 2, (2 false negative results).Sensitivity (95% CI) of the oral fluid based OraQuick® HIV-1 & 2 test was determined as 98.96% (97.98% to 99.96%)and the specificity was determined as 100% **(Table 2).**

Table 2. Comparison of results of EIA, Oralquick and Western blot tests

Screening	HIV +ve N (%)	HIV -ve N (%)	False +ve N (%)	False -ve N (%)	Total
Confirmatory Enzyme immune assay	192(68.3)	86(30.6)	3(0.01)	NA	281(100.0)
Oraquick	190(67.6)	89(31.7)	0(0)	2(0.7)	281(100.0)
Western blot	192(98.5)	3**(1.5)	NA	NA	195***(100.0)

• The HIV testing algorithm does not require WB confirmatory testing once the EIA (enzyme immune-assay) is non-reactive. **From the false positive results of three EIA screening tests. ***The remaining 86 EIA non-reactive samples did not require WB confirmatory testing according to the conventional algorithm.

Discussion

The interest generated by the development of oral fluid based rapid assays for screening HIV infection is tempered by concern for the accuracy of these assays. Several population-based studies of HIV screening in Africa using saliva or oral fluids not only gave satisfactory results that compared favorably with conventional benchmark serum-based assays[4,12,19-20, 25,27] but also indicate that the time is ripe for acceptance and wide-scale use of oral fluid based assays especially OraQuick® oral assays for HIV screening.

The results of our evaluation of OraQuick® HIV I/2 assay in a random sample of a Nigerian population, giving sensitivity(95% CI)of 98.96%(97.98% to 99.96%)and specificity of 100%, confirmed and replicated previous results of accuracy of oral fluid based HIV assays especially, Oraquick® rapid HIV assay[4,19,20,,27]. This may help revolutionize the screening of the Nigerian population and facilitate early detection and management of HIV infection. Similarly, enhanced voluntary screening and self testing is expected among many subjects afraid of any invasive procedures and the hard-to-reach population, where poverty and ignorance play prominent roles in the spread of HIV infection.

We did not have any false positive result in our sample population using OraQuick® oral tests and our experience is similar to the findings and high levels of concordance for HIV positive samples and the near absence of false positives in the studies by Kakizawa et al[21]in an Indian population study and Malamud and Friedman[22]. However, when false positive and discordant results were noted with OraQuick® oral tests[23] and where confirmatory testing was done on discordant samples, nearly one in five turned out to be HIV-infected[24], hence the recommendation for the use of serum or plasma-based confirmatory tests for reactive rapid oral fluid based HIV screening tests[24].

In our study, regarding false positive reactive tests, OraQuick® oral tests appeared to give more accurate results. We observed three false positive results with serum based EIA screening tests all in three male subjects, while both oral fluid sample tested with OraQuick® and conventional serum confirmatory tests correctly were non reactive in these cases. Outside of probable procedural and interpretation errors or cross reactions, we were unable to explain the factors responsible for the three false positive reactive serum based EIA screening tests among the 3 male subjects in this study.

We were also unable to properly explain the factors responsible for the occurrence of two false negative tests in two women aged 20years and 30 years using oral fluid based OraQuick®, in this study while both EIA and serum confirmatory tests were reactive. However, the false negatives may not be unconnected with a possible procedural error in sample collection or application of the device intraorally to areas of HIV antibody secretion or instrument failure due to storage and defect. Previous studies have also reported one false negative result in an Indian study[26] and 3 false negative results in a British study[9], which blamed observer error for the observed 3 false negative results.

When there is apparent conflict with the results of OraQuick® HIV I/2 assay or discordant results with oral fluid testing a repeat of OraQuick® HIV I/2 assay is recommended. Alternatively, the combinations of OraQuick® HIV I/2 assay and another serum based assay in an HIV testing algorithm[25] have proved to be beneficial in resolving discordant cases.

Limitations in this study include the small sample size, absence of specifying HIV infective subtypes in the diagnosis, high cost and not readily available OraQuick® HIV I/2 assay kits.

Conclusion

It is concluded from this study that use of OraQuick® HIV1 & 2 assays in HIV antibodies detection in saliva give sensitivity and specificity results that compares favorably with serological assays for HIV 1 & 2 antibodies detection.

Competing interests: The opinions and conclusions expressed in this study are those of the authors only. No funding or part in the writing of the manuscript was received from OraSure Technologies, Inc., other agencies or from any institution. The tests kits were supplied and paid for by the authors. The authors therefore declare that they have no competing interests.

Acknowledgement

We acknowledge with thanks the suggestions and cooperation of Dr A.S. Akanmu of the Department of Hematology & Blood Transfusion of the University of Lagos in the course of carrying out this study.

References

1 Rossi G. Saliva-based methods for the diagnosis of HIV infections. RecentiProg Med 1998; 89: 383-384.
2 Martinez P M, Torres, AR, Ortiz-de-Lejarazu R, Montoya A, Martin J F, Eiros J M. Human immunodeficiency virus testing by enzyme-linked fluorescent and western blot assays using serum, gingival-crevicular transudate, and urine samples. J Clin Microbiol 1999; 37: 1100 - 1106.
3 Granade TC, Phillips SK, Parekh B, Gomez P, Kitson-Piggott W, Oleander H, Mahabir B, Charles W, Lee-Thomas S. Detection of antibodies to human immunodeficiency virus type 1 in oral fluids: a large-scale evaluation of immuno-assay performance. ClinDiagn LabImmunol 1998; 5: 171 - 175.
4 Ettiegne-Traore V, Ghys PD, Maurice C, Hoyi-Adonsou YM, Soroh D, Adom, ML, Teurquetil MJ, Diallo MO, Laga M, Greenberg AE. Evaluation of an HIV saliva test for the detection of HIV-l and HIV-2 antibodies in high-risk populations in Abidjan, Cote d'Ivoire. Int J STD AIDS 1998; 9:173-74.
5 Nishanian P, Aziz N, Chung J, Detels R, Fahey J L . Oral fluids as an alternative to serum for measurement of markers of immune activation. ClinDiagn LabImmunol 1998; 5: 507-512.
6 Schramm W, Angulo GB, Torres PC, Burgess-Cassler A. A simple saliva-based test for detecting antibodies to human immunodeficiency virus. ClinDiagn LabImmunol 1999; 6: 577 - 580.
7 Delaney KP, Branson BM, Uniyal A, Kerndt PR, Keenan PA, Jafa K, Gardner AD, Jamieson DJ, Bulterys M. Performance of an oral fluid rapid HIV-1/2 test: experience from four CDC studies. AIDS 2006; 20:1655-1660.

8 Debattista J, Bryson G, Roudenko N, Dwyer J, Kelly M, Hogan P, Patten J. Pilot of non-invasive (oral fluid) testing for HIV within a clinical setting. Sex Health 2007; 4: 105-109.

9 ZelinJ, Garrett N, Saunders J, Warburton F, Anderson J, Moir K, Symonds M, Estcourt C; North East London Sexual Health Network Research Consortium. An evaluation of the performance of OraQuick ADVANCE Rapid HIV-1/2 Test in a high-risk population attending genitourinary medicine clinics in East London, UK. Int J STD AIDS 2008; 19:665-667.

10 Mortimer PP, Parry JV. Detection of antibodies to HIV in saliva: a brief review ClinDiagn Virol 1994; 2: 231 – 243.

11 .Coates R, Millson M, Myers T, Rankin J, McLaughlin B, Major C, Rigby J, Mindell W . The benefits of HIV antibody testing of saliva in field research. Can J Public Health 1991; 82: 397 – 398.

12 .Matee MI, Lyamuya EF, Simon E, Mbena EC, Kagoma C, Samaranayake LP, Scheutz F. Detection of anti-HIV IgG antibodies in whole saliva by GACELISA and Western blot assays. East Afr Med J 1996; 73: 292-294.

13 Sy FS, Rhodes SD, Choi ST, Drociuk D, Laurent A A, Naccash RM, Kettinger LD . The acceptability of oral fluid testing for HIV antibodies. A pilot study in gay bars in a predominantly rural state. Sex. Transm Dis 1998; 25: 211-215.

14 Major C J, Read S E, Coates R A, Francis A, McLaughlin B J, Millson M., Shepherd F, Fanning M, Calzavara L, MacFadden D .Comparison of saliva and blood for human immunodeficiency virus prevalence testing. J Infect Dis 1991; 163: 699 – 702.

15 McKeganey N, Barnard M, Leyland A, Coote I, Follet E. Female street working prostitution and HIV infection in Glasgow. Brit Med J 1992; 305: 801-804.

16 Peralta L, Constantine N, Griffin-Deeds B, Martin L, Ghalib K. Evaluation of youth preferences for rapid and innovative human immunodeficiency virus antibody tests. Arch Pediatr Adolesc Med 2001; 155: 838-843.

17 Bauserman RJ, Ward, MA, Eldred L, Swetz A. Increasing voluntary HIV testing by offering oral tests in incarcerated populations. Am. J. Public Health 2001; 91: 1226-1229.

18 Tess BH, Granato C, Parry JV, Santos VA, Lago TG, Newell ML, Dunn DT, Rodrigues LC . Salivary testing for human immunodeficiency virus type 1 infection in children born to infected mothers in Sao Paulo, Brazil. The Sao Paulo Collaborative Study for Vertical Transmission of HIV-1. Pediatr Infect Dis J 1996; 15: 787-790.

19 Fylkesnes K, Kasumba K. The first Zambian population-based HIV survey: saliva-based testing is accurate and acceptable. AIDSids 1998; 26: 540-541.

20 Grant RM, Piwowar E M, Katongole-Mbidde E, Muzawalu W, Rugera S, Abima J, Stramer S L, Kataaha P, Jackson B . Comparison of saliva and serum for human immunodeficiency virus type 1 antibody testing in Uganda Using a rapid recombinant assay. ClinDiagn LabImmunol 1996; 3: 640-644.

21 Kakizawa J, Ushijima H Oka S, Ikeda Y, Schroder HC, Muller WE. Detection of human immunodeficiency virus-l DNA, RNA and antibody, and occult blood in inactivated saliva: availability of the filter paper disk method. Acta Paediatr Jpn 1996; 38: 218-223.

22 Malamud D, Friedman HM. HIV in the Oral cavity: virus, Viral inhibitory Activity and Antiviral Antibodies: A Review. Crit Rev Oral Biol Med 1993; 4: 461 – 466.

23 Centers for Disease Control and Prevention (CDC) . False-positive oral fluid rapid HIV tests--New York City, 2005-2008; 20:660-665.

24 Wesolowski LG, Mackellar DA, Ethridge SF, Zhu JH, Owen SM, Sullivan PS. Post Marketing Surveillance Team. Repeat confirmatory testing for persons with discordant whole blood and oral fluid rapid HIV test results: findings from post marketing surveillance. PLoSONE 2008.3:e1524.doi;10.1371/journal.pone. 0001524

25 Dalila Zachary, Lawrence Mwenge, Monde Muyoyeta, Kwame Shanaube, Albertus Schaap,Virginia Bond, Barry Kosloff, Petra de Haas and Helen Ayles. Field comparison of OraQuick ADVANCE Rapid HIV-1/2 antibody test and two blood-based rapid HIV antibody tests in Zambia. BMC Infectious Diseases. 2012, 12:183-185.

26 Pai NP, Barick R, Tulsky JP, Shivkumar PV, Cohan D, Kalantri S, Pai M, Klein MB, Chhabra S. Impact of round-the-clock, rapid oral fluid HIV testing of women in labor in rural India.PLoS Med 2008; 6:5:e92.

27 Pascoe SJ, Langhaug LF, Mudzori J, Burke E, Hayes R, Cowan FM: Field evaluation of diagnostic accuracy of an oral fluid rapid test for HIV, tested at point-of-service sites in rural Zimbabwe. AIDS Patient Care STDS 2009; 23:571 – 576.