EVALUATION OF ORAQUICK® HIV-1/2 AS ORAL RAPID TEST

Celine Nguefeu NKENFOU*[1,2], Japhette Esther KEMBOU [',3] Edith Saounde TEMGOUA[1], Appolinaire DJIKENG[4], Linda Mekue MOUAFO[',3], Elvis Ndukong NDZIE[',3], Irenee DONKAM[2] Vitorrio COLIZZI[5] and Martin SanouSobzé[3]

[1]"Chantal Biya" International Reference Centre (CBIRC), Yaounde, Cameroon, [2]Higher Teachers' Training College, University of Yaounde I, Cameroon, [3]Faculty of Science, University of Dschang, Cameroon, [4]Bioscience eastern and central Africa - International Livestock Research Institute Hub (BecA-ILRI Hub), Nairobi, Kenya, [5]University of Tor Vergata, Italy
* E-mail: nkenfou@yahoo.com

Abstract

As Cameroon scales up its national HIV/AIDS control program, evaluating the performance of commercially available tests for accurate and cost effective diagnostics becomes essential. A cross-sectional study assessed the performance of an HIV oral rapid test. A total of 1520 participants consented to participate in the study. After counselling, they were tested for HIV using the national algorithm followed by OraQuick. Results of the national algorithm were compared to those of OraQuick, for sensitivity, specificity, positive predictive and negative predictive values. 62% of participants were male, and 1% was reported HIV-positive following the national algorithm. The OraQuick test had 93% sensitivity, 99% specificity, 99.93% NPV and 90% PPV (95% CI, Kappa 0.965). Though more expensive (2-6x) compared to the national algorithm tests, oral mucosal transudate-based test demonstrated good performance. Therefore, it could be implemented in resource-constrained settings if subsidized and could increase participation since less invasive with no blood accident exposure.

Key words: OraQuick, OMT-based test, Diagnostic kits, National algorithm

Introduction

HIV serology has evolved since 1980's, rising from 1st to 4th generation of diagnostic tests. Most of the kits used are based on HIV antibodies detection on blood samples. Taking blood samples during population-based surveys can be problematic: skin puncture increases the complexity of the test and the risk of needle-stick injuries to staff. In some populations, blood collection is not well accepted for religious or cultural reasons. For example, Gregson et al. (2002) found that fear of Satanism was a common reason for refusing to provide Dried Blood Spot (DBS) samples in an epidemiologic survey in Zimbabwe. In children, newborns, immune-compromised and obese individuals in whom getting blood samples can be particularly difficult (Alemnji et al., 2009), the use of blood as the sole medium for testing for HIV can be problematic; but this could be eased by using other body fluids such as oral fluid (Delaney et al., 2006; Sangare et al., 1997) because its collection involves non-invasive techniques, unlike phlebotomy and fingertip pricking. This could be more adaptable for field use (Constantine and Zink, 2005; UNAIDS/WHO, 2001) and screening of high risk and far-to-reach populations (Keenan *et al.*, 2005) or in resource-constrained settings with improved participant acceptability.

Performing blood based assays usually required skilled personnel. In addition there is a significant waiting time for results to be communicated back to either the patient or the treating physician. This leads to an increase in anxiety for patients as well as significant loss of participants to follow-up for results collection. Evaluating oral fluid test kit for potential use as rapid point-of-care testing (Ndembi *et al.,* 2011) and means of their implementation as components of HIV control initiatives and programs in Cameroon presents several advantages especially for a larger national coverage, thus allowing a real estimation of the global prevalence and a more adequate and well-targeted prevention and treatment to ensure disease regression as well as mortality reduction due to HIV/AIDS. The aim of this study was therefore to evaluate the sensitivity, specificity of OraQuick® Rapid HIV-1/2 Antibody Test on oral fluid collected from participants coming from different areas of Cameroon, attending the 2011 Cameroon national universities games.

Material and methods:
Study design and sample population

A cross-sectional study was carried out during the May 2011 University games, to evaluate an oral mucosa transudate (OMT)-based rapid kit (OraQuick® Rapid HIV-1/2 Antibody Test from Orasure Technologies, Inc. Bethlehem, PA 18015 USA) during a free screening campaign on participants attending voluntary counselling and testing services. The screening was carried out on 1520 participants, from diverse areas of Cameroon. They were briefed on the objectives of the study in a mass-counselling. Participants with known HIV negative status; unknown HIV status; and willingness to sign an informed consent form were recruited for the study. To evaluate the oral fluid kit, we followed the national algorithm for voluntary screening, based on the UNAIDS testing strategy II. The recommended algorithm involved the use of a first

screening test based on blood sample. In case this yielded a positive result, a second confirmatory test (also based on blood sample) was applied and the results served as Gold standard for the evaluation. This was then followed by the application of the test under evaluation (based on oral fluid).

Ethical considerations

Our study received the approval of the NEC (National Ethical Committee, authorisation n° 215/CNE/SE/2012) of Cameroon. It is only after the mass counselling that participants who accepted to be screened were enrolled in the study. Participants were also ensured of confidentiality, post-accompanying counselling (in case of a positive result) as recommended by the UNAIDS/WHO policy statement on HIV testing. Confidentiality was ensured by assigning identification numbers to the participants of the study. The post-accompanying counseling was especially done for those tested positives, in order to morally and psychologically assist them and refer them to accredited and competent HIV treatment centers.

Data and sample collection

Questionnaires were first administered to participants in order to collect socio-cultural information. Each participant was first assigned an identification number. This was followed by matched test on blood and oral fluid. A drop of whole blood was needed for the standard test. The OMT specimens were collected using the collection device provided within OraQuick® Rapid HIV-1/2 Antibody Test kit. All the samples were tested at the screening site.

Quality and control assurance

Blood based kits (Determine® HIV-1/2 Ag/Ab Combo as first test and HIV (1+2) Antibody (Colloidal Gold) (KHB Shanghai Kehua Bio-engineering Co. Ltd) as confirmatory test) were performed by experienced and well-trained laboratory technicians. The medical staff involved was trained for the use of the oral fluid test, before this was applied; and it was also applied following the recommended manufacturers procedure. Results on test strips were read by more than 2 laboratory technicians.

Statistical analysis

The sensitivity, specificity, positive and negative predictive values of OraQuick® Rapid HIV-1/2 Antibody Test with oral fluid were calculated and compared to the results of the gold standard Determine HIV-1/2 Ag/Ab Combo rapid test and HIV (1+2) Antibody (Colloidal Gold).
Sensitivity was calculated as the number of HIV-positive samples detected by the test under evaluation, divided by the total number of HIV-positive samples confirmed by the gold standard, multiplied by 100. **Specificity** was calculated as the number of HIV-negative samples detected by the kit under evaluation, divided by the total number of HIV-negative samples identified by the gold standard, multiplied by 100. **The positive predictive value** (PPV) was calculated as the true positives (TP) divided by the sum of true positives (TP) and false positives (FP). **The negative predictive value** (NPV) was calculated as the true negatives (TN) divided by the sum of false negatives (FN) and true negatives (TN). Analyses were done in Microsoft Excel 2010 and 95% Confidence Intervals (CI) were calculated in the statistical package for social sciences (SPSS) analysing program (PASW Statistics 18). Level of significance was tested at 5% probability.

Results

Of the 1520 participants recruited, 941 (62%) were males, all aged between 13 and 49. Five hundred and seventy nine (579) (38%) were females, and 66% of participants were students from secondary schools, most of them resident of Dschang. Fourteen (14) (1%) samples were confirmed HIV-positive by the gold standard against 1506 (99%) participants confirmed HIV-negative.

The OraQuick® HIV-1/2 Rapid OMT-based test detected a total of 14 positive samples, of which 13 were true positives and 1 was a false positive; thus the sensitivity and positive predictive value of 93% and 92.86% respectively. Equally, of the 1506 negative samples confirmed by the gold standard, 1505 samples were detected HIV-negative by OraQuick® HIV-1/2. Thus the occurrence of 1 false negative result with the OraQuick® HIV-1/2 Rapid OMT-based test, giving a specificity of 99.93% and a negative predictive value of 99.9 %. The performance of OraQuick test under evaluation in comparison to the gold standard couple is summarised in the table below A kappa concordance of 0.965 (p = 0.035) was also observed.

Discussion

The complexity of some HIV serodiagnostic tools has always contributed to limit the current efforts to scale-up HIV control and prevention initiatives and programs. HIV serodiagnosis particularly in resource limited settings has been hindered by several factors, including the need for trained personnel, high cost of equipment and reagents, and lack of

electricity and laboratory infrastructure (UNAIDS/WHO, 1998). The development of rapid tests has brought solution to most of the above mentioned drawbacks, but continuous improvement is needed.

Table 1: Performance of the OraQuick test.

Test kits	True positive	True negative	False positive	False negative	Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)
Gold standard (*Determine* + "*Shanghai*"	14	1506	0	0	100 (98.5-100)	100 (99.2-100)	100 (98.5-100)	100 (99.2-100)
Test under evaluation (OraQuick® HIV-1/2 Rapid OMT	13	1505	1	1	93.0 (66.0-99.0)	99.9 (99.0-100)	92.9 (66.0-99.0)	99.9 (99.9-100)

CI, confidence interval; **Se**, sensitivity; **Sp**, specificity; **PPV**, positive predictive value; **NPV**, negative predictive value.

Results of this study demonstrated that oral fluid testing with OraQuick® HIV-1/2 Ab Rapid OMT test presents sensitivity and specificity acceptable in the range required by WHO. In our population, the test presents a specificity of 99.9% and a sensitivity of 93%. These values are comparable to the gold standard as demonstrated by the kappa concordance of 0.96 (p = 0.035) observed in our study. Such results are reflective of the prevalence observed in the country (4.3%) (NIS, 2012), since it has been found that prevalence of the disease largely affects performance of rapid tests (CDC/WHO/APHL, 2001). For example, similar field evaluations of the same kit gave sensitivity and specificity of 99.3% and 99.6% in Los Angeles (Delaney *et al*., 2006) where HIV prevalence was 5.1% and in East London (5.73% of prevalence) where it gave 93.64% and 99.87% as sensitivity and specificity respectively (Zelin *et al.*, 2008) meanwhile in Zimbabwe where the prevalence was estimated at 29.8%, a sensitivity and a specificity of 100% (Sophie *et al*., 2009) have been observed when using the oral fluid test.

The high viral diversity of HIV reported in Cameroon could have an impact on HIV test kits performance, since 2 discordant results were reported, which lowered the test performance. In the first case, one of the samples was found reactive to the gold standard test; meanwhile it was not reactive with the oral fluid kit. In fact, Aghokeng *et al.* (2009) as well as Ndembi *et al.* (2011) reported that some HIV serological assays fail to detect some HIV strains such as HIV-1 group O and N, which are particularly found in Cameroon. False negative can also occur with *OraQuick* when used to screen asymptomatic patients with unknown HIV status (Delaney *et al*., 2006; Bhore.*et al*., 2003), particularly those with early HIV infection (Stekler *et al*., 2007) or when used in patients receiving highly active antiretroviral therapy. These are individuals infected with HIV-1 or HIV-2 and receiving highly active antiretroviral therapy (HAART) and who have fluctuating levels of antibodies (O`Connell *et al*., 2003).

In the second case, one sample was reactive with *OraQuick* while it was not with the gold standard. This is called false positive. It tends to lower the specificity of a test and can be explained by some factors resulting from the patient's physiological state. These are: multiparous women, rheumatoid factor, Epstein Barr virus, HAV, HBV, a state of treatment with interferon, antigrippal viral vaccine, hyper-gammaglobulinemia, and others (*OraQuick* info package insert).

The cost/efficacy analysis showed that even though expensive, this kit is safer, easier to use, non-invasive, and offers a better comfort to patients and medical staff, thus limiting risks of blood exposure, compared to other available tests. Many countries based their HIV testing programs on HIV blood-based tests which are considered gold standards for HIV testing. This explains why in our study, 85% of our participants claimed to have never heard about testing using oral fluid before and they were therefore skeptical about the use of this, for HIV screening. However, this kit presents advantages which make it very suitable to be implemented in outreach medical settings of Cameroon, and for hard to reach populations.

A limiting factor to our means and tentative to bring explanations on these false positive and false negative case observed was the fact that samples were not conserved for further laboratory analyses. However, occurrence of false positive results when using this kit, reminds us the paramount importance of test confirmation, when carrying out HIV screening either with blood or oral fluid.

The field evaluation of OraQuick® HIV-1/2 Ab Rapid test brings out the fact that this test presents comparable specificity and sensitivity as well as many advantages rendering it suitable to be implemented in medical outreach settings of Cameroon as well as to hard to reach populations, regarding its friendly use character. This also suggests that, if included in the Cameroonian national algorithm for HIV testing, it could greatly impact on HIV control initiatives and programs, by increasing participation at screening sites and allowing a larger national coverage for better disease prevalence estimation. It will surely result in a well and better targeted disease control program, for a rapid, more adequate and well-designed treatment to ensure disease regression as well as mortality reduction. A large scale evaluation of oral fluid testing and a determination of its impact on HIV prevalence and control program in some outreach areas of Cameroon and beyond are therefore recommended. As to the Cameroonian ministry of public health it is recommended to negotiate a partnership with the manufacturer or to establish a sponsorship with some financial partners to lower the price of the kit and render it affordable to populations.

Acknowledgements

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