Rapid oral fluid HIV antibody testing in exposed babies: A diagnostic study

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ABSTRACT

Aims: The usefulness of rapid oral fluid HIV antibody tests has rarely been evaluated in exposed babies.

Study Design: A diagnostic survey comparing the performance of OraQuick oral HIV antibody test and Determine the routine rapid blood screening test.

Place and Duration of Study: University College Hospital, Ibadan and Nigerian Institute of Medical Research, Lagos, between May 2010 and April 2011.

Methodology: The study involved children aged less than 18 months referred for screening in two large HIV care programmes in Nigeria using OraQuick (Test A) and Determine (Test B). The testing was blinded and HIV status was confirmed using DNA PCR.

Results: A total of 94 children were studied with ages ranging from 0.13 to less than 18months. Out of the 94 parallel tests, when compared with DNA PCR, there were 7 (7.5%) discordant results. Test A gave one false positive, one false negative and no indeterminate result. Test B gave four false positive, one false negative and two indeterminate results. Test A had a sensitivity of 93.3%, specificity of 98.7%, positive predictive value of 93.3% and negative predictive value of 98.7% compared with Test B which had 90.0%, 92.9%, 60.0% and 98.7% respectively. Among the caregivers 88 (93.6%) preferred oral fluid testing to blood as it is painless and easy to perform.

Conclusion: Compared with Determine, OraQuick correlates better with DNA PCR in detecting the absence of infection in HIV exposed babies. Given this performance, it may be useful in expanding testing in HIV exposed children in settings where there are challenges with early infant diagnosis.

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INTRODUCTION

Oral fluids have been employed to measure antibodies to infectious agents such as measles and hepatitis viruses [1]. Oral fluid based point-of care HIV tests have also been developed, based on detection of HIV antibodies in oral fluid of patients infected by HIV. These tests have been found to be convenient and also ensure quick results [2]. The development of rapid tests generally has improved the access to HIV testing as results are available within 30 minutes [3]. The use of oral fluids for HIV testing offers other advantages over that of blood [4]. Sample collection is safer thereby reducing occupational risk of exposure to HIV; in addition the ease of sample collection is an advantage as it is minimally invasive. In children, obtaining venous blood is difficult and requires expertise and finger pricks are painful. The HIV testing protocol for exposed infants who were enrolled in the routine Prevention of Mother-to-Child Transmission (PMTCT) programme from birth in many paediatric HIV programmes in the Nigeria involves DNA PCR blood test at six weeks and repeated at three months (where possible). Otherwise, the initial DNA PCR test is carried out at first presentation and the second test at least one month apart from the first. At each of these stages the turnaround time is not short as a result of various logistic challenges.

Studies carried out among adults have demonstrated oral fluid HIV testing to be accurate in many settings. A high risk adult population in the UK was tested for HIV with OraQuick and the results were compared with an enzyme immunoassay [5]. A test sensitivity of 100% was obtained with OraQuick. In another study carried out to validate the performance of rapid tests in 5 clinical trial sites in Africa and one site in the United States, OraQuick and Determine had overall sensitivities of 99.3% and 99.8% and specificities of 99.3% and 99.4% respectively [6]. In the United States, there were concerns about the performance of oral fluid-based OraQuick test as a result of false positive results that were reported. A field investigation was carried out which did not identify a cause for the increase in false-positive oral-fluid results, and the incidence study detected no false-positive results [7].

The sensitivity and specificity of a given HIV serological assay in children may not be the same as that reported in adult populations. An HIV exposed child might have maternal antibodies, but if uninfected is unlikely to have the antigen. Additional assessment of the performance characteristics of the available serological assays is required in paediatric populations. Currently, there are few published data on the use of oral fluid HIV tests in children [8]. Furthermore, there are very few studies comparing oral fluid tests with another rapid antibody tests in children. Most other studies compared oral fluid test with enzyme linked immunosorbent assay (ELISA) which has now been replaced by rapid antibody testing in the routine screening for HIV in many resource constrained countries [9]. In

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developing countries where early infant diagnosis with DNA PCR has remained a challenge, it is important to evaluate possible alternatives.

A very small percentage of infants born to HIV infected women are tested within the first 2 months of life [10]. Diagnosis of HIV in children less than 18 months with DNA PCR is a challenge in many countries in Africa even where the Early Infant Diagnosis (EID) programme which utilizes Dried Blood Spot (DBS) technique has been adopted. Multiple steps are involved in DBS for PCR testing; from sample collection to storage, transportation, laboratory analysis and return of results. A recent review of early infant HIV-1 diagnosis programmes in resource limited settings showed high losses to follow-up at each step in the EID cascade [11]. Point-of-care testing using a rapid test is therefore required as it could circumvent most of the logistic problems associated with collection and transportation of DBS samples. This will reduce the rates of loss to follow-up such that the diagnosis could be made during the initial presentation affording early initiation of treatment in positive children. Incorporating initial screening with rapid HIV tests into the conventional testing algorithm to screen-out HIV-uninfected infants was found to be cost effective and would reduce programme costs in resource limited settings [12].

The objective of the study was to evaluate the usefulness, caregiver acceptability and preference for oral fluid HIV testing with OraQuick compared with finger prick blood testing with Determine which is the routine rapid test currently used for screening in Nigeria.

MATERIALS AND METHODS

This was a diagnostic study, carried out in the Paediatric HIV programmes of the University College Hospital, Ibadan and the Nigerian Institute of Medical Research (NIMR), Lagos. These health institutions are located in the South-Western zone of Nigeria and their HIV care programmes are large, accepting referrals from many states both within and outside the zone. The subjects were children aged less than 18 months of unknown HIV status who presented in the two health care facilities and were being followed up in the PMTCT programme or presenting for the first time with symptoms suggestive of HIV infection or requiring HIV tests as part of the Provider Initiated Testing and Counselling (PITC) initiative, [13]. Those referred to the study facilities for HIV screening from other health care facilities were also included.

Research Definitions:

- HIV exposed baby: A baby born to an HIV infected mother and may be negative or positive
- Perinatal HIV Infection: An exposed infant who has at least two positive HIV DNA PCRs.

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The study assistant designated to perform the oral fluid HIV testing was trained on the use of OraQuick (Test A) - OraSure Technologies Inc, - prior to the beginning of the study and was blinded to the HIV status of the children when performing the test. Demographic data were collected on both the children and their caregivers. Children who had commenced antiretroviral drugs were excluded from the study. All the caregivers of the children had pre and post-test counseling. Information was also collected on whether or not mother had prevention of mother to child transmission and Antiretroviral (ARV) prophylaxis in pregnancy.

Determine HIV 1/2 (Test B) - manufactured for Abbott Laboratories by ABBOTT JAPAN CO., LTD. Minto-Ku, Tokyo, Japan - is a rapid immunochromatographic test used on plasma, whole blood or serum to detect antibodies to HIV types 1 and 2. Test A also detects antibodies in the aforementioned fluids but in addition is used to detect antibodies to HIV-1 and HIV-2 in oral fluid. The children of consenting caregivers who gave informed consent had finger prick blood taken for HIV-1/2 antibody using Test B and were also tested with Test A on oral fluid. Both kits were used according to the manufacturers' instructions. Oral fluid was collected by the trained study staff according to pack instructions where the upper and lower gums were swabbed (at least once exposing the flat pad against gum lines). The test kit was inserted into the developer vial and results read after 20 minutes. Test A (oral fluid) rapid test was performed in parallel with Test B rapid blood HIV test. Verification of the rapid test results was done by the laboratory technician in the HIV programmes who was trained on rapid testing. A test was interpreted as positive if there was a clear test band in addition to the control band, negative if the test band was absent and indeterminate (weakly positive) if the test band was faint. Venous blood samples were collected on the same day for DNA PCR for the confirmation of the HIV status of the children.

Following the performance of the tests, one-on-one interviews were conducted with each caregiver to assess acceptability and preference having observed the two specimen collection methods (oral fluid vs. finger prick blood), these were documented.

Data were entered into a microcomputer and analyzed using the SPSS 12.0 for Windows. Continuous variable estimates were expressed as mean (±SD) and median while categorical variables were in proportions, ratios and percentages. The sensitivity, specificity, positive and negative predictive values were calculated for both Test A and Test B. Indeterminate (weakly positive) tests were counted as positive since they raise a suspicion of presence of HIV antibodies.

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RESULTS

A total of 94 children were tested. The ages of the children ranged from 0.13 to 17 months with a median of 6.0 months. There were 47 (50.0%) males and an equal number of females (50.0%). Out of the 94 children, the rapid tests were 92.6% concordant with HIV DNA PCR as 79 (84.0%) children were negative while 15 (16.0%) were positive.

Test A and Test B results compared with reference standard (DNA PCR)

Out of the 94 parallel tests of Test A and Test B, when compared with the gold standard (DNA PCR), there were 7 (7.5%) discordant results. Tables 1, 2a and 2b show that compared with HIV DNA PCR, Test A gave one false positive, one false negative and no indeterminate result. Test B gave four false positive, one false negative and two indeterminate results which were regarded as positive. DNA PCR was negative in six out of the seven discordant test results. When the six children were evaluated clinically, they had no symptoms suggestive of HIV disease; however, the only child who was positive by DNA PCR (but negative by both rapid tests) was symptomatic. Her CD4 count was 1329 /µl (15%) while the viral load was 2.4 million copies per ml. In addition to these, her mother did not receive ARV prophylaxis in pregnancy.

Table 1: Discordant results of Test A and Test B compared with DNA PCR

Serial No	Age (months)	Sex	ARV prophylaxis in mother	Test A	Test B	DNA PCR
1	3	Male	Yes	Negative	Positive	Negative
2	6	Female	No	Negative*	Negative*	Positive*
3	6	Female	Yes	Negative	Positive	Negative
4	9	Female	Yes	Negative	Indeterminate	Negative
5	12	Male	Yes	Negative	Positive	Negative
6	14	Male	Yes	Negative	Positive	Negative
7	14	Male	Yes	Positive	Indeterminate	Negative

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Table 2a: Test A compared with the gold standard DNA PCR

		DNA PCR		Total
		+	-	
Test A	+	14	1	15
	-	1	78	79
Total		**15**	**79**	**94**

Table 2b: Test B compared with the gold standard DNA PCR.

		DNA PCR		Total
		+	-	
Test B	+	9	6	15
	-	1	78	79
Total		**10**	**84**	**94**

Test A compared with Determine test results

The performance of Test A compared with Test B is shown in table 3. Test A had a sensitivity of 93.3%, specificity of 98.7%, PPV of 93.3% and NPV of 98.7% compared with Test B that had 90.0%, 92.9%, 60.0% and 98.7% respectively.

Table 3: Performance of Test A compared with Test B using DNA PCR as gold standard (n=94)

Performance	**Test B**		**Test A**	
	Estimate	**[95 % CI]**	**Estimate**	**[95 % CI]**
Sensitivity (%)	90.0	[59.6- 98.2]	93.3	[70.2-98.8]
Specificity (%)	92.9	[85.3-96.7]	98.7	[93.2-99.8]
PPV (%)	60.0	[35.7-80.2]	93.3	[70.2- 98.8]
NPV (%)	98.7	[93.2- 99.8]	98.7	[93.2- 99.8]

Acceptability and preference of oral fluid vs. finger prick blood testing by caregivers

The mean age of the 94 caregivers was 33.0 ±7.3yrs. The relationship to the child and level of education of the caregiver showed that mothers constituted 90.4% (85) of the caregivers, 67 (71.0%) of whom had at least secondary education.

Acceptability of oral fluid HIV test was 100% as all caregivers accepted the use of the kit. However, oral fluid testing was preferred by 88 (93.6%) caregivers. The reasons given for its preference were; painless to the child 79 (89.5%), ease of testing 8 (9.1%) and being novel 1 (1.4%). The remaining 6 (6.4%) caregivers who preferred blood testing believed that blood would be more reliable and accurate in testing for HIV.

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DISCUSSION

The study showed that there was discordance between the two rapid tests and the DNA PCR confirmatory test. The discordance between the rapid tests and DNA PCR could be explained by the peculiar immunology of HIV in children in which they acquire maternal HIV antibodies trans-placentally. These antibodies may persist for as long as 18 months of age or longer. Schupbach et al in their study in Switzerland reported that traces of HIV-reactive IgG persisted as long as 21months [14]. Furthermore, the levels and clearance time of the persisting maternal antibodies may vary among infants. Moodley et al reported 100% maternal antibody clearance by 15months, [15] while Jendis et al reported that HIV specific IgG was detected in 100% of their cohort by 12months and was still positive in 83% by 13 to 18 months. Weak HIV-specific IgG were also observed in the later study above the age of 15 months with no other signs of HIV infection, suggesting that the demonstration of antibodies in children beyond this age did not necessarily indicate HIV infection [16].

HIV infected individuals on Antiretroviral Therapy (ART) have been known to have false negative results with rapid tests as was the case in a South African study carried out among adults by Scott et al [17]. The present study excluded children who were on ART; therefore the one case of false negative result for both Test A and Test B could not be explained by use of ART. This child with false negative rapid test results who was positive by DNA PCR was aged 6 months and was symptomatic. Observer errors have also been implicated in another study. When OraQuick was evaluated in a high-risk population in the United Kingdom; reduced sensitivity was obtained and was adduced to false-negatives considered to be due to observer error. This was rectified by further training and correcting this error which resulted in increased test sensitivity to 100%. The authors then concluded that the observed test performance of OraQuick compared well with Enzyme linked Immunosorbent Assay (EIA) and with other rapid tests [5]. Observer error was not considered in our study as the two rapid tests were carried out in parallel and quality control measures were in place such that only the trained study assistant consistently carried out the tests which were independently verified by the routine staff. In our study the cause of the false negative and indeterminate rapid test results is therefore not obvious.

In our study, among the seven test results that were discordant between the rapid tests and DNA PCR, Test B had six false positives and only one false negative, while Test A had one false positive and one false negative. That Test B gave more false positives than Test A may suggest that the former is a better screening test as it is more proficient in detecting persisting maternal antibodies. The high antibody detection rate of Test B may be an advantage as the identification of exposed infants will serve to identify their positive mothers which will in turn, facilitate provision of treatment and

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care to women, and prevent transmission of HIV during subsequent pregnancies. From the point of view of Test A giving less false positive results, it appears to be a better diagnostic test. There is a potential for this characteristic to be explored further as it offers hope for use as HIV point-of-care rapid test with available results on the same day of presentation. There are obvious advantages to this as it will be cost effective and more convenient compared to the current modalities employed in PCR-testing.

Whether the discordance in the detection of antibodies by Test A and Test B resulted from the variation in antibody levels in blood as supposed to oral fluid or variation in individual children, this could not be confirmed as antibody estimation was beyond the scope of the present study. As the two rapid tests used in this study are based on antibody detection, their performance could also have been compared against another antibody detection test which would be the gold standard. However, there are no reliable HIV-1–specific antibody assays to differentiate between maternal and autologous antibody in children [18]. Use of ELISA was not considered as gold standard in this study for the above reason and also as it is no longer used routinely in screening children, its use being limited by its long turnaround time, cost and the need for skilled technologists [8]. This study rather aimed at comparing these two rapid tests with DNA PCR which is the gold standard as it detects viral particles. Nevertheless, the study carried out among 105 children in South Africa by Feucht et al studied had shown excellent correlation between OraQuick and ELISA [19] They compared the use of OraQuick test in oral fluid and whole blood both of which were in turn compared with ELISA. They reported that Oraquick oral fluid and whole blood results compared well with HIV ELISA result as 100% sensitivity, specificity, negative and positive predictive values were recorded for both tests. It is to be noted though that the ages of the children in their study ranged between 16 and 139 months.

The acceptability of 100% in the present study was not surprising as a similar finding was reported in another study in which HIV test was performed among secondary school students in Tanzania [20]. The OraQuick oral fluid-based test was also found to be highly preferred by the participants in an Indian study [21]. When the STD clinics in New York offering on-site rapid tests replaced finger-stick whole-blood testing with oral fluid testing, they recorded increased uptake of HIV testing [22]. As minimal skill is required in carrying out oral fluid testing, this would significantly improve access to testing in the health facilities and community settings. This is particularly relevant in resource-constrained countries with poor laboratory infrastructure, lack of skilled phlebotomists and laboratory technologists. In the case of HIV Counseling and Testing programmes among the paediatric age group, oral fluid testing would appeal to caregivers as it is less invasive and is quick. This is important in facilitating screening for early identification of infected children. With the high acceptance of oral testing among caregivers, access to testing can be expanded in children and will be valuable in the

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follow up of babies in PMTCT programme as well as in the PITC strategy. However, such a test should be highly sensitive and specific.

The World Health Organization (WHO) recommends that HIV serological assays used for the purpose of clinical diagnostic testing should have a minimum sensitivity of 99% and specificity of 98% [8]. The observed sensitivities for Test A and Test B were 93.3% and 90.0% respectively in the children less than 18 months which both fell short of the above recommendation. The specificities for both tests were high, though only Test A met up with the recommendation as it had a specificity of 98.8% compared with Test B which recorded 96.4%. With regards to the diagnostic utility and practical use of these rapid tests in the Early Infant Diagnosis programme, the negative predictive value will be more appropriate in this instance as it can tell how likely an exposed baby with a negative test result will actually not have HIV. The positive predictive value is also useful as it tells how likely an exposed baby with a positive test result will actually have HIV. As the NPV of both Test A and Test B rapid tests is high (98.7%), the tests would perform well in excluding a diagnosis of HIV in an exposed baby. The PPV for Test A was higher (93.3%) compared with Test B (60.0%), meaning that Test A is a better predictor of the likelihood of an exposed baby to have HIV. Though the study suffers from small number of HIV positive cases which could weaken the comparisons, this may not apply judging from the confidence intervals around the sensitivity, specificity, positive predictive value and negative predictive value.

Conclusion

In this study, the less sensitive detection of HIV antibodies by OraQuick correlates better with the absence of infection. It is possible to make a case that for exposed babies, perhaps in the absence of symptoms and where the mother has received adequate ART prophylaxis, negatives by the OraQuick would be a pointer to an uninfected infant who may not require confirmation by DNA PCR test. This may be a cost effective alternative that may be useful in expanding testing in HIV exposed children in settings where there are challenges with the HIV DNA test. A cost/benefit analysis of this approach would be very helpful, given the ease of carrying out the test and its acceptability.

CONFLICT OF INTERESTS: The oral fluid HIV testing kits were sourced from OraSure technologies Inc. and were donated unconditionally. No further funding or other form of support was obtained from the company for the research.

AUTHORS' CONTRIBUTIONS

RO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. ND, BB and KO participated in the study design and protocol. BB also managed the analyses of the study. All authors read and approved the final manuscript.

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ETHICAL APPROVAL

Ethical approval for the study was obtained from the Oyo state Health Research and Ethics Committee.

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