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Evaluation of saliva-based diagnostic test kit for routine detection of antibodies to HIV

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Summary
The study was carried out to determine the reliability of a saliva based test kit for routine detection of HIV antibodies. 150 paired plasma and saliva samples were collected from 50 patients who were known to be positive for HIV-I and 100 others whose HIV serostatus were previously unknown. All the plasma samples were tested for HIV antibodies using Novopath Immunoblot Technique (as the gold standard), Wellcozyme (Murex) ELISA, Latex Agglutination Test (Capillus) and SeroCard Kit. The saliva samples were screened for HIV antibodies using SalivaCard Test Kit. All the 50 known positive patients tested positive when retested with immunoblot and 9 of 100 whose serostatus were unknown also tested positive giving a total of 59 positive results and 91 negative results. Of the 59 positive results, 59, 57, 58 and 47 were correctly identified as true positives by Wellcozyme, Capillus, SeroCard and SalivaCard respectively. Of the 91 negatives, 90, 91, 90 and 85 were correctly identified as true negatives respectively.
Sensitivities in the same order were 100%, 97%, 98.3% and 79.7% whilst specificities were 98.9%, 100%, 98.9% and 97.8%. Whereas evaluation parameters for Wellcozyme, Capillus and SeroCard test kits met the criteria for licensure of a test kit as a routine test method for HIV antibody detection, the SalivaCard values fell far short of the stipulated criteria. The Sensitivity, Test Efficiency and Positive Predictive Values of 79.7%, 88% and 67.8% respectively obtained for SalivaCard are too low and the test kit cannot be recommended for routine use as HIV antibody detection kit.

Keywords: *Saliva, HIV-antibodies, Specificity, Sensitivity, Positive Predictive Value, Negative Predictive Value.*

Resumé
L'étude a été faite pour déterminer la précision d'un Kit de dépistage basé sur la salive pour la détection routinière desanticorps VIH. 150 paires de plasma et de salive ont été prélévéde 50 malades dont la séropositivité en VIH-1 était connue and 100 autres dont les séroprévalances de VIH n'étaient connu au préalable. Tous les prélèvements ont été examiné pourles anticorps VIH en utilisant la technique Novopath Immunoblot (comme lanorme d'or), Wellcozyme (Murex) ELISA, Latex Ag glutination Test (Capillus) et Kit de Serocard. Lesprélèvements de salive ont subit un dépistage pour des anticorps en utilisant le kit de dépistage SalivaCard. Tous les 50 séroposif du VIH ont teste posifit encore après avoir subit un autre testavecl'immunoblot et 9 parmi les 100 dont la séroprévalance n'était pas connu ont aussi testé positif donnant un total de 59 résultats positifs et 91 négatifs. Des 59 résultats, 59, 57, 58 et 47 ont été correctement identifiés comme des vrais positifs par Wellcozyme, Capillus, SeroCard et SalivaCard respectivement
Des 91 négatifs, 90,91, 90 et 85 ont été identifiés correctement comme étant respectivement de vrais. Dans le même ordre de sensitivité était 100%, 97%, 98.3% et 79.7% tandis que les spécificités étaient 98.9%, 100%, 98.9% et 97.8%.Tandis queles paramètres d'évaluation de Kit de dépistage de Wellcozyme, Capillus et SeroCard étaient conform auxcritaires d'octroi de licence d'un Kit de dépistage servant de méthode d' analyse routinière de la détection desanticorps VIH, le Saliva Card ne répondait pas à ces critières.la sensitivité, l'efficacité de dépistage et les positives valeurs prédictives de 79, 7%, 88% et 67,8% respectivement obtenus pour le Saliva Card sont très bas et leKit de dépistage ne peut être recommendé pour une détection routinière d'anticorps de VIH.

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Introduction

Between 1981 when the first cases of AIDS were described in the United States of America [1,2] and 1985, diagnosis of HIV/AIDS was mainly presumptive as has been described elsewhere [3]. The discovery of the causative agents of AIDS in 1983 [4] opened the doorway for development of laboratory techniques for confirmatory diagnosis of HIV infection.

The initial tests developed were ELISAs (Enzyme Linked Immunosorbent Assays) because these types of tests had been used successfully for the detection of the presence of other infectious viral agents [5], Soon after the licensure of ELISA for routine screening of blood for the detection of HIV antibodies in 1985 [6], other techniques became available such as Gelatin Particle, Latex [7] and Red Cell [8] Agglutination tests. Thereafter, dot-blot assays and newer generation ELISAs which incorporate the use of recombinant or synthetic peptide antigens (rather than crude viral lysate antigens for the detection of viral antibodies) were developed. As technology evolved, so did the newer tests, both in performance and in the ease of administration. As such, the newest generation of screening tests are claimed to be so sensitive as to reduce the serologic window period to about 3 to 4 weeks [9].

Major drawbacks of all these tests, however, are that they require an invasive procedure (bleeding with needles and syringes) to obtain materials (blood) for testing. Several health workers have become infected as a result of accidental needle injury following such procedure [10]. An approach aimed at reducing the risk of occupational exposure is the development of home based [9] and office based [11] test systems whereby the blood is obtained by the use of a lancet blade and a filter strip with blotted blood, which is mailed in a protected envelope to the laboratory using an anonymous code [9].

The discovery of HIV antibodies in the saliva [12,13,14] and the urine [15] implies that serological testing for the diagnosis of HIV/AIDS can be carried out without recourse to any invasive procedure whether needle or lancet. An added advantage of the use of saliva or urine as test specimen is that they are easy and less expensive to collect [9]. A number of test methods based on saliva are now being marketed. They have been reported to have sensitivity as high as 99.9% [9,16]. Doubts

exist, however, as to whether all the available kits will have reliability measure as high as this despite the fact that the concentration of IgG in the saliva is well above 0.5mg/L, the level necessary for detection of HIV antibodies [9]. As such, continuous evaluation and re-evaluation exercises are required as a measure of reliability for these test kits.

Reports of some of these evaluation exercises have shown that saliva dependent serologic testing for detection of HIV antibodies may be significantly less specific and less sensitive compared with plasma or serum dependent testing [17,18]. Recently, SalivaCard, a saliva-based test kit was introduced into Nigeria. Evaluation of the performance characteristics of this novel test kit in terms of sensitivity, specificity, test efficiency and positive and negative predictive indices in the Nigerian population is the object of the present communication. Some of the well established test kits such as Wellcozyme (Murex) and Capillus (Latex Agglutination Test) were also re-evaluated for comparison. A SeroCard Test Kit from the same manufacturer as the SalivaCard (Trinity Biotech Plc, Dublin, Ireland) was also evaluated.

Materials and methods

Paired plasma and saliva samples were collected from patients attending Haematology Outpatients Clinic of the Lagos University Teaching Hospital after informed consent was obtained. The patients were in two groups - those whose HIV serostatus were known (Western blot confirmed positive) and those whose serostatus were yet to be determined.

All of the plasma samples were tested for HIV antibodies using:

1) Wellcozyme (Murex) HIV-I and II Kit (WellcozymeKit no. K895010)
2) Capillus HIV-I and II (Cambridge Riotech Ltd.) Lot No BO: 7408
3) SeroCard HIV-I and II (Trinity Biotech Plc, Dublin, Ireland) Lot No. D2153

All assays were carried out according to the manual instructions of the manufacturers. Western blot test was run on all the plasma samples using Novopath HIV-I Immunoblot (Bio-Rad lot no. 9710968) as the reference test [19]

The saliva samples were obtained using a collection device (orapette) supplied by the manufacturer. The orapette consists of:

1) Receiver
2) Rayon ball
3) Plunger

The rayon ball is a pad, which is used to scrape the gum and the cheek until wet and then placed in the receiver. The plunger is after this, placed in the receiver to squeeze out the saliva which is allowed to drop onto the sample port of a pre-prepared SalivaCard. The test was done immediately after saliva collection. There was no need for storage or transportation.

The SalivaCard has four ports - two sample ports, a non-specific peptide binding (NSB) reaction port and a test reaction (TR) port coated with synthetic HIV 1 and 2 peptides. The card incubated at ambient temperature for two minutes during which saliva moves from corresponding sample ports to NSB and TR ports where antibody in the saliva complex with peptides. Following a washing step, alkaline phosphatase conjugated antihumanglobulin is added to the reaction ports and the card incubated again at room temparature for another two minutes. After a second washing, BCIP substrate solution is added and the card incubated again for five minutes before result is read. Development of a blue colour at the TR port and no colour at NSB port indicate a positive result whilst no colour or light blue colour at both TR and NSB ports indicate negative result. Development of dark blue colour at NSB irrespective of any colour at TR port invalidates the result and the test is repeated.

Evaluation was carried out by determining:

a) False Positive Results (FPR)
b) False Negative Results (FNR)
c) True Positive Results (TPR)
d) True Negative Results (TNR)

The results of Novopath Immunoblot were used as the standard [19] and evaluation parameters:

a) Sensitivities
b) Specificities
c) Test Efficiencies (TE)
d) Negative Predictive Value (NPV)
e) Positive Predictive Value (PPV)

were calculated for each assay kit. NPV and PPV calculations were based on National Seroprevalence value of 5.5% [20]. Table 1 shows the method of calculation of these evaluation parameters. Values of specificities, sensitivities and predictive indices were compared using chi-square test.

Table 1: Method of calculation of specificity, sensitivity and test efficiency.

Test result	HIV antibodies Present	Absent	Total
Positive	A (TPR)	B (FPR)	(A + B)
Negative	C (FNR)	D (TNR)	(C + D)
Total	A + C	B + D	A+B+C+D

TPR = True positive result FPR = False positive result
TNR = True negative result FNR = False negative result

$$\text{Sensitivity} = \frac{A}{A+C} \times \frac{100}{1}$$

$$\text{Specificity} = \frac{D}{D+B} \times \frac{100}{1}$$

$$\text{Test Efficiency} = \frac{A+D}{A+B+C+D} \times \frac{100}{1}$$

Results

A total of 150 patients were bled and their saliva collected. Of these, 50 were already confirmed HIV-I positive and were attending HIV/AIDS Clinic at Haematology Outpatients in LUTH. All were immunoblot positive on retesting. Out of the 100 patients whose serostatus were previously unknown, nine (9) were confirmed to be positive for HIV-I by immunoblot assay giving a total of 59 positive results and 91 negative results.

Evaluation Results

Wellcozyme Kit (Murex)

All the 59 positive results by immunoblot were correctly identified by this kit as positive (TPR) giving No FNR. Of the 91 negative results, 90 were correctly identified as negative (TNR) giving one FPR. As such, sensitivity, specificity and test efficiency were found to be 100%, 98.9% and 99% respectively. The calculation of predictive indices based on National Seroprevalence Rate of 5.5% is presented in table 2. The PPV and NPV were respectively 84.1% and 100%.

Capillus (Latex Agglutination Kit)

With this kit, 57 of the 59 positive results were correctly identified as positive with two (2) FNR while all the 91 negative

results were correctly identified - No FPR. Sensitivity, Specificity and TE were respectively 97%, 100% and 98.7%. The PPV and NPV were 100% and 99.8% respectively (table 2).

recommended test method in most routine laboratories, although the test procedure consumes a lot of time (at least two hours). Wellcozyme is reputed for yielding 100% sensitivities in many

Table 2: Calculation of PPV and NPV based on HIV seroprevalence of 55/1000 in Nigeria[20]

Calculation parameters	Assay kits				
	Immunoblot	Wellcozyme	Capillus	SeroCard	SalivaCard
Senstivity % (S)	100	100	97	98.4	79.7
Specificity % (SP)	100	98.9	100	98.9	97.8
Test true positive (TTP) = PTPN(55) x S	55	55	53.4	54	43.8
False negative (FN) = PTPN - TTP	0	0	1.6	1	11.2
Test true Negative (TTN) = PTNN(945) x SP	945	934.6	945	934.0	924.8
False positive (FP) = PTNN - TTN	0	10.4	0	10.4	20.8
Positive predictive Value % = $\frac{TTP \times 100}{TTP + FP}$	100	84.1	100	83.9	67.8
Negative predictive Value % = $\frac{TTN \times 100}{TTN + FN}$	100	100	99.8	99.9	98.8

PTPN = Prevalence of True Positive in Nigeria =55/1000
PTNN = Prevalence of True Negative in Nigeria = 945/1000

eroCard Kit

his kit correctly identified 58 of the 59 positive results with ne FNR. It identified 90 of the 91 negative results correctly ith one FPR. It has a test sensitivity, specificity and effiiency of 98.3%, 98.9% and 98.7% respectively. The PPV and PV were 83.9% and 99.9% respectively.

alivaCard Kit .

his kit had the highest FNR, 47 of the 59 positive results being rrectly identified with 12 FNR. It also had the highest false sitive result of six (6), 85 of the 91 negative results being rrectly identified. Its sensitivity (79.7%) and specificity 7.8%) were significantly different from that of immunoblot ith assigned value of 100%. $P<0.05$ for each chi-square test. he PPV and NPV were 67.8% and 98.8% respectively (table

Iscussion

ensitivity and specificity are two important parameters used the determination of reliability of a test kit for the detection of IV antibodies. Whereas the sensitivity is designed to elimiate false negative results by being able to detect very small nount of the antibodies in a given specimen, specificity is esigned to eliminate false positive results by being able to disriminate between HIV antibodies and other similar antibodies. or transfusion service, specificity may be sacrificed for sensivity. This ensures that no blood with the slightest amount of IV antibodies is undetected. At worst, some pints that are ly negative will also be discarded as being positive.

he Wellcozyme test kit perfectly meets this criterion and is the evaluation studies [21,22]. In this study, Wellcozyme, Capillus and SeroCard with sensitivities of 100%, 97% and 98.4% and specificities of 98.9%, 100% and 98.9% respectively, have all met the criteria for Licensed ELISA Kits [23]. Latex Agglutination Test (Capillus) [24] and SeroCard [22] have been found to give comparable results with immunoblot tests in previous evaluation studies.

For Saliva Card, all evaluable parameters, except specificity, had values, which fell far short of the required values for Licensure for routine use as test kit for HIV antibody detection [23] The reason for these low values may not be unconnected with the low concentration of immunoglobulins in the saliva. The concentration of IgA in saliva is 87% of that in the plasma. However IgG concentration in the saliva is much lower being approximately 1/1000th of its serum concentrations (14mg/L in saliva compared with 14,700mg/L in plasma [25]). Since HIV antibodies are IgG, the low concentration of this immunoglobulin in the saliva may explain the unacceptably high number of FNR (12 out of 59).

The low levels of IgG immunoglobulins in the saliva may not totally explain our present results because a level of IgG, much lower than 14mg/L (0.5mg/L), has been reported to be detectable by sensitive test kits [9]. Archibald [16] screened saliva samples using Western blot procedure and found 95% sensitivity and 100% specificity - values much higher than our present findings of 79.7% and 97.8% respectively. A report [26] from Cote d'Ivoire where 75 saliva samples were evaluated for HIV antibodies using GAC ELISA yielded a sensitivity of 100% and specificity of 97.7%. Thus, the discrepancy between the present and previous reports may be accounted for

by differences in test kits. It is possible that the SalivaCard contains low antigen concentration on the reaction port. Low antigen concentration may result in prozone phenomenon whereby antigen-antibody reaction is impaired as a result of disproportionate concentrations of antigens and corresponding antibodies [17]. Test efficiency of 88% for SalivaCard indicated the inability of this test kit to correctly identify all true positive and true negative test results. Although NPV of 99% implies that a person who tested negative by SalivaCard has a probability of 0.99 of being truly negative, an individual who tested positive by this kit has a probability of 0.68 of being truly positive in a population with high HIV seroprevalence of 5.5% [20] This is unacceptable and the kit should not be recommended for routine screening of HIV antibodies as it is now.

References

1 Gottlieb MS, Shroff R, Schanker H.M; *et.al*: Pneumocystis Carinii Pneumonia and Mucosal Candidiasis in previously healthy homosexual men. N Eng J Med 1981; 301:1425-31.

2 CDC: Undifferentiated Non-Hodgkin's Lymphoma among homosexual males. United States. MMWR 1982; 31:227-30.

3 Akanmu AS and Akinsete I.: Diagnosis of HIV/AIDS: Evolution, Problems and Applicability of diagnostic methods in Nigeria. 1998; Nig Med J; Suppl. 34, 43-49.

4 Gallo R C, Salahuddin S Z, Popovic M; *et.al*: Frequent detection and isolation of cytopathic retro viruses (HTLV-III) from patients with AIDS and risk for AIDS. Science 1984; 224:500-503

5 Saag M S: AIDS Testing, Now and in the Future In: Sande MA, Volderberg P A, eds. The Medical Management of AIDS. 3rd edn. Philadelphia. W.B. Saunders 1992. pp 33-53

6 CDC Update: Serologic Testing for antibody to human immuno- deficiency virus. MMWR 1988; 33:833-40.

7 Riggins CH, Beltz GA, Hung CH.; *et.al*: Detection of antibodies to human immunodeficiency virus by Latex Agglutination with recombinant antigen. J Clin Microbiol 1987; 25:1772-73

8 Kemp BE, Rylatt DB, Bundesen PG; *et.al*: Autologous red cell agglutination assay for HIV-1 antibodies. Simplified test with whole blood. Science 1988; 241:352-54.

9 Bartler J G: Laboratory Tests In: Medical Management of HIV infection. Baltimore Maryland. John Hopkins's University Press 1998.

10 Bartler JG.: Natural History and Classification: Mecha nisms of Transmission. Ibid. Pgs 9-12.

11 Gellert GH, Moore DF., Weismuller PC, Greenwood R, and Maxwell R M.: Office based system for HIV antibody (letter). N Eng J Med 1993; June 10, 328:211.

12 Soto-Ramirez LE, Hemández-Gomez L and Sifuentes-Orsornio J.: Detection of specific antibodies in gingival and crevicular transudate by enzyme-linked immunosorbent assay for diagnosis of human immuno- deficiency virus type-1 infection. J Clin Microbiol 1992; Nov 30. 11. 2780-83.

13 Urquia M, Rodrigue-Archilla A, Gonzalez-Moles MA., and Ceballos A.: Detection of anti-HIV antibodies in Saliva. J Oral Pathol Med 1993; April 22. (4)153-56.

14 Hunt AJ., Connel J and Christofinis G.: The testing of Saliva samples for HIV-1 antibodies. Reliability in non-clinic setting. Genitourin Med 1993. 69(1):29-30.

15 Connell JA, Parry JV., Mortimer P.P., *et.al*: Preliminary report: accurate assays for anti-HIV in urine. AIDS 1990; 335:1366-69

16 Archbald DV and Hebert CA.: Salivary detection of HIV-1 antibodies using recombinant HIV-1 peptides. Viral Immunol 1991; 4:17-22.

17 Constantine NT, Callahan JD and Watts DM.: Urine and Saliva Tests In: Mitchel S., Gringle R. Eds. HIV Testing & Quality Control. A guide for laboratory personnel. Durham Family Health International 1991. Pgs 32-33.

18 Morris DF.: False Positive Salivary HIV Test (letter) BMJ 1992; Oct 31. 305(6861):1093-4

19 CDC Update: Serologic Testing for HIV-1 antibody - United States 1988 and 1989; MMWR 1990. 39(22) 380-83.

20 National AIDS/HIV/STD Control Programme. FMOH and Services. 1999 HIV/Syphilis Sentinel Seroprevalence Survey in Nigeria. Technical Report.

21 Rai A, Kuman S and Prabhakaran P.K.: Evaluation of different commercial kits for HIV/HTLV-II. J Commun Dis 1991. 23(2):149-53.

22 Chaiari U, Sirivichagakul S, Phamuphak P, Panmony E and Ubolyam S.: Comparison of the sensitivity of various anti-HIV tests in early sero- conversion sera. Asian-Pac J Allergy-Immunol 1991; Dec. 9. (2)95-100.

23 Robert Z, Cordes DO, Michael e and Ryars DO.: HIV Testing. Post-graduate Medicine 1995; 985 No. 5.

24 Van-de-Perre P, Nzaramba D, Allen S, Riggin EH., Sprechier-Gold Berger and Butzner J.P.: Comparison of six serological assays for human immunodeficiency virus antibody infections in developing countries. J Clin Microbiol 1988; 26(3) 552-56.

25 Tamashiro H and Constantine H.T.: Serological diagnosis of HIV infection using oral fluid samples. Bull World Health Organisation 1994; 72(1) 135-43

26 Gershy-Damet G.M, Koffi K and Abougal: Salivary diagnosis of human HIV-1 and 2 infection in Cote-d'Ivoire using two assays. Trans R Top Med Hyg 1992; Nov-Dec 86(6) 70-71.