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EVALUATION OF FALSE POSITIVITY AND CROSS REACTIVITY IN THE INVESTIGATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ANTIBODIES

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ABSTRACT

This study evaluated the causes of false positive Human Immunodeficiency Virus test results (*F*+HIV), cross reactivity of HIV antibodies with other non HIV antibodies, and efficiency of the serial and parallel testing algorithms. 100 blood samples randomly collected from clients attending the Heart to Heart HIV counseling and testing unit of FMC Umuahia, were screened using the rapid ELISA and Enzyme Immuno Assay (EIA) tests. Discordant HIV results were screened for Anti Streptolycin O (ASO), Rheumatoid factor (RF) and Hepatitis B surface antigen (HBsAg). Of the 100 samples, 73 were negative to HIV antibodies, 11 positive, and 16 discordant results. EIA confirmed 8 of 16 discordant results negative, 5 indeterminate and 3 positive. *F*+ results were 33%, and false negatives were 4%. A marked percentage of samples exhibited cross reactivity with ASO (8;62%), HBsAg (3;23%), and RF (2;15%). An Odds Ratio (OR) of 33:0 (95% CI 13.8-26.2), showed that Determine rapid test kit is 33 times more likely to give a false positive HIV result than Unigold rapid test kit. The parallel algorithm showed better efficiency than the serial. This study showed that *F*+HIV test result is prevalent, and cross reactivity is the plausible cause of *F*+HIV test results.

Keywords: Cross- reactivity, false positive, HIV, Umuahia.

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INTRODUCTION

Chronic infection with Human Immunodeficiency Virus (HIV) induces a progression of the depletion of $CD4^+$ cells in individuals infected with the virus (Kashala *et al*., 1994). The resultant effect of this depletion is a weak immune system that gives room for opportunistic infections. Thus, early and correct diagnosis of the HIV virus is necessary to minimize the depletion of CD4+ cells.

HIV screening tests and confirmation algorithms according to Walensky et al. (2008) are crucial because in this era of potent anti retroviral therapy, timely and correct diagnosis of HIV infection in the emergency wards and other testing sites are critical to ensure maximal treatment benefits. Walensky *et al.* (2008) reported that even highly accurate tests may be reactive in the absence of disease, especially when prevalence of the disease is low; thereby giving rise to false positive results. This could create psychological as well as financial problems for patients.

Wrong diagnosis has caused a lot of problems in diagnostic laboratories worldwide, and the psychological trauma that accompanies a wrong diagnosis cannot be quantified. The psychological trauma that accompanies wrong diagnosis was evident in the report of Dielenberg (2011) presented in The Star online news publication titled "*Patient wrongly diagnosed with HIV awarded RM 150,000*". In the report, a father of five in Malaysia ran away to an unknown destination after a private hospital misdiagnosed him as HIV positive. Gardner (2010) noted that "trials have had problems recruiting people because of the stigma and repercussions" that accompanies a positive HIV test result.

False-positives to HIV protein have been documented (Ng, 1991) and the rising cases of false positive HIV (*F*+HIV) results with the ELISA, and indeterminate HIV results with the western blot assay has been linked to the reaction of HIV tests with many different diseases and conditions other than HIV (Cross-reactivity) (Treanor, 2006). Cross-reactivity is said to be a specific phenomenon in immunology and though cross-reacting antigens differ, they share common determinants and reaction is specific in respect to these determinants.

However, about 70-80% of the immunoglobulins produced by B-cells in response to an antigen are said to be non-specific (Koliadin, 1998). Proteins are renowned for their specificity of function. There is some evidence that many proteins, from enzymes to antibodies, are functionally confused and react indiscriminately (James and Tawfik, 2003). Cross-reaction occurs when an antibody directed against one protein, also reacts with another different protein against which that antibody is not directed (Jayapal, 2007). Cross reactivity of antibodies is a very crucial one because the bane of wrong diagnosis partially depends on it. Hence, the possibility of cross reactivity and false positivity should be considered when testing for HIV antibodies.

The national guidelines for HIV diagnosis is either a serial or a parallel algorithm. This is carried out with rapid diagnostic tests (RDTs), usually based on the ELISA technique. According to Shanks (2012), RDTs are screening tests not designed for definitive diagnosis, but very essential for blood transfusion screenings and emergency cases. RDTs are however, said to yield false positive results (Shanks, 2012).

This study aims at evaluating the false positivity of HIV test results and cross reactivity of HIV antibodies with other non HIV antibodies, such as Anti Streptolysin O (ASO), Rheumatoid factor (RF) and Hepatitis B Surface Antigen (HBsAg), using rapid Determine HIV 1/2 test and Unigold test kit. Hence, this study shall evaluate the sensitivity and specificity of these test kits and determine the efficiencies of the parallel and serial testing algorithms.

MATERIALS AND METHODS

Study Area: The study was conducted at the Federal Medical Centre Umuahia, located in the State capital situated in Umuahia North Local Government Area of Abia State Nigeria. The Umuahia North local Government Area is bounded by Umuahia South, Aba North, Bende, and Okigwe Local Government Areas. The indigenous language is Igbo.

Inclusion criteria: Adult males and females of 18 years and above attending the Heart to Heart HIV counseling and testing clinic at the time of the study, whose HIV statuses were not known, were recruited for this study.

Exclusion criteria: Children below the age of 18 years, pregnant women, confirmed cases of HIV, and clients who recently received any form of vaccination were excluded.

Ethical consideration: Ethical approval was sought and granted by the Research and Ethics Committee of Federal Medical Centre Umuahia, while informed consent was obtained from willing participants.

Duration of study: the study was conducted within a five week period (June/July, 2012).

Study Design: Diagnostic test performance assessment within the framework of a cohort study.

Sample size and method of sampling: Based on the estimated false positive HIV results of 7% (Shanks, 2012) and the level of confidence (95%), the sample size was calculated to be $99.92 \approx 100$, using the formula $n= t^2 x P(1-P) / m^2$ (UNICEF, 2005). [Where n= Desired sample size, t= Desired confidence level (95%) = 1.96., P= Estimated prevalence of false positive HIV test results, m = Degree of accuracy desired (Margin of error) (0.5% ie 0.05)]

Hence, a sample size of 100 was used for the study. 25 samples were collected the first week, 15 the second week, 20 the third week, 15 the fourth week, and 25 the fifth week.

Sample collection: A total of 100 blood samples were randomly collected into serum separator vacutainer tubes from clients attending the Heart to Heart HIV Counseling and testing unit of the Federal Medical Center (FMC) Umuahia, Abia State, Nigeria, after obtaining informed consent. 2ml venous blood samples were drawn from each subject, and the samples serially labeled and allowed to cloth. The sera were then collected into clean sterile cryovials and stored in the refrigerator at 2-6 ^{O}C until samples were analyzed.

Method of sample analysis: HIV testing was performed with Determine (Abbott Diagnostic Division, Netherlands) and Unigold (Trinity Biotech plc, Ireland) using the National guidelines (Serial and parallel Algorithms). Samples that gave discordant HIV screening results with the two kits were further subjected to Enzyme immunoassay (EIA) testing, to confirm the true sera-status of the samples using the Immuno-Comb 11 HIV 1&2 Comb-Firm kit (Orgenics).

The samples that gave discordant results were also screened for ASO, RF and HBsAg. All assays were carried out as described by the manufacturers. The true sera-status of samples was established using the following algorithm: Samples reactive by EIA twice were considered positive while samples that were non

reactive by EIA twice were considered negative (FMOH, 2006).

Data analysis: Statistical analysis was done using the Odds Ratio (OR) test to calculate the odds or likelihood of having a false positive result. The sensitivity and specificity analyses were determined to know the level of sensitivity and specificity of the test kits. OR value >1 was considered to be statistically significant.

RESULTS

Table 1 shows the HIV ELISA Screening Reaction by Determine and Unigold Test kits. While seventy three (73) samples reacted negative to HIV antibodies with both test kits, eleven (11) samples reacted positive with both kits. Thirteen (13) samples reacted positive to HIV antibodies by Determine kit, but reacted negative by Unigold test kit while three (3) samples reacted positive to HIV by Unigold, but reacted negative by Determine.

Table 2 shows the Confirmatory reactions for HIV discordant subjects and follow-up with ASO, RF and HBsAg screening tests. EIA confirmed the 73 samples that reacted negative by both kits as negative. EIA confirmed 11 samples that reacted positive to both kits as positive. However, EIA confirmed 8 of the 16 discordant results as negative, 5 as indeterminate, with only a single band positive for glycoprotein 120 (gp120) and 3 as positive.

Table 3 shows the percentage representation of the sensitivity and specificity of the rapid test kits. The sensitivity and specificity of Determine were 84% and 90% respectively. While the sensitivity and specificity of Unigold was both 100% excluding the indeterminate cases. Percentage false positives with Determine test kit was 33%, and Percentage false negative was 4%

Statistical test results showed the Odds ratio of 33:0 indicating that Determine test kit is 33 times more likely to give a false positive result than Unigold. Also, determine is 33 times more likely to cross-react with non HIV antibodies than Unigold

Figure 1 shows the cross reactivity of HIV antibodies with ASO, RF. and HBsAg. Of the 16 discordant results, 8 (62%) samples reacted with ASO, 3 (23%) with HBsAg, and 2 (15%) with RF. However, the 3 positive samples by Unigold, but negative by Determine were none reactive to these other tests.

In this study, the 8 of the 13 discordant cases were considered to be false positives, and the 3 of the 76 discordant considered false negatives. The 8 false-positives (S20, S27, S30, S37, S44, S69, S75 and S93) had a positive reaction with ASO; 2 of the 5 indeterminate (S 6 and S72) had a positive reaction with RF, while 3 of the indeterminate (S40, S52 and S82) had a positive reaction with HBsAg.

Table 1: HIV ELISA Screening Reaction with Determine and Unigold Test kits

	ELISA Test		
Total no of samples	Determine Test	Unigold Test	Result
73	-	-	Negative
13	+	-	Discordant
11	+	+	Positive
03	-	+	Discordant

Key: - = Non reactive; + = Reactive

DISCUSSION

The results of this study has further confirmed that false positivity and cross reactivity in HIV investigation exist and hence a cause for concern that should be given attention. The 33% false positive results recorded in this study, though higher, are in accordance with the findings of Shanks (2012), who reported a false positive HIV result rate of 7% and the study of Scarano (2011) who reported a 0.2 percent false positive HIV result with ELISA tests. Furthermore, Arora (2003) reported that various structural components of *Streptococcus pyogenes* exhibit antigenic cross reaction with different tissues

of the human body. Pregnancy, particularly multiple pregnancies and some disease conditions such as leprosy, malaria and tuberculosis have also been implicated in false positive HIV test results.

Table 2: Confirmatory reaction for HIV discordant subjects and follow-up with ASO, RF and HBsAg

Sample No	ELISA		EIA (HIV 1&2 CombFirm)					HIV Result	ASO	RF	HBsAg	Inference
Sample No	Determine Test	Unigold Test	P24	P31	gp120	gp41	gp36	HIV Result	ASO	RF	HBsAg	Inference
S6	+	-	-	-	+	-	-	Ind	-	+	-	Ind
S20	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S27	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S30	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S31	-	+	+	-	+	+	-	Pos	-	-	-	F-ve
S37	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S40	+	-	-	-	+	-	-	Ind	-	-	+	Ind
S44	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S52	+	-	-	-	+	-	-	Ind	-	-	+	Ind
S69	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S72	+	-	-	-	+	-	-	Ind	-	+	-	Ind
S75	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S80	-	+	+	+	+	+	-	Pos	-	-	-	F-ve
S82	+	-	-	-	+	-	-	Ind	-	-	+	Ind
S93	+	-	-	-	-	-	-	Neg	+	-	-	F+ve
S97	-	+	+	-	+	+	-	Pos	-	-	-	F-ve

Key : ASO = Antistreptolysin O Titre; RF = Rheumatoid factor; HBsAg = Hepatitis B surface antigen; - = Non reactive; + = Reactive; Ind = Indeterminate; Pos= Positive; Neg= Negative; F+ve = False positive; F-ve = False negative.

Table 3: Percentage representation of the sensitivity and specificity of the rapid test kits.

Test Kits	Total no tested	Total +ve	Total -ve	False +ve (%)	False – ve (%)	Sensitivity (%)	Specificity (%)	Odds Ratio
Determine	100	24	76	33	04	84	90	33:0
Unigold	100	14	86	0	0	100	100	0:0

Key: +ve = positive; -ve = negative;

Scarano (2011) also reported in the article "*Causes of a False Positive HIV Test*" that *Rethinkingaids.com* noted a report by the American Medical Association, where in a preliminary health department data from August- November 1999; 32 pregnant women were diagnosed as HIV positive. However, a confirmatory test showed that 17 of the women were actually negative to the HIV virus. This is in accordance with our findings whereby of the 27 samples that reacted positive to HIV antibodies initially, only 14 were confirmed to be true positives.

With the Odds Ratio of 33:0 (95% CI 13.8-26.2), it is evident that there is a good number of false positive HIV cases in the Umuahia metropolis of Nigeria. This result is not markedly different from that recorded by Walensky et al. (2008), where they recorded positive likelihood ratio of 8 to 32 with Ora Quick HIV screening test. In their Universal Screening for HIV infection in the Emergency Room (USHER) trial study in the emergency department with Ora Quick HIV test, of the 31 patients who initially tested positive to HIV antibodies, only five were found to be HIV-positive after undergoing a

confirmatory test to establish the true sero-status of the Ora-Quick tests.

Cross reactivity with ASO, RF and HBsAg with HIV antibodies as observed in this study, resulting in false positive HIV result, corroborate the reports of Scarano (2011), where cross reactivity was observed in persons who had prior infections with hepatitis B, malaria or tuberculosis. Other persons, who had received flu vaccinations, had autoimmune diseases, and multiple pregnancies were also implicated in stimulating the production of antibodies that cross reacted with HIV antigens, thereby resulted in false positive HIV test (Scarano, 2011).

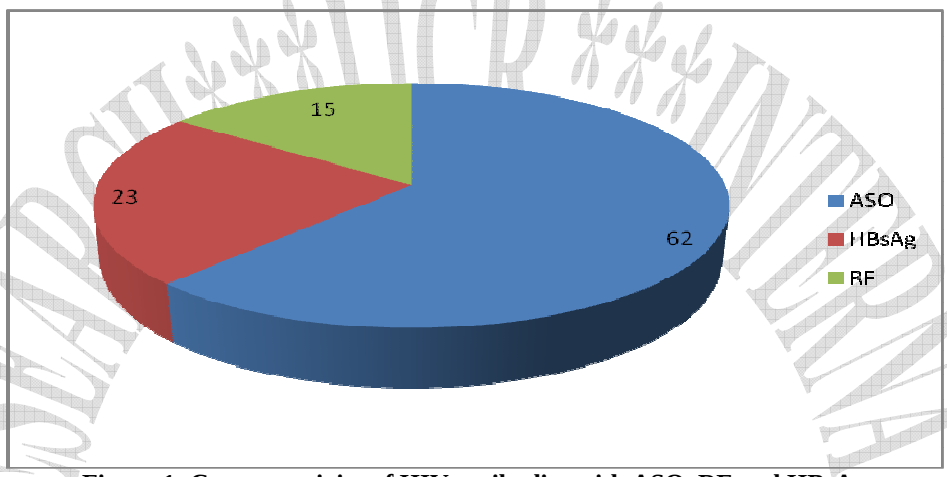

Figure 1: Cross reactivity of HIV antibodies with ASO, RF.and HBsAg

The sensitivity and specificity results of Unigold in this study met the expected results of the evaluation studies of Delaney et al. (2011), where sensitivity and specificity of Unigold was 100%. However, the sensitivity and specificity results of Determine did not meet the evaluation of 99.4% sensitivity and 99.6% specificity recorded by van den Berk et al. (2003), but was rather lower than anticipated. This study showed that three positive reactions by Unigold test kit were not detected by determine test kit when the parallel algorithm was applied. This gave the parallel algorithm an edge over the serial because with the serial algorithm, the first line test (Determine) was negative. Going by the algorithm no further testing was required.

In conclusion therefore, with the percentage of false positives and cross reactivity recorded in this study, it is advisable that medical diagnostic laboratories should not rely on a single HIV testing result, but a confirmatory test be carried out using antigen based testing kits to avoid litigations; as a fall out of false positive results as in the Negeswara case whereby the sum of RM 100,000 was awarded for medical negligence and Rm 50,000 for defamation when he was misdiagnosed of being HIV positive (Dielenberg, 2011). In order to achieve this, it is recommended that a confirmation test be added to improve the test algorithm. Also, adding, and improving quality control, establishing an external quality control assessment scheme. Finally, quality assurance programs should be established to oversee the quality of tests results produced by the public and private sector to ensure that reliable and accurate results are released by laboratories.

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AUTHORS' CONTRIBUTIONS

Amechi B.O. is the principal investigator involved in the design of the study. Chikwendu, C.I. was involved in sample collection and screening, while Osagie, R.N., analyzed and interpreted data.