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RESEARCH LETTER

Detection of highly divergent HIV-1 in clinical specimens using rapid HIV serologic assays

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1 | INTRODUCTION

HIV continues to be a major global public health issue, and there are still to be many challenges hindering the effort to control and eradicate infection. According to recent estimates, 14.2% of people living with HIV in the United States have not been diagnosed as being HIV positive. HIV diagnosis is a primary step in the effort to end the HIV epidemic as it allows access to prevention and treatment. Thus, considerable efforts have been mounted to address the performance and reliability of HIV testing. Rapid diagnostic tests (RDTs) that detect the presence of antibodies against HIV-1/2 and/or HIV p24 antigen are the preferred assays for routine diagnosis of HIV because they are fast, easy to use and accurate. To be approved for commercial use, WHO and FDA guidelines on HIV testing recommend that the performance of RDTs should meet a sensitivity and specificity ≥98%.[1] Nevertheless, investigations have shown that the performance of RDTs may be challenged by various factors such as the genetic variability of HIV, coinfection or the presence of endogenous/exogenous substances.[2-4] Ongoing monitoring of commercial assays for HIV diagnosis is helpful and recommended, notably because of the increasing prevalence of divergent HIV-1 variants worldwide.

The HIV epidemic in Cameroon, a west central African country, is characterized by broad genetic diversity. Although recent reports indicate that the epidemic in this region is dominated by HIV-1 CRF02_AG. Cameroon exhibits a high number of cocirculating HIV-1 subtypes, a high intra-subtype diversity, and the possibility of an ongoing evolution with the occurrence of new recombinant viruses.[5-7] To address the concern related to the impact of HIV diversity on diagnosis, we investigated the ability of FDA-approved rapid serologic assays to detect divergent HIV strains in clinical specimens obtained from Cameroon.

2 | METHODS

2.1 | Specimens

Specimens used in this study were stored, deidentified plasma specimens collected from HIV positive and negative individuals in Cameroon. According to the record, specimens were collected between 2006 and 2017, and were tested for HIV antibodies at the site of collection using the Determine HIV 1/2 rapid antibody assay (Abbott, Wiesbaden, Germany), the assays in routine use in Cameroon for HIV diagnosis. Of 954 plasma specimens received, 705 (73%) were classified as HIV-1 positive and 249 (27%) as HIV-1 negative based on testing in Cameroon. In our laboratory, these specimens were retested with six FDA-approved RDTs (Table 1).

2.2 | Laboratory assays

Testing was performed according to manufacturers' instructions by trained laboratory staff. Specimens with reactive results for each of the assays used were classified as HIV-1 reactive, HIV-2 reactive, HIV-1/HIV-2 reactive, and HIV nonreactive. In the event of an indeterminate result, testing of related specimens was repeated before the result was recorded.

2.3 | Ethical considerations

This study was approved by the FDA Institutional Review Board, the US HHS/Food, and Drug Administration Research in Human Subjects Committee, and the exempt reference number is 01-044B. Specimens used had all been previously obtained for another purpose and were deidentified for the purpose of this study.

3 | RESULTS

Demographic and clinical data were available for most specimens. HIV-infected plasma specimens included in this study were mostly from females (72%). At the sampling date, the median age was 35 (range from 14 to 77 years old) and 70% were naïve of any antiretroviral treatment. The average HIV-1 RNA viral load was 86 445 copies/mL (range: <40-1 537 201 copies/mL) and the median CD4 cell count was 360/mm^3 (range: <40-1 537 201). Based on partial genotyping (*pol*, *gag or env* sequences), the plasma specimens harbored various divergent HIV-1 strains including five subtypes (F2, A1, G, D, and A2), several CRFs (02_AG, 22_01A1, 01_AE, 06_cpx, 09_cpx, 11_cpx, 13_cpx, 18_cpx, and 19_cpx,) and a few unclassified strains.

TABLE 1 Characteristics of selected FDA-approved RDTs

Immunoassay	Trade name	Manufacturer
HIV-1/HIV-2 antibody detection	OraQuick ADVANCE Rapid HIV-1/2 antibody test	OraSure Technologies
	Uni-Gold Recombigen HIV-1/2	Trinity Biotech
	Clearview HIV 1/2 STAT-PAK	Chembio Diagnostic Systems
	Clearview COMPLETE HIV 1/2	Chembio Diagnostic Systems
	Multispot HIV-1/HIV-2 rapid test	Bio-Rad Laboratories
Antigen/antibody detection	Alere Determine HIV-1/2 Ag/Ab Combo	Alere Scarborough

Abbreviation: RDTs, rapid diagnostic tests.

Results of testing showed that depending on the RDTs used, antibodies against HIV-1/2 were detected in 97% to 99.7% of specimens previously shown to be antibody-positive (Table 2). Testing of HIV negative specimens showed varying levels of specificity depending on the individual RDTs used and the results shown in Table 2.

All specimens previously determined to be HIV-1 antibody-positive in Cameroon, when tested were antibody-positive/antigen-negative when tested using the Alere HIV-1/2 Ag/Ab Combo assay (N = 392). Among these HIV-1 positive specimens, the Ag/Ab combo test detected no p24 Ag positive specimen. Further, specimens that were reactive with the Alere Ag/Ab combo test were also 99% concordant with the OraQuick, Uni-Gold Recombigen and Clearview COMPLETE, and 100% with Multispot HIV-1/HIV-2 and Clearview HIV 1/2 STAT-PAK. Similar results were observed with specimens that tested nonreactive.

Multispot HIV-1/HIV-2 rapid test, the only HIV-1 and HIV-2 discriminatory rapid test included in our study, detected antibody against HIV-1 in more than 99% of specimens shown to be previously reactive. In the remaining 1% (6 of 608), we observed an indeterminate HIV-1/HIV-2 profile, suggesting potential coinfection with HIV-1 and HIV-2 or cross-reactivity. These latter specimens were negative according to testing in Cameroon. Four of the six specimens were reactive using the other RDTs in our study. The lack of reactivity of the remaining two specimens may be a confirmation of their HIV-1 status given that they were weakly reactive with the Multispot assay.

Overall, results obtained from the selected RDTs used in our study in determining HIV status were highly concordant. Taking

TABLE 2 Results of testing using selected RDTs

RDTs used (No. of specimens tested)	Test result (No. of specimens tested)	Pre-screen HIV positive; % (N)	Pre-screen HIV negative; % (N)
Orasure Oraquick HIV-1/2 (929)	Nonreactive (247)	11.7 (29)	88.3 (218)
	Reactive (682)	98.5 (672)	11.5 (10)
Uni-Gold HIV1/2 (929)	Nonreactive (239)	11.7 (28)	88.3 (211)
	Reactive (690)	97.5 (673)	2.5 (17)
Multispot HIV-1/2 (827)	Nonreactive (219)	9 (20)	91 (199)
	Reactive (608)	97 (591)	3 (17)
STAT-PAK HIV-1/2 (827)	Nonreactive (226)	9 (20)	91 (206)
	Reactive (601)	98 (591)	2 (10)
Sure Check HIV-1/2 (891)	Nonreactive (233)	10 (24)	90 (209)
	Reactive (658)	98 (648)	2 (10)
Alere HIV-1/2Ag/Ab (435)	Nonreactive (43)	35 (15)	65 (28)
	Reactive (392)	99.7 (391)	0.3 (1)

Abbreviation: RDTs, rapid diagnostic tests.

results from all our selected RDTs, with the exception of the Alere HIV-1/2 Ag/Ab Combo assay, the rate of false-positive results at the collection site was between 9% and 13%. The rate of false-negative results was lower and ranged from less than 1% to 3% (Table 2).

4 | DISCUSSION

FDA-approved rapid serologic tests for the diagnosis of HIV have shown excellent performance characteristics[8,9] for HIV diagnosis in patients. However, HIV genetic diversity is a significant concern with respect to virus diagnosis. In this study, detection of antibodies against HIV-1/2 was confirmed in a majority of the specimens from Cameroon originally classified as HIV-1 positive. The rate of false-positive results at the collection site of up to 13% was comparable to the 12% previously reported in Cameroon,[2] and may be explained by the performance characteristics of the rapid test used. It may also be due to intercurrent infections prevalent in the West African setting that could cause cross-reactivity leading to potential false-positive results. False-negative results are more likely to be associated with recent or acute HIV infection which is not detected by antibody assays.[3] However, this could not be verified in our study as there were no p24 antigen positive specimens detected using the Alere antigen/antibody rapid assay which is the only combo assay used, suggesting that the specimens in our collection were likely from patients with well-established HIV infection.

In this study, we observed a high degree of concordance of the results between the selected RDTs we analyzed suggesting their substantial ability to detect HIV infection in clinical specimens of divergent strains. Overall, FDA-approved rapid HIV serologic tests showed good performance with diverse HIV-1 strains and the impact of virus diversity on their ability to aid in the diagnosis of HIV infection is likely to be low. The current practice of using multiple tests to confirm infection further helps to ensure a high degree of accuracy of rapid HIV serologic assay.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Christelle Mbondji-Wonje, Indira Hewlett
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All authors have read and approved the final version of the manuscript.

Christelle Mbondji-Wonje had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

Christelle Mbondji-Wonje affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and, that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request and in compliance with the FDA policy.

DISCLAIMER

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

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