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## Evaluation of four rapid tests for diagnosis and differentiation of HIV-1 and HIV-2 infections in Guinea-Conakry, West Africa

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### ABSTRACT

With both HIV-1 and HIV-2 prevalent in Guinea-Conakry, accurate diagnosis and differentiation is crucial for treatment purposes. Thus, four rapid HIV tests were evaluated for their HIV-1 and HIV-2 diagnostic and discriminative capacity for use in Guinea-Conakry. These included SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc.), Genie II HIV1/HIV2 (Bio-Rad), First Response HIV Card Test 1-2.0 (PMC Medical) and Immunoflow HIV1-HIV2 (Core Diagnostics). Results were compared with gold standard tests (INNO-LIA HIV-1/II Score) and NEW LAV BLOT II (Bio-Rad). Four hundred and forty three sequential stored HIV-positive serum samples, of known HIV-type, were evaluated. Genie II HIV1/HIV2, Immunoflow HIV1-HIV2 and SD Bioline HIV 1/2 3.0 had 100% sensitivity (95% CI, 98.9–100%) while for First Response HIV Card Test 1-2.0 this was 99.5% (95% CI, 98.2%–99.9%). In terms of discriminatory capacity, Genie II HIV1/HIV2 identified 382/384 (99.5%) HIV-1 samples, 49/52 (95%) HIV-2 and 7/7 (100%) HIV-positive untypable samples. Immunoflow HIV1-HIV2 identified 99% HIV-1, 67% HIV-2 and all HIV-positive untypable samples. First Response HIV Card Test 1-2.0 identified 94% HIV-1, 64% HIV-2 and 57% HIV-positive untypable samples. SD-Bioline HIV 1/2 3.0 was the worst overall performer identifying 65% HIV-1, 69% HIV-2 and all HIV-positive untypable samples.

The use of SD Bioline HIV 1/2 3.0 (the current standard in Guinea-Conakry) as a discriminatory HIV test is poor and may be best replaced by Immunoflow HIV1-HIV2.

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### 1. Introduction

HIV testing remains the cornerstone of national HIV programs, being the entry point to HIV/AIDS prevention, care and treatment. In resource-limited settings, rapid HIV tests offer an innovative, non-sophisticated and robust alterna-

tive to enzyme-linked immunoassays (ELISA) and western blot (WB) testing. This is because they are relatively simple to use, do not need trained laboratory technicians or specific infrastructure, the results are available in about 15 minutes, point-of-care decision making is possible and the approach is convenient for both patient and clinician.<sup>1</sup> Furthermore, algorithms based on a combination of two or more simple rapid assays have been shown to have diagnostic accuracy comparable to the gold standard testing strategy with the exception of individuals in the seroconversion phase.<sup>2–6</sup>

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In countries where HIV-1, HIV-2 and dual infection (HIV-1+ HIV-2) co-exist, it is important to accurately diagnose which specific type of HIV the person is infected with. This is crucial to ensure that patients are initiated on appropriate antiretroviral (ARV) regimens. Accurate diagnosis of HIV-2 is particularly important as HIV-2 is intrinsically resistant to non-nucleoside reverse transcriptase inhibitors,<sup>7,8</sup> one of the pillar (first line regimen) drugs used in ARV regimens for the treatment of HIV-1. Guinea-Conakry is one of such countries where both HIV-1 and HIV-2 co-exist. In Guinea-Conakry, Médecins sans Frontières (MSF) run an HIV/AIDS program in close collaboration with the Ministry of Health. This being a referral centre for HIV/AIDS care, HIV testing is performed with a serial algorithm using two distinct rapid HIV assays: the Determine HIV-1/2 assay (Abbott Laboratories, Tokyo, Japan) and the SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc, Kyonggi-do, South Korea). Concern about the atypically high number of reported HIV-positive untypable infections using this testing algorithm have led to doubts about the accuracy of SD Bioline HIV 1/2 3.0 as a discriminatory test for HIV-1 and HIV-2. Given this and the dearth of published information on the capacity of HIV rapid tests to differentiate between HIV-1 and HIV-2<sup>3,9–11</sup> we evaluated the performance of the currently used discriminatory rapid HIV test (SD Bioline HIV 1/2 3.0) and three other rapid tests for their HIV-1 and HIV-2 diagnostic and differentiation capacity using a panel of serum samples from Guinea-Conakry.

## 2. Materials and methods

### 2.1. Study setting and serum sample selection

This study was conducted in Matam, Guinea-Conakry in collaboration with the Institute of Tropical Medicine (ITM), Antwerp, Belgium between November 2007 and January 2008. Following HIV testing using specific testing algorithms, sequential samples of pooled positive serum specimens were selected on the basis of their known HIV serological type: 404 samples were collected in Guinea-Conakry (250 HIV-1, 150 HIV-positive untypable and 4 HIV-2 samples, classified on the basis of the routine National HIV testing algorithm as described below), and 41 HIV-2 samples (classified according to the AIDS Reference Laboratory (ARL) testing algorithm as shown in Figure 1) were provided by ITM out of their laboratory collection, to increase the power of the study.

In Guinea-Conakry, the HIV/AIDS program uses a serial testing algorithm with two distinct rapid HIV assays for HIV diagnosis: the Determine HIV-1/2 assay (Abbott Laboratories) and the SD Bioline HIV 1/2 3.0 (Standard Diagnostics Inc.). Sera that react negatively with the Determine test are considered true HIV negative and are not investigated further. Sera that are reactive with the Determine HIV-1/2 but negative with the SD Bioline HIV 1/2 3.0 are considered discordant (i.e. do not fulfill the criteria for being HIV negative or HIV positive). A positive HIV diagnosis is made when

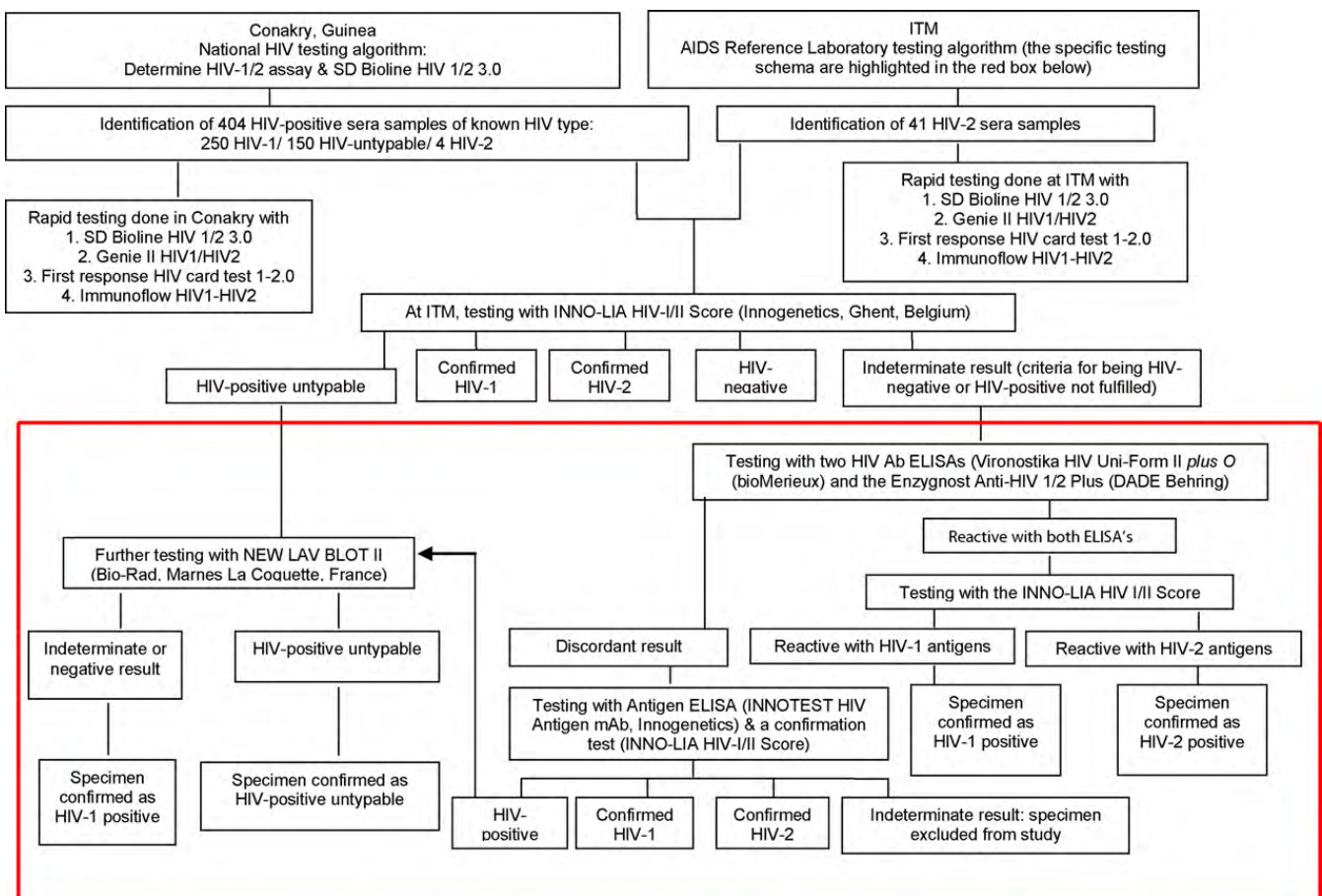


Figure 1. A flow diagram showing the HIV testing schema used to diagnose and confirm HIV serotypes.

**Table 1**  
Characteristics of four selected HIV rapid tests

	RAPID TESTS			
	Genie II HIV1/HIV2	SD Bioline HIV 1/2 3.0	Immunoflow HIV1-HIV2 HIV1-HIV2	First Response HIV Card Test 1-2.0
<b>Manufacturer</b>	Bio-Rad, Marnes-la-Coquette, France	Standard Diagnostics Inc, Kyonggi-do, South Korea	Core Diagnostics, Birmingham, UK	PMC Medical, India Pvt Ltd, Mumbai, India
<b>Assay Type</b>	Immunochromatographic assay, lateral flow	Immunochromatographic assay, lateral flow	Immunochromatographic assay, lateral flow	Immunochromatographic assay, lateral flow
<b>Specimen type</b>	serum / plasma	whole blood / serum / plasma	whole blood / serum / plasma	whole blood / serum / plasma
<b>Storage conditions</b>	2–8 °C	4–30 °C	4–30 °C	4–30 °C

the assays are concordantly positive and then the SD Bioline HIV 1/2 3.0 enables HIV type to be determined (HIV-1, HIV-2 or HIV positive untypable – when HIV type can not be identified).

## 2.2. Selection of rapid HIV assays for evaluation

Four rapid HIV tests were selected for evaluation of their ability to diagnose and discriminate between HIV-type: SD Bioline HIV 1/2 3.0, Genie II HIV1/HIV2 (Bio-Rad, Marnes-la-Coquette, France), First Response HIV Card Test 1-2.0 (PMC Medical, India Pvt Ltd, Mumbai, India) and Immunoflow HIV1-HIV2 (Core Diagnostics, Birmingham, UK) (Table 1). The rapid HIV assays included in our study were selected on the basis of their local availability, procedural simplicity, cold chain requirements and their previously reported ability to discriminate between HIV-1 and HIV-2.

## 2.3. Rapid HIV testing and comparison with the gold standard

Rapid HIV testing, with the four selected HIV assays, was performed on the 404 serum samples in Guinea-Conakry and the 41 HIV-2 samples at ITM. All assays were performed as recommended by the manufacturer by one well trained operator and visual interpretations of the results were made independently by two readers. If there was a discrepant test result, the two readers agreed on a final result. Different testing operators performed and interpreted the rapid tests in Guinea-Conakry and at ITM.

The 404 serum samples in Guinea-Conakry were transported to ITM under cold-chain where they underwent confirmatory testing together with the 41 HIV-2 samples already at ITM (see Figure 1). Samples were first tested with INNO-LIA HIV-I/II Score (Innogenetics, Ghent, Belgium) to check that they were true HIV-positive and to also discriminate between HIV-1 and HIV-2. Where an indeterminate result was obtained (i.e. where the criteria for being HIV-negative or HIV-positive were not fulfilled), further characterisation was done using the AIDS Reference Laboratory (ARL) confirmation testing strategy (see Figure 1). When an HIV-positive untypable result was obtained with the INNO-LIA HIV-I/II Score, the confirmatory New LAV BLOT II assay (Bio-Rad, Marnes-la-Coquette, France) was performed. An HIV-positive untypable result might be found if the patient is infected with both viruses,

or the patient is infected with HIV-1 and their antibodies cross-react with the HIV-2 antigen or the patient is infected with HIV-2 and their antibodies cross-react with the HIV-1 antigen.<sup>12–19</sup> If the subsequent New LAV BLOT II result was indeterminate or negative, the specimen was confirmed as HIV-1 positive. However if the New LAV BLOT II result still came back as HIV-positive untypable, then diagnosis of HIV type could not be made. Further confirmation by DNA polymerase chain reaction (PCR) on peripheral blood mononuclear cell (PBMC) blood was not possible in the setup of this evaluation. All tests were performed according to the manufacturer's recommendations and interpreted accordingly.

## 2.4. Data collection and statistical analysis

Data collection sheets at the laboratory in Guinea-Conakry and Antwerp, Belgium were used to record results of the rapid tests. The sensitivity of each rapid test was determined by comparing the results for detection of HIV infection (all HIV types included) with those obtained using the gold standard testing algorithm. The degree of agreement between the rapid assays and gold standard testing at discriminating between HIV type, was assessed by use of the Kappa statistic with values graded as follows: 0.81–1.0: almost perfect agreement; 0.61–0.80: substantial agreement; 0.41–0.60: moderate agreement; 0.21–0.40: fair agreement; 0.01–0.20: slight agreement; and <0.01: poor agreement. The level of significance was set at  $P=0.05$ . Data were all transferred to Microsoft Excel® and analyzed using Stata IC 10 (Stata Corporation, Texas, USA).

## 3. Results

There were a total of 445 serum samples selected for this study including 404 from Guinea-Conakry and 41 from Antwerp. Two samples from Guinea-Conakry were deemed indeterminate by the INNO-LIA HIV-I/II and by further confirmatory testing, and were therefore excluded from further analysis. Of the 443 samples included in the analysis, 384 were HIV-1 positive, 52 HIV-2 positive and 7 HIV-positive untypable as confirmed by the INNO-LIA HIV-I/II and NEW LAV BLOT II tests.

Genie II HIV1/HIV2, Immunoflow HIV1-HIV2 and SD Bioline HIV 1/2 3.0 each demonstrated a sensitivity of 100% (95% CI, 98.9–100%) in detecting HIV infection (all types included) while First Response HIV Card Test 1-2.0 had a



**Table 2**  
Diagnostic accuracy of four rapid assays in discriminating between HIV-1 and HIV-2

Diagnostic accuracy	HIV Rapid Tests			
	Genie II HIV1/HIV2	SD Bioline HIV-1/2 3.0	Immunoflow HIV1-HIV2	First Response HIV Card Test 1-2.0
<b>HIV-1 (n=384)</b>				
No. of samples identified as HIV-1	383	250	380	365
No. correctly identified	382 (99.5%)	250 (65.1%)	380 (99.0%)	362 (94.3%)
No. incorrectly identified as:				
HIV-2	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
HIV-positive	2 (0.5%)	134 (34.9%)	4 (1.0%)	22 (5.7%)
Kappa statistic	0.97	0.33	0.96	0.78
Measure of agreement	99.3% ( $P < 0.001$ )	69.8% ( $P < 0.001$ )	99.1% ( $P < 0.001$ )	94.4% ( $P < 0.001$ )
<b>HIV-2 (n=52)</b>				
No. of samples identified as HIV-2	49	36	35	33
No. correctly identified	49 (95.2%)	36 (69.2%)	35 (67.3%)	33 (63.5%)
No. incorrectly identified as:				
HIV-1	1 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
HIV-positive	2 (3.8%)	16 (30.8%)	17 (32.7%)	17 (32.7%)
HIV-negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (3.8%)
Kappa statistic	0.97	0.80	0.78	0.75
Measure of agreement	99.3% ( $P < 0.001$ )	96.4% ( $P < 0.001$ )	96.2% ( $P < 0.001$ )	95.7% ( $P < 0.001$ )
<b>HIV-positive untypable (n = 7)</b>				
No. of samples identified as HIV-positive untypable	11	157	28	43
No. incorrectly identified as:				
HIV-1	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (42.9%)
HIV-2	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Kappa statistic	0.77	0.06	0.38	0.14
Measure of agreement	99.1% ( $P < 0.001$ )	66.1% ( $P < 0.001$ )	95.3% ( $P < 0.001$ )	90.5% ( $P < 0.001$ )

sensitivity of 99.5% (95% CI, 98.2–99.9%), having incorrectly identified two HIV-2 positive specimens as HIV-negative. Table 2 shows the diagnostic accuracy of the rapid HIV tests at discriminating between HIV-1, HIV-2 and HIV-positive untypable samples. Overall, the Genie II HIV1/HIV2 was the most accurate discriminating assay identifying 99.5% HIV-1 samples, 95.2% HIV-2 and all (100%) of the HIV-1/2 samples. This assay demonstrated a high degree of agreement with the gold standard (almost perfect agreement for the diagnosis of HIV-1 and HIV-2 (Kappa statistic 0.97 for both HIV types) and substantial agreement for diagnosis of HIV-positive untypable samples (Kappa statistic 0.77). SD Bioline failed to identify 35% HIV-1 samples and 31% HIV-2 samples, misidentifying them as HIV-positive untypable. Overall, SD Bioline HIV 1/2 3.0 was the worst performer in its discriminative capacity, demonstrating only fair agreement with the gold standard for the accurate diagnosis of HIV-1 (Kappa statistic 0.33) and only slight agreement for the accurate diagnosis of HIV-positive untypable samples (Kappa statistic 0.06). Immunoflow HIV1-HIV2 correctly identified almost all the HIV-1 samples (99%) while First Response HIV Card Test 1-2.0 identified 94% of these samples. Both of these assays failed to identify about the same proportion of HIV-2 samples (Immunoflow HIV1-HIV2, 33% and First Response HIV Card Test 1-2.0, 36%) wrongly identifying them as HIV-positive untypable. Both the Immunoflow HIV1-HIV2 and the First Response HIV Card Test 1-2.0 demonstrated a high measure of agreement with the gold standard for accurately diagnosing HIV-1 and HIV-2 (Kappa statistics  $\geq 0.75$ , with Immunoflow HIV1-HIV2 performing slightly better than First Response HIV Card Test 1-2.0) but were relatively poorer at accurately

diagnosing HIV-positive untypable samples. Apart from Genie II HIV1/HIV2 identifying one out of 52 (1.9%) HIV-2 samples as HIV-1, none of the other assays misidentified HIV-1 as HIV-2 or vice versa. SD Bioline HIV 1/2 3.0 was the worst overall performer in its discriminative capacity.

#### 4. Discussion

This is one of the first evaluation studies on the diagnostic accuracy of locally available rapid HIV assays in discriminating between HIV-1 and HIV-2 in a West African country where both HIV types are prevalent. The findings show that SD Bioline HIV 1/2 3.0 has a relatively poor HIV discriminatory capacity. Initial concerns in Guinea-Conakry over the atypically high number of HIV-positive untypable infections being diagnosed with SD Bioline HIV 1/2 3.0 (wrongly interpreted in the field as dual HIV-1 + HIV-2 infections) were also confirmed in this study: about a third of all the true HIV-1 and HIV-2 specimens were misclassified by this rapid assay as being HIV-positive untypable.

In countries like Guinea where HIV-1, HIV-2 and dual infections co-exist, the public health implications of using this test for HIV discrimination are significant. Up to 30% of patients with HIV-1 could be wrongly diagnosed as having dual HIV-1+2 and will thus not be placed on a standard first-line ART regimen containing non-nucleoside reverse transcriptase inhibitors (NNRTI)<sup>7,8</sup> as is normal practice. This is because HIV-2 is known to be intrinsically resistant to NNRTIs. The preferred ARV regimen in such patients is a more costly protease inhibitor (PI) containing regimen associated with greater complicity of administration

(needs cold chain), pill counts and more side effects.<sup>20</sup> This could lead to reduced adherence which in turn generates viral resistance and compromises future treatment options. Furthermore, in a context of scaling up ART to thousands of individuals, unnecessarily placing up to 30% of the ART naïve HIV-1 population on a PI containing regimen could eventually compromise the efficacy of the standard choice of second-line ART regimens in patients that fail standard first-line ART.

Of the four rapid assays evaluated, the Genie II HIV1/HIV2 was found to be most accurate for discriminating between HIV-1 and HIV-2 (this finding has not always been confirmed in other settings<sup>9</sup>) but did misidentify an HIV-2 sample as being HIV-1 which is a problem given the treatment issues around HIV-1 and HIV-2. However, use of the Genie II HIV1/HIV2 in Guinea-Conakry would be particularly compromised by the fact that the test requires sera (instead of whole blood) and is subject to cold chain availability, both of which are important operational considerations at peripheral facilities.<sup>21–24</sup> The First Response HIV Card Test 1-2.0 HIV test is also not recommended as it had a sensitivity that was below the WHO recommended minimum threshold of 99.5%.

Genie II HIV1/HIV2 may be the most appropriate assay of choice for a two test algorithm in laboratories which have cold chain access and centrifuge equipment. However, in those laboratories without, Immunoflow HIV1-HIV2 would probably be the most reliable assay of choice as it can be stored at room temperature and can be used on whole blood specimens. Immunoflow HIV1-HIV2 also correctly diagnosed 99% of HIV 1 specimens and 67% of HIV-2 specimens. Although 33% of HIV-2 specimens were incorrectly diagnosed as HIV-positive untypable, from a clinical perspective this is not a problem as both HIV-2 and HIV-positive untypable patients will be placed on PI containing ARV regimens. In any case if accurate discriminatory diagnosis of dual infection is desired this could be done through the use of a third rapid test such as Genie II HIV1/HIV2 or a confirmation test (Western blot) or by polymerase chain reaction (PCR).<sup>25–29</sup> PCR, however, will not always solve the problem due to the frequent low proviral load for HIV-2.

Our study has two specific limitations: (i) the specificity of the rapid assays was not measured as no HIV-negative samples were included in the study (this is important to elucidate as any selected second line rapid assay should demonstrate a high specificity) and (ii) dual HIV-1+2 infection was not confirmed among the HIV-positive untypable samples by NEW LAV BLOT II.

In the urban setting of Guinea-Conakry, the use of SD Bioline HIV 1/2 3.0 as a discriminatory HIV test in a two test diagnosis algorithm is inadequate and may be best replaced by Immunoflow HIV1-HIV2.

**Authors' contributions:** PC, ND, MD, KF, GB KTS and RZ were involved with conception and design of the study. ND, KF and GB conducted the laboratory analysis. KTS and RZ did the analysis and wrote the first draft of the manuscript. All authors reviewed the manuscript critically and improved the intellectual content. PC is guarantor of the paper.

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**Conflicts of interest:** We have no conflicts of interest to declare.

**Ethical approval:** Ethical approval was received from the National Ethics Committee of Guinea-Conakry and from ITM.

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