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Performance of Bio-Rad HIV-1/2 Confirmatory Assay in HIV-1, HIV-2 and HIV-1/2 dually reactive patients - comparison with INNO-LIA and immunocomb discriminatory assays

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ABSTRACT

Background: Being able to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection is imperative for the appropriate selection of antiretroviral therapy (ART) in regions with high HIV-2 endemicity.
Objectives: To evaluate Bio-Rad Geenius HIV-1/2 Confirmatory Assay against INNO-LIA HIV 1/2 Score and ImmunoComb HIV 1/2 BiSpot with an emphasis towards ability to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection.
Material and Methods: 131 samples from ART naïve HIV infected patients in Guinea-Bissau were selected retrospectively and tested with Geenius, INNO-LIA and Immunocomb. HIV-1/2 RNA were measured in all samples and HIV-1/2 DNA in 59 samples.
Results: The Geenius reader typed 62 samples as HIV-1 reactive, 37 samples as HIV-2 reactive and 32 samples as HIV-1/2 dually reactive. Geenius manual reading classified 10% more samples as HIV-1/2 dually reactive (n = 35). INNO-LIA typed 63 samples as HIV-1 reactive, 36 samples as HIV-2 reactive and 32 samples as HIV-1/2 dually reactive while Immunocomb classified a large proportion of samples as HIV-1/2 dually reactive (n = 45). The measurement of agreement of the Geenius reader compared with INNO-LIA and Immunocomb was 92.4% and 84.0% respectively while the measurement of agreement of Geenius manual reading compared with INNO-LIA and Immuncomb was 93.1% and 89.3% respectively.
Conclusions: Geenius has similar performance characteristics as INNO-LIA, and performs considerably better than Immunocomb, for differentiating between HIV types. This is especially true when using the Geenius reader while manual reading of the Geenius assay seemed to overestimate the numbers of HIV-1/2 dually reactive samples.

1. Background

An accurate laboratory diagnosis of HIV is a cornerstone of national HIV programs and essential for patient management and subsequent treatment. In June 2014 the US Centers for Disease Control and Prevention presented an updated diagnostic algorithm. It consists of a combined screening assay simultaneously detecting HIV-1/2 antibodies and p24 antigen, followed by confirmation and HIV-type differentiation by a serological test. If the HIV-type differentiation assay is nonreactive or indeterminate HIV-1 nucleic acid amplification test (NAAT) is recommended (Centers for Disease Control and Prevention and Association of Public Health Laboratories, 2014). Verification of a serologically suspected HIV-2 infection is not straightforward as many HIV-2 infected patients have undetectable or significantly lower HIV-2 RNA plasma viral load compared to HIV-1 plasma viral load (Andersson et al., 2000). Thus a negative result does not exclude HIV-2 infection.

Abbreviations: ART, antiretroviral therapy; IQR, Interquartile range; NAAT, nucleic acid amplification test

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Furthermore HIV-2 genomic testing is often not available in low resource settings and HIV-type discrimination relies on serological tests (Jespersen et al., 2014).

HIV-2 is inherently resistant to non-nucleoside reverse transcriptase inhibitors, commonly used as a first-line antiretroviral treatment (ART) of HIV-1 in sub-Saharan Africa (Visseaux et al., 2016). Therefore it is important to be able to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection to avoid HIV-2 and HIV-1/2 dually infected patients to be wrongly classified as HIV-1 positive and put on inappropriate ART. HIV-2 is most prevalent in West Africa but minor HIV-2 epidemics have been established in countries in southern Europe with historical relations to West Africa and through immigration to India and the United States of America (Visseaux et al., 2016). Globally, Guinea-Bissau has the highest prevalence of HIV-2 but over the last 30 years prevalence rates of HIV-2 have been declining while the HIV-1 prevalence has been increasing (Mansson et al., 2007; Olesen et al., 2018). Consequently both HIV viruses co-exist with a number of intra-patient coinfections.

ImmunoComb HIV 1/2 BiSpot (Orgenics, Yavne, Israel) (Mansson et al., 2007, 2009; Tchounga et al., 2016, 2014) and INNO-LIA HIV 1/2 Score (Fujirebio, Ghent, Belgium) (Chaillet et al., 2010; Zbinden et al., 2016) are two serological tests that have been widely used for HIV confirmation and HIV-type discrimination. As the production of Immunocomb now has ceased this has prompted us to evaluate a new strategy for HIV confirmation and HIV-type discrimination in a setting with high numbers of HIV-2 and HIV-1/2 infected individuals.

Geenius HIV 1/2 Confirmatory Assay (Bio-Rad, Marnes-al-Coquette, France) is a new confirmatory test for HIV discrimination which has previously been evaluated with regard to discriminatory capacity between HIV-1 and HIV-2 (Abbate et al., 2014; Fordan et al., 2017; Friedrichs et al., 2015; Herssens et al., 2014; Malloch et al., 2013; Montesinos et al., 2014; Tinguely et al., 2014). However, these evaluations have included no or only a few samples of HIV-1/2 dually reactive patients. The objective of the present study was to evaluate Geenius against INNO-LIA and Immunocomb with an emphasis towards ability to discriminate between HIV-1, HIV-2 and HIV-1/2 dual infection.

2. Material and methods

2.1. Setting

Patient sera from ART naïve individuals attending the outpatient ART centre at the national Hospital Simão Mendes in Guinea-Bissau and included in the Bissau HIV Cohort were used in this study. The Bissau HIV Cohort has been described previously (Jespersen et al., 2014, 2015). At the ART centre free-of-charge HIV testing is performed daily and in individuals with a positive HIV test a CD4 cell count measurement were taken the subsequent days. If the patients gave their consent plasma and cell suspension were frozen and stored in Guinea-Bissau until shipment to a biobank in Aarhus, Denmark.

2.2. Sample selection

For this evaluation we used a subset of samples used in a previous study presenting a comparison between INNO-LIA and Immunocomb (Hønge et al., 2018). The 239 samples in the previous study were retrospectively selected from a biobank, stratified by HIV-type to ensure equal numbers of HIV-1, HIV-2 and HIV-1/2 dually infected patients based on the initial HIV-type discrimination performed in Bissau (Hønge et al., 2018). Based on availability of material, a total of 131 plasma samples of the original 239 samples were used in the present study. The samples were originally collected in Guinea-Bissau between September 2007 and May 2012 (Hønge et al., 2018).

2.3. Geenius HIV 1/2 confirmatory assay

Geenius assay is a single-use immunochromatographic test with the following HIV antigens attached to a membrane strip on a cartridge (HIV-1: p31, gp160, p24, gp41 and HIV-2: gp36, gp140). The cartridges were visualized and interpreted automatically using Geenius reader S/N DP3B002818 and Mini Pc Lenovo (Geenius Reader). In addition the cartridges were read manually by two independent observers adhering to the manufacturer's instructions where "*Even a faint band must be considered as reactive*". According to the manufacturer's instructions the Geenius assay was interpreted as HIV-1 reactive if two HIV-1 bands with at least one ENV (gp160 or gp41) were present. HIV-2 reactivity was assumed if both HIV-2 bands (gp36 and gp140) were visible. HIV-1/2 dual reactivity (labelled 'untypable' in the manufacturer's instructions) was assumed if both HIV-2 bands and the two HIV-1 ENV (gp160 and gp 41) bands and/or GAG (p24) and/or POL (p31) were visible, here termed HIV-1/2 dual reactive.

It is also noteworthy that the Geenius reader use an algorithm taking band intensity into account while the instruction for manual reading is simplified and strict binary interpretation of bands is recommended.

2.4. INNO-LIA, Immunocomb, HIV-RNA and DNA measurement

As this study used a subset of samples from a previous study the methods have previously been described (Hønge et al., 2018). In short, INNO-LIA test strips were scanned and interpreted by a software algorithm (LiRAS for Infectious Diseases V3.00) after a 16-hour incubation. Samples reactive for both HIV-1 specific and HIV-2 specific antibody lines are classified as "*Positive for HIV antibodies (untypable)*". Immunocomb is a multi-spot rapid test which takes 36 min to complete. Dual spot reactivity was interpreted subjectively according to the package insert "*In cases of HIV-1/HIV-2 coinfection, two spots of equal intensities have been observed*". HIV RNA analyses were performed on all samples. Quantification of HIV-1 RNA was performed using Abbott m2000 system (Abbott RealTime HIV 1, version 9.00; Abbott Molecular Inc, Abbott Park, IL USA) while quantification of HIV-2 RNA was performed using an in-house method (Hønge et al., 2018). HIV-1 and HIV-2 DNA was extracted from a cell suspension employing EZ1 DNA Blood Kit (Qiagen, Hilden Germany) (Hønge et al., 2018). The cell suspension consisted of whole venous blood drawn in EDTA tubes, centrifuged, and with the plasma layer aspirated. The cell suspension was then frozened without any further preparation and later thawed before extraction. Primers and probes were applied as reported by Gueudin et al (Gueudin et al., 2008), but a plasmid was not used and thus our results were qualitative. Total reaction volume was 40 µL. HIV DNA was analyzed in available samples typed HIV-1/2 dually reactive and in samples where Geenius, INNO-LIA and Immunocomb gave divergent results. In two samples characterized as HIV 1/2 dually reactive by Immunocomb but not by INNO-LIA or Geenius, there was not enough material to perform DNA analysis rendering the total amount samples analysed for DNA to 59 samples.

2.5. Statistics

For categorical variables Fisher´s exact test was used and for continuous variables means were presented and analyzed with two-sample t-test. Kappa score was used to compare agreement between Geenius and INNO-LIA and Immunocomb and between the different Geenius reading options. The relation between immune status and confirmed/unconfirmed HIV-1/2 dually reactive samples was analyzed with logistic regression. Data was analyzed using STATA IC 13 (Stata Corp, Texas USA).

Table 1
Demographic data stratified by Serologic test and HIV-typing.

	INNO-LIA[a]			Geenius Reader			Geenius Manual Reading[b]			Immunocomb[a]		
	HIV-1	HIV-2	HIV-1/2	HIV-1	HIV-2	HIV-1/2	HIV-1	HIV-2	HIV-1/2	HIV-1	HIV-2	HIV-1/2
	N = 63	N = 36	N = 32	N = 62	N = 37	N = 32	N = 65	N = 31	N = 35	N = 63	N = 23	N = 45
Women n (%)	43	26	26	41	27	27	44	21	30	42	16	37
	(68.3)	(72.2)	(81.3)	(66.1)	(73.0)	(84.4)	(67.7)	(67.7)	(85.7)	(66.7)	(69.6)	(82.2)
Men n (%)	20	10	6	21	10	5	21	10	5	21	7	8
	(31.8)	(27.8)	(18.8)	(33.9)	(27.0)	(15.6)	(32.3)	(32.3)	(14.3)	(33.3)	(30.4)	(17.8)
Age stratification (yrs) n (%)												
15-24	7	1	2	6	1	3	7	1	2	7	1	2
	(11.1)	(2.8)	(6.3)	(9.7)	(2.7)	(9.4)	(10.8)	(3.2)	(5.7)	(11.1)	(4.4)	(4.4)
25-34	24	6	14	24	8	12	24	5	15	24	2	18
	(38.1)	(16.7)	(43.8)	(38.7)	(21.6)	(37.5)	(10.8)	(16.1)	(42.9)	(38.1)	(8.7)	(40.0)
35-44	20	11	8	20	12	7	20	10	9	19	8	12
	(31.8)	(20.6)	(25.0)	(32.3)	(32.4)	(21.9)	(30.8)	(32.3)	(25.7)	(30.2)	(34.8)	(26.7)
45-54	7	5	8	8	5	7	9	5	6	8	4	8
	(11.1)	(13.9)	(25.0)	(12.9)	(13.5)	(21.9)	(13.9)	(16.1)	(17.1)	(12.7)	(17.4)	(17.8)
≥55	5	13	0	4	11	3	5	10	3	5	8	5
	(7.9)	(36.1)	(0.0)	(6.5)	(29.7)	(9.4)	(7.7)	(32.3)	(8.6)	(7.9)	(34.8)	(11.1)
CD4 cell count (cells/μL) n (%)												
≤199	24	10	17	26	11	14	26	9	16	24	7	20
	(38.1)	(27.8)	(53.1)	(41.9)	(29.7)	(43.8)	(40.0)	(29.0)	(45.7)	(38.1)	(30.4)	(44.4)
200-349	16	11	9	14	11	11	16	9	11	16	6	14
	(25.4)	(30.6)	(28.1)	(22.6)	(29.7)	(32.3)	(24.6)	(29.0)	(31.4)	(25.4)	(26.1)	(31.1)
350-499	8	7	2	8	7	2	8	6	3	8	5	4
	(12.7)	(19.4)	(6.3)	(12.9)	(18.9)	(6.3)	(12.3)	(19.4)	(8.6)	(12.7)	(21.7)	(8.9)
≥500	15	8	4	14	8	5	15	7	5	15	5	7
	(23.8)	(22.2)	(12.5)	(22.6)	(21.6)	(15.6)	(23.1)	(22.6)	(14.3)	(23.8)	(21.7)	(15.6)
HIV-1 RNA detected n (%)	62	0	30	61	1	30	64	0	28	62	0	30
	(98.4)	(0.0)	(93.8)	(98.4)	(2.7)	(93.8)	(98.5)	(0.0)	(80.0)	(98.4)	(0.0)	(66.7)
HIV-2 RNA detected n (%)	0	21	13	0	23	11	0	19	15	0	14	20
	(0.0)	(58.3)	(40.6)	(0.0)	(62.2)	(34.4)	(0.0)	(61.3)	(42.9)	(0.0)	(60.9)	(44.4)
HIV-1 DNA detected n (%)	8	0	28	7	0	29	10	0	26	8	0	28
	(100.0)	(0.0)	(87.5)	(100.0)	(0.0)	(90.6)	(100.0)	(0.0)	(74.3)	(100.0)	(0.0)	(65.1)[c]
HIV-2 DNA detected n (%)	0	19	18	0	20	17	0	14	23	0	8	29
	(0.0)	(100.0)	(56.3)	(0.0)	(100.0)	(53.1)	(0.0)	(100.0)	(65.7)	(0.0)	(100.0)	(67.4)[c]
Samples not confirmed HIV-1/2 dually reactive by HIV-1/2 RNA/DNA detection n (%)			14 (43.8)			15 (45.5)			17 (48.6)			25 (58.1)[c]
HIV-1/2 dually reactive samples only positive for HIV-1 DNA and/or RNA n (%)			12 (37.5)			13 (40.6)			10 (28.6)			12 (27.9)[c]
HIV-1/2 dually reactive samples only positive for HIV-2 DNA and/or RNA n (%)			2 (6.3)			2 (6.3)			7 (20.0)			13 (30.2)[c]
Median (IQR) CD4 for confirmed HIV-1/2 dually reactive samples			231.5 (130.0-414.0)			224.0 (130.0-340.0)			231.5 (130.0-414.0)			231.5 (130.0-414.0)
Median (IQR) CD4 for uncorfirmed HIV-1/2 dually reactive samples			129.5 (93.0-206.0)[d]			206.0 (93.0-327.0)			164.0 (93.0-296.0)			179.0 (121.0-274.0)

[a] This study used a subset of samples from a previous study presenting a comparison between INNO-LIA and Immunocomb (Hønge et al., 2018).
[b] According to the inlet instruction even the faintest band were considered as reactive.
[c] Two samples typed as HIV-dually reactive by Immunocomb not analysed for HIV-DNA and excluded in the analysis, both negative in HIV-RNA.
[d] Logistic regression comparing the CD4 cell count between confirmed and unconfirmed HIV-1/2 dually reactive samples adjusted for sex and age, Odds ratio 1.01 (p = 0.03).

3. Results

3.1. Characteristics

Samples from 131 ART naïve patients, of whom 95 were women, were included in the study. Median age was 37 years (interquartile range [IQR] 30–46) and the median CD4 cell count at the date of sample collection was 245 cells/μL (IQR 126–433 cells/μL). See Table 1 for complete demographic data.

3.2. Geenius results and comparison with HIV RNA and DNA detection

The Geenius reader indicated that 62 (47.3%) samples were HIV-1 positive, 37 (28.2%) were HIV-2 positive and 32 (24.4%) were HIV-1/2 dually reactive, while manual reading according to the inlet instructions classified 10% more samples as HIV-1/2 dually reactive (n = 35) (Table 2). There was no disagreement between the two independent observers who read Geenius according to the inlet instructions.

HIV-1 RNA and HIV-2 RNA were measured in all samples and HIV DNA was measured in 59 samples, including all but two of HIV-1/2 dually reactive results in any of the assays (Table 1). All samples typed as HIV-1 reactive by automatic and manual reading options had undetectable HIV-2 RNA/DNA levels. All samples typed as HIV-2 reactive by the two Geenius reading options had undetectable HIV-1 RNA/DNA levels except one sample which was incorrectly typed HIV-2 reactive by the Geenius Reader. Neither HIV RNA nor DNA was consistently detectable in HIV-1/2 dually reactive patients. For the Geenius reader 15/32 (46.9%) of samples could not be confirmed by NAAT, and 17/35 (48.6%) for manual reading according to the inlet instructions.

3.3. INNO-LIA results and comparison with Geenius

Overall there was a good agreement varying between 92.4%–93.9% (Table 2). For the INNO-LIA HIV-1 and HIV-2 reactive samples none

Table 2
Comparison between INNO-LIA, Geenius reader, Geenius manual reading and Immunocomb.

INNOLIA[a]	Geenius reader	Geenius Manual reading[b]	Immunocomb[a]
HIV-1 (n = 63)			
No. Of samples identified as HIV-1	62	65	63
Agreement between INNOLIA and Geenius/Immunocomb	60 (952%)	63 (100.0%)	62 (98.4%)
Geenius/Immunocomb result if disagreement			
HIV-2	0 (0.0%)		0 (0.0%)
HIV-1/2	3 (4.8%)		1 (1.6%)
HIV-2 (n = 36)			
No. Of samples identified as HIV-2	37	31	23
Agreement between INNOLIA and Geenius/Immunocomb	34 (94.4%)	30 (83.3%)	23 (63.9%)
Geenius/Immunocomb result if disagreement			
HIV-1	0 (0.0%)	0 (0.0%)	0 (0.0%)
HIV-1/2	2 (5.6%)	6 (16.7%)	13 (36.1%)
HIV-1/2 (n = 32)			
No. Of samples identified as HIV-1/2	32	35	45
Agreement between INNOLIA and Geenius/Immunocomb	27 (84.4%)	29 (90.6%)	31 (68.9%)
Geenius/Immunocomb result if disagreement			
HIV-1	2 (6.2%)	2 (6.3%)	1 (1.6%)
HIV-2	3 (9.4%)	1 (3.1%)	13 (28.9%)
Kappa statistics	0.88	0.89	0.82
Measurement of agreement	92.4%	93.1%	88.6%

[a] This study used a subset of samples from a previous study presenting a comparison between INNO-LIA and Immunocomb (Hønge et al., 2018).
[b] According to the inlet instruction even the faintest band were considered as reactive.

had detectable both HIV-2 RNA/DNA and HIV-1 RNA/DNA, respectively. Among samples typed as HIV-1/2 dually reactive by INNO-LIA, 14/32 (43.8%) could not be confirmed by NAAT (Table 1). In 10 samples there were discordant results between the Geenius reader and INNO-LIA, NAAT was in concordance with the Geenius reader in four of these samples. NAAT did not disapprove any INNO-LIA results while one sample typed as HIV-2 reactive by the Geenius reader had detectable HIV-1 RNA which in clinical practice would have direct implication on ART regimen selection.

3.4. Geenius results as compared with Immunocomb results

The agreement between Geenius and Immunocomb was lower than between Geenius and INNO-LIA. The measurement of agreement between Immunocomb compared to the Geenius reader and manual reading was 84.0% and 89.3% respectively. The difference was attributed to the difficulty in discriminating between HIV-2 and HIV-1/2 dually reactive. Among samples typed as HIV-1/2 dually reactive by Immunocomb, 25/43 (58.1%) could not be confirmed by NAAT. Of these 25 samples, 14 samples were typed by the Geenius reader as HIV-2 reactive and had undetectable HIV-1 RNA and/or HIV-1 DNA (2 samples were not analysed for HIV DNA but were typed as HIV-2 reactive by INNO-LIA as well). These 14 individuals were compared to individuals typed as HIV-2 monoreactive by both the Geenius reader and Immunocomb (n = 22) in regard to age, gender, CD4 cell count and presence of HIV-2 viremia, without any detected differences.

4. Discussion

In this study, we evaluated the performance of Geenius for the discrimination between HIV-1, HIV-2 and HIV-1/2 dual infection against two other confirmatory tests. We examined clinical samples from the field of which a high number were typed as HIV-2 and HIV-1/2 dually reactive. HIV RNA and HIV DNA analyses further strengthen this study.

It is challenging to discriminate between HIV monoinfection and HIV-1/2 dual infection in settings where both viruses co-exist, due to cross-reactivity in serological tests. Thus, the gold standard for detection of HIV-1/2 dual infection is through NAAT. These procedures are however not generally available in resource-limited settings and consequently it is important to have as accurate serological tests as possible. Recently a point-of-care molecular test (Alere q HIV-1/2 Detect, Alere Detect) for HIV-1 and HIV-2 RNA detection was evaluated and the authors concluded that this test is a potential diagnostic tool in resource limited settings (Chang et al., 2017).

Previous studies (Andersson et al., 1997; Walther-Jallow et al., 1999; Gautheret-Dejean et al., 2015) have found that Immunocomb to some degree overestimates the number of HIV-1/2 dually reactive results but not reaching the extent seen in our material. This is most likely due to inter-reader variability which have been reported previously (Hønge et al., 2018). In contrast we had two independent observers reading Geenius according to the inlet instructions with full agreement. Misclassification of HIV-2 and HIV-1/2 dual reaction fortunately has no implication on ART regimen selection with current drug regimens but could have personal implications as HIV-1/2 dually infected individuals progress faster to AIDS than HIV-2 infected individuals (Esbjornsson et al., 2012; Esbjörnsson et al., 2014). Geenius performs considerably better than Immunocomb and both the Geenius reader and manual reading of Geenius showed good concordance with INNO-LIA.

Visual reading of the Geenius assay adhering to the manufacturer's instructions however seemed to overestimate the number HIV-1/2 dually infected samples compared with the Geenius reader.

Before choosing a particular test one must take into account the advantages and disadvantages of the different assays. Geenius comes at approximately the same financial cost per sample as INNO-LIA and offers other advantages such as easy handling where only limited laboratory experience is needed and reading within 30 min making it more suitable in resource limited settings. In contrast INNO-LIA requires overnight incubation and continuous power supple for 16 h. The Geenius reader and manual reading results in relation to INNO-LIA differed regarding HIV-1/2 dual reactivity (agreement 84.4%% and 90.6% respectively). As INNO-LIA has not been a rational choice in most locations with higher HIV-2 endemicity, Geenius would be a more plausible, if still not inexpensive, approach to serological differentiation. In a setting with predominately HIV-1 monoinfections, the Geenius reader and visual reading of the Geenius assay seem to perform equally and provide the option to use the assay without the Geenius reader cutting costs. However in a setting such as Guinea-Bissau with a high number of expected HIV-1/2 dually reactive samples, our results indicate that you would obtain better results using the Geenius assay with the automatic reader.

The findings in this study are subject to several limitations. First and

foremost it was not designed as a complete sensitivity and specificity assessment of the Geenius assay. In 37.8–41.7% of samples typed as HIV-2 positive by INNO-LIA and the different Geenius reading options HIV-2 RNA could not be detected which however is consistent with previous findings where undetectable HIV-2 RNA levels has been reported in 36–46% of ART naïve HIV-2 patients (Gottlieb et al., 2002; Chang et al., 2012; Ekouevi et al., 2015). Samples from some patients classified as HIV-1/2 dually reactive by Geenius and INNO-LIA could not be confirmed by RNA/DNA analyses. This could be due to suppression of the less dominant HIV type (Raugi et al., 2013). The low proportion of samples with confirmed HIV DNA could be due to low leukocyte number and consequently low concentration of DNA in our material, but unfortunately information on cell count was not available. The use of digital droplet PCR might have been more sensitive for HIV DNA leading to more HIV-1/2 dually reactive samples being confirmed. Another possibility would be misclassification by INNO-LIA and Geenius.

In summary Geenius performs considerably better than Immunocomb for differentiating between HIV-type and has similar performance characteristics as INNO-LIA in a setting with high numbers of HIV-2 and HIV-1/2 infected individuals, especially when using the Geenius reader. However, the cost of tests and an automatic reader can be problematic in resource-limited settings such as West Africa where HIV-2 and HIV-1/2 dual infections are most prevalent.

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Competing interests

The authors taken part in this study have declared that they have no disclosures regarding funding or conflicts of interest with respect to this manuscript.

Ethical approval

The present study was approved by the National Ethics Committee of Guinea-Bissau, (Parecer NCP/No. 15/2007)

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