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Performance evaluation of the Bio-Rad Geenius HIV 1/2 supplemental assay

Wei Luo[a], Vickie Sullivan[a], Tara Smith[a,b], Philip J. Peters[a], Cynthia Gay[c], Emily Westheimer[d], Stephanie E. Cohen[e], S. Michele Owen[a], Silvina Masciotra[a,*]

[a] Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, United States
[b] Oak Ridge Institute for Science and Education, United States
[c] University of North Carolina, Chapel Hill, NC, United States
[d] New York City Department of Health & Mental Hygiene, New York City, NY, United States
[e] San Francisco Department of Public Health, San Francisco, CA, United States

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ABSTRACT

Background: In the US, the HIV diagnostic algorithm for laboratory settings recommends the use of an HIV-1/HIV-2 differentiation supplemental assay after an initial reactive antigen/antibody (Ag/Ab) assay result. Since the discontinuation of the Multispot HIV-1/HIV-2 Rapid Test (MS), the Geenius HIV-1/2 Supplemental assay (Geenius) is the only FDA-approved supplemental differentiation test.

Objective: We compared the performance of Geenius to MS and Western Blot (WB).

Study design: The relative seroconversion plasma reactivity of Geenius and MS was assessed using a 50% cumulative frequency analysis from 17 HIV-1 seroconverters. In addition, previously characterized plasma specimens, 186 HIV-1 positive, 100 HIV-2 positive, and 93 Ag/Ab-positive/HIV-1 RNA-negative, were tested with Geenius v1.1 software. McNemar's test was used for paired comparison analysis. A subset of 48 specimens were retested with the upgraded Geenius v1.3 software.

Results: In HIV-1 seroconverters, the relative seroconversion reactivity was 2.5 and 2 days before the first positive HIV-1 WB for Geenius and MS, respectively. In HIV-1 positive samples, Geenius performed similarly to HIV-1 WB ($p = 0.1687$) and MS ($p = 0.8312$). In HIV-2 positive samples, Geenius underperformed compared to HIV-2 WB ($p = 0.0005$) and MS ($p = 0.0012$). When using the upgraded software among the HIV-1 positive and Ag/Ab-reactive/HIV-1 RNA-negative samples, gp140 reactivity decreased without affecting characterization of HIV-2 samples.

Conclusions: With HIV-1 samples, Geenius, WB and MS performance was similar as supplemental tests. The updated Geenius software reduced false gp140 reactivity, but had no impact on identifying true HIV-2 infections. Further evaluation will assess the impact of the Geenius software update on final diagnostic interpretations

1. Background

In 2014 the US Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) updated recommendations for the laboratory diagnosis of HIV infection [1]. The guideline recommends that specimens with a reactive result on antigen/antibody (Ag/Ab) combination immunoassay should be tested with a US Food and Drug Administration (FDA)-approved antibody immunoassay that differentiates HIV-1 and HIV-2 antibodies. This algorithm replaced the use of HIV-1 Western blot (WB) for confirmation of HIV infection, although it is still FDA-approved and available in the US.

The Bio-Rad Multispot HIV-1/HIV-2 Rapid Test (MS, Bio-Rad Laboratories, Redmond, WA) was the only FDA-approved differentiation supplemental test available in the US until October 2014 when the Geenius HIV 1/2 Supplemental Assay (Geenius, Bio-Rad Laboratories, Redmond, WA) received FDA-approval. Geenius is currently the only supplemental differentiation test available in the US since the discontinuation of MS in 2016.

Geenius is a single-use immunochromatography assay designed for the confirmation and differentiation of antibodies to HIV-1 and HIV-2 in serum, plasma and whole blood samples. The dual path lateral flow cartridge contains HIV-1 (p31, gp160, p24, p41) and HIV-2 (gp36, gp140) antigens bound to a membrane solid phase. The Geenius assay uses a small reader for test interpretation and can be completed within 30 min [2]. In contrast to MS and WB, Geenius has eight possible final assay interpretations: HIV Antibody-negative, HIV-1-positive, HIV-2-

* Corresponding author at: 1600 Clifton Road NE MS: A-25, GA, 30329, United States.
E-mail address: svm6@cdc.gov (S. Masciotra).

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positive, HIV-2-positive with HIV-1 cross reactivity, HIV-positive untypable, HIV-1 indeterminate, HIV-2 indeterminate, and HIV indeterminate.

A comparative evaluation of MS and Geenius has been reported using Canada's HIV testing algorithm [3]. In addition, Geenius has been evaluated in Europe, Asia, and Israel in comparison to other confirmatory tests, including WB, and was found to perform well at confirming HIV diagnosis [4–7]. However, an evaluation comparing the performance of Geenius with MS and WB in the context of the CDC/APHL HIV laboratory diagnostic algorithm with a sample set with known acute infections has not been performed. In this study we compared the performance of Geenius to MS and WB in previously characterized samples primarily collected from individuals in early stages of HIV-1 infection.

2. Study design

2.1. HIV testing

2.1.1. Bio-Rad Multispot HIV-1/HIV-2 rapid test

The procedure was performed following the assay package insert [8] and test results were interpreted visually. Test interpretations for the MS supplemental assay include nonreactive, HIV-1-indeterminate, HIV-1-positive, HIV-2-positive, HIV-positive but undifferentiated as HIV-1 or HIV-2. All undifferentiated specimens were also tested using the recommended specimen dilution procedure (1:10) or further (1:100) to differentiate HIV-1 and HIV-2 antibodies.

2.1.2. Bio-Rad Geenius HIV 1/2 supplemental assay

The Geenius assay was performed according to the package insert [2] for plasma specimens. All cassettes were read by an automated reader utilizing software v1.1. To reduce the occurrence of HIV-2 indeterminate results, the automated reader software was updated in 2017 to raise the cut-off value of the HIV-2 gp140 band (software v1.3). To evaluate the impact of the new cutoff value, plasma samples from 14 HIV-1, 30 HIV-2 and four ARC-positive/HIV-1 NAT-negative individuals, that could be affected by the change, were retested with the updated software (v1.3) [9].

2.1.3. Bio-Rad GS HIV-1 western blot

The Bio-Rad GS HIV-1 Western Blot (HIV-1 WB) was performed according to the package insert and the results were interpreted as negative, indeterminate, or positive [10].

2.1.4. MP diagnostics HIV-2 blot 1.2 western blot

The MP Diagnostics HIV-2 Blot 1.2 Western Blot (HIV-2 WB) is a WB assay using bound antigenic proteins from partially purified inactivated HIV-2 [11]. It is a Conformité Européene (CE)-marked product (MP Biomedical Asia Pacific Pte. Ltd) but is not FDA-approved. The CDC laboratory validated the assay for HIV-2 diagnosis under CLIA regulations.

2.2. Sample sets

All specimens used in this study were unlinked from personal identifiers and this study was determined by the CDC to be research not involving human subjects.

2.2.1. Commercial HIV-1 seroconversion plasma specimens

To assess assay performance in detecting early infection, well-characterized HIV-1 seroconversion panels from the US were obtained from Zeptometrix, Inc. (Buffalo, NY) and BBI-SeraCare Diagnostics [12,13]. Each panel contained at least one specimen that was HIV-1 WB-positive (17 seroconverters with 166 total specimens) and all panels had at least one specimen that was HIV-1 nucleic acid test (NAT)-only positive.

2.2.2. Specimens collected during the STOP study

A subset of plasma specimens from the CDC Screening Target Populations to Interrupt On-going Chains of HIV Transmission with Enhanced Partner Notification (STOP) study was included in this evaluation [14–16]. This prospective multi-site (New York City, California, North Carolina) study evaluated methods to detect acute HIV-1 infections. During the study, participants were screened with OraSure OraQuick ADVANCE Rapid HIV-1/2 Antibody Test or Alere Clearview HIV-1/2 STAT-PAK on fingerstick whole blood, and EDTA whole blood was collected for further testing. HIV-1 NAT (Abbott RealTime HIV-1 assay; Chicago, IL, USA or Hologic Aptima HIV-1 RNA Qualitative Assay, Marlborough, MA) and Abbott ARCHITECT HIV Ag/Ab combo assay (ARC, Abbott Diagnostics, Abbott Park, IL), MS and HIV-1 WB were performed locally. Subsets of 279 and 263 plasma specimens had NAT/ARC/MS and NAT/ARC/HIV-1 WB results, respectively. A subset of 88 ARC-positive/HIV-1 NAT-negative samples were also tested with the Bio-Rad GS HIV1/2 Ag/Ab Combo assay (BRC; Bio-Rad Laboratories) at CDC to assess false reactivity.

2.2.3. HIV-2 antibody-positive plasma specimens

The 100 HIV-2 Ab-positive specimens included 16 from the US and 84 from Ivory Coast (Boca Biolistics, Inc, Coconut Creek, FL) [12]. The HIV-2 specimens characterized in the field were further tested at CDC with BRC, Geenius, andHIV-2 WB. Sequence analysis of the integrase region revealed that a third of samples from Ivory Coast were genotypes A, B and A/B recombinants [12].

2.3. Data analysis

Serial plasma specimens from 17 HIV-1 seroconverters were used to estimate the relative seroconversion reactivity of Geenius and MS as a supplemental assay. The 50% cumulative frequency analysis estimated the days at which 50% of the specimens tested became positive relative to the first positive WB.

The McNemar's test, a pair comparison statistical test, was used to analyze the differences in reactivity (positive vs. other test results) between MS and Geenius for the STOP and HIV-2 samples. A p value less than 0.05 was considered statistically significant. Samples with results from both the v1.1 and v1.3 Geenius reader software were compared to evaluate the impact of the updated software.

3. Results

3.1. Early HIV-1 seroconversion reactivity

Fig. 1 shows the relative reactivity of both the Geenius v1.1 and MS assays compared to other assay results previously obtained using the same commercial seroconversion panels [13,17]. The sequence of test reactivity was expressed as the number of days before the first HIV-1 WB-positive result. MS and Geenius assays were reactive 2 and 2.5 days before HIV-1 WB, respectively.

3.2. Test concordance among STOP samples

3.2.1. Geenius v1.1 vs. HIV-1 WB in HIV-1 positive samples

The comparison of 170 ARC-positive/HIV-1 NAT-positive with HIV-1 WB and Geenius results is shown in Table 1. Among 110 (64.7%) HIV-1 WB-negative/-indeterminate specimens, likely early infections, Geenius detected four as HIV-2 indeterminate with gp140 only reactivity. Among the 60 HIV-1 WB- positive, 47 (78.3%) were Geenius HIV-1-positive. Overall concordance between Geenius and HIV-1 WB was 75.3% (128/170).The paired comparison between Geenius and HIV-1 WB of HIV-1 positive vs. other results showed no statistical difference ($p = 0.1687$).

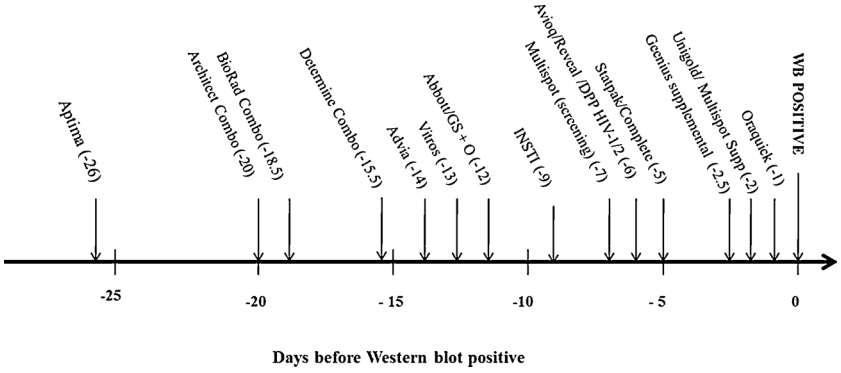

Fig. 1. Sensitivity of assay reactivity during early HIV-1 infections as number of days before first positive western blot (WB) when 50% of specimens tested with each test became positive.

The names, abbreviations, and sources of the HIV assays previously evaluated[11,12] are as follows: APTIMA HIV-1 Quantitative RNA assay (Aptima, Gen-Probe, Inc., San Diego, CA); ARCHITECT® HIV Ag/Ab Combo assay (Architect; Abbott Diagnostics, Wiesbaden Germany; CE marked version was used as the US version was not available when testing was conducted); GS HIV Combo Ag/Ab (Bio-Rad Combo; Bio-Rad Laboratories, Redmond, WA); Determine™ HIV-1/2 Ag/Ab Combo (Determine Combo (rapid test); Alere Medical Co., Ltd. Scarborough, ME); GS HIV-1/HIV-2 *PLUS* O EIA (GS + O; Bio-Rad Laboratories); VITROS anti-HIV 1 + 2 assay (Vitros; Ortho-Clinical Diagnostics, Buckinghamshire, UK); ADVIA Centaur HIV 1/O/2 enhanced assay (Advia; Bayer, Tarrytown, NY); Abbott HIVAB HIV-1/2 (rDNA) EIA (Abbott; Abbott Laboratories, Abbott Park, IL); Avioq HIV-1 Microelisa system (Avioq; Avioq, Inc, Rockville, MD); INSTI (INSTI™ HIV-1/HIV-2 Rapid Antibody test, BioLytic, Canada); Multispot HIV-1/HIV-2 rapid test (Multispot; Bio-Rad Laboratories); Clearview HIV-1/2 STAT-PAK rapid test (Statpak; Inverness Medical, Princeton, NJ); Clearview COMPLETE HIV-1/2 rapid test (Complete; Inverness Medical); CHEMBIO DPP® HIV-1/2 (DPP; CHEMBIO Diagnostics Systems, Inc. Medford, NY); Reveal G2 and G3 Rapid HIV-1 rapid antibody tests (Reveal G2 or G3 ; MedMira Laboratories, Inc.; Halifax, Nova Scotia, Canada); OraQuick ADVANCE Rapid HIV-1/2 antibody test (Oraquick; OraSure Technologies, Inc.; Bethlehem, PA); Uni-Gold Recombigen HIV rapid test (Unigold; Trinity Biotech USA, St. Louis, MO); Geenius HIV1/2 Supplemental Assay (BioRad Combo; Bio-Rad Laboratories). These assays have manufacturer reported point estimates for sensitivity and specificity ranging from 99.60% to 100.00% and 98.60% to 99.90%, respectively. The Genetic Systems HIV-1 WB (Bio-Rad Laboratories) and Cambridge Biotech HIV-1 WB (Maxim Biomedical Inc., Rockville, MD) have been shown to give concordant interpretations in studies conducted to qualify use in our clinical laboratory and were used interchangeably.

Table 1
Comparison of Geenius, HIV-1 Western blot, and Multispot Reactivity with HIV-1 Infections.

		Geenius final assay interpretations			
	Total	HIV NEG	HIV-1 IND	HIV-1 POS	HIV-2 IND
ARC-POS/WB-NEG	78	75	2	1	0
ARC-POS/WB-IND	32	17	6	5	4*
ARC-POS/WB-POS	60	5	8	47	0
ARC-POS/MS-NEG	103	88	6	6	3*
ARC-POS/MS-IND	14	5	4	4	1*
ARC-POS/MS-HIV-1-POS	68	4	7	57**	0
ARC-POS/MS-UND	1	0	0	1	0

HIV infections were confirmed with nucleic acid amplification testing; ARC, Abbott ARCHITECT Ag/Ab assay; WB, HIV-1 Western blot; MS, Multispot; NEG, negative; IND, indeterminate; POS, positive; UND, undifferentiated; * gp140 only reactivity observed with software version v1.1 was lost when retesting samples with v1.3, three changed interpretation to HIV antibody negative and one to HIV indeterminate; ** eight specimens required 1:10 dilution following the MS protocol.

Table 2
Comparison of Geenius, HIV-1 Western blot, and Multispot Performance on Abbott ARCHITECT HIV Ag/Ab Combo Assay-reactive, but HIV-1 NAT Negative Samples.

	Geenius final assay interpretations				
	Total	HIV NEG	HIV-1 IND	HIV-1 POS	HIV-2 IND
ARC-POS/WB-NEG	72	67	2	0	3*
ARC-POS/WB-IND	14	13	0	1	0
ARC-POS/WB-POS	1	1	0	0	0
ARC-POS/MS-NEG	89	85	1	0	3*
ARC-POS/MS-IND	2	0	1	1	0
ARC-POS/MS-HIV-2 POS	2	2	0	0	0

ARC, Abbott ARCHITECT Ag/Ab assay, WB, HIV-1 Western blot; MS, Multispot; NAT, HIV-1 nucleic acid test; NEG, negative; IND, indeterminate; POS, positive; *when samples were retested with Geenius software v1.3 two samples were HIV antibody negative.

3.2.2. Geenius v1.1 and MS in HIV-1 positive samples

The comparison of 186 ARC-positive/HIV-1 NAT-positive samples tested with MS and Geenius is shown in Table 1. Among 117 (62.9%) MS-negative/-indeterminate, likely early HIV-1 infections, Geenius detected four samples as HIV-2 indeterminate with gp140 only reactivity. Among the 69 MS-HIV-1 positive/-HIV undifferentiated samples, likely established infections, 58 (84%) were Geenius HIV-1-positive. Overall concordance between Geenius and MS was 80.1% (149/186).The paired comparison between MS and Geenius performance as supplemental tests in the diagnostic algorithm (HIV-1 positive vs. other results) showed no statistical difference ($p = 0.8312$). Geenius was HIV-1 positive in all eight samples which required the MS dilution protocol and in one MS-undifferentiated sample that required NAT to confirm infection.

3.2.3. Geenius v1.1 analysis in ARC-positive/HIV-1 NAT-negative samples

ARC-positive plasma specimens that were HIV-1 NAT-negative and possibly ARC-false positive results were tested with Geenius and HIV-1 WB (n = 87) and Geenius and MS (n = 93) and results are shown in Table 2. HIV-1 WB was negative in 72/87 (82.8%), MS was negative in 89/93 (95.7%) and Geenius was negative in 87/93 (93.5%) of HIV-1 NAT negative specimens. There was no agreement in positivity between HIV-1 WB and Geenius, and MS and Geenius in this sample set. Overall, Geenius classified three samples as HIV-2-indeterminate with gp140 only reactivity. In addition, to further address false reactivity 88 of 93 ARC-positive samples were tested with BRC and six were BRC-repeatedly reactive. Of those six, one was HIV-1 WB-indeterminate/MS HIV-1-indeterminate/Geenius HIV-1-positive and one was HIV-1 WB-negative/MS HIV-1-indeterminate/Geenius HIV-1 indeterminate. The remaining four samples were WB and MS negative.

3.3. Test concordance among HIV-2 infections

3.3.1. Geenius v1.1 vs. HIV-2 Western blot

The comparison between Geenius and HIV-2 WB in 100 HIV-2 samples is shown in Table 3. Of 99 HIV-2 WB-positive samples, 85 (85.9%) samples were Geenius HIV-2 Ab-positive including 50 HIV-2 positive with HIV-1 cross-reactivity. The remaining 14 Geenius v1.1 results that required NAT to confirm infection included nine (9.1%) HIV untypable, one (1%) HIV-2 indeterminate, one (1%) HIV indeterminate, and one (1%) HIV negative from the Ivory Coast and two (2%) HIV-2 indeterminate from the U.S. One HIV-2 WB indeterminate was also Geenius HIV-negative, but MS HIV-2 positive. The paired comparison

Table 3
Comparison of Geenius, HIV-2 Western blot, and Multispot Reactivity with HIV-2 Antibody-positive Specimens.

	Geenius final assay interpretations						
	Total	HIV NEG	HIV IND	HIV-2 IND	HIV-2 POS	HIV-2 POS XR HIV-1	HIV UNTYP
BRC-POS/WB-HIV-2 POS	99	1	1	3*	35	50	9**
BRC-POS/WB-HIV-2 IND	1	1	0	0	0	0	0
BRC-POS/MS-HIV-2 POS	99	2	1	3*	35	49	9**
BRC-POS/MS-UND	1	0	0	0	1	0	0

BRC, Bio-Rad GS HIV1/2 Ag/Ab Combo assay; WB, HIV-2 WB; MS, Multispot; NEG, negative; IND, indeterminate; POS, positive; XR HIV-1, cross-reactivity with HIV-1; UNTYP, untypable; UND, undifferentiated; HIV-2 positive and HIV-2 positive with cross-reactivity with HIV-1 are considered HIV-2 infections per package insert; *one specimen required 1:10 dilution following the MS protocol and when samples were retested with Geenius software v1.3 one was HIV-2 positive; ** four specimens required 1:10 dilution and two 1:100 dilution following the MS protocol.

(HIV-2 positive vs. other results) showed that HIV-2 WB confirmed more HIV-2 positive than Geenius ($p = 0.0005$).

3.3.2. Geenius v1.1 vs. MS

The comparison between Geenius and MS in 100 HIV-2 samples is shown in Table 3. Of 99 MS HIV-2-positive samples, 84 (84.9%) were Geenius HIV-2 Ab-positive including. 49 HIV-2 positive with HIV-1 cross-reactivity. Six of nine Geenius untypable and one of four Geenius HIV-2 indeterminate samples were MS HIV-undifferentiated initially and required the MS dilution protocol for confirmation. Like with HIV-2 WB, the paired comparison (HIV-2 positive vs. other results) showed that MS performed better with more confirmed HIV-2 samples than Geenius as a supplemental test ($p = 0.0012$).

3.4. Comparison of Geenius V1.1 and V1.3

To address the impact of the new gp140 cutoff, a subset of 48 samples were tested with Geenius software versions v1.1 and v1.3 (Table 4). All Geenius HIV-2 indeterminate results among non-HIV-2 samples had only gp140 reactivity with v1.1. When retested with v1.3, only one sample remained HIV-2-indeterminate with only gp140 reactivity, five became HIV Ab-negative and one HIV-indeterminate. In contrast, among HIV-2 samples HIV-2 indeterminate results had only gp36 reactivity in v1.1 and v1.3. After retesting, one HIV-2-indeterminate and two HIV-untypable samples with v1.1 became HIV-2-positive with HIV-1 cross-reactivity with v1.3. Samples were better classified with v1.3 than v1.1.

4. Discussion

Supplemental HIV diagnostic assays are crucial for the serologic confirmation of HIV-1 and HIV-2 infections. We evaluated the Geenius HIV 1/2 supplemental test as a replacement for the MS HIV-1/HIV-2 Rapid Test in the CDC/APHL laboratory diagnostic testing algorithm using a combination of HIV-1 and HIV-2 specimens first testing reactive with an Ag/Ab combo test. In addition, we compared the performance of Geenius to HIV-1/HIV-2 WB since they are still used in several laboratories in the US. To the best of our knowledge, our study is the first in the US comparing the relative seroconversion reactivity of the Geenius and MS HIV 1/2 supplemental assays to first HIV-1 WB-positive using longitudinal specimens from HIV-1 subtype B seroconverters. Results show that both supplemental assays, Geenius and MS, become reactive a few days before the first HIV-1 WB-positive. Among specimens from a study designed to identify acute infections [16], there were no significant differences between Geenius and MS or HIV-1 WB in the proportion of specimens identified as HIV-1 positive. Thus, we conclude that the three assays perform similarly with HIV-1 samples.

Similar performance results were also reported by two other US studies [18,19] and one Canadian study [3] when comparing Geenius with MS. Two US studies reported a 100% agreement between Geenius and MS in 250 established HIV-1 infections, with only four specimens testing Geenius untypable or undifferentiated. However, there was 99.7% agreement when HIV-1 positive samples were identified using the current diagnostic algorithm [18,19]. The Canadian study showed 100% agreement in sensitivity for both MS and Geenius, and 96.3% and 99.1% in specificity for MS and Geenius, respectively. The agreement between MS and Geenius in our HIV-1 sample set, that included 62.9% early HIV-1 infections, was lower than what was previously reported from studies that included only a small number of samples from acute HIV-1 infections [4].

The similar results between Geenius and HIV-1 WB are consistent with studies performed in Europe and Asia [4,6,7]. In a study from

Table 4
Comparison of Geenius V1.1 and V1.3 Results in a Sample Subset.

	Geenius final assay interpretations							
	HIV NEG	HIV IND	HIV-1 IND	HIV-1 POS	HIV-2 IND	HIV-2 POS	HIV-2 POS XR HIV-1	HIV UNTYP
HIV-1 infections								
Geenius v1.1	1	0	9	0	4	0	0	0
Geenius v1.3	4	1	7	2	0	0	0	0
HIV-2 infections								
Geenius v1.1	1	0	0	0	3	7	10	9
Geenius v1.3	0	1	0	0	2	7	13	7
ARC-POS/HIV-1 NAT-NEG								
Geenius v1.1	1	0	0	0	3	0	0	0
Geenius v1.3	3	0	0	0	1	0	0	0

ARC, Abbott ARCHITECT Ag/Ab assay; NAT, nucleic acid test; NEG, negative; IND, indeterminate; POS, positive; XR HIV-1, cross-reactivity with HIV-1; UNTYP, untypable; UND, undifferentiated.

Belgium, 44 HIV BLOT 2.2- positive samples were all confirmed by Geenius [6]. A larger study from Italy [4] also demonstrated similar results for Geenius and New LAV I and II, a different WB test from Bio-Rad, where both assays confirmed 378 of 406 ARC-positive samples. Combined, these international studies show that Geenius can be an acceptable alternative for MS and WB for confirmation of HIV-1 infection.

In contrast, when compared to previous studies reporting both Geenius and MS accurately differentiating HIV-1 and HIV-2 Abs [3,18], our study found that Geenius performance was lower. Geenius detected HIV-2 antibodies in fewer specimens known to be HIV-2 antibody-positive compared to MS and HIV-2 WB. One possible explanation could be the effect of long-term storage at -80 °C before Geenius testing.

The addition of three new test result interpretations produced by Geenius compared to MS might increase the number of HIV NATs needed to confirm infections using the HIV diagnostic algorithm, especially to rule out HIV-2 infections. The new HIV-2-indeterminate result was observed in non-HIV-2 positive samples with only HIV-2 gp140 reactivity when using Geenius v1.1. In contrast, among HIV-2 antibody-positive samples reactivity to only the HIV-2 gp36 antigen was observed. An increased number of HIV-2-indeterminate results with Geenius was observed in another study [19] in which 13 of 161 HIV-negative samples were Geenius HIV-2-indeterminate due to reactivity to gp140 only. After this study was completed, Bio-Rad updated their software (V1.3) to increase the gp140 cutoff and potentially decrease the number of HIV-2 indeterminate results due to gp140 only reactivity. When we retested samples using the upgraded Geenius software V1.3, we found the new gp140 cutoff reduced HIV-2 indeterminate results among non-HIV-2 samples, but it did not impact the identification of true HIV-2 infections. Although this evaluation is on a limited sample size and more studies are needed to evaluate the impact of the software V1.3, these results are very promising.

In summary, in using the 2014 CDC/APHL recommended laboratory diagnostic algorithm the Geenius HIV 1/2 supplemental assay performed similarly to the discontinued MS and to HIV-1 WB for identifying HIV-1 infections. Geenius underperformed compared to MS and HIV-2 WB with HIV-2 infections. Reflex to HIV NATs allows identification of acute HIV infections and the upgraded Geenius software may reduce the need for NAT among samples with non-specific gp140 reactivity.

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Competing interest

No financial disclosures were reported by the authors of this paper.

Ethical approval

The study was approved by CDC through a research determination in accordance with federal human participants' protection regulations and CDC policies and procedures.

Disclaimer

The findings and conclusions in this report are ours and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of brand names is for identification purposes and does not imply endorsements by the US Department of Health and Human or the Centers for Disease Control and Prevention.

CRediT authorship contribution statement

Wei Luo: Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Vickie Sullivan:** Investigation, Data curation, Writing - original draft. **Tara Smith:** Investigation, Data curation. **Philip J. Peters:** Conceptualization, Resources, Writing - original draft. **Cynthia Gay:** Resources, Data curation, Writing - original draft. **Emily Westheimer:** Resources, Data curation, Writing - original draft. **Stephanie E. Cohen:** Resources, Data curation, Writing - original draft. **S. Michele Owen:** Writing - review & editing, Supervision, Funding acquisition. **Silvina Masciotra:** Conceptualization, Methodology, Validation, Formal analysis, Visualization, Data curation, Writing - review & editing, Supervision, Project administration.

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