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ORIGINAL ARTICLE

Evaluation of a Rapid Confirmatory Human Immunodeficiency Virus Test: Overall Performance and Band Analysis

Yassmine Amor [1, 2], Amal Zouaki [1, 2], Jalila Zirar [1, 2], Ghizlane El Amin [1, 2], Nora Touyar [1, 2], Myriam Seffar [1, 2], Sessouma Abou [1, 2], Sami Belkhettab [1, 3], Wafae Ammouri [1, 3], Hakima Kabbaj [1, 2]

[1] *Mohamed V University, Faculty of Medicine and Pharmacy, Rabat, Morocco*
[2] *Ibn Sina University Hospital Center, Specialties Hospital, Central Laboratory of Virology, Rabat, Morocco*
[3] *Department of Internal Medicine, Faculty of Medicine and Pharmacy of Rabat, Ibn Sina University Hospital Center, Mohammed V University, Rabat, Morocco*

ABSTRACT

Background: **Accurate human immunodeficiency virus diagnosis is required to treat and prevent transmission. HIV western blot (MP-WB) has major drawbacks such as prolonged turnaround times, and high percentages of indeterminate results. To address these issues, the CDC in 2014 recommended the use of an immunological test capable of differentiating anti-HIV-1 antibodies from anti-HIV-2 antibodies for HIV diagnosis. The Geenius™ HIV 1/2 Confirmatory Assay/Bio-Rad is a rapid, single-use immunochromatographic test designed to confirm and differentiate HIV antibodies.**

Methods: **This study, conducted at the Central Virology Laboratory of the Rabat Specialty Hospital, Morocco, evaluated the performance of the Geenius test as an alternative to MP-WB for confirming HIV-1 diagnoses over 24 months. 116 samples were included, 113 patient samples with repeatedly reactive HIV screening test (HIV Combo Ag/Ab) and 3 external quality evaluation programs. Samples were tested by Geenius™ HIV-1/2 confirmatory assay and MP-WB. A nucleic acid amplification test (NAAT) was performed for discordant cases.**

Results: **Geenius and MP-WB mostly agreed in 95% of the overall, with a Cohen's kappa coefficient at 0.728, indicating substantial agreement. Geenius also produced fewer indeterminate results compared with MP-WB (16.67% vs. 83.33%), mainly in low-reactive samples. At the band level, the highest performances were seen with Gp160 and Gp41, showing concordance rates of 97% and 99%, respectively, with Cohen's kappa coefficient of 0.866 and 0.955. In contrast, the p24 and p31 bands demonstrated lower performances.**

Among six discordant results, five had low HIV Combo index values and were NAAT confirmed negative. A single high screening ratio and positive NAAT discordant case was classified as acute HIV infection. Excluding indeterminate Geenius results, the Geenius assay displayed 99% sensitivity, 100% specificity, 100% PPV, and 90.9% NPV, which justifies its better diagnostic performance compared to MP-WB.

Conclusions: **The Bio-Rad Geenius HIV-1/2 confirmatory test represents a reliable, rapid, and less labor-intensive alternative to MP-WB, offering a simplified approach for confirming HIV diagnoses, with good sensitivity for gp41 and gp160 bands, and can be integrated into diagnostic algorithms.**

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Correspondence:
Yassmine Amor
Central Laboratory of Virology
Faculty of Medicine and Pharmacy of Rabat
Ibn Sina University Hospital Center
Mohammed V University
Rabat
Morocco

Phone: +212 661680240
Email: yassmineamor02@gmail.com

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INTRODUCTION

Accurate laboratory diagnosis of human immunodeficiency virus (HIV) infection is essential for identifying individuals who could benefit from antiretroviral therapy and for reducing the risk of HIV transmission. Despite low HIV prevalence in the general Moroccan population, a focused epidemic is found among key populations at higher risk of exposure [1]. Revised algorithms for Human Immunodeficiency Virus confirmatory testing have been recommended by various institutions worldwide. The Centers for Disease Control and Prevention (CDC) in the United States (US) updated its recommendations for HIV confirmation in 2014, such that after an initial fourth-generation HIV Ag/Ab screening test, the use of HIV western blot was replaced by a HIV-1/HIV-2 antibody differentiation assay as a supplemental test for HIV confirmation [2].

Geenius™ HIV 1/2 is a single use immunochromatographic assay that uses recombinant and specific synthetic peptides. It decreases reporting time, identifies patients with acute HIV infection earlier, and improves patient care [3,4] while western blot (MP-WB) testing takes several hours of technical time, requires training and expertise in data interpretation. Moreover, it may produce indeterminate results and delay reporting, and may be misinterpreted in patients with HIV-2 infection due to antigenic cross-reactivity [5].

This study assessed both the global performance of the Bio-Rad Geenius HIV 1/2 assay and its individual band analysis in confirming HIV-1 infection.

MATERIALS AND METHODS

Samples

This retrospective study was conducted at the Central Virology Laboratory of the Specialty Hospital of Rabat, Ibn Sina University Hospital Center, Morocco. We included in this study all patients whose serum was repeatedly reactive at HIV Ag/Ab Combo/Alinity test during the study period, which lasted 24 months, from June 2022 to June 2024. The patients were from various clinical departments of the Ibn Sina University Hospital Center.

Serum was collected in dry tubes, with or without a separation gel. The Geenius™ HIV ½, the MP Diagnostics HIV Blot 2.2 (MP-WB) were performed in the same sample. In case of discordant result, an additional EDTA tube sample was requested for HIV Nucleic Acid Amplification Test (HIV-1 NAAT). Samples were stored at -20°C until analysis.

The HIV Ag/Ab Combo Test

The HIV Ag/Ab Combo is a microparticle chemiluminescent immunoassay (CMIA) performed on the Alinity /Abbott analyzer. It is used for the qualitative simultaneous detection of the HIV p24 antigen and antibodies against Human Immunodeficiency Virus Type 1 and/or Type 2 (HIV-1/HIV-2) in human serum or plasma. A sample is considered non-reactive if the result is < 1.0 s/co (signal-to-cutoff) and reactive if the result is ≥ 1.0 s/co. When a sample was reactive, it was rechecked after centrifugation at 10,000 g for 10 minutes [6].

Geenius™ HIV 1/2 Confirmatory Assay / Bio-Rad

The Geenius™ HIV 1/2 Confirmatory Assay (Geenius) is a qualitative immunochromatographic test designed for the confirmation and differentiation of specific antibodies against HIV-1 and HIV-2 in samples of capillary whole blood, venous whole blood, serum, or plasma. It is a single-use test and does not require multiple samples or series testing. This test includes six "test" bands (numbered 1 to 6) corresponding to recombinant antigens of HIV-1 (p31, gp160, p24, gp41) and HIV-2 (gp36, gp140) fixed on a solid-phase membrane, along with a control band "C" to ensure the validity of the test. The test takes approximately 40 minutes to perform and requires 5 µL of serum or 15 µL of whole blood [7].

According to Bio-Rad criteria, a result is interpreted as positive for HIV-1 when there are two bands with at least one corresponding to an envelope (ENV) protein. It is considered negative if no bands are detected, and indeterminate if the band pattern does not meet the criteria for a positive result [7].

MP Diagnostics HIV Blot 2.2/MP

The MP Diagnostics HIV Blot 2.2/MP Biomedicals (western blot) is a qualitative immunoenzymatic test designed for the confirmation of specific antibodies against HIV-1 and HIV-2 in human serum or plasma. The test uses specific antigens of HIV-1 and a synthetic peptide specific to HIV-2. The reading is performed visually by the operator. Each test strip includes internal control bands to validate test performance (positive, weakly positive and negative control serum) that are also tested alongside six patient samples to confirm the reliability of results [8].

Interpretation of the western blot follows manufacturer guidelines, where a result is considered negative if no viral specific bands are present or if only p17 antibodies are detected without any other bands. A result is HIV-1 positive when there is detection of two envelope (ENV) bands (gp160, gp41, gp120) along with either one GAG band (p17, p24, p55) or one POL band (p31, p51, or p66). A result is interpreted as HIV-1 indeterminate if viral specific bands are present but the pattern does not fulfill the criteria for a positive result [8].

Xpert® HIV-1 Viral Load

The Xpert® HIV-1 Viral Load test is an *in vitro* reverse transcription polymerase chain reaction test for the de-

tection and quantification of HIV-1 RNA in human plasma collected from patients infected with HIV-1 using automated GeneXpert systems. The test can quantify HIV-1 RNA within a range of 40 to 10,000,000 copies/mL. In our study, it was only performed for discordant Geenius and WB-MP results [9].

Statistical analysis

The statistical analysis of data was performed with JAMOVI V 2.5.6.0 statistical software. The results were expressed as percentage and effective for qualitative variables and as median and quartile for quantitative variables. The comparison between Geenius HIV1/2 and western blot was evaluated by the Cohen's kappa coefficient (< 0.20 slight, 0.21 - 0.4 fair, 0.41 - 0.60 moderate, 0.61 - 0.8 substantial, 0.81 almost perfect). The sensitivity and specificity were determined after excluding Geenius indeterminate result.

RESULTS

According to our inclusion criteria, a total of 116 specimens were selected. Among these, 113 from patients who tested reactive twice on the HIV screening test (HIV Combo Ag/Ab), and three external quality evaluation (EQE) samples. The screening test signal-to-cutoff (S/CO) ratios had a median value at 727 S/CO, with an interquartile range of 532 - 887 S/CO and a total range of 1.02 - 1,362 S/CO. The overall agreement between the Geenius and western blot assays was 95%, with a Cohen's kappa value of 0.728, indicating substantial agreement (Table 1).

When evaluating the concordance of individual bands, Geenius assay showed high concordance with MP-WB for Gp160 and Gp41, with agreement rates of 97% and 99%, and kappa values of 0.866 and 0.955, respectively, indicating excellent agreement. However, performance was lower for p24 and p31, with agreement rates of 72% and 70%, and kappa values of 0.292 and 0.377, respectively, indicating only fair to moderate agreement (Figure 1).

Among the six discordant results, five cases (83.3%) exhibited low HIV Combo index values (< 10 S/CO). Of these, one patient had an indeterminate Geenius result, and tested negative by both MP-WB and HIV-1 NAAT. The remaining four patients were all indeterminate by MP-WB and negative by Geenius and HIV-1 NAAT (Table 2). One discordant case involved a patient with a high HIV Combo Ag/Ab index value, whose sample was negative by Geenius and indeterminate by MP-WB. This profile was consistent with an acute HIV-1 infection (Table 2).

As for Geenius performances compared with MP-WB, the assay demonstrated excellent overall diagnostic accuracy, with a sensitivity of 99%, specificity of 100%, a positive predictive value (PPV) of 100%, and a negative predictive value (NPV) of 90.9%. These findings show the high reliability of the Geenius test in correctly identifying cases (Table 3). At the individual antigen level, Gp160 and Gp41 showed excellent performance, while p24 and p31 had limited diagnostic accuracy (Table 3).

DISCUSSION

For over 20 years, the western blot has served as the primary confirmatory test for HIV. While it is specific and reliable, it is also known to be labor-intensive, time-consuming, and subjective, requiring technical expertise for interpretation. Additionally, it can sometimes yield indeterminate results, causing delays in providing final diagnoses [10].

Bio-Rad developed a new confirmatory and differentiation test, the Geenius HIV-1/2 Confirmatory Assay, which was approved by the Food and Drug Administration in October 2014 [11]. Since then, it has been evaluated in many studies [10,12,13], including a few studies comparing between Geenius and MP-WB [10]. Our results showed an overall sensitivity and specificity of the Geenius HIV1/2 based on MP-WB and HIV-1 NAAT at 99% and 100.0%, respectively. The only false-negative result (Table 3) occurred in a patient with an acute HIV-1 infection.

Reported sensitivities and specificities in previous studies ranged from 99.3 - 100% and 96.3 - 100%, respectively [10,12,13]. The sensitivity could be lower in studies including specimens of seroconversion or acute infection [10,14].

Montosonis et al. compared the performance of Geenius with that of MP-WB. The study included 160 specimens and reported an overall sensitivity of 92% for the Geenius assay, compared with 89% for the MP-WB. In HIV seroconversion samples, MP-WB yielded more indeterminate but fewer negative results than Geenius. After excluding early seroconversion samples, the sensitivity of both Geenius and MP-WB reached 100%. These findings highlight the value of NAAT in cases of seroconversion [10].

Kondo et al. evaluated the Geenius HIV-1/2 Confirmatory Assay against the conventional western blot assays (HIV-1 WB and HIV-2 WB) using 166 HIV-1 positive samples (146 from patients with established HIV-1 infection and 20 from patients with acute infection), five HIV-1 seroconversion and 140 HIV negative samples from the Japanese population. Although the sensitivity of Geenius and MP-WB was not significantly different (99.3% vs. 98.6%) for samples from established HIV-1 infections, Geenius gave seven positive results in 20 MP-WB negative or indeterminate samples from acute HIV-1 infections and provided positive results earlier than MP-WB in two of five seroconversion panels, showing that Geenius is more sensitive than MP-WB. For 140 HIV-1 negative samples including 10 false-positive samples, Geenius gave 136 negative and MP-WB gave 112 negative results, showing that Geenius is more specific than MP-WB [15].

In band interpretation, agreement between the Geenius

Table 1. Overall agreement between Geenius HIV ½ assay and western blot.

Geenius assay				
		negative	positive	indeterminate
Western blot	negative	6	0	1
	positive	0	104	0
	indeterminate	5	0	0
	agreement %	95		
	Cohen's kappa	0.728		

Table 2. Specimens with discordant results between Geenius HIV ½ assay and western blot.

Patients	HIV Combo Ag/Ab (S/CO)	Geenius HIV ½ assay	Western blot	Discordant band	HIV-1 NAAT	Interpretation
1	1.25	indeterminate	negative	P31	negative	HIV negative
2	1.02	negative	indeterminate	P24	negative	HIV negative
3	1.48	negative	indeterminate	P24	negative	HIV negative
4	4.21	negative	indeterminate	P24	negative	HIV negative
5	9.45	negative	indeterminate	Gp160	negative	HIV negative
6	167	negative	indeterminate	P24	positive	HIV positive

S/CO: signal to cutoff value, NAAT: nucleic acid amplification test.

Table 3. Overall performances of Geenius HIV1/2 assay based on Western Blot *.

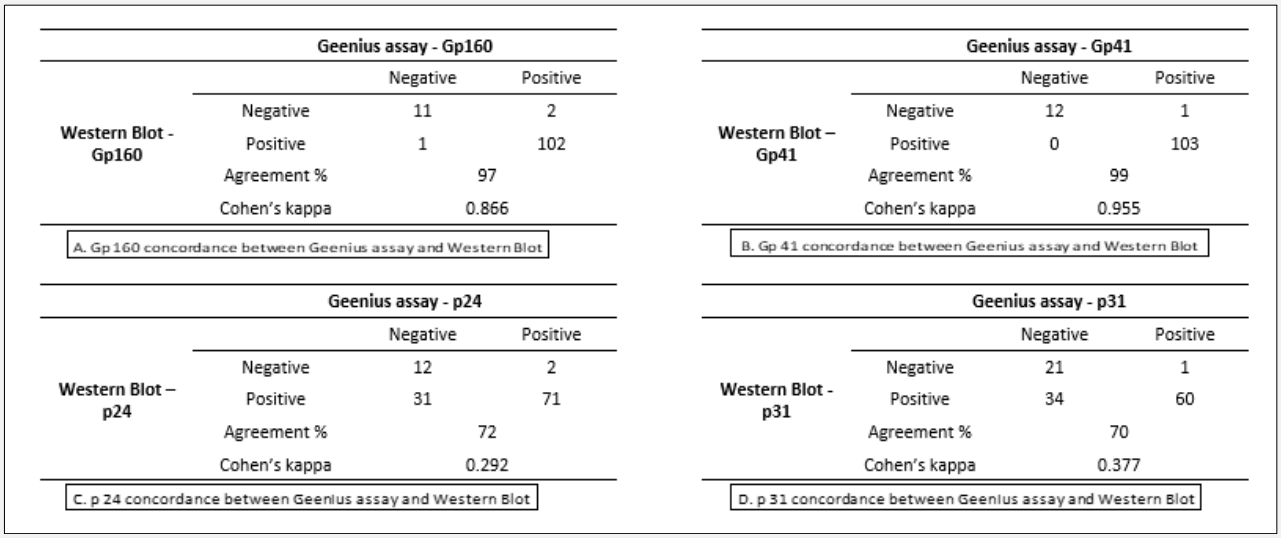
	TP (N)	FP (N)	TN (N)	FN (N)	Sensibility (%)	Specificity (%)	NPV (%)	PPV (%)
Global	104	0	10	1	99	100	90.9	100
Gp160	104	0	11	1	99	100	91.7	100
Gp41	104	0	11	1	99	100	91.7	100
P24	73	0	11	32	69.5	100	25.6	100
P31	60	1	10	45	57.1	90.9	18.2	98.4

TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative, NPV: Negative Predictive Value, PPV: Positive Predictive Value.
*** The only Geenius HIV ½ Assay indeterminate result was excluded.**

assay and western blot was moderate. The assay showed excellent concordance for the Gp160 and Gp41 bands but demonstrated lower sensitivity for the p31 and p24 bands. However, this lack of sensitivity for these two bands does not affect the final interpretation of the results.

The study of E. Tuaillon et al. showed good performance for Geenius™ in confirming HIV infection during the early phase of infection, good sensibility for the detection of Gp41 and Gp160 bands, but lower performance to distinguish between acute from early chronic HIV-1 infection due to a lower capacity to detect p31 and p24 [16], which aligns with our results. These findings suggest that the detection of the p31 band should be improved by the manufacturer to facilitate the staging of early HIV infection [16].

Geenius HIV-1/2 Confirmatory Assay showed more advantage over MP-WB, as it provides fewer indeterminate results than MP-WB particularly in low reactive samples. In our study, Geenius had only one indetermi-



		Geenius assay - Gp160	
		Negative	Positive
Western Blot - Gp160	Negative	11	2
	Positive	1	102
	Agreement %	97	
	Cohen's kappa	0.866	

A. Gp 160 concordance between Geenius assay and Western Blot

		Geenius assay - Gp41	
		Negative	Positive
Western Blot – Gp41	Negative	12	1
	Positive	0	103
	Agreement %	99	
	Cohen's kappa	0.955	

B. Gp 41 concordance between Geenius assay and Western Blot

		Geenius assay - p24	
		Negative	Positive
Western Blot – p24	Negative	12	2
	Positive	31	71
	Agreement %	72	
	Cohen's kappa	0.292	

C. p 24 concordance between Geenius assay and Western Blot

		Geenius assay - p31	
		Negative	Positive
Western Blot - p31	Negative	21	1
	Positive	34	60
	Agreement %	70	
	Cohen's kappa	0.377	

D. p 31 concordance between Geenius assay and Western Blot

Figure 1. Individual bands concordance between Geenius HIV 1/2 and western blot assays.

nate result due to p31 compared to five with MP-WB (80% p24 and 20% Gp160).

With the recent introduction of the Geenius assay, studies have shown that incorrectly reported "indeterminate" but true negative cases using MP-WB could be resolved by Geenius, suggesting increased test specificity for the Geenius assay [10]. Chui Ching Wong compared Geenius performances to that of MP-WB and showed that this HIV 1/2 differentiating assay was able to resolve MP-WB HIV indeterminate cases without seroprogression as being negative to HIV, and was also able to confirm more early-stage cases as positive [17].

Furthermore, Geenius provides results sooner than western blot which is more advantageous for laboratories since it reduces both the time to train the operator and to report results. In addition, it allows testing of individual patient samples without requiring batch processing, enabling more flexible and timely diagnosis [18].

When comparing to MP-WB, Geenius demonstrates advantages in reliability within quality management, as previously highlighted [10]. Individual samples are analyzed in a separate closed cassette, minimizing the risk of contamination. Barcoding of sample and cassette minimizes the risk of error, while electronic capture and storage by the system ensure traceability. The application of an automatic reader also minimizes the risk of interpretive error [10].

CONCLUSION

This study highlights the significant advantages of the Geenius HIV 1/2 test in confirming HIV-1 infection, as it shows good sensibility for the detection of gp41 and gp160 bands and good agreement when compared to MP-WB assay. Moreover, it significantly reduces the number of indeterminate results.

Geenius also offers practical advantages including faster result reporting and reduced technical complexity making it more favorable to laboratory procedures. Its single-use, closed cassette format minimizes the risk of handling errors and cross-contamination. Although its performance has not been evaluated in patients positive for HIV-2, in the Moroccan context of low HIV-2 prevalence, this study suggests that Geenius is a promising alternative to MP-WB for HIV confirmatory diagnosis. These findings align with global trends and recommendations advocating for faster, more accurate, and patient-centered diagnostic approaches.

Data Availability:

The data used to support the results of this study are included within the article.

Source of Funds:

No funding was received for conducting this study.

Declaration of Interest:
The authors declare that there are no conflicts of interest regarding the publication of this article.

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