Geenius™ HIV 1/2 Supplemental Assay • Internal Performance Evaluation

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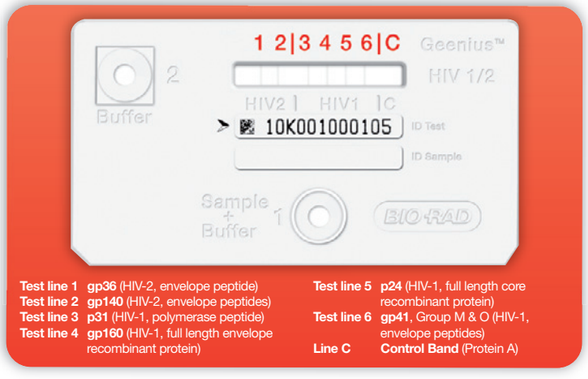
Introduction

The Geenius HIV 1/2 Supplemental Assay is a single-use immunochromatographic test for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 (HIV-1, Group M and O) and Types 2 (HIV-2) in fingerstick whole blood, venous whole blood, serum or plasma samples.
The Geenius HIV 1/2 Supplemental Assay is intended for use as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by screening procedures.
Geenius provides automatic reading and interpretation of the results in less than 30 minutes.
It is under CE marking process.
The goal of this study performed by Bio-Rad was to evaluate the performance of this new assay compared to confirmation and/or differentiation assays. Sensitivity and specificity were evaluated using routine blood bank samples, hospitalized patient samples, known positive samples and seroconversion panels.

Principle of the Test

The Geenius HIV 1/2 Supplemental Assay employs HIV-1 and HIV-2 antigens bound to the membrane and colloidal gold Protein A.
It contains 6 test lines and 1 control band which are numbered on the cassette.

Figure 1 • Geenius Cassette

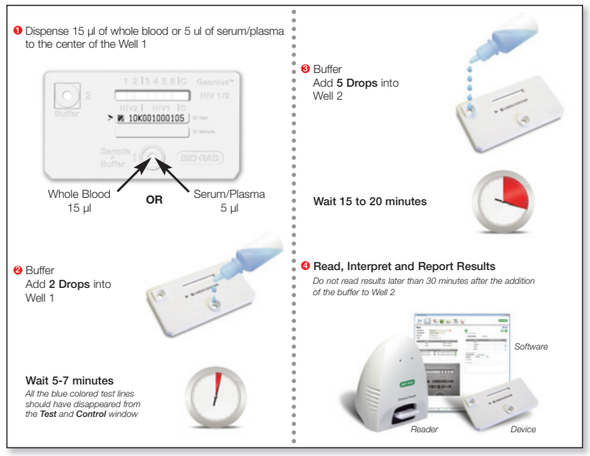


In a reactive sample, the antibodies are captured by the antigens immobilized in the **test** area (test lines 1 to 6). The colloidal gold Protein A binds to the captured antibodies, producing pink/purple lines.
In the absence of HIV antibodies, there are no pink/purple lines in the **test** area.

The sample and conjugate continue to migrate along the membrane and produce a pink/purple line in the **control area (C)** containing Protein A. This procedural control serves to demonstrate that sample and reagents have been properly applied and have migrated through the device.

Procedure

Figure 2 • Geenius Protocol



Interpretation Criteria

Table 1 • Package Insert Validation Criteria

Positivity Criteria	Geenius HIV 1/2 Supplemental Assay
HIV-1 Positive	1 ENV HIV-1 + GAG or 1 ENV HIV-1 + POL or 2 ENV HIV-1
HIV-2 Positive	2 ENV HIV-2
Negative	No band

Table 2 • Package Insert Global Assay Interpretation

HIV-2 Result	HIV-1 Result	Assay Interpretation
Negative	Negative	**HIV Negative**
Indeterminate	Negative	**HIV-2 Indeterminate**
Negative	Indeterminate	**HIV-1 Indeterminate**
Indeterminate	Indeterminate	**HIV Indeterminate**
Negative	Positive	**HIV-1 Positive**
Indeterminate	Positive	**HIV-1 Positive**
Positive	Negative	**HIV-2 Positive**
Positive	Indeterminate	**HIV-2 Positive**
Positive	Positive	**HIV-2 Positive with HIV-1 cross reactivity*** **HIV Positive Untypable***

** Differentiation features managed by proprietary algorithm.*

Specificity

This study was performed on blood bank routine samples (n = 430) and hospitalized patient samples (n = 100). These samples were chosen among serum and venous blood. No false positive results were found: specificity was calculated at 100%. Within this set of negative samples, the rate of indeterminate results was 3.5% for blood bank routine samples and 2% for hospitalized patient samples. With GS HIV-1 Western Blot, typically 15-25% of negative samples are HIV-1 WB indeterminate.

Table 3 • Specificity Results (n = 530)

	Blood bank donors		Hospitalized patients
	Serum samples	Whole blood samples	Serum samples
Negative	224	191	98
Indeterminate	6	9	2
Positive	0	0	0

Sensitivity

Sensitivity on HIV-1 Positive Samples

135 HIV-1 positive samples from group M and 5 HIV-1 positive samples from group O were tested.
100% were found HIV-1 positive. None of these HIV-1 positive samples were found HIV-2 positive.

Sensitivity on Seroconversion Panels

A total of 32 seroconversion panels (154 samples) were tested.
On 13/32 panels, Geenius showed higher sensitivity than GS HIV-1 Western Blot Assay, which corresponds to a total of a 65 samples found HIV-1 positive with Geenius versus 49 samples found HIV-1 positive with GS HIV-1 Western Blot Assay.

Table 4 • Results on Seroconversion Panels

	GS HIV-1 Western-Blot	Geenius HIV 1/2 Supplemental Assay
Negative	63	73
Indeterminate	42	16
HIV-1 Positive	49	65
Total	154	154

Sensitivity on HIV-2 Positive Samples

A total of 232 HIV-2 positive samples were tested.
100% were found positive. 150 (65%) of these HIV-2 positive samples were also found HIV-1 positive.
With Geenius software powered by proprietary algorithms, 221 samples (95%) were HIV-2 positive or HIV-2 positive with HIV-1 cross-reactivity. The number of non-differentiated samples decreased to 11 (5%).
With Multispot assay the number of non-differentiated samples is 11 (5%) using the dilution protocol.

Table 5 • HIV-2 Sensitivity Results (n = 221)

	Geenius HIV 1/2 Supplemental Assay	Multispot (dilution protocol)
Negative	0	0
Indeterminate	0	NA
HIV-2 Positive	82	221
HIV-2 Positive (with HIV-1 cross-reactivity)	139	NA
HIV Positive Untypable	11	11

Results on HIV 1/2 Co-Infected Samples

4 co-infected HIV-1 and HIV-2 samples perfectly characterized by HIV-1 and HIV-2 PCR, were tested.

With Geenius they were all correctly classified as non-differentiated, compared to Multispot which misclassified 2 out of 4 co-infected samples.

Table 6 • Results on HIV 1/2 Co-Infected Samples (n = 4)

	Geenius HIV 1/2 Supplemental Assay	Multispot HIV-1/HIV-2 Rapid Test
Sample 1	HIV Positive Untypable	Untypable
Sample 2	HIV Positive Untypable	HIV-2
Sample 3	HIV Positive Untypable	HIV-1
Sample 4	HIV Positive Untypable	Untypable

Conclusion

This evaluation suggests excellent performance in terms of specificity and sensitivity (on HIV-1/2 positive and seroconversion panels) for this new confirmation and differentiation HIV-1/2 assay. Moreover, the use of Geenius™ Software allows HIV-1/2 differentiation in more than 90% on HIV-2 samples.

The **Geenius™ HIV 1/2 Supplemental Assay** is the first:
- unitary assay with automatic reading and interpretation combining robustness, simplicity and complete traceability
- HIV Supplemental Assay providing the results in less than 30 minutes
- unitary confirmation assay validated on whole blood

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