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Comparison of the Performance of *Vio*One™ HIV Profile™ and Geenius™ HIV-1/2 Supplemental Assays

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ABSTRACT

Background: Currently, the immunoblot-based Geenius™ HIV-1/2 Supplemental Assay (Geenius; Bio-Rad Laboratories, Inc.) is the only FDA-approved HIV-1 and -2 antibody (Ab) confirmatory and differentiation assay used in the recommended HIV testing algorithm in the U.S. *Vio*One™ HIV Profile™ Supplemental Assay (*Vio*One; Avioq, Inc.) is a research use-only enzyme-linked immunosorbent assay (ELISA) developed for the same testing purposes. This study was conducted to compare the performance and workflow of Geenius and *Vio*One.

Methods: Residual plasma specimens (200 consecutive prospectively collected and 87 previously stored clinical samples, and 13-member AccuSet™ HIV-1/2 Performance Panel [SeraCare Life Sciences, Milford, MA]) were tested with the *Vio*One per manufacturer's instructions for comparison to known Geenius results with or without accompanying results of Architect HIV Ag/Ab Combo assay. Intra- and inter-assay reproducibility of *Vio*One were evaluated by observing the Ab reactivity to HIV-1 and -2 antigens used in the assay. Mean hands-on and hands-off time durations were determined from 3 separate assay runs (12 samples per run, including 2 assay controls) each of *Vio*One and Geenius for workflow comparison.

Results: Agreement of HIV-1 and -2 Ab interpretive results for all samples tested with *Vio*One and Geenius are shown in the table below, along with frequencies of minor (negative vs indeterminate HIV-1 or -2 Ab status) and major (positive vs negative HIV-1 or -2 Ab) discordances.

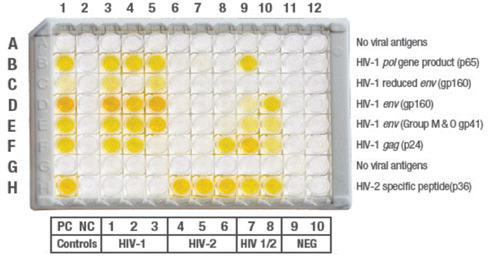
Sample type	*N*	Complete agreement	Minor discordances	Major discordances
Consecutive prospective samples	200	192 (96%)	7	1
Previously stored samples	87	44 (51%)	35	8
AccuSet panel	13	7 (54%)	5	1

Overall, indeterminate Ab results was observed less frequently with *Vio*One (1%) than Geenius (17%) among all samples tested. Intra- and inter-assay reproducibility of *Vio*One were 100% for an HIV Ab-negative, HIV-1 Ab-positive, and HIV-2 Ab-positive plasma specimens. Mean hands-on and total time durations of 67 and 187 min were needed to perform *Vio*One, compared with 68 and 88 min, respectively, for Geenius.

Conclusions: *Vio*One yielded HIV-1 and -2 Ab interpretive results comparable to those of Geenius among prospectively collected clinical plasma specimens. While hands-on time durations are comparable between the two assays, *Vio*One required significantly longer total duration than Geenius for assay completion.

INTRODUCTION

The immunoblot-based Geenius™ HIV-1/2 Supplemental Assay (Geenius; Bio-Rad Laboratories, Inc., Redmond, WA) is the only FDA-approved HIV Ab supplemental assay for use in the current recommended laboratory testing algorithm for the detection of HIV infection in the U.S. The *Vio*One™ HIV Profile™ Supplemental Assay (Avioq, Inc., Research Triangle Park, NC) is a research use-only assay developed for the same testing purposes. It is an enzyme-linked immunosorbent assay (ELISA) using recombinant HIV-1 *env, pol,* and *gag* gene products and an HIV-2 specific transmembrane peptide (gp36) as antigens individually coated onto the wells of microwell plates (see below).



We conducted a prospective study to determine the reproducibility and accuracy of *Vio*One in comparison to Geenius among plasma specimens, and to compare the hands-on, hands-off, and total time durations to perform both assays.

METHODS

Clinical specimens: Residual plasma specimens (87 previously stored and 200 consecutive prospectively collected clinical samples, and 13-member AccuSet™ HIV-1/2 Performance Panel [SeraCare Life Sciences, Milford, MA]) were used.

Specimen testing: Geenius and *Vio*One were performed according to manufacturers' instructions for use, with or without previous Architect HIV Ag/Ab Combo assay testing.

Reproducibility of *Vio*One was evaluated with intra-assay testing of 10 replicates each of HIV Ab-negative, HIV-1 Ab-positive, and HIV-2 Ab-positive plasma specimens and 10 inter-assay runs of replicates of the same 3 specimens.

Hands-on and hands-off time durations were determined from 3 separate assay runs (12 samples per run, including 2 assay controls) each of VioOne and Geenius for workflow comparison.

Table 1. Comparison of results among 87 previously stored clinical plasma specimens

VioOne result	Geenius result								
	HIV1+ HIV2-	HIV1+ HIV2 Ind	HIV1- HIV2-	HIV1 Ind HIV2-	HIV1 Ind HIV2 Ind	HIV1 Ind HIV2+	HIV1- HIV2 Ind	HIV1- HIV2+	HIV1+ HIV2+
HIV1+ HIV2-	16	10		4 [a]					2 [a]
HIV1+ HIV2 Ind									
HIV1- HIV2-			15	5	3		10		
HIV1 Ind HIV2-				1					
HIV1- HIV2+						7		6	2 [a]
HIV1+ HIV2+									6

-, Negative; +, Positive; Ind, Indeterminate.
[a] Geenius results showed gp41 or gp160 band in 4 samples, whereas VioOne detected patterns of p65 + gp160 + rgp160 + gp41 + p24, gp160 + gp41, gp160 + gp41 + p24, or gp160 + gp41.
[b] In both specimens, Geenius results showed gp36, gp140, p31, gp150, and gp41, but VioOne detected p65, gp160, rgp160, gp41, and p24.
[c] Geenius results of both specimens showed gp36, gp140, p31, gp160, p24, gp41 bands for HIV1, whereas VioOne detected only gp36 and p24 in both specimens.

Table 3. Comparison of results on the AccuSet™ HIV-1/2 Performance Panel members

Panel member	Geenius band result	Geenius interpretation	VioOne marker result	VioOne interpretation
1	gp140, p31, gp160, p24, gp41	HIV-1 Ab+; HIV-2 Ab Ind	p65, rgp160, gp160, gp41, p24	HIV-1 Ab+; HIV-2 Ab-
2	p31, gp160, gp41	HIV-1 Ab+; HIV-2 Ab-	p65, rgp160, gp160, gp41, p24	HIV-1 Ab+; HIV-2 Ab-
3	p31, gp160, gp41	HIV-1 Ab+; HIV-2 Ab-	p65, rgp160, gp160, gp41	HIV-1 Ab+; HIV-2 Ab-
4	p31, gp160, p24, gp41	HIV-1 Ab+; HIV-2 Ab-	p65, rgp160, gp160, gp41, p24	HIV-1 Ab+; HIV-2 Ab-
5	gp160, gp41	HIV-1 Ab+; HIV-2 Ab-	p65, gp160, gp41, p24	HIV-1 Ab+; HIV-2 Ab-
6	p31, gp160, gp41	HIV-1 Ab+; HIV-2 Ab-	p65, rgp160, gp160, gp41, p24	HIV-1 Ab+; HIV-2 Ab-
7	No band	HIV-1 Ab-; HIV-2 Ab-	No marker	HIV-1 Ab-; HIV-2 Ab-
8	gp36, gp140, p31, gp160, gp41	HIV Ab+, untypable	gp36, p24	HIV-1 Ab-; HIV-2 Ab+
9	gp36, gp140, p24	HIV-1 Ab Ind; HIV-2 Ab+	gp36, p24	HIV-1 Ab-; HIV-2 Ab+
10	gp36, gp140, p31, p24, gp41	HIV-1 Ab-R; HIV-2 Ab+	gp36, p24	HIV-1 Ab-; HIV-2 Ab+
11	gp36, gp140, p31	HIV-1 Ab Ind; HIV-2 Ab+	gp36	HIV-1 Ab-; HIV-2 Ab+
12	gp36, gp140, p31	HIV-1 Ab Ind; HIV-2 Ab+	gp36	HIV-1 Ab-; HIV-2 Ab+
13	gp36, gp140	HIV-1 Ab-; HIV-2 Ab+	gp36, p24	HIV-1 Ab-; HIV-2 Ab+

These bands are unique to the respective assays.
Minor discordant interpretation between the 2 assays.
Major discordant interpretation between the 2 assays.

Table 2. Comparison of results among 200 prospectively collected clinical plasma specimens

VioOne result	Geenius result				
	HIV1- HIV2-	HIV1+ HIV2-	HIV1 Ind HIV2-	HIV1+ HIV2 Ind	HIV1- HIV2 Ind
HIV1- HIV2-	118				2
HIV1+ HIV2-	1 [a]	73		4	
HIV1 Ind HIV2-	1		1		

[a] Abbott Architect HIV Ag/Ab assay was positive with S/CO = 14.99; Roche cobas HIV-1 assay result were "Undetected" on 2 occasions 1 month apart.

Table 4. Reproducibility of *Vio*One HIV Profile Assay

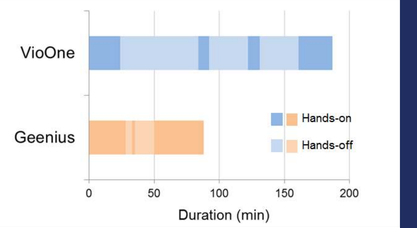
Sample	Geenius result	Intra-assay		Inter-assay	
		N	No. positive (%)	*N*	No. positive (%)
HIV Ab-negative	No band	10	0	10	0
HIV-1 Ab-positive	p31, gp160, p24, gp41	10	10 [a]	10	10 [a]
HIV-2 Ab-positive	gp36, gp140	10	10 [b]	10	10 [b]

[a] VioOne reactive markers detected: p65, rgp160, gp160, gp41, and p24.
[b] VioOne reactive markers detected: gp36 and p24.

Table 5. Reproducibility of *Vio*One-Specific HIV Ab

Sample	*N*	%CV of S/CO ratios on individual HIV-1/-2 Ab					
		HIV-1 p65	HIV-1 reduced gp160	HIV01 gp160	HIV-1 gp41	HIV-1 p24	HIV-2 p36
Intra-assay							
HIV-1 Ab-positive	10	0.8%	2.6%	1.1%	2.9%	1.6%	
HIV-2 Ab-positive	10					6.6%	2.6%
Inter-assay							
HIV-1 Ab-positive	10	9.6%	11.6%	9.3%	9.3%	11.8%	
HIV-2 Ab-positive	10					27.9%	10.7%

Figure 1. Comparison of hands-on, hands off, and total time duration for testing 12 samples



RESULTS

- 50.6% agreement (44 / 87) of results was obtained for Geenius and *Vio*One on the previously stored clinical plasma specimens, with major discordances occurring in 9.2% (8 / 87) of results (Table 1). Minor discordances were observed in 40.2% (35 / 87) of results, most likely due to differences in the freeze-thaw cycles of the specimens tested.
- 96% agreement (192 / 200) of results was obtained for the 2 assays in testing the prospective collected, consecutive clinical plasma specimens (Table 2). Major discordance was observed in only 1 specimen. Differences in freeze-thaw cycles and HIV antigens used probably accounted for the minor discordances (7 / 200) between the 2 assays.
- 53.8% agreement (7 / 13) of results was observed for Geenius and *Vio*One in testing the 13-member AccuSet™ HIV-1/2 Performance Panel (Table 3).
- Overall, indeterminate HIV-1 or HIV-2 Ab results were observed more frequently with Geenius (51/300, 17%) than *Vio*One (3/300, 1%), most likely due to the HIV antigens used and the interpretation of the epitope-specific antibodies detected.
- *Vio*One intra-assay and inter-assay reproducibility was 100% (Table 4) with low %CV (Table 5).
- Hands-on time duration was comparable between *Vio*One and Geenius (67 vs 68 min), but the former assay required longer total duration for assay completion (187 vs 88 mins) (Figure 1).

CONCLUSIONS

*Vio*One™ HIV Profile™ Supplemental Assay is reliable for detection and confirmation of HIV antibodies in clinical plasma specimens, in comparison to the Geenius assay. However, *Vio*One took longer duration for assay completion than Geenius.

Acknowledgement

We thank Avioq, Inc., for providing the *Vio*One™ assay reagents and technical support for this study and the Mayo Clinic Hepatitis / HIV Laboratory staff.

Evaluation of the Avioq VioOne HIV Profile Supplemental Assay as an alternative to the Bio-Rad Geenius HIV 1/2 Supplemental Assay

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Background

The recommended algorithm for HIV diagnosis distributed by the Centers for Disease Control and Prevention (CDC) (CDC 2018) states that clinical laboratories should perform a screening immunoassay for the detection of HIV-1/2 antibodies, an antibody differentiation assay, and potentially a nucleic acid test (NAT) for the confirmation of acute HIV infection.

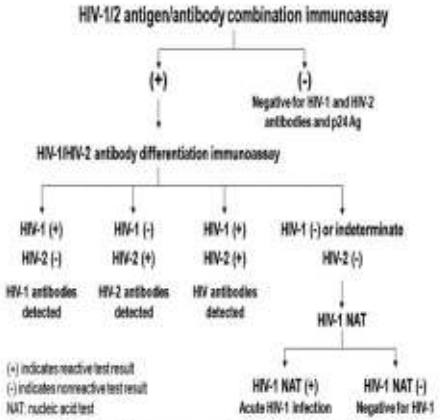

Figure 1. The CDC recommended algorithm for laboratory diagnosis of HIV infection.

Methods

In this study, 130 clinical serum specimens previously tested using the Bio-Rad Geenius Supplemental Assay were retested using the Avioq VioOne HIV Profile Supplemental Assay. Results from both platforms were compared to examine the performance of the Avioq VioOne HIV Profile Supplemental Assay.

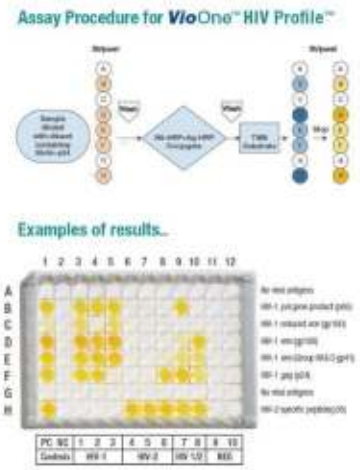

Figure 2. Overview of the VioOne HIV Profile assay procedure.

Figure 3. Bio-Rad Geenius Supplemental Assay cartridge.

Results

Results from the Bio-Rad Geenius HIV 1/2 Supplemental Assay and the Avioq VioOne HIV Profile Supplemental Assay passed accuracy criteria (>90%) with 99% concordance. There was only one discrepant result between the Geenius and the HIV Profile Assay.

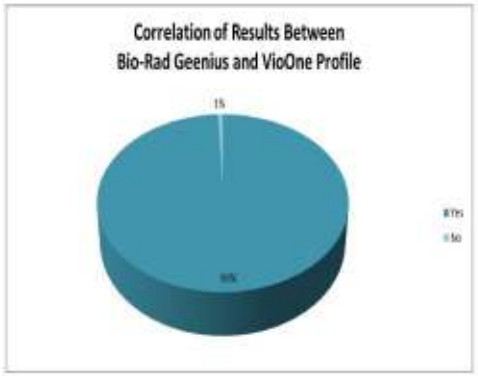

Figure 4. Correlation of results between both supplemental assays.

Conclusion

These studies comparing the Bio-Rad Geenius HIV 1/2 Supplemental Assay to the Avioq VioOne HIV Profile Supplemental Assay were satisfactory. Pending FDA approval, the Avioq VioOne Supplemental Assay is a suitable alternative testing method to the Bio-Rad Geenius Supplemental Assay for HIV differentiation and supplemental testing.

References:

Centers for Disease Control and Prevention, 2018. Quick reference guide: recommended laboratory HIV testing algorithm for serum or plasma specimens. *CDC Stacks. Updated January.*

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