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Evaluation of Bio-Rad Geenius HIV-1 and -2 Assay as a Confirmatory Assay for Detection of HIV-1 and -2 Antibodies

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The performance of the new Bio-Rad Geenius HIV-1/2 confirmatory assay was compared with that of the Chiron RIBA HIV-1/HIV-2 strip immunoblot assay using 166 samples from HIV-1-, HIV-2-, or HIV-1/2-positive and HIV-negative individuals and two quality control panels. Our results indicate that the Geenius assay is a suitable alternative for confirmatory HIV testing.

International guidelines for the diagnosis of human immunodeficiency virus type 1 (HIV-1) and HIV-2 recommend the use of multistep algorithms for identifying early HIV infections and for confirming and differentiating between established HIV-1 and HIV-2 infections (1, 2). The current diagnostic algorithm in our laboratory requires screening with a fourth-generation HIV Ag/Ab Combo assay followed by testing of reactive samples with the Chiron RIBA HIV-1/HIV-2 strip immunoblot assay (RIBA) and with the INNOTEST HIV antigen MAb test (Innogenetics) (HIV antigen test) (3). RIBA is a labor-intensive strip assay that relies on visual interpretation of reactivity to viral antigens and synthetic peptides (4). Bio-Rad Laboratories developed the Geenius HIV-1/2 confirmatory assay (Geenius assay) as a new single-use immunochromatographic assay designed for the confirmation and differentiation of antibodies to HIV-1 and HIV-2 in whole blood, venous blood, serum, or plasma samples (5). The CE-marked Geenius assay has automatic reading and interpretation and can be completed within 30 min.

This study evaluated the Geenius assay as an alternative to RIBA using multiple sets of clinical samples and external quality control panels.

The Geenius assay was evaluated by testing 75 serum samples from patients known to be HIV positive or HIV negative, 79 consecutive routine samples with reactive Combo assay results, and 12 samples from external quality control panels and by comparing the results with RIBA results.

Seventy-five samples were retrospectively selected based on the HIV infection status of the patients, defined as positive (confirmed HIV-positive follow-up samples) or negative (one or more HIV-negative follow-up samples). This group included 55 single samples from 25 patients with established HIV infection (23 with HIV-1, 1 with HIV-2, and 1 with HIV-1/HIV-2 double infection), 25 HIV-negative individuals, and 5 patients in the acute phase of infection with ongoing HIV-1 seroconversion (based on a confirmed HIV antigen test). Paired serum samples ($n = 20$) from 10 patients with confirmed primary HIV infection (positive HIV antigen test and negative RIBA in the first sample and a confirmed HIV-positive follow-up sample using RIBA, the HIV antigen test, or HIV RNA) were tested with the Geenius assay.

Furthermore, 79 consecutive routine clinical samples, admitted to the laboratory from June to August 2013, were analyzed in parallel with RIBA and the Geenius assay. The results from follow-up testing were not available at the time of data compilation; therefore, the confirmed HIV status of most of these patients was unknown.

Finally, two external quality control panels obtained from Equalis in 2011 and 2013, consisting of 12 samples (4 HIV-1 positive, 2 HIV-2 positive, 6 HIV negative), were retested with the Geenius assay.

All selected serum samples were tested with the Geenius assay according to Bio-Rad's three-step protocol (6).

For the 75 retrospectively selected clinical samples, the Geenius assay and RIBA produced concordant results in 25 samples from patients with established HIV infection and in 24 of 25 samples from HIV-negative patients (Table 1). The only discordant result was obtained from a patient for whom the Geenius assay was indeterminate while RIBA was negative and supplemental and follow-up testing was negative. RIBA and the Geenius assay gave concordant results for 76 of 79 routine clinical samples concurrently tested by both assays (Table 1). Three samples were indeterminate in RIBA but positive ($n = 1$) or negative ($n = 2$) in the Geenius assay. The two samples with negative Geenius results tested negative for HIV antigen, and the sample with a positive Geenius result was not tested for HIV antigen. Follow-up testing was not completed by the time the data were collected for this study. Twelve samples from quality control panels were tested with the Geenius assay and gave results matching the anticipated results (data not shown).

We analyzed 25 samples from 15 patients in the acute phase of HIV-1 infection (Tables 1 and 2). Discordances between the Geenius and RIBA results were observed in nearly half of these samples (12 of 25). In these samples, RIBA was often indeterminate, whereas the Geenius assay was either negative or positive. No obvious difference in the sensitivities of the two assays was discovered. Thus, for 10 samples that were indeterminate in RIBA, the

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TABLE 1 Comparison of results from the RIBA and Geenius HIV assays

Sample type and HIV status	*n*	No. with each RIBA result			No. with each Geenius result		
		Negative	Indeterminate	Positive	Negative	Indeterminate	Positive
Retrospective clinical samples[a]							
HIV-1 positive	23	0	0	23	0	0	23
HIV-2 positive	1	0	0	1	0	0	1
HIV-1 and HIV-2 positive	1	0	0	1	0	0	1
HIV negative	25	25	0	0	24	1	0
Acute HIV infection[b]	5	0	5	0	2	1	2
Acute HIV seroconversion panel[b]							
1:e sample	10	9	1	0	8	0	2
2:e sample	10	0	5	5	4	0	6
Prospective clinical samples	79	35	3	41	37	0	42
Total	154	69	14	71	75	2	77

[a] HIV status for the retrospectively analyzed positive samples was based on results from follow-up testing confirming HIV infection with RIBA or HIV RNA/DNA. HIV status for the retrospectively analyzed negative samples was based on negative results for both HIV antigen assay tests and follow-up tests. These include samples from 10 blood donors.

[b] Acute-phase HIV infection status was based on indeterminate or negative RIBA results, a positive HIV antigen assay result, and documented HIV seroconversion in follow-up samples.

Geenius assay was positive for 5 samples and negative for 5 samples. Furthermore, one sample was negative in RIBA and positive in the Geenius assay, while a second sample was positive in RIBA and negative in the Geenius assay (Table 2).

TABLE 2 Results for seroconversion sample panel

Patient no.	Sample no.	Date (days[b])	Sample result[a]			
			HIV antigen	NT	RIBA	Geenius
1	1	0	Pos	Pos	Neg	Neg
	2	+4	Pos		Ind	Pos
2	1	0	Pos	Pos	Neg	Neg
	2	+7	ND		Pos	Pos
3	1	0	Pos	Pos	Neg	Neg
	2	+12	Pos		Pos	Pos
4	1	0	Pos	Pos	Neg	Neg
	2	+24	Pos		Pos	Pos
5	1	0	Pos	Pos	Neg	Pos
	2	+14	Neg		Pos	Pos
6	1	0	Pos	Pos	Neg	Neg
	2	+4	Pos		Pos	Neg
7	1	0	Pos	Pos	Neg	Neg
	2	+11	Pos		Ind	Neg
8	1	0	Pos	Pos	Neg	Neg
	2	+7	Pos		Ind	Neg
9	1	0	Pos	Pos	Neg	Neg
	2	+8	Pos		Ind	Neg
10	1	0	Pos	Pos	Ind	Pos
	2	+41	ND		Ind	Pos

[a] NT, neutralization test for confirmation of HIV antigen; Neg, negative; Ind, indeterminate; Pos, positive; ND, not done.

[b] Number of days between first and second samples.

In this evaluation, the Geenius assay proved to be a reliable alternative to RIBA and should perform satisfactorily when applied to our HIV diagnostic algorithm. Our results showed a high concordance between RIBA and Geenius assay results in terms of sensitivity and specificity for HIV-negative individuals and patients with established HIV infection. However, for patients with ongoing primary HIV infection, discordant results were more common, with the Geenius assay giving fewer indeterminate results than RIBA in this limited material. Similar results were reported by Malloch et al. (7), who compared the performance of the Geenius assay with that of the Bio-Rad multispot HIV-1/2 rapid test. The sensitivities of antibody-confirmatory tests in early infection are limited by the natural delay of antibody production, so early HIV infections still need to be diagnosed with Combo screening combined with HIV antigen and/or HIV RNA testing.

The Geenius assay has a simple protocol, bar-coded single-use test cassettes (which minimize the risk for sample-patient mix-ups), and an automated reader that allows for the objective evaluation of results, eliminating subjective laboratory personnel interpretations. Furthermore, the ease of use and short analysis time allow for daily implementation, which is more difficult to achieve with RIBA.

The following limitations of our study should be taken into consideration. The manufacturer of the Geenius assay recommends using samples that have not gone through five or more freeze-thaw cycles (6). Many of the samples in our seroconversion panel had gone through multiple freeze-thaw cycles for other studies, which may have influenced the results. We could not fully evaluate the ability of the Geenius assay to detect HIV-2 and double HIV-1 and HIV-2 infections, because such infections are very rare in Sweden. However, for the few clinical and external quality panel samples with HIV-2, the results matched the expected outcomes. The Geenius assay was developed to be flexible and allow for use with different sample types (e.g., whole blood, plasma), but we investigated the performance with serum samples only. However, Minard et al. (5) showed that the assay has a high specificity in alternative sample types (plasma, venous blood, capillary blood) as well.

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