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Evaluation of the Bio-Rad Geenius HIV 1/2 Assay as an Alternative to the INNO-LIA HIV 1/2 Assay for Confirmation of HIV Infection

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The Bio-Rad Geenius HIV 1/2 assay was evaluated as an alternative to the INNO-LIA HIV 1/2 assay for the confirmation of HIV infection in 198 serum samples reactive to 4th-generation HIV enzyme immunoassays (EIAs). The Geenius assay correctly identified 85% of the samples, compared to 75% identified by the INNO-LIA assay, reduced the number of indeterminate results, and shortened the overall turnaround time.

For more than 20 years, the standard algorithm for the diagnosis of HIV infection in Israel has remained a sequential multistep process. Screening was done in several authorized HIV laboratories using two different third-generation enzyme immunoassays (EIAs) which detect both IgM and IgG anti-HIV antibodies, followed by a confirmatory assay performed in the Israeli National HIV Reference Laboratory (INHRL) using the INNO-LIA HIV 1/2 score line immunoassay (Innogenetics, Ghent, Belgium) (1). The INNO-LIA assay results form the basis of the national HIV registry, updated yearly by the Ministry of Health (2). In September 2012, 4th-generation EIAs that detect the p24 antigen and IgM and IgG anti-HIV-1/HIV-2 antibodies but are unable to differentiate between them (3–5) were introduced, decreasing the window of time between infection and the ability to detect it by screening (6). The INNO-LIA test that employs recombinant and peptide-based HIV-1 and HIV-2 antigens can differentiate between HIV-1 and HIV-2 infections, and although it is unable to identify IgM antibodies or the p24 antigen, it was considered the most specific test and therefore remained the confirmatory test for HIV infection (7). It is a nonautomated assay that has a turnaround time of nearly 24 h and requires 3 to 4 h of manual work. As the number of suspected cases of acute HIV infection has increased, the need for a more sensitive and rapid confirmatory test has become evident.

Recently, the Bio-Rad multispot HIV-1/HIV-2 rapid test (Bio-Rad Laboratories, Hercules, CA) was approved by the FDA (in March 2013) as the confirmatory test after a repeatedly reactive 4th-generation HIV immunoassay and was suggested as a replacement to the Bio-Rad viral lysate Western blot assay in the new algorithm published by the Clinical and Laboratory Standards Institute (8). This multispot assay detects and differentiates HIV-1 and HIV-2 antibodies in serum and plasma and was reported to confirm HIV infections at a proportion similar to that of the Western blot assay (9, 10). The Bio-Rad Geenius HIV 1/2 confirmatory assay is a newer test which can also be used for the confirmation and differentiation of HIV-1 and HIV-2 infection. Each sample (whole blood, serum, or plasma) is processed separately in a closed cassette where recombinant or synthetic peptides specific for HIV-1 (gp41, gp160, p31, p24) or HIV-2 (gp36, gp140) antigens are applied as discrete bands. It has a dual-path platform technology, and the antibodies bind to the appropriate antigen before a detection reagent is added (11). The result is available within 30 min following a three-step protocol. The Geenius HIV 1/2 assay was approved in Europe for the diagnosis of HIV infection and received a CE mark in February 2013. Recently, the Geenius assay was compared to the multispot assay and was found to be a suitable alternative to the multispot assay in the second-stage HIV algorithm (12). However, direct comparison of the Geenius to a line immunoassay, such as the INNO-LIA assay, was not performed.

Our study evaluated the performance of the Bio-Rad Geenius HIV 1/2 confirmatory assay as an alternative to the INNO-LIA assay in a range of samples reactive on screening immunoassays submitted to the INHRL for confirmation of HIV infection. Of 820 serum samples collected between September 2012 and December 2013, 198 representatives of positive, negative, and indeterminate INNO-LIA results were used. For each individual, HIV infection status was deemed positive if a sample or any of the following samples from the same individual were confirmed to be HIV-1/2 positive by the INNO-LIA assay or negative if the sample was negative with the INNO-LIA assay and a following sample was nonreactive in the screening assays or if previous and following samples collected during a period of ≥6 months were repeatedly reactive in the HIV screening tests and consistently indeterminate by the INNO-LIA assay in the absence of any clinical signs or symptoms of HIV infection. Samples were eligible for the study if they were found to be repeatedly reactive in either the Architect HIV Ag/Ab combo (Abbott Diagnostics, Abbott Park, IL, USA) or the Vidas HIV DUO Ultra (bioMérieux, Marcy l'Etoile, France) 4th-generation EIAs (175 samples) or if they were found to be reactive following screening in the Israeli blood bank (18 samples, tested by AxSYM HIV 1/2 GO; Abbott, Germany) and if a sufficient serum volume remained. Five proficiency test samples (Labquality, Helsinki, Finland)—two HIV-1 positive, two HIV-1 negative, and one HIV-2 positive—were also included. Results of the INNO-LIA assays, which were performed according to the

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TABLE 1 Performance of INNO-LIA and Geenius assays for true HIV-positive[a] ($n = 129$) and -negative ($n = 69$) individuals

True HIV status	INNO-LIA result	No. of individuals with indicated Geenius assay result		
		Positive ($n = 100$)	Indeterminate ($n = 11$)	Negative ($n = 18$)
Positive ($n = 129$)	Positive ($n = 86$)	84	2	0
	Indeterminate ($n = 21$)	16	4	1
	Negative ($n = 22$)	0	5	17
		Positive ($n = 0$)	Indeterminate ($n = 1$)	Negative ($n = 68$)
Negative ($n = 69$)	Positive ($n = 0$)	0	0	0
	Indeterminate ($n = 6$)	0	0	6
	Negative ($n = 63$)	0	1	62

[a] HIV-1 positive, HIV-2 positive, and indeterminate HIV results in Geenius or INNO-LIA assays were all considered HIV positive.

manufacturer's instructions using an overnight protocol (7), were available for 191 samples prior to commencement of the current study. These samples were stored at −20°C until their use with the Geenius assay. INNO-LIA testing was performed concomitantly with the Geenius assay on seven fresh samples. The Geenius assay was performed according to the manufacturer's instructions (11). Positive and negative controls were included with each batch of samples in the INNO-LIA and the Geenius assays. The samples were blindly tested. The work was approved by the Sheba Medical Center institutional review board (approval 0778-13-SMC). The Geenius assay was repeated if the results were discordant with the INNO-LIA results and with the HIV infection status of the individual.

The results of the Geenius and INNO-LIA assays for 129 individuals infected with HIV and for 69 individuals not infected with HIV were compared (Table 1). Overall, the percentage of samples with correct assay results scoring either positive (from HIV-infected individuals) or negative (from HIV-uninfected individuals) was significantly higher with the Geenius assay (85% [168/198] versus 75% [149/198] with the INNO-LIA assay; $P = 0.017$).

The Geenius assay gave a reduced number of samples with negative or indeterminate scores from HIV-positive individuals and thus is more sensitive than the INNO-LIA assay in identifying new HIV infections. The Geenius assay also gave a reduced number of samples from HIV-negative individuals with indeterminate tests due to nonspecific reactions. Indeed, the performance of the Geenius assay was superior to that of the INNO-LIA assay in all parameters tested, although the overall agreement between the assays was good (kappa = 0.87) (Table 2).

TABLE 2 Summary of the performances of INNO-LIA and Geenius assays[a]

Parameter	Result (% [95% CI]) of:	
	INNO-LIA	Geenius
Sensitivity[b]	83 (76–88)	86 (79–91)
Specificity[c]	91 (82–96)	99 (92–100)
Positive predictive value[b]	95 (91–99)	99 (95–100)
Negative predictive value[c]	74 (64–84)	79 (69–86)
Test Performance	85 (79–90)	90 (84–93)
Kappa	0.87	

[a] When evaluating sensitivity, specificity, positive predictive value, negative predictive value, and test performance, all indeterminate interpretations were considered to be correct for HIV-positive individuals and incorrect for HIV-negative individuals (14).
[b] HIV-1 positive, HIV-2 positive, and HIV indeterminate were all considered HIV positives in the sensitivity and positive predictive value calculations.
[c] Specificity and negative predictive value were based on the ability of the test to identify HIV-1 or HIV-2 negative individuals as HIV negative.

The Geenius assay provides other advantages over the INNO-LIA assay. It minimizes the risk for contamination by employing a separate closed device for each sample. The use of a bar code for the sample and the cassette reduces mistakes. The digital capture and storage of the image and the results allow traceability. Subjectivity between lab personnel is minimized by the use of an automated reader. Finally, the direct cost of the Geenius assay is competitive with that of the INNO-LIA assay.

Conversely, similar to the INNO-LIA and multispot assays (10), the Geenius assay did not confirm all 4th-generation reactive results. HIV RNA testing is highly recommended for resolving discordant screening and confirmatory results in cases of suspected acute infection (13). Another limitation of this study is that, since infection with HIV-2 is rare in Israel, only two HIV-2 cases were evaluated; thus, our ability to evaluate the impact of HIV-2 diagnosis is limited.

We conclude that the Geenius assay is superior to the INNO-LIA assay for the confirmation of HIV-1 infection. HIV RNA testing should be utilized when discrepant results are obtained, especially in cases of suspected acute infection.

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