Evaluation of the Bio-Rad Geenius HIV 1/2 assay as an alternative to the INNO-LIA HIV 1/2 score for the confirmation of HIV infection

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Running Head: Geenius for confirmation of HIV infection

Key words: HIV1/2 infection, Geenius, INNO-LIA, confirmation

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Abstract
The Bio-Rad Geenius HIV 1/2 assay was evaluated as an alternative to the INNO-LIA HIV 1/2 assay for confirmation of HIV infection in 198 serum samples reactive with HIV 4th generation EIA assays. Geenius identified correctly 85% of the samples compared to 75% by INNO-LIA, reduced the number of indeterminate results and shortened the overall turnaround time.
For over 20 years, the standard algorithm for diagnosis of human immunodeficiency virus (HIV) infection in Israel has remained a sequential multi-step process. Screening was done in several authorized HIV laboratories, using two different third generation enzyme immunoassays (EIA) 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 I/II score line immunoassay (Innogenetics, Ghent, Belgium) (1). The INNO-LIA results form the basis for the national HIV registry updated yearly by the Ministry of Health (2). In September 2012, 4th generation EIA assays that detect 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 period between time of 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 though it is unable to identify IgM antibodies or p24 antigen it was considered the most specific test and therefore remained the confirmatory test for HIV infection (7). It is a non-automated assay with turnaround time of nearly 24 hours requiring 3-4 hours of manual work. As the number of suspected cases of acute HIV infection increased, a 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, California) was approved by the FDA (March 2013) as the confirmatory test after a repeatedly reactive 4th generation HIV immunoassay and was suggested as a replacement to 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 similar proportion to the Western blot assay
(9, 10). The Bio-Rad Geenius HIV1/2 Confirmatory assay is a newer test which can
also be used for 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 antigens (gp36, gp140) are applied as discrete bands. It has a Dual Path
Platform technology and the antibodies bind to the appropriate antigen before
detection reagent is added (11). The result is available within 30 min following a
three step protocol. The Geenius HIV1/2 assay was approved in Europe for the
diagnosis of HIV infection and received CE-mark on February, 2013. Recently,
Geenius was compared to the Multispot and was found to be a suitable alternative
to Multispot assay in the second stage of HIV algorithm (12). However, direct
comparison of the Geenius to a line immunoassay like the INNO-LIA was not
performed.

Our study evaluated the performance of the Bio-Rad Geenius HIV1/2 confirmatory
assay as an alternative to the INNO-LIA in a range of samples reactive on screening
immunoassays submitted to the INHRL for confirmation of HIV infection. 198 of 820
serum samples collected between September 2012 and December 2013,
representatives of positive negative and indeterminate INNO-LIA results were used.
For each individual, HIV infection status was determined according to the following
scheme: positive, if a sample or any of the following samples from the same
individual were confirmed to be HIV1/2 positive by INNO-LIA; negative, if the sample was INNO-LIA negative and a following sample was non-reactive in the screening assays or if previous and following samples, collected during a period of at least six months, were repeatedly reactive in the HIV screening tests and consistently indeterminate by INNO-LIA, 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 (Architect; Abbott Diagnostics, Abbott Park, IL, USA) or Vidas HIV DUO ULTRA (Biomérieux, Marcy-l’Etoile, France), 4th generation EIA assays (175 samples), or if they were found to be reactive following screening in the Israeli blood bank (eighteen samples, tested by AxSYM HIV 1/2 GO, ABBOTT, Germany), and if sufficient sera volume remained. Five proficiency test samples (Labquality, Helsinki, Finland) - two HIV-1 positives, two HIV-1 negative and one HIV-2 positive, were also included. Results of INNO-LIA assay, which was performed according to the instructions of the manufacturer using an overnight protocol (7), were available for 191 samples prior to commencement of the current study. These samples were stored frozen at -20°C until used by Geenius. INNO-LIA testing was performed concomitantly with the Geenius assay on seven fresh samples. Geenius assay was performed according to the manufacturer’s instructions (11). Positive and negative controls were included with each batch of samples in both the INNO-LIA and the Geenius assays. The samples were blindly tested. The work was approved by the Sheba Medical Center Institutional review board (0778-13-SMC). Geenius assay was repeated if results were discordant with the INNO-LIA results and with the HIV infection status of the individual.
The results of Geenius and INNO-LIA for 129 individuals infected with HIV and for 69 individuals not infected with HIV (Table 1) were compared. Overall, the percentage of samples with correct assay result, scoring either positive (from HIV infected individuals) or negative (from HIV uninfected individuals) was significantly higher in Geenius (75%, 149/198, by INNO-LIA while 85%, 168/198, by Geenius, p-value 0.017).

Geenius reduced the number of samples scoring negative or indeterminate from HIV positive individuals, thus being more sensitive to identify new HIV infections. Geenius also reduced the number of samples from HIV negative individuals testing indeterminate due to non-specific reactions. Indeed, the performance of Geenius was superior to INNO-LIA in all parameters tested though the overall agreement between the assays was good (kappa=0.87, Table 2).

Geenius provides other advantages over INNO-LIA. It minimizes the risk for contamination by employing a separate closed device for each sample. The use of barcode of both sample and 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 automated reader. And finally, the direct cost of the Geenius assay is competitive to INNO-LIA.

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

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

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References


Table 1 Performance of INNO-LIA and Geenius assays for true HIV positive (n=129) and negative (n=69) individuals

<table>
<thead>
<tr>
<th>INNO-LIA</th>
<th>Geenius</th>
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<tbody>
<tr>
<td></td>
<td>True HIV Positives (n=129)</td>
</tr>
<tr>
<td></td>
<td>Positive (n=100)</td>
</tr>
<tr>
<td>Positive</td>
<td>84</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
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<thead>
<tr>
<th>INNO-LIA</th>
<th>Geenius</th>
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<tbody>
<tr>
<td></td>
<td>True HIV Negatives (n=69)</td>
</tr>
<tr>
<td></td>
<td>Positive (n=0)</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
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</table>

2 HIV-1 positive, HIV-2 positive and HIV untypeable in Geenius or INNO-LIA assays were all considered as HIV positives.
Table 2: Summary of the performance INNO-LIA and Geenius

<table>
<thead>
<tr>
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<th>INNO-LIA% (95% CI)</th>
<th>Geenius% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>83 (76-88)</td>
<td>86 (79-91)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>91 (82-96)</td>
<td>99 (92-100)</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>95 (91-99)</td>
<td>99 (95-100)</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>74 (64-84)</td>
<td>79 (69-86)</td>
</tr>
<tr>
<td><strong>Test Performance</strong></td>
<td>85 (79-90)</td>
<td>90 (84-93)</td>
</tr>
<tr>
<td><strong>Kappa (k)</strong></td>
<td></td>
<td>0.87</td>
</tr>
</tbody>
</table>

*a* When evaluating sensitivity, specificity, positive predictive value, negative value, test performance, all indeterminate interpretations were considered to be correct for HIV positives, and incorrect for HIV negative individuals (14).

*b* HIV-1 positive, HIV-2 positive and HIV untypeable 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 negatives as HIV negative.