

## Comparison of Two Rapid Human Immunodeficiency Virus (HIV) Assays, Determine HIV-1/2 and OraQuick Advance Rapid HIV-1/2, for Detection of Recent HIV Seroconversion<sup>∇</sup>

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Received 8 April 2008/Returned for modification 3 June 2008/Accepted 23 July 2008

**Modified protocols of two rapid tests were compared with a less sensitive (LS) (detuned) enzyme immunoassay (EIA) for their abilities to distinguish recent human immunodeficiency virus (HIV) seroconversion from long-term infections. The results for samples from 100 HIV-positive patient that had previously been tested by the Vironostika LS EIA had a 97% concordance with the results of the Determine HIV 1/2 assay and 93% concordance with those of the OraQuick HIV 1/2 assay.**

The monitoring of individuals for determination of the incidence of human immunodeficiency virus (HIV) infection is important for public health surveillance and prevention programs. The less sensitive (LS) enzyme immunoassay (EIA) (or the serologic testing algorithm for recent HIV seroconversion [STARHS]) has made possible the serologic diagnosis of incident HIV infection in individual patients as well as estimation of the incidence of HIV in populations (1–3, 5). The Vironostika LS EIA has been validated by the U.S. Centers for Disease Control and Prevention (CDC) and is now widely applied in the United States and internationally to estimate the incidence of HIV type 1 (HIV-1) and to recruit subjects with early HIV infection into clinical trials. However, the Vironostika LS EIA is no longer available as of April 2008, according to the manufacturer. Therefore, a replacement assay will need to come from one of the currently available HIV-1/2 assays or a new assay will need to be developed for estimation of the incidence of HIV-1/2 infections.

Advances in HIV diagnostic technologies have resulted in the development of simple rapid tests (RTs) for antibody detection. We compared two RTs (the Determine HIV-1/2 assay [Abbott Laboratories, Abbott Park, IL] and the OraQuick Advance HIV-1/2 assay [OraSure Technologies, Inc., Bethlehem, PA]), modified as suggested by the CDC (6) to the Vironostika LS EIA (bioMerieux Inc, Durham, NC), using 100 consecutive samples submitted for assessment of clinical trial eligibility. STARHS was performed with confirmed HIV-positive serum samples by following standard CDC protocols and the algorithm for the Vironostika LS EIA. The Vironostika LS EIA is a second-generation assay that uses a viral lysate as the capture antigen. The Determine HIV-1/2 assay uses HIV-1 synthetic peptide gp41 and recombinant antigens gp41 and gp120, HIV-2 synthetic peptide gp36 and recombinant antigen gp36, and HIV-1 subtype O recombinant antigens gp41 and

gp120. The antigens used in the OraQuick assay are synthetic peptides representing the HIV envelope region.

To identify persons recently infected with HIV, STARHS uses the modified protocol of the FDA-approved standard EIA by increasing the specimen dilution and decreasing the sample volume and substrate incubation times to render the assay less sensitive. The Vironostika LS EIA detects HIV infection approximately 170 days (95% confidence interval = 163 to 183 days) after the initial infection by using a standard optical density (SOD) cutoff of  $\leq 1.0$ . A blood sample that is reactive by a sensitive EIA and that is positive by Western blotting but nonreactive by the LS EIA (with a value less than or equal to the SOD cutoff) identifies a person in the period of early HIV infection, when the antibody titer is increasing but has not peaked. We were using the Vironostika LS EIA to determine eligibility for a clinical trial that required HIV-infected individuals with recent infection ( $\leq 6$  months from seroconversion); for that clinical trial, we used an SOD cutoff of  $\leq 0.75$  to minimize the likelihood that people with established infection would be enrolled in the trial.

Both RTs are manually performed, visually read, qualitative immunoassays for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma, or whole blood. For the Determine HIV-1/2 LS assay, the samples were diluted in two steps: 5  $\mu$ l of sample was diluted in 195  $\mu$ l of whole plasma from a healthy individual plus (1:40), followed by a second dilution of 10  $\mu$ l into 240  $\mu$ l of pooled true human serum (SeraCare Life Sciences, Milford, MA), for a final dilution of 1:1,000. A 50- $\mu$ l aliquot of this final dilution was applied directly to the fibrous pad of the test strip, and the test was visually read after 15 min of incubation at room temperature. For the OraQuick HIV-1/2 LS assay, the predilution was achieved by adding 5  $\mu$ l of specimen to 195  $\mu$ l of pooled true human serum (1:40). Twenty microliters of the prediluted specimen was added to 800  $\mu$ l of OraQuick Advance Rapid HIV-1/2 assay buffer (200  $\mu$ l was removed from 1 ml buffer, and 20  $\mu$ l of the prediluted sample was added to achieve final dilution of 1:1,640). To ensure the precision of the sample dilutions, the volumes were measured with precision pipettes in place of the loops supplied with the kits. The Flat-Pad device

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<sup>∇</sup> Published ahead of print on 6 August 2008.

TABLE 1. Concordance between Vironostika LS EIA and modified LS RTs

| Assay, result                   | No. of samples with the indicated reactivity by the following assay <sup>a</sup> : |             |                |             |
|---------------------------------|--|-------------|----------------|-------------|
|                                 | Determine HIV-1/2 LS assay   |             | OraQuick assay |             |
|                                 | Reactive   | Nonreactive | Reactive       | Nonreactive |
| Vironostika LS EIA, reactive    | 66   | 3           | 63             | 6           |
| Vironostika LS EIA, nonreactive | 0  | 31          | 1              | 30          |

<sup>a</sup> Reactive indicates a long-term infection, and nonreactive indicates a recent seroconversion.

from the kit was then inserted into the vial, and the test result was read after 20 min of incubation at room temperature.

The results of both the RTs were read as described in the manufacturer's package insert. They were scored as nonreactive, weakly reactive, and reactive. Samples with no visual band were classified as nonreactive and were reported as recent seroconversions. The samples with a light band were classified as weakly reactive, and those with a definite band were classified as reactive; however, both weakly reactive and reactive samples were reported as being from individuals with long-term infections. The results obtained with 100 samples that had previously been tested by the Vironostika LS EIA are shown in Table 1. Overall, the results of the Determine HIV-1/2 LS test had 97% concordance with the results of the Vironostika LS EIA, while the results of the OraQuick LS assay gave a concordance of 93% (Table 1). The three discordant samples found in the Determine HIV-1/2 LS (detuned) assays had Vironostika LS EIA SODs that ranged from 1.006 to 1.516 (Table 2). In contrast, the six falsely nonreactive specimens found by the less sensitive OraQuick LS assay had Vironostika LS EIA SODs that ranged from 1.022 to 6.208. Two specimens (specimens D and E) were nonreactive by both detuned RTs but scored reactive by the Vironostika LS EIA, with SODs of 1.516 and 1.107, respectively. One specimen had a falsely reactive OraQuick LS (detuned) assay result, although the Vironostika LS EIA SOD was only 0.583. It has previously been reported that the Determine HIV-1/2 RT is more sensitive than some of the other RTs (4).

Our results suggest that the Determine HIV-1/2 LS assay would give results that are more comparable to those of the Vironostika LS EIA than the OraQuick LS assay would for the identification of recent infections. These RTs provide results in minutes, use minimal laboratory equipment, and have been used in resource-limited settings. The Determine HIV-1/2 assay is approved for use internationally but not in the United States, whereas the OraQuick Advance Rapid HIV-1/2 assay is approved by the FDA for use in the United States.

Application of these effective RTs for the identification of

TABLE 2. Details of the discordant results found by testing for HIV antibody by the LS assays

| Sample | Vironostika LS EIA SOD | Result by <sup>a</sup> : |                            |                   |
|--------|------------------------|--------------------------|----------------------------|-------------------|
|        |                        | Vironostika LS EIA       | Determine HIV-1/2 LS assay | OraQuick LS assay |
| A      | 6.208                  | R                        | R                          | NR                |
| B      | 2.292                  | R                        | R                          | NR                |
| C      | 1.660                  | R                        | R                          | NR                |
| D      | 1.516                  | R                        | NR                         | NR                |
| E      | 1.107                  | R                        | NR                         | NR                |
| F      | 1.022                  | R                        | R                          | NR                |
| G      | 1.006                  | R                        | NR                         | R                 |
| H      | 0.583                  | NR                       | NR                         | R                 |

<sup>a</sup> R, reactive, which indicates a long-term infection; NR, nonreactive, which indicates a recent seroconversion.

recent HIV seroconversion will likely facilitate studies designed to derive incidence estimates in different parts of the world, especially in resource-limited settings. One drawback of the use of RTs tests for the estimation of recent infection for surveillance purposes is that they do not lend themselves to high-throughput testing. Because these RTs for the detection of HIV are easy to use, they are sometimes performed by personnel with limited or no formal laboratory training. These modified LS assays should be performed by trained laboratory personnel, as they involve serial dilution of the samples with precision pipettes, visual scoring of the bands, and interpretation of the results. Standardization of RTs for the detection of HIV as a tool to estimate the incidence of HIV will require additional studies.

This work was supported by a grant from the National Institute of Allergy and Infectious Diseases for the UNC Center for AIDS Research (grant P30AI50410).

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