

Modification of Rapid Human Immunodeficiency Virus (HIV) Antibody Assay Protocols for Detecting Recent HIV Seroconversion

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Assay protocols of three rapid human immunodeficiency virus (HIV) assays, OraQuick-1/2, SeroStrip-1/2, and Determine-1/2, were modified to detect recent HIV seroconversion using a higher dilution of serum specimens. Optimal predilution of specimens resulted in negative test results during early periods of seroconversion (about 6 months), when antibody levels were low. A total of 269 seropositive specimens from routine HIV type 1 testing and from commercial sources (low-titer and seroconversion panels) were tested, and results were recorded as negative (score = 0) or positive using intensity scores from 0.5 (weak positive) to 4 (strongly positive). The same specimens were previously tested by a less sensitive (LS) enzyme immunoassay (EIA), Abbott 3A11-LS, and were classified as recent or long-term infections based on the standardized optical density (SOD) cutoff of 0.75. Overall concordance of >94% was observed between 3A11-LS and modified rapid tests (RT-LSs) for detecting and distinguishing recent HIV seroconversion from long-term HIV infection (κ statistics = 0.894 to 0.901). Moreover, intensity scores on RT-LSs correlated well with median 3A11-LS SOD values ($R^2 > 0.98$). Our results indicate that rapid HIV tests can be modified to detect recent seroconversion with results comparable to those from less sensitive EIA.

Estimating human immunodeficiency virus type 1 (HIV-1) incidence is important for identifying populations with high rates of current transmissions, for directing resources and prevention programs, and for assessing the future of the HIV epidemic. Many methods of incidence estimation, including longitudinal studies, back calculation, p24 antigen enzyme immunoassay (EIA), and viral RNA testing have been utilized in the past; however, these methods are either difficult, costly and/or problematic due to a variety of reasons (13). New advances have provided a serological testing algorithm for identifying recent HIV seroconverters using either a sensitive or a less sensitive (LS) EIA (5, 12), immunoglobulin G-capture BED-EIA (10) or avidity based approach (14). These methods can identify recent HIV seroconverters within about 6 months after seroconversion and have the ability to estimate HIV-1 incidence using a single cross-sectional study (1, 4, 5). However, these EIAs require standard immunoassay equipment and several hours to complete. This could be of concern in resource-limited settings where most diagnostic testing occurs using rapid HIV tests (RTs).

Advances in HIV diagnostic technology have resulted in the development of simple RT assays. These tests provide results in minutes, use minimal laboratory equipment, and have been used in a variety of international settings since their introduction (3, 6–8, 17). Many resource-limited settings that utilize RTs because of their ease of use and cost effectiveness unfortunately do not have the resources to determine HIV incidence. Having the ability to cost-effectively and accurately de-

termine HIV incidence, especially in resource-limited high incidence regions, would provide a valuable epidemiological tool for assessing that region's HIV epidemic. This study was conducted to determine whether simple HIV RTs could be modified as less sensitive assays in order to differentiate recent HIV seroconversion from long-term infection

MATERIALS AND METHODS

Serum specimen sets. Three sets of HIV-1-antibody-positive specimens (total $n = 269$) were used to evaluate three rapid HIV antibody tests using a less sensitive protocol (RT-LSs). A set of HIV-1-antibody-positive specimens was selected from routine diagnostic testing ($n = 157$). All 157 specimens were tested by the EIA-Western blot algorithm and were confirmed to be seropositive. HIV-antibody-positive specimens ($n = 42$) from 11 seroconversion panels (panels AB, AD, AF, D, H, I, P, T, U, V, and W) from Boston Biomedica Inc. (Boston, MA) were also evaluated using the RT-LS method along with a third set of specimens ($n = 70$) comprised of low- and mixed-titer panels (PRB-102, PRB-104, PRB-201) also from Boston Biomedica Inc. Definitive HIV-1 subtypes of infected donors were not known; all HIV-positive sera were from the United States and therefore were likely to be subtype B. All 269 sera were positive when tested by regular rapid test protocols.

Testing procedure. Three RT assays were evaluated in this study: Determine HIV-1/2 (Abbott Laboratories, Abbott Park, IL), OraQuick HIV-1/2 (Orasure Technologies, Inc., Bethlehem, PA), and SeroStrip HIV-1/2 (Chem-Bio, Inc., Medford, NY). All three RTs are visually scored, lateral-flow assays that contain all of their respective reagents on an absorbent fibrous pad. Due to our inability to manipulate the reagents, these assays were modified only through dilution of the specimen. All specimens were prediluted in HIV-negative normal human plasma (NHP) to maintain the physiologic concentration of total immunoglobulin G. Initially, a known panel of five specimens with varying levels of HIV-1 antibodies was diluted twofold from 1:2 through 1:50,000 to empirically determine the optimal dilution to distinguish recent from long-term infection for each assay. Two sera were classified as recent, and three were classified as long-term infections by the 3A11-LS. The optimal specimen dilution was selected by comparing it to 3A11-LS data and was 1:1,000 for Determine-LS (Det-LS), 1:1,600 for OraQuick-LS (Ora-LS), and 1:5,000 for SeroStrip-LS (Sero-LS). When the RT was performed at the dilution stated, recently infected individuals gave negative results. For Det-LS, the optimal dilution was prepared in two steps to

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TABLE 1. Concordance (%) between 3A11-LS and modified less sensitive rapid-test protocols for classifying recent or long-term HIV-1 infections

Rapid test	% Concordance with 3A11-LS for the following panels:			Total (n = 269)
	HIV-1 positive (n = 157)	Seroconversion (n = 42)	Low titer (n = 70)	
Det-LS	91.7	100.0	98.6	94.8
Ora-LS	94.3	95.2	97.1	95.2
Sero-LS	94.9	92.9	97.1	95.2

increase accuracy. Five μl were added to 195 μl of NHP (1:40) followed by a second dilution of 10 μl into 240 μl of NHP to achieve final dilution of 1:1,000. A 50- μl aliquot of diluted specimen was applied directly onto the test strip. For Ora-LS, the predilution was achieved by adding 5 μl of specimen to 195 μl of NHS (1:40). A 20- μl aliquot of prediluted specimen was added to 800 μl of OraQuick buffer (final 1:1,600), and the test was performed according to the manufacturer's instructions. For Sero-LS, 2 μl was pipetted into 98 μl of NHP (1:50). A 2- μl aliquot of prediluted sample was added to 198 μl of SeroStrip buffer (final 1:5,000), and the test was performed as per instructions. The loops provided with the last two RT devices were not used when performing the LS protocols.

All three RTs were visually read by trained personnel using a scale of 0 to 4, 0 being nonreactive and 4 being strongly reactive (0 = negative, 0.5 = weak +, 1.0 = +, 2.0 = ++, 3.0 = +++, 4.0 = ++++). The specimens found to be nonreactive (score = 0) were classified as recent, while those with a score of 0.5 or higher were classified as long-term infections. Each assay was read within the specified time period, as per manufacturers' instructions, and with sufficient lighting.

Reference method and analysis. All low- and mixed-titer specimens and all HIV-1-antibody-reactive specimens were previously tested by the 3A11-LS EIA (Abbott Laboratories, Abbott Park, IL) described by Janssen et al. (5). Concordance between an RT-LS and the 3A11-LS was calculated by the proportion of total specimens with the same classification by both assays. Agreement between an RT-LS and the 3A11-LS assay was assessed by calculating the kappa statistics. The accuracy of each RT to detect recent infection was calculated by dividing the number of recent infections with the RT-LS by the number of recent infections determined by the 3A11-LS. For each RT-LS protocol, intensity scores were compared to the median standardized optical density (SOD) values of 3A11-LS at each level.

RESULTS

Concordance between 3A11-LS results and rapid tests for classifying both recent and long-term infections in individual sets of specimens ranged from 91.7% to 100% (Table 1), with overall concordance being 94.8% for Det-LS and 95.2% for both Ora-LS and Sero-LS. A total of 116 (43.1%) of 269 specimens were classified as recently infected (SOD < 0.75) by the 3A11-LS assay. Det-LS classified 122 (45.4%), Ora-LS classified 107 (39.8%), and Sero-LS classified 113 (42.0%) specimens as recently infected, demonstrating that the accuracy of the RT-LS approach for identifying incident infections is within 10% of the number obtained by the 3A11-LS assay.

Agreement between the two methods, Det-LS and 3A11-LS, was further analyzed by a 2 by 2 table as shown in Fig. 1A. A total of 112 specimens were classified as recent, while 143 specimens were classified as long-term by both methods; only 14 (5.2%) specimens had discordant test results (kappa = 0.984; proportion agreement = 0.94). Since RTs are read visually, we compared the intensity score for the RT-LS with the median 3A11-LS SOD results at each level. There was a strong correlation ($R^2 > 0.98$) between the median 3A11-LS SOD and Det-LS scores (Fig. 1B). Concordance between Ora-LS

3A11-LS	Determine-LS	
	Recent	Long-Term
Recent	112	4
Long-Term	10	143

Proportion agreement=0.94, Kappa=0.894

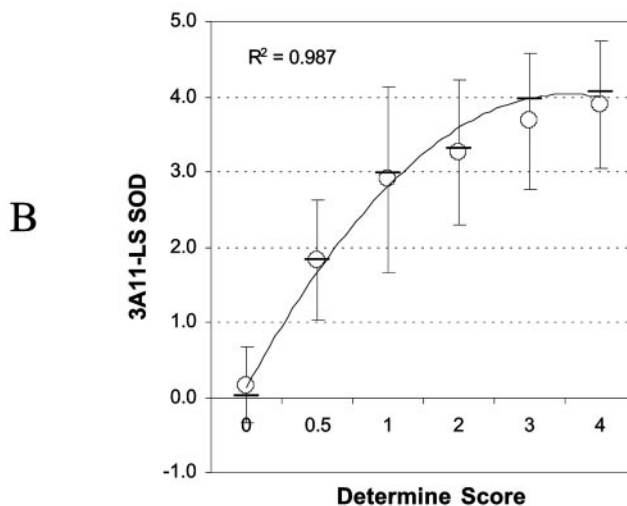


FIG. 1. Comparison of Det-LS and 3A11-LS results. (A) A 2-by-2 table showing concordant and discordant results by both methods. Kappa statistics and percent agreement are shown. (B) Concordance of Det-LS intensity score and median 3A11-LS SOD (vertical lines) at each level. Bar represents 1 standard deviation around the mean (open circles). A second-order polynomial best-fit line is shown with the correlation coefficient.

and 3A11-LS is shown in a 2 by 2 table (Fig. 2A); both methods classified 105 specimens as recent and 151 specimens as long-term, while 13 (4.8%) specimens were classified as discordant (kappa = 0.9; proportion agreement = 0.95). Moreover, the Ora-LS intensity score and median 3A11-LS SOD were strongly correlated (Fig. 2B) ($R^2 > 0.98$). Similar analysis between Sero-LS and 3A11-LS demonstrated (Fig. 3A) that 108 specimens were classified as recent and 148 as long-term by both methods, with only 13 (4.8%) of specimens having discordant results (kappa = 0.901; proportion agreement = 0.95). Again, a strong positive correlation was observed between the intensity score of Sero-LS and median 3A11-LS SOD values (Fig. 3B) ($R^2 > 0.98$).

DISCUSSION

Rapid HIV testing is a cost-effective method for diagnosing HIV-1 infection in resource-limited settings. Using RTs to estimate incidence would provide an important advantage in guiding prevention programs. Currently, either commercial HIV-1 EIAs have been modified (5, 12, 14) or new EIAs have been devised (10, 11) to detect recent HIV-1 seroconversion. The less sensitive EIAs, in particular, distinguish early from

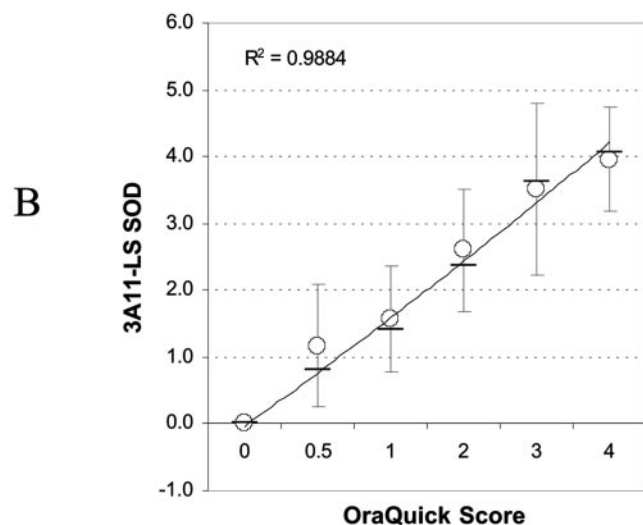
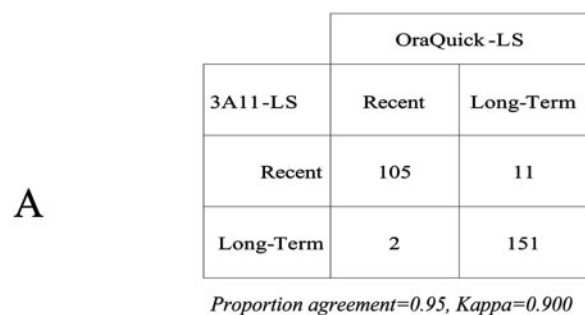


FIG. 2. Comparison of Ora-LS and 3A11-LS results. (A) A 2-by-2 table showing concordant and discordant results by both methods. Kappa statistics and percent agreement are shown. (B) Concordance of OraQuick-LS intensity score and median 3A11-LS SOD (vertical lines) at each level. Bar represents 1 standard deviation around the mean (open circles). A second-order polynomial best-fit line is shown with the correlation coefficient.

long-term infections based on differing antibody titers, when the assay is done at a high dilution (1/20,000). We show here that HIV-1 RT procedures can be modified similarly by a predilution step and can be employed to distinguish recent from long-term infections, with overall results similar to those of less sensitive EIA (3A11-LS). The agreement between 3A11-LS and RT-LS was >90% for individual sets of specimens and was about 95% for all specimens tested (kappa = about 0.9). Since RTs are visual qualitative tests, we scored the intensity of bands if HIV antibodies were detected. There was a strong correlation between the intensity scores of each of the RTs and median 3A11-LS SOD values ($R^2 > 0.98$) (Fig. 1B, 2B, and 3B).

Modifications of RT protocols involving predilutions and subsequent final dilutions, where applicable (e.g., OraQuick and SeroStrip), were performed using precision pipettes rather than the loops supplied with the kits. This was done to increase the accuracy of the results and to evaluate if the rapid tests could be used successfully for this purpose. Precision pipetting may not be practical in settings where rapid tests are being widely used for HIV diagnosis. If the RT-LS testing is to be performed using the loop, the accuracy and reproducibility

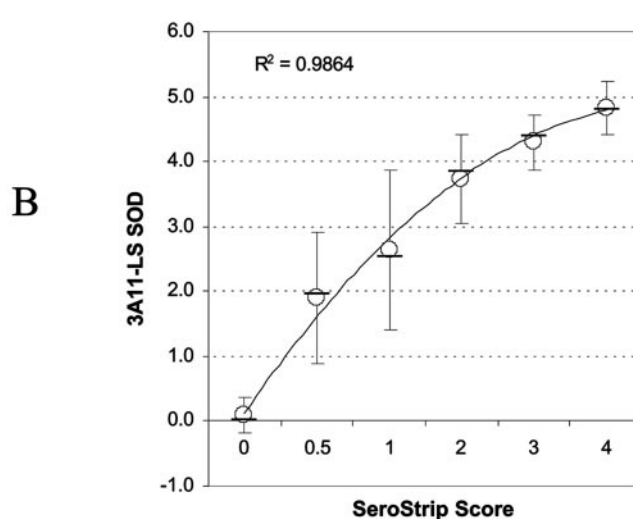
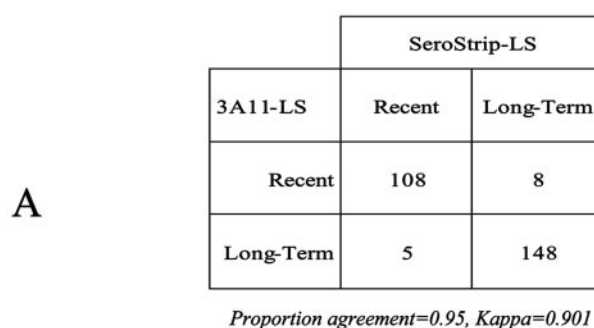


FIG. 3. Comparison of Sero-LS and 3A11-LS results. (A) A 2-by-2 table showing concordant and discordant results by both methods. Kappa statistics and percent agreement are shown. (B) Concordance of SeroStrip-LS intensity score and median 3A11-LS SOD (vertical lines) at each level. Bar represents 1 standard deviation around the mean (open circles). A second-order polynomial best-fit line is shown with the correlation coefficient.

may be somewhat lower. However, for the purpose of estimating population incidence with the intent to monitor trends, this approach may be valuable whereas EIAs may not be practical. Because the RT-LS methods are visually interpreted, it was not possible to quantify the coefficient of variation. In general, the reproducibility of results was quite high (>95% concordant) when a subset of specimens was retested (data not shown).

Recently, Constantine et al. (2) reported the use of a modified UniGold Recombigen HIV rapid test to detect recent HIV-1 infections. However, the recommended modification gave a significantly high number of false recent infections in persons known to be infected for a long time. Therefore, its use was recommended only in an algorithm with a less sensitive EIA to increase the predictive value of detecting recent infection. Our results demonstrate that all three rapid tests, Determine-1/2, OraQuick-1/2, and SeroStrip-1/2, can be used to detect recent HIV-1 seroconversion with results quite comparable to those obtained by a less sensitive EIA protocol. Although the actual window period for detecting recent seroconversion is not determined for these tests, concordance of our results suggests that it is similar to that of 3A11-LS (about 130 days) (5). It is to be noted that we have examined the corre-

lation between RT-LS and 3A11-LS, using classification by 3A11-LS as the gold standard. Apart from the 42 positive specimens from 11 seroconversion panels, most of which cover the early period after seroconversion, we have not used longitudinal specimens that span the window period.

Definitive determination of the window period would require further testing using a large number of incident panels with longer follow up. Moreover, the frequency of misclassification in patients with AIDS has not been determined.

While the results in this study are promising, there may be reasons for caution. Since these assays are read visually, the appropriate amount of light was found to be critical for the readout. This can be of concern in resource-limited settings. Constantine et al. (2) observed that the reading time can be critical in order to optimize sensitivity when using the modified UniGold assay. We followed the manufacturer's recommendations for each test while reading results.

All of the specimens used in this study were from U.S. donors who are likely to be infected with HIV-1 subtype B. Both the 3A11-LS and Vironostika-LS assays have been demonstrated to have variable performance in different subtypes (9, 16); this variability has been attributed to the use of subtype-B-derived antigens in the assays (10). RT-LS will likely have the same limitations and may perform differently in specimens from individuals infected with other subtypes. Therefore, widespread application of this approach is not advisable without further evaluation in settings where non-B subtypes are prevalent. However, we have shown that, in principle, careful modification of RT protocols can be made to distinguish recently infected individuals from those infected for longer periods.

Ideally, a rapid assay should be developed using multiple subtype antigen, such as the BED synthetic peptide (10), and optimized for the detection of recent infection. Our previous data suggest that it is important to incorporate a multisubtype antigen for the detection of recent infections. Such an assay is likely to perform similarly among populations infected with different subtypes and can be used worldwide.

The use of less sensitive HIV rapid antibody tests may provide a cost-effective method in establishing incidence. Utilization of this method could enhance the epidemiological tools in resource-limited settings, in high-incidence regions, and in point-of-care testing facilities. In addition, the development of a rapid test that incorporates HIV-1 diagnosis as well as the detection of HIV-1 incident infection in a single device may be very useful for the simultaneous measure of prevalence and incidence in the population. Our study here is a first step showing the conceptual feasibility of such an approach.

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