Performance of the Liaison XL Murex HIV Ab/Ag Test on Clinical Samples Representing Current Epidemic HIV Variants

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Screening for HIV infection has improved since the first immunoassays. Today, diagnosis of HIV infection can be performed with fourth-generation tests that track both the patient’s antibodies and HIV antigen. The objective of this study was to evaluate the clinical sensitivity and specificity of the new DiaSorin Liaison XL Murex HIV Ab/Ag assay compared to another fourth-generation assay, the Abbott Architect assay. This work was performed on a large panel of 900 samples, including negative (n = 493) and HIV-positive (n = 407) representatives of HIV-1 group M subtypes and circulating recombinant forms (CRFs), HIV-1 group O, and HIV-2 variants. The results highlight the high specificity (98.9%) and sensitivity (100%) of this new fourth-generation assay, which are consistent with its use for the screening and diagnosis of HIV infections with the current circulating strains.

Since the discovery of HIV, many tests have been marketed for the diagnosis of HIV infection, and the performance of these tests has been extensively evaluated (1–3). First-, second-, and third-generation assays, which detect only antibodies against HIV, lack the sensitivity to detect primary infections and may fail to detect recent non-B subtype infections or divergent variants (1, 4). Fourth-generation tests, which detect antibodies against both the virus and the viral antigen p24, are now recommended in France and elsewhere (5, 6) due to their great potential for detecting early infections. Indeed, their utilization reduces the diagnostic window by 1 to 3 weeks, depending on the generation of the test in the comparison (7–9). However, any diagnostic test also has to be able to detect all circulating HIV variants. HIV is currently divided into two types, HIV-1 and HIV-2, according to the genetic diversity; these types are further subdivided into four groups (M to P) for HIV-1 and nine groups (A to I) for HIV-2 (10, 11). Pandemic HIV-1 group M is also subdivided into nine nonrecombinant subtypes (A to D, F to H, and J to K) and numerous circulating recombinant forms (CRF) (http://hiv-web.lanl.gov/) and unique recombinant forms. We reported recently that the HIV Ag/Ab Combo Architect assay (Architect) (Abbott, Delkheim, Germany) performed well in detecting currently described HIV variants in both European and African populations (12). A new 4th-generation assay, the DiaSorin (Saluggia, Italy) Liaison XL Murex HIV Ab/Ag (Liaison XL), was recently developed. However, few studies have investigated the performance of this assay (1, 13, 14), and no data are available about its ability to recognize different HIV variants, except for data about the p24 antigen (1).

Thus, we compared the performance of the new test to that of the Abbott Architect assay in a large panel of samples representative of different clinical stages of HIV infection and of HIV genetic diversity and for HIV-negative status.

MATERIALS AND METHODS

Nine hundred clinical samples were collected at the Charles-Nicole Hospital, Rouen, France, including samples obtained during routine diagnostic activities at the hospital and samples sent to our laboratory because it is associated with the National Reference Center on HIV. Of these, 502 samples were prospective fresh blood samples from hospitalized patients that were obtained for HIV testing, and 398 were HIV-positive frozen plasma samples from laboratory collections.

The 900 samples were collected from 873 patients (sequential samples for patients [n = 14] or same-day samples for controls [n = 13]), with a mean age of 38.9 years (range, 1 to 95 years) and a sex ratio (male/female) of 1.05. The viruses were characterized for group and subtype by molecular (n = 214) or serotyping (n = 166) analysis as described previously (15, 16). The genetic diversity of HIV in this sample set was as follows: 208 HIV-1 group M subtype B viruses (molecular analysis [n = 81] and serotyping [n = 127]), 133 HIV-1 group M subtype non-B viruses (A [n = 15], C [n = 8], D [n = 6], F [n = 10], G [n = 9], H [n = 5], I [n = 1], CRF01_AE [n = 6], CRF02_AG [n = 37], CRF06_cpx [n = 5], CRF09_cpx [n = 1], CRF11_cpx [n = 4], CRF14_BG [n = 3], CRF17_BF [n = 1], CRF18_cpx [n = 1], CRF37_cpx [n = 1], CRF42_BF [n = 1], three nontypeable mosaic forms, and 16 non-B serotypes that were characterized by serotyping because of the absence of viral replication preventing molecular characterization). The subtype of 11 samples could not be determined by serotyping assays, but seroreactivity showed that they were HIV-1 group M. Nine samples of HIV-1 group O and 19 samples of HIV-2 (group A [n = 5], group B [n = 2], and serotype HIV-2 [n = 12]) were also included to represent the current epidemic of HIV variants.

Among the HIV-1 group M samples, 13 samples belonged to 12 individuals with a primary or recent infection (defined as follows: Architect Abbott HIV Ag/Ab Combo positive, HIV antigen Vidas HIV P24 II [bio-Mérieux, Marcy-l’Etoile, France] positive, and/or Western blot negative or indeterminate by New LAV BLOT 1 [Bio-Rad, Marnes-la-Coquette, France]).

The DiaSorin Liaison XL uses chemiluminescence immunoassay technology. The assay combines a monoclonal antibody and the recombinant antigens HIV-1 gp41 (group M and group O) and HIV-2 gp35 to deter-
mine the presence of the p24 antigen of HIV-1 and specific antibodies to both HIV-1 (group M and group O) and HIV-2. The test is configured for an automated random-access instrument (Liaison XL), with a run time of 46 min. Sample/cutoff (S/CO) ratios of ≥1 are considered reactive and indicate the presence of anti-HIV immunoglobulin and/or p24 antigen. A separate read then identifies the positive fraction: antibodies, antigen, or both.

The Abbott Architect assay is also a chemiluminescent magnetic microparticle-based immunoassay, with a run time of 28 min. The assay is used to determine the presence of HIV-1 p24 antigen and antibody to HIV-1 group M, HIV-1 group O, and HIV-2 using a randomized, random-access instrument. Recombinant antigens and synthetic peptides derived from HIV transmembrane proteins (TMP) of HIV-1 group M, HIV-1 group O, and HIV-2 are used to detect antibodies, and anti-p24 monoclonal antibodies are used to detect antigens. S/CO ratios of ≥1 are considered reactive and indicate the presence of anti-HIV immunoglobulin and/or p24 antigen, although the test does not distinguish between antigen and antibody reactivities.

Samples were processed according to the manufacturer’s recommendations, with retesting of reactive samples in duplicate; the samples that gave discordant results with the two assays were analyzed further. Complementary serological assays were performed, including the Vidas HIV Duo Quick (bioMérieux) and the Murex HIV Ag/Ab combination (DiaSorin), both of which detect antigens and antibodies, and Western blotting with the New LAV BLOT 1 kit and New LAV BLOT 2 kit (Bio-Rad) and Vidas HIV P24 II (bioMérieux) to detect the p24 antigen of HIV-1. Molecular tests were also carried out on each discordant sample to identify a possible early step of primary infection: the viral load was assessed by the Abbott RealTime HIV-1 assay, and the RT and protease genes of HIV-1 group M were examined by reverse transcription (RT)-PCR when sufficient volume was available.

RESULTS

Among the 502 prospective samples from the hospitalized population, 487 were found negative and 9 were found positive by the two tests (Table 1). The results of the Liaison XL assay for HIV-negative samples were homogeneous, with a mean of 0.270 (range, 0.196 to 0.896) for antibody (Ab) reactivity and a mean of 0.319 (range, 0.253 to 0.709) for antigen (Ag) reactivity. Six samples showed discordant results between the Liaison XL and Architect assays. Five of these samples were found positive with Liaison XL, with indexes close to the cutoff for the Ab signal (1.12 to 4.26; n = 4) or close to the cutoff for the Ag signal (1.04; n = 1). All of these samples were confirmed to be negative by the complementary serological tests (Vidas HIV Duo Quick-Murex HIV Ag/Ab combination, Western blotting, and Vidas HIV P24 II) and the molecular tests; they were thus considered false-positive results for Liaison XL. One sample was negative in Liaison XL (Ab = 0.242; Ag = 0.332) but positive in Architect (8.56). This sample came from a 77-year-old woman who had shown repeated false reactivities in previous analyses for several years. The results of the complementary tests were all negative, leading to the interpretation of false-positive reactivity in Architect.

Among the 398 positive samples from the collection, 397 were found positive by the two tests. The discordant sample corresponded to a patient at an early stage of primary infection with a group M subtype B strain. Two sequential samples were available for this patient: the first one, at the time of diagnosis during acute infection, was positive in Liaison XL (Ab = 1.24; Ag = 19.4) and Architect (15.86), and a second sample was collected 6 days after the patient was started on highly active antiretroviral therapy (HAART). The second sample gave the discordant result and was negative in Abbott but positive in Liaison XL (Ab = 6.69; Ag negative) (of note, this sample had an Abbott RealTime HIV-1 load of 3.8 log copies/ml). Thus, in this atypical case, both tests detected HIV in the first sample due to the presence of Ag (indicated by the Ag value of the Liaison XL), but only the Liaison XL was positive at the early step of the seroconversion phase (indicated by the positive Ab value).

The other samples from patients with primary or recent HIV infections were all positive in Liaison XL; seven samples were found positive for both antibodies and antigen, five samples were found positive for antibodies only, and one sample was positive for antigen only. The 19 HIV-2 samples were found positive in Liaison XL with a Ab S/CO mean of 84.8 (range, 39.2 to 143), similar to that found for all HIV-1 group M-positive samples (96.4 [range, 3.71 to 140]). The mean Ab S/CO of HIV-1 group O samples was lower than that of group M samples at 35.4 (range, 5.2 to 62.8).

From these data, the sensitivity of the Liaison XL HIV was 100% (95% confidence interval [CI], 98.83 to 100%), and the specificity was 98.9% (95% CI; 97.5 to 99.6%). The positive predictive value of the Liaison XL HIV was 98.7%, and the negative predictive value was 100%.

DISCUSSION

In this study, we examined the performance of the Liaison XL HIV assay with a large set of 900 samples. These samples were representative of HIV-negative status, of different clinical stages of HIV infection, and of the genetic diversity of the current epidemic HIV, including variants of HIV-2 and HIV-1 group O. The Liaison XL HIV correctly detected all HIV-positive samples and detected HIV in a sample collected during a treated primary infection. To our knowledge, this is the second automated 4th-generation test, along with the Vidas HIV Duo Ultra (bioMérieux), that can separate signals from antibody and antigen reactivities. This ability to distinguish between signals gives a quick indication of the date of infection before the completion of confirmatory tests (such as Western blotting or immunoblotting) and enables primary infections to be identified more rapidly and easily. There is currently no consensus regarding the possibility of replacing the HIV p24 test with this type of combined test, the lack of a confirmation assay based on neutralization being problematic if the findings are inconclusive (8).

We did not observe any correlations that may otherwise enable the HIV serotype to be suspected, which contrasts with our previous findings with the HIV Ag/Ab Combo Architect kit (12). Nonetheless, HIV-1 group O samples and group M samples showed

### Table 1: Overall results (hospitalized patients and collection) for the Liaison XL and Architect assays according to the final status

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<th>No. of samples</th>
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<td></td>
<td>HIV negative status</td>
<td>(n = 493)</td>
<td>HIV positive status</td>
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<td>Liaison XL negative</td>
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<td>Liaison XL positive</td>
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* Atypical sample collected from a patient treated at the stage of primary infection.

* Nine new infections diagnosed in the prospective samples of hospitalized patients combined with the 397 samples from the HIV-positive collection.
distinct reactivities (means, 35.4 and 96.4, respectively) in Liaison XL HIV. These differences probably emphasize the higher intra-group antigenic diversity of group O than of group M within the immunodominant epitope region of the envelope protein (which serves as a target for detection) (4, 17). Alternatively, these differences may be due to a low abundance of antibodies produced by these patients, although this aspect needs to be explored.

Of note, 25 samples showed very low Ag or Ab reactivity (between the threshold of 1 and a value of 5). Among them, 20 corresponded to true HIV-negative samples and 5 were false-positive results. Ab indexes were also particularly low for two patients in the primary HIV infection stage (1.09 and 1.24), although antigenic reactivity was present (5.36 and 19.4), which excluded the interpretation of a false-positive result. This finding clearly demonstrates the benefit of distinguishing between Ag and Ab reactivities in diagnostic tests.

Overall, our results confirm the excellent clinical sensitivity of Liaison XL HIV that was determined recently by Alonso et al. (14). However, a major limitation of their paper was the absence of data on the ability of Liaison XL HIV to detect infections with different HIV variants. Our results indicate that Liaison XL is suitable to detect infections with HIV genetic variants that are currently in circulation. Alonso et al. found a specificity of 99.2% for Liaison XL HIV and reported fewer false-positive results with the test than with Architect. However, we found more false-positive results with Liaison XL HIV, and the specificity of Liaison XL HIV was 98.9%, which is slightly lower than that reported by Alonso et al. This was mostly due to samples with values very close to the cutoff. We also found true-positive samples with similar low values, which corresponded mostly to early stages of the infection, but also to group O HIV variants. These observations highlight the need for caution during the interpretation of indexes around the cutoff and the need for complementary analysis to avoid misinterpretation (18).

In conclusion, we have demonstrated the high specificity and the effectiveness of the Liaison XL Murex HIV Ag/Ab assay in a large panel of clinically positive samples representing current epidemic HIV variants, underlining the high performance of the assay.

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REFERENCES


