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## SHORT COMMUNICATION

# Performance of the Liaison® XL Murex recHTLV-I/II Immunoassay in the Detection of HTLV-1/2 Antibodies in Serum

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### SUMMARY

***Background:*** **Human T-cell lymphotropic virus type 1 and 2 (HTLV-1/2) immunoassays are used for blood screening from blood products, milk, and organ donors.**
***Methods:*** **We assessed the performance of the DiaSorin Liaison® XL murex recHTLV-I/II immunoassay relative to the Abbott Architect® rHTLV-I/II immunoassay and with the Innogenetics immunoblot as confirmation.**
***Results:*** **A panel of HTLV positive (n = 66) and negative (n = 30) sera was tested in both techniques within the same freeze/thaw cycle. The specificity and sensitivity of DiaSorin immunoassay were 100% and 78.8%, respectively. Abbott and DiaSorin immunoassays showed a correlation in chemiluminiscent signals to cutoff (S/CO) (Pearson r = 0.92). Half of the samples (34/66) from the seropositive panel were not confirmed by immunoblot (S/CO < 5 in both techniques).**
***Conclusions:*** **Our data confirmed that the DiaSorin Liaison® XL murex recHTLV-I/II immunoassay is an effective platform for HTLV screening. Due to false-positive reaction, especially for samples with low S/CO, each seropositive sample should be confirmed by immunoblot.**
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### KEY WORDS

automated platform, false positive, human T-cell lymphotropic virus, immunoassay, immunoblot

### INTRODUCTION

The Human T-cell lymphotropic virus type 1 (HTLV-1) was the first human retrovirus identified in 1980 [1]. Human T-cell lymphotropic virus type 1 and 2 (HTLV-1/2) are distributed worldwide, infecting approximately 15 - 20 million individuals for type 1 virus. HTLV-1 is known to be endemic in Southeastern Japan, several Caribbean countries, sub-Saharan Africa, South America, and the Middle East, while HTLV-2 is naturally endemic in natives from Africa and aborigines of America [2-4]. If approximately 95% of HTLV-I infected individuals remain asymptomatic for their entire life, about 5% of the infected patients will develop HTLV-induced disease. Numerous diseases have been associated with the

virus, including adult T-cell leukemia/lymphoma, HTLV-associated myelopathy/tropical spastic paraparesis, uveitis, dermatitis, and various other inflammatory conditions. HTLV-2 has been detected in patients with some neurological disorders [4-9].

HTLV infection requires cell-to-cell contact with infected T cells [10]. Virus transmission may occur via sexual contact, mother-to-child, or transfusion with infected blood [5]. Screening for HTLV-1/2 in blood banks is mandatory in several countries [5,11]. Blood screening for HTLV-1 is performed by an enzyme immunoassay (EIA) or particle agglutination test. These tests showed low specificity; therefore, positive results should be confirmed by immunoblot [12-14]. HTLV-DNA detection by in-house PCR is used to confirm positive and equivocal immunoblot [5,13].

The aim of this study was to compare the performance of the DiaSorin Liaison® XL murex recHTLV-I/II immunoassay relative to the Abbott immunoassay on a panel of HTLV positive sera.

## MATERIALS AND METHODS

### Samples

Ninety-six sera tested as positive (n = 66) and negative (n = 30) by the Abbott assay routinely used in our laboratories (Saint-Louis Hospital, Paris, France, and Strasbourg Hospital, Strasbourg, France). These sera were used to compare the performance of the DiaSorin assay relative to the Abbott assay. The seropositive panel was selected to cover a wide range of signal-to-cutoff ratio (S/CO: $1 \leq n = 36 < 30$; $30 \leq n = 10 < 100$; $n = 20 \geq 100$). The 96 sera were selected from registered collection of samples frozen and stored at -20°C. For the present study, samples were tested simultaneously by both techniques on the same day within the same freeze/thaw cycle.

Positive samples were confirmed by immunoblot. A positive sample by immunoassay was considered as false positive when it was associated to a negative immunoblot.

### DiaSorin immunoassay

The Liaison® XL murex recHTLV-I/II immunoassay is performed on the Liaison® XL analyzer (DiaSorin, Saluggia, Italy). These systems automate and integrate a two-step chemiluminescence immunoassay (CLIA) of the target antibodies against HTLV (HTLV-1/2 p21 recombinant antigen, HTLV-1 and HTLV-2 gp46 synthetic peptides) in serum samples. During the first incubition, HTLV antibodies present in samples bind to coated magnetic particles. Then, a conjugate linked to an isoluminol derivate reacts with this complex. Subsequently, a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured as relative light units (RLU). A ratio of the sample RLU level over the calibrator established cutoff RLU (S/CO) is then calculated. A threshold of 1.0 S/CO is suggested by the manufacturer.

### Abbott immunoassay

For comparison, all specimens were tested using the Abbott Architect® rHTLV-I/II immunoassay (Abbott Laboratories, Wiesbaden, Germany), as recommended by the manufacturer. This is an automated-system of detection by chemiluminescent microparticle immunoassay (CMIA) of HTLV-1/2 gp46 synthetic peptides and HTLV-1 p21 recombinant antigen. In this technique, samples are combined with antigen coated paramagnetic microparticles. After washing, an acridinium-labeled anti-human antibody conjugate is added. A trigger solution is then combined with the reaction mixture. Subsequently, the chemiluminescent signal is measured and expressed as RLU and a S/CO is then calculated. The positivity threshold is set at 1.0 S/CO according to the manufacturer.

### HTLV immunoblot

The specificity of positive HTLV1/2 EIA assays was confirmed using the INNO-LIA HTLV-I/II immunoblot assay (Innogenetics, Ghent, Belgium).

The strips included *gag* (p19-I/II, p24-I/II) and *env* (gp46-I/II, p21-I/II) antigens. The lack of bands or the presence of a single one (p19 I/II, p24 I/II or gp46 I/II) denote negative results, one band (p21-I/II) or two bands (except p21-I/II) indicated equivocal results, and two bands including *gag* (p19-I/II and p24-I/II) and *env* (p21-I/II, gp46-I and gp46-II) bands, indicated HTLV positivity. The presence of p19-I and gp46-I signs for HTLV-1 infection. Samples containing gp46-II or a gp46-II band intensity that was greater than those of p19-I and gp46-I were considered HTLV-2 seropositive [15].

### Statistical analysis

Seropositive samples with quantitative S/CO results of both the Abbott Architect® rHTLV-I/II and the DiaSorin Liaison® XL murex recHTLV-I/II Assay were compared using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). We described the results of the seropositive panel in each technique (Abbott *versus* DiaSorin, Abbott *versus* Immunoblot, DiaSorin *versus* Immunoblot). We also assessed correlation in S/CO values between DiaSorin and Abbott using a linear regression.

## RESULTS

### Specificity

None of the 30 negative samples were found positive thus the specificity of Liaison® XL murex recHTLV-I/II was 100% (95% CI, 88.4 to 100.0).

**Table 1. Qualitative results for DiaSorin, Abbott, and immunoblot (Innogenetics) assays.**

**A**

| | | Abbott | | |
|---|---|---|---|---|
| | | Negative | Positive | Total |
| DiaSorin | Negative | 3 | 11 | 14 |
| | Positive | 3 | 49 | 52 |
| | Total | 6 | 60 | 66 |

**B**

| | | Abbott | | |
|---|---|---|---|---|
| | | Negative | Positive | Total |
| Immunoblot (Innogenetics) | Negative | 6 | 23 | 29 |
| | Equivocal | 0 | 3 | 3 |
| | Positive | 0 | 34 | 34 |
| | Total | 6 | 60 | 66 |

**C**

| | | DiaSorin | | |
|---|---|---|---|---|
| | | Negative | Positive | Total |
| Immunoblot (Innogenetics) | Negative | 12 | 17 | 29 |
| | Equivocal | 2 | 1 | 3 |
| | Positive | 0 | 34 | 34 |
| | Total | 14 | 52 | 66 |

**Table 2. Description of equivocal immunoblot results with banding patterns [positive (+) or negative (-)] and S/CO of both chemiluminescent immunoassays.**

| Patient | EIA (S/CO) | | Immunoblot, Innogenetics | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Abbott | DiaSorin | p19-I/II | p24-I/II | gp46-I/II | p21-I/II | p19-I | gp46-I | gp46-II |
| 1 | 3.59 | 68.00 | + | + | - | - | - | - | - |
| 2 | 1.54 | 0.71 | + | - | + | - | - | + | - |
| 3 | 2.41 | 0.93 | + | - | + | - | - | + | - |

**EIA - Enzyme immunoassay.**
**S/CO - Sample over cutoff.**

## Clinical sensitivity

The 66 positive samples selected on Abbott were retested in the same freeze/thaw cycle with both Abbott and DiaSorin assays (Table 1A). Forty-nine concordant positive results were recorded with both assays. In both techniques, the HTLV immunoassay was positive for 49 samples and negative for 3 plasmas (on re-test), leading to overall concordance of 78.8% (52/66).

Taking into account that samples were initially selected on the Abbott platform, the DiaSorin assay detected HTLV antibodies in 52/66 (78.8%) samples, and the Abbott assay detected HTLV antibodies in 60/66 (90.9%) samples. Six samples turned seronegative upon retesting in the Abbott assay. Blot analysis yielded positive results for 34 (52%) of the 66 selected samples initially reactive (all HTLV-1). This difference corresponded to 23 and 17 samples that were found reactive with the Abbott and the DiaSorin assays, respectively, but seronegative with the immunoblot assay (Table 1B, 1C). Also, three samples (one reactive with both DiaSorin and Abbott techniques and two reactive in Abbott only) were qualified as equivocal on immunoblot analysis (Table 2). None of these equivocal cases was retested due to insufficient sample volume.

Quantitative analysis was performed for 66 clinical samples selected on Abbott with S/CO measurements by both systems. Correlation between the DiaSorin and the Abbott assay was performed using a linear regression on the 66 samples. Figure 1 shows the correlation between both assays with a Pearson's r of 0.92

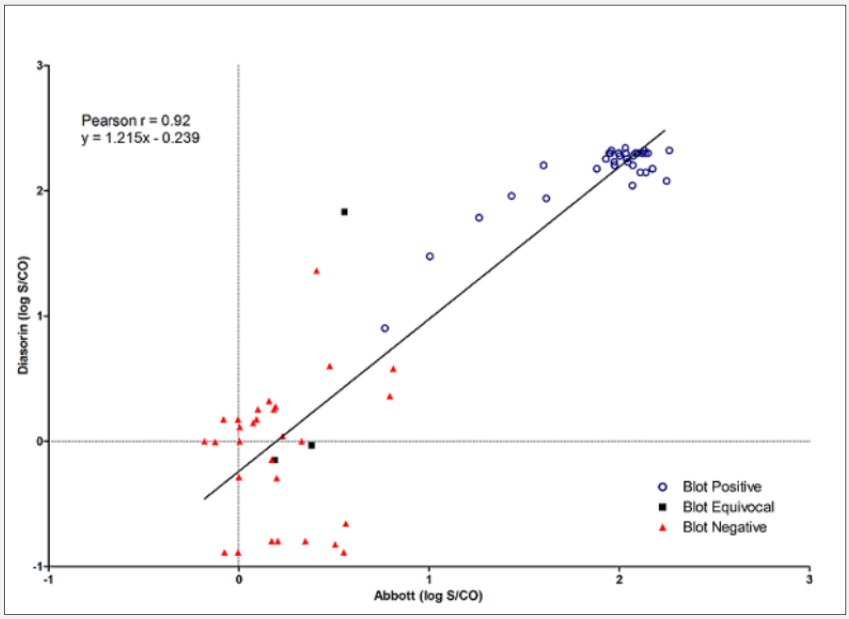


**Figure 1. Linear regression of S/CO measurements with DiaSorin and Abbott immunoassay.**

**Samples are differentiated between immunoblot positive (ȼ, n = 34), equivocal (n, n = 3) and negative ( ▲, n = 29).**

($p < 0.0001$). The median S/CO for seropositive but immunoblot negative samples was of 1.00 (range, 0.13 - 23) and 1.49 (range, 0.66 - 6.68) for DiaSorin and Abbott platforms, respectively. The median S/CO for seropositive and immunoblot positive samples was of 180.00 (range, 8.00 - 220) and 108.84 (range, 5.86 - 183.32) for DiaSorin and Abbott platforms, respectively. So, reactive samples with a S/CO < 5 in both techniques were all negative by immunoblot.

## DISCUSSION

The detection of HTLV poses a significant challenge to effective blood screening of organ, blood or milk donors. Additionally, few automated immunoassay platforms are available to test routinely gathered sera for HTLV infection as well as other viral infections.

Using HTLV seronegative and representative seropositive clinical samples, we showed that the performance of the DiaSorin Liaison® XL murex recHTLV-I/II immunoassay was close to that of the Abbott Architect® rHTLV-I/II immunoassay. Indeed, both techniques showed a remarkable correlation in S/CO values.

One study explored the performance of the DiaSorin assay finding a high sensitivity of the platform [16]. We assessed the clinical performance of the DiaSorin automated immunoassay on a large seropositive panel and the concordance between EIA tests and immunoblot that is far more specific.

Interestingly, half of the samples from the EIA reactive panel were not confirmed for HTLV-1/2 infection by immunoblot. As these discrepant sera showed S/CO values under 5 in both DiaSorin and Abbott immunoassays, we suggest manufacturers might reconsider the S/CO positivity limit. Third generation EIA kits show high S/CO values, independently of the immunoblot results [17]. If their sandwich format and their ability to bind cross-reactive antigens offer a high sensitivity to third generation EIA tests, they may be responsible for false-positive results as well.

Assuming that samples were initially selected on Abbott, we found a slightly higher proportion of false positive results with the Abbott technology than DiaSorin. Unfortunately, the three equivocal blot samples could not be tested by PCR techniques [18]. Numerous cases of seroindeterminate samples have been documented in which sera are reactive by EIA but displayed an incomplete banding pattern on confirmatory immunoblot. Reported prevalence of seroindeterminates varied from 0.023% to 20% according to the country of origin and at-risk population [5,13]. Although the clinical significance of HTLV-1/2 seroindeterminates is unclear, several hypotheses have been suggested such as a defective

virus, low copy numbers, punctual mutations, cross-reactivity to other retroviruses or a novel virus or a malaria parasite [5,13,19-20].
Only few available assay platforms, as the DiaSorin Liaison® XL and Abbott Architect platforms, provide all required markers for blood donor screening, especially as regards HTLV-1/2 screening

## CONCLUSION

The DiaSorin Liaison® XL murex recHTLV-I/II immunoassay is an effective platform for HTLV screening. Due to false-positive reaction, especially for samples with low S/CO levels, each seropositive sample should be confirmed by immunoblot. For blood donors with a reactive EIA assay and a negative immunoblot, HTLV-DNA detection by PCR could be tested.

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**Ethical Approval:**
Not required.

**Declaration of Interest:**
We report no competing interests.

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