Evaluation of the new Multi-HTLV serological assay: Improvement for HTLV-2 detection

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BACKGROUND

Despite the high specificity presented by the complementary serological tests used for HTLV infection, they have different rates of sensitivity and can generate inconclusive, discordant or false-negative results, especially when applied to HTLV-2 positive individuals. For these cases, more specific molecular techniques are indicated, which do not have available commercial protocols, making it difficult to implement them on a large scale or in peripheral locations.

The goal of this study was to evaluate a new complementary serological methodology (Multi-HTLV/Infinity Biomarkers), developed based on conventional ELISA protocols. In addition to being cheaper and easier to perform, its ability to simultaneously detect circulating antibodies against a set of selected and validated antigens, allows the study of the specific serological signatures of each patient and the evolution of the disease (Figure 1).

Figure 1. Multi-HTLV antigens map

METHODS

• This was a cross-sectional study that included participants over 18 years of age who had been regularly followed up for more than 20 years at the Instituto de Infectologia Emílio Ribas (IIEIR) in São Paulo, Brazil;
• All volunteer samples was performed after screening by enzyme-linked immunosorbent assay with HTLV viral lysate (Gold ELISA HTLV-1+2I, REM), followed by confirmation by WB (HTLV Blot 2.40, MP Biomedical) and molecular assays (qPCR and PCR RFLP);
• A total of 240 plasma samples were tested by qPCR and used to calculate the sensitivity and typing accuracy of the Multi-HTLV: 127 were positive for HTLV-1, 112 for HTLV-2, and seven for both types;
• Thereafter, the non-parametric Mann-Whitney U test was used to calculate the concordance between the qPCR test and Multi-HTLV in 12 samples with discrepant and inconclusive qPCR results.

RESULTS

Based on concordance of positivity with qPCR results (regardless of typing), Multi-HTLV obtained sensitivity of 97% (95% CI: 0.92-0.98) for HTLV-1 and 94% (0.87-0.96) for HTLV-2.

However, due to a decrease shown for the accuracy of typing (from 98% for HTLV-1 and 94% for HTLV-2, this test obtained only 95% (95% CI: 0.90-0.97.81) of perfect agreement with the qPCR results for HTLV-1 and 86% (78.04-91.01) for HTLV-2 (Figure 2).

Evaluating the performance of Multi-HTLV under atypical conditions, using samples with doubtful or false-negative qPCR results. It was noted that the sum of antigen reactivity was significantly higher in qPCR positive samples than in doubtful or false negative qPCR (Figure 3A). However, even with this decrease, the Multi-HTLV was able to correctly identify 4 (out of 5) samples with doubtful results. In addition, this test correctly identifying all HTLV-2 positive samples with false-negative qPCR results (Figure 3B).

OUTCOMES IMPACT

Improved diagnostic tests that lead to better identification of an infection in patients or blood donors play a fundamental role in public health, helping create strategies to fight infectious diseases. Furthermore, they are effective when used for early identification of the infection, consequently enabling better care and monitoring of those infected. However, in the 30 years since the development of the LIA, no significant progress has been made in improving the effectiveness of the tests currently used for HTLV-2.

CONCLUSIONS

Our findings in a significant number of HTLV-positive samples, showed that the Multi-HTLV assay is reliable, easy-to-perform, and can be used in locations without infrastructure for molecular testing. In addition, this assay is a potential alternative that fills the gap generated by samples with discrepant results in screening or confirmatory serological tests, mainly in HTLV-2 seropositive individuals, as well as open new avenues to study the specific serological signatures of each patient and follow the evolution of the disease.

ADDITIONAL KEY INFORMATION

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Conflict of Interest declaration

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Ethical Statements

This study was approved by the Ethics Committees of the IMTSR Deliberative Council and Instituto de Infectologia Emilio Ribas (CAAE: 86379218.6.1001.0061).