Transactions of the Royal Society of Tropical Medicine and Hygiene (2009) **103**, 420—422

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/trst

SHORT COMMUNICATION

Comparison of three ELISAs for the routine diagnosis of human T-lymphotropic virus infection in a high-prevalence setting in Peru

Kristien Verdonck[a,b,*], Elsa González[a,c], Fernando Maldonado[a], Doris Agapito[a], Sonia Van Dooren[d], Anne-Mieke Vandamme[d], Alfonso Silva-Santisteban[a], Guido Vanham[b,e], Daniel Clark[a,f], Eduardo Gotuzzo[a,g]

[a] *Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Lima 31, Peru*
[b] *Virology Unit, Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium*
[c] *Departamento de Medicina, Facultad de Medicina, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Lima 31, Peru*
[d] *Rega Institute, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium*
[e] *Department of Biomedical Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, 2610 Wilrijk (Antwerp), Belgium*
[f] *Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Lima 31, Peru*
[g] *Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Av. Honorio Delgado 262, Lima 31, Peru*

Received 10 August 2008; received in revised form 1 December 2008; accepted 1 December 2008
Available online 19 January 2009

KEYWORDS
Human T-lymphotropic virus 1;
Human T-lympho-tropic virus 2;

Summary To compare three human T-lymphotropic virus (HTLV) ELISAs in a high-prevalence setting, we recruited 300 adults: 125 relatives of HTLV-infected subjects and 175 patients with possible diagnoses of HTLV-associated diseases. Sera were tested with Platelia, Murex, and Ortho ELISA. Samples with positive or discordant ELISA underwent confirmatory Inno-Lia testing. Inno-Lia gave 85/300 HTLV-1-positive and 1/300 HTLV-2-positive results. The positive predictive value was 98% for Platelia, and 100% for Murex and Ortho. Six samples had discordant

* Corresponding author. Present address: Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, Lima 31, Peru. Tel.: +51 1 4823910; fax: +51 1 4823404.
E-mail address: tverdonck@itg.be (K. Verdonck).

0035-9203/$ — see front matter
doi:10.1016/j.trstmh.2008.12.002

Diagnosis;
ELISA;
Predictive value of tests;
Peru

ELISA; Murex gave one false-negative result, Ortho two and Platelia one. In high-prevalence settings, it is recommended to test samples with two ELISAs before considering them HTLV-seronegative.

1. Introduction

Control strategies for human T-lymphotropic virus (HTLV) infection rely on accurate diagnostic tests. An estimated 90% of people living with HTLV do not develop associated diseases and are unaware of their carrier status. HTLV-1 infection may, nevertheless, cause severe complications, including adult T-cell leukaemia/lymphoma and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1] HTLV-2 has been linked to HAM/TSP, arthritis and pulmonary disorders.[2] In Peru, an estimated 1—2% of the population carry HTLV-1; HTLV-2 has been reported rarely.[3]

Several ELISAs are available for HTLV testing. To eliminate false-positive reactions and to discriminate HTLV-1 from HTLV-2, confirmatory testing with line immuno-assay, western blot, immunofluorescence assays or molecular methods is recommended.[1] However, confirmatory tests are expensive and not always accessible in developing countries; therefore, HTLV diagnosis is often based on ELISA only.

Various studies have evaluated the accuracy of ELISA in sera panels and samples of candidate blood donors.[4] Evaluations in high-prevalence settings are scarce. One study from a Brazilian reference laboratory, in which the HTLV-1 prevalence was 6%, found that none of the evaluated ELISAs detected all infected samples.[5]

The purpose of this study was to compare three ELISAs in a high-prevalence setting under routine conditions. We determined positive predictive value and inter-ELISA agreement.

2. Materials and methods

We consecutively recruited 300 individuals of $\geq$18 years old who attended the Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, for HTLV screening. They were relatives of HTLV-infected subjects or had signs suggestive of HTLV-associated diseases. After obtaining informed consent, trained health workers interviewed the participants and took a blood sample. Each serum sample was distributed over four cryovials, which were stored at $-20\,^{\circ}C$ until use. The maximum number of freeze—thaw cycles was two.

The following HTLV ELISAs were evaluated: Platelia (Bio-Rad Laboratories, Marnes la Coquette, France), Murex (Murex Biotech Limited, Dartford, UK), and Ortho (Ortho-Clinical Diagnostics, Amersham, UK). Platelia is a first-generation assay based on HTLV-1 lysate. Murex and Ortho are third-generation ELISAs using recombinant and/or synthetic antigens. After the start of this study, the production of Platelia was stopped; Platelia results are complete for 221/300 samples. Inno-lia HTLV I/II Score (Innogenetics, Ghent, Belgium) was used as a confirmation test and to distinguish HTLV-1 from HTLV-2. The indications of the manufacturers were followed for all serology tests. Inno-lia was done only on samples with positive or discordant ELISA results. One trained biologist did all serology tests; she was blinded for the other test results.

Using Inno-lia as a reference, we determined positive predictive values. Confidence intervals were calculated with the Wilson score method without continuity correction. We described discordant ELISA results, and calculated kappa values to evaluate inter-ELISA agreement.

Test results and appropriate counselling were provided for the participants.

3. Results

From April 2006 to February 2007, 300/335 (90%) eligible subjects were enrolled. Mean age was 44 years (SD 16); 167 (56%) were women. One hundred and twenty-five participants (42%) were relatives of HTLV-infected people and 175 (58%) had possible diagnoses of HTLV-associated diseases (strongyloidiasis: 64; suspected HAM/TSP: 42; other diseases: 69).

Murex gave 85 repeatedly reactive results and Ortho 84. The positive predictive value for Murex and Ortho was 100% (95% CI 96—100): all positive results were confirmed with Inno-Lia. There were 43 repeatedly reactive results with Platelia, and the positive predictive value was 98% (95% CI 88—100). Inno-Lia gave, in total, 85 positive results for HTLV-1 and one for HTLV-2.

There were six inter-ELISA discordances (Table 1). In 1/5 samples with discordant ELISA and determinate Inno-Lia results, Murex did not coincide with Inno-Lia (false-negative result). Ortho gave incorrect results in 2/5 samples (two false-negative) and Platelia in 2/3 (one false-negative and one false-positive result).

In the evaluation of inter-method agreement, Platelia and Murex coincided in 220/221 samples (99.5%; $\kappa = 0.99$). Platelia and Ortho gave 219/221 concordant results (99.1%; $\kappa = 0.97$). Murex and Ortho coincided in 297/300 samples (99.0%; 83 positive and 214 negative results, $\kappa = 0.98$).

4. Discussion

The inter-ELISA agreement was high (kappa values $\geq$0.95). Murex and Ortho gave no false-positive results, but false-negative results occurred with all three ELISAs. In this study population, following international guidelines (i.e. defining a sample as seronegative after one negative ELISA) could have led to a diagnostic error in 3/300 subjects. Testing sera with two different ELISAs could detect such errors. A retrospective study in Brazil led to the same conclusion.[5]

The three ELISAs require the same laboratory equipment and time. The cost per test, including ELISA kits and necessary additional materials, is US$2.9 for Platelia, US$3.8 for Murex and US$4.0 for Ortho.

Table 1 ELISA and Inno-lia results for six samples with ELISA discordances

Motive test	Platelia 1	Platelia 2	Murex 1	Murex 2	Ortho 1	Ortho 2	Inno-lia	Conclusion
Relative	Positive	Not done	Negative	Not done	Negative	Not done	Indeterminate (p19 and p24 positive)	–
Relative	Positive	Positive	Negative	Negative	Negative	Negative	Negative (p19 positive, no other bands)	Platelia 1 and 2 false positive
Strongyloidiasis	Positive	Not done	Negative	Not done	Negative	Negative	Negative (no bands)	Platelia 1 false positive
Relative	Not done	Not done	Positive	Positive	Negative	Positive	HTLV-1 positive (all bands present)	Ortho 1 false negative
Strongyloidiasis	Positive	Not done	Positive	Positive	Negative	Positive	HTLV-1 positive (all bands present)	Ortho 1 false negative
Relative	Negative	Not done	Positive	Negative	Positive	Positive	HTLV-1 positive (p19, gp21, gp46 and gp46-1 positive)	Platelia 1 and Murex 2 false negative

Our findings should not be transferred to settings with a lower HTLV-1 prevalence or in which only healthy subjects are tested. On the other hand, the fact that we included consecutive patients instead of using referred samples or dilution series gives an idea of the actual frequency of diagnostic errors.[4]

Whereas many reports from low-prevalence settings have pointed out problems with false-positive ELISA results, this study shows that the occurrence of false-negative results should be a matter of concern.

Authors' contributions: EGot, GV, KV, EGon, DC and FM designed the study protocol; EGot, ASS, EGon and KV enrolled the participants; DA carried out the serological tests; KV, EGon, DC and FM analyzed the data; SVD, AMV, GV and DA gave advice on diagnostic testing before the start of the study and contributed to the interpretation of the results; KV and EGon drafted the manuscript. All authors read and approved the final manuscript. KV, EGon and DC are guarantors of the paper.

Acknowledgements: We thank our nurse and health workers Yessica Ramos, Viviana Quintana, Elsa Campos and Juana Huertas, as well as our collaborating physicians Fanny Ita and Erick Mayer.

Funding: This study was financed by the Directorate-General of Development Co-operation of the Belgian Government through a project of the Flemish Interuniversity Council (VLIR) and through the Framework Agreement with the Institute of Tropical Medicine of Antwerp.

Conflicts of interest: None declared.

Ethical approval: The study protocol was approved by the Institutional Review Board of the Universidad Peruana Cayetano Heredia (IRB 00001014).

References

1. Verdonck K, Gonzalez E, Van Dooren S, Vandamme AM, Vanham G, Gotuzzo E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007;**7**: 266—81.
2. Roucoux DF, Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus type II. *AIDS Rev* 2004;**6**: 144—54.
3. Alarcon JO, Friedman HB, Montano SM, Zunt JR, Holmes KK, Quinnan GVJr. High endemicity of human T-cell lymphotropic virus type 1 among pregnant women in Peru. *J Acquir Immune Defic Syndr* 2006;**42**:604—9.
4. Thorstensson R, Albert J, Andersson S. Strategies for diagnosis of HTLV-I and -II. *Transfusion* 2002;**42**:780—91.
5. Jacob F, de los Santos-Fortuna E, Azevedo RS, Caterino-de-Araujo A. Performances of HTLV serological tests in diagnosing HTLV infection in high-risk population of São Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 2007;**49**:361—4.