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Summary

Background and objective: A performance evaluation of a latex agglutination test (LAT) manufactured by the Laboratory of Hemoderivados dependent, of the National University of Cordoba, for antibody to Hepatitis B surface antigen (HBsAb), was carried out and compared with a commercial enzyme immunoassay. **Material and methods:** Testing 1,320 Cordoba's National University Blood Bank donor and immunized workers from Hemoderivados Laboratory were tested for the presence and levels of HbsAb with LAT and compared with commercial EIA (ABBOTT LABS, North Chicago, Illinois-USA) and RIA (SORIN/Biomedica-Divisione Diagnostica-Italy). **Results:** This tests showed 100% sensitivity and comparable specificity (LAT 99.5%, EIA 99.7% among donor samples). **Conclusion:** We conclude that LAT is a specific and sensitive test for HBsAb; it is simple to perform and does no require sophisticated equipment. Hence, it is suitable for mass screening of blood donors in a third world country like Argentina.

Resumen

Objetivos: Determinar el desempeño de un test de aglutinación látex (LAT) para determinar anticuerpos antiantígeno de superficie de la hepatitis B desarrollado en el Laboratorio de Hemoderivados (UNC-Hemoderivados) dependiente de la Universidad Nacional de Córdoba. **Material y métodos:** Se determinó la presencia y el nivel de anticuerpos AcHBs en muestras de 1,320 donantes voluntarios del Banco de Sangre de la Universidad Nacional de Córdoba y personal de UNC-Hemoderivados inmunizados contra el virus de la hepatitis. Para ello se empleó un test de aglutinación látex (LAT) y reactivos comerciales para EIA (ABBOTT Labs, North Chicago, Illinois-USA) y RIA (SORIN/Biomedica Divisione Diagnostica-Italy). **Resultados:** Tanto el LAT como el EIA mostraron 100% de sensibilidad y una especificidad comparable (LAT 99.5%, EIA 99.7%). **Conclusión:** El LAT es una técnica específica y sensible para determinar AcHBs; es simple y no requiere de equipos sofisticados para su desarrollo. Por lo tanto, es aplicable en la búsqueda poblacional de donantes de plasma específico antihepatitis B, sobre todo en países como la Argentina donde, a pesar de contar con centros que disponen de tecnologías modernas, existen regiones en las que es difícil acceder a ellas.

Introduction

In the last few years, due to its high specificity and sensitivity, ELISA was chosen as the test for the determination against the surface antigen of Hepatitis B Virus (HBsAB) in human plasma or serum for fractionation to produce Hepatitis B immunoglobulin (HBIg). This test has a very good specificity and sensitivity.

The EIA requires expensive equipment and trained personnel to be performed,[1-7] The latex agglutination test (LAT) overcomes these problems,[8,9] and it involves a one step reaction of the reagent with the antibody (Ab). The latex particles are sensitized with HBsAg, so that results can be interpreted visually.[1-12] The LAT has been evaluated in our Blood Banks (Cordoba University Blood Bank, Hemotherapy Institute and Neonatology University Hospital)[10,11] and found to be reliable as a screening test. In the third world, and particularly Argentina, there is also a real need for a simpler, specific, sensitive and cheap test in the Blood Bank's Laboratories, specially in field studies for plasma procurement to produce HBIg where trained personnel and equipment are not always available to perform EIA or RIA.

This work reports the performance of the LAT under our country conditions and compares it with the EIA of ABBOT Labs.

Material and methods

This work was done at the UNC-EMODERIVADOS Laboratory and the Blood Banks of the Cordoba National University.

HBsAb was determined in 1,320 samples of human plasma collected. Among them, 55 samples were from immunized workers from Hemoderivados Labs and 8 from positive control patients with high HBsAb titers. Clinical data, including sexual behavior, were collected from each donor. Further evaluation was also carried out on 651 samples of human plasma whose positive or negative Hepatitis B condition had been previously known.

Commercial EIA (Abbott Laboratories, North Chicago, Illinois, USA) and our LAT were used in the screening test; as well as a reference RIA test (SORIN/Biomedica Divisione Diagnostica-Italy).

The principle of the LAT/HBAB-HEMO DERIVADOS is agglutination of the HbsAg-coated particles with HBsAb present in human plasma or serum samples. Samples (25 µL) were mixed with the same volume of a 2% suspension of sensitized particles on a slide. Slides were gently and continuously shaken for 5 minutes at 25ºC. Agglutinated particles formed only in HBsAb positive samples.[12] Samples were tested in duplicate by both LAT and EIA. RIA was utilized to check all samples with an initial HBsAb positive result. A sample was reported as positive if found to be repeatedly reactive in a second assay. False positives samples were those found reactive by the screening test, but negative by RIA test.

Plasma samples were separated from the other blood components, aliquoted in 1 mL and stored frozen (-20ºC) until tested.

Results

Of the 1,257 samples tested, 62 (4.9%) and 60 (4.8%) were repeatedly positive by EIA and LAT respectively; 58 (4.6%) of these samples were reactive by RIA. Forty-six of the 55 immunized workers from Hemoderivados Lab. were found repeatedly positive by both screening tests. All samples of the testing serum were found to be repeatedly positive by LAT, EIA and RIA test *(table I)*.

The 651 samples of known HBsAb status showed comparable results *(table II)*.

In the screening of blood donors EIA showed 2 more false-positive results than in the LAT, and in the samples of known HBsAb status EIA showed 3 more false-positive results than in the LAT *(tables I and II)*.

LAT and EIA tests showed an sensitivity of 100%, while the specificity of bath tests was > 99% *(table III)*.

Table I. Comparative assay of 1,320 samples by LAT and EIA tests.

Category	Samples	LAT		EIA		RIA	
		Positive	Negative	Positive	Negative	Positive	Negative
Blood donors	1,257	62	1,195	60	1,197	58	1,199
Positive testing	8	8	0	8	0	8	0
Immunized workers	55	46	0	46	0	46	0

Table II. Comparative evaluation of 651 sera, whose positive or negative HBV condition had been previously known.

HBsAg status	Samples	LAT	EIA	RIA
Negative	599	0	0	0
Positive	40	40	40	40
Weak positive	5	5	5	5
False positive	7	2	5	0

Table III. Sensitivity and specificity values of LAT and EIA.

Sample group	Assay	Sensitivity	Specificity
Donors	EIA	100% (60/60)	95.07% (1,195/1,257)
	LAT	100% (60/60)	95.23% (1,197/1,257)
Serum panel	EIA	100% (45/45)	99.17% (601/606)
	LAT	100% (45/45)	99.66% (604/606)

Discussion

The present practice is to use EIA, for the detection of HBsAb and then to verify the EIA positive serum with RIA test.[6,7,13-16]

The currently available EIA requires infrastructure and trained personnel. In the developing countries, this infrastructure is not commonly available in rural areas, in these cases the LAT represents an interesting alternative.

This study shows that detection of HBsAb by LAT and EIA is similar. The false positivity rate of LAT was similar to that of the EIA. The LAT correctly identified all the positive samples including the weak positives of the serum panel.

We found that this LAT is a very simple and economical method for the detection of HBsAb in the specific gammaglobulin; plasma procurement (title > 5 UI/mL).[17,18] In most region of Argentina for sure EIA and Nucleic Acid Testing of samples can be implemented. This LAT test has application in some areas, specially rural areas where presently no HBsAb testing facilities are available; if the data regarding its sensitivity and specificity are confirmed by others services.

This test is suitable for mass screening of blood donors and patients in a Third World country like Argentina or MERCOSUR community.

We conclude that the LAT is a specific and sensitive test for HBsAb. It is simple to perform does not require sophisticated equipment, is cost effective and saves technician time.

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