

Comparison of two automated chemiluminescence tests for the detection of antibodies against the hepatitis C virus

Comparação de dois testes automatizados por quimioluminescência para a detecção de anticorpos contra o vírus da hepatite C

Comparación de dos pruebas automatizadas por quimioluminiscencia para la detección de anticuerpos contra el virus de la hepatitis C

Felicidade Mota Pereira

Laboratório Central de Saúde Pública do Estado da Bahia, Salvador, Bahia, Brasil

Maria Alice Sant'Anna Zarife

Laboratório Central de Saúde Pública do Estado da Bahia, Salvador, Bahia, Brasil

Leonardo Assis Bertollo

Laboratório Central de Saúde Pública do Estado da Bahia, Salvador, Bahia, Brasil

ABSTRACT

INTRODUCTION: A correct diagnosis of hepatitis C virus (HCV) infection is important because treatment is highly expensive and has severe side effects. The assays for the detection of antibodies against HCV (anti-HCV) have undergone several changes, and third-generation tests have been widely used due to their increased sensitivity and specificity. **OBJECTIVE:** In this study, we aimed to compare two commercially available, automated third-generation chemiluminescence tests for the detection of anti-HCV antibodies. **METHODS:** We analyzed 67 samples from the serum databank from the Laboratório Central de Saúde Pública do Estado da Bahia (LACEN-BA). The reagents examined were the automated ARCHITECT[®] anti-HCV assay (Abbott Diagnostics, Wiesbaden, Germany) and Elecsys[®] anti-HCV assay (Roche Diagnostics, Basel, Switzerland) tests and the confirmatory Recombinant Immunoblot Assay (RIBA) test (Chiron RIBA VHC 3.0 SIA, Chiron Corporation, Emeryville, CA, USA). **RESULTS:** The Architect anti-HCV assay indicated 18 positive samples, whereas 39 were negative and ten were indeterminate. The Elecsys anti-HCV assay indicated 47 negative and 20 positive samples and no indeterminate samples. Of the ten indeterminate samples indicated by the Architect anti-HCV assay, five were negative by the RIBA test and five were indeterminate. All indeterminate samples indicated by the RIBA test were reactive against the c33 protein. **CONCLUSION:** The agreement between the two tests (62 samples) was 91.9%. The Elecsys anti-HCV assay appears to be less sensitive than the Architect anti-HCV, particularly for the detection of the c33 protein shown by the RIBA test. In addition, the Architect anti-HCV assay indicated more indeterminate results out of the negative samples confirmed by the RIBA test, which suggests a lower specificity than the Elecsys anti-HCV assay.

Keywords: Hepatitis C; Hepatitis C Antibodies; Laboratory Test.

Correspondence / Correspondência / Correspondencia:

Felicidade Mota Pereira

Rua Waldemar Falcão, 123. Bairro: Candeal

CEP: 40296-710 Salvador-Bahia-Brasil

Tel: +55 (71) 3276-1721

E-mail: felizmp@yahoo.com.br

Translated by / Traduzido por / Traducido por:

American Journal Experts

INTRODUCTION

The diagnosis and monitoring of hepatitis C virus (HCV) infection are based on two types of tests: a serological test that detects HCV antigen-specific antibodies and assays that detect viral RNA or HCV core antigens¹. However, anti-HCV antibody detection methods do not distinguish between active infections and resolved infections. In addition, false negative results are common because the window period (seroconversion of anti-HCV) is long and lasts between 45 and 68 days².

False positive results for anti-HCV antibody tests are a well-known problem. This may occur due to interfering factors such as high gamma globulin levels, nephrotic syndrome, liver diseases, autoimmune diseases, viral or parasitic infections or pregnancy in women³. The Centers for Disease Control and Prevention (CDC) estimates that for immunocompetent individuals, approximately 35% of the anti-HCV ELISA immunoassay (EIA) results are false positives⁴.

Currently, the detection of anti-HCV antibodies is routinely performed using two primary methodologies: EIAs and chemiluminescence immunoassays (CLIAs)⁵. EIAs detecting anti-HCV antibodies have undergone several stages of development. The third generation test (EIA III) is the most widely used because of its higher sensitivity and specificity, which is due to the greater reactivity to the NS3 region of the virus and the incorporation of the C100 epitopes and antigens from the NS5 region of the viral genome⁶. However, automated CLIAs have been replacing EIAs because of their practicality, accuracy and greater than 98% specificity; importantly, the sensitivity of automated CLIAs are similar to that of EIAs⁶.

The presence of anti-HCV antibodies does not always indicate the existence of a current infection; instead, it may represent a false positive result or be indicative of previous resolved acute hepatitis, which corresponds to an immunological scar⁶. Thus, despite the high specificity of the third generation tests, confirmatory tests must still be performed on samples with low sample/cut-off (S/CO) ratios to avoid false positive results⁷.

Currently, CLIAs are routinely used in large testing laboratories. Although they are gradually replacing EIAs, a limited number of studies on the comparison and evaluation of these automated CLIAs have been published⁸.

The objective of this study was to compare two third-generation, fully automated, commercially available CLIAs for the detection of anti-HCV antibodies that are routinely used in the *Laboratório Central de Saúde Pública do Estado da Bahia - LACEN-BA* (Public Health Central Laboratory of Bahia State).

MATERIALS AND METHODS

We tested 67 samples selected from the LACEN-BA databank. When analyzed previously, 18 samples tested positive for anti-HCV antibodies, 39 tested negative and 10 exhibited indeterminate results. The LACEN-BA routinely uses the Architect Anti-HCV Assay Kit (Abbott Diagnostics, Wiesbaden, Germany). In this study, this kit was used as the reference and was compared to the Elecsys Anti-HCV Assay Kit (Roche Diagnostics, Basel IA, Switzerland). A confirmatory recombinant immunoblot assay (RIBA) (Chiron RIBA HCV 3.0 SIA; Chiron Corporation, Emeryville, CA, USA) was performed on samples with indeterminate results.

The manufacturers' instructions were followed for all tests performed. The Architect anti-HCV assay is a CLIA performed on fully automated equipment (Abbott Architect i4000). Although also performed using completely automated equipment (Roche Modular E 170), the Elecsys anti-HCV assay uses electrochemiluminescence (ECL) technology.

RIBAs utilize strips impregnated with synthetic and recombinant peptides (c100, c33, c22 and NS5) that are applied on separate lines in a solid phase¹.

The characteristics of the two automated tests are summarized in table 1. In both methodologies, the emitted signal light is directly proportional to the antibody titer present in that sample.

Table 1 – Characteristics of the two automated tests used for the detection of anti-HCV antibodies

Test	Reagents	Principle	Sample volume (µL)	Calibration	Reagent stability	Reaction time (min)
Elecsys anti-HCV	Advance preparation	ECLIA	40	Per kit – 4 points	3 days in the equipment	18
Architect anti-HCV	Ready to use	CLIA	20	Per lot – 4 points	Up to 25 days in the equipment	29

The results of both tests are based on a cut-off (CO) value of 1.0. Architect anti-HCV assay results are expressed by the ratio of the sample's optical density to the cut-off measure (S/CO), while Elecsys anti-HCV assay results are presented as index values. The LACEN-BA considers sample results to be anti-HCV antibody positive when they are four times higher than the CO value (> 4.0), negative when they are < 0.9, and indeterminate when they are between 0.9 and 4.0 for both tests. The CO value was established because of its higher concordance (95%) with the RIBA test positive results obtained using the Chiron RIBA test.

RESULTS

According to the Architect anti-HCV assay, 18 samples were positive, 39 were negative and 10 were indeterminate; in contrast, 20 samples were positive and 47 were negative according to the Elecsys anti-HCV assay. The Elecsys anti-HCV assay did not report any indeterminate results. These data are summarized in table 2.

Table 2 – Correlation between the results of the Architect anti-HCV assay and the Elecsys anti-HCV assay, in absolute numbers

Elecsys anti-HCV	Architect anti-HCV			Total
	Positive	Indeterminate	Negative	
Positive	18	2	-	20
Indeterminate	-	-	-	-
Negative	-	8	39	47
Total	18	10	39	67

Conventional signal used. - Numerical data not equal to zero due to rounding.

The 10 samples with indeterminate results from the Architect anti-HCV assay had S/CO ratios ranging from 1.12 to 3.86. Of these samples, five were negative and five were indeterminate according to the Chiron RIBA. When analyzed using the Elecsys anti-HCV assay, eight samples were negative and two were positive. Thus, of the 67 samples analyzed, 44 were negative, 18 were positive and 5 exhibited indeterminate results according to the Chiron RIBA. All samples with indeterminate Chiron RIBA results exhibited reactivity to the c33 protein of HCV. The laboratory results of the samples classified as indeterminate by the Architect anti-HCV assay, as well as the results of the RIBA test, are summarized in table 3.

Table 3 – Sample/cut-off ratio values for samples that tested as indeterminate according to the Architect anti-HCV assay compared to the results of the Elecsys anti-HCV assay and the RIBA test

Sample	Architect anti-HCV (S / CO)	Elecsys anti-HCV (index)	RIBA			
			c100	c33	c22	NS5
1	1.56	0.025	-	-	-	-
2	1.12	0.019	-	+	-	-
3	2.00	0.015	-	-	-	-
4	2.06	0.144	-	+	-	-
5	3.86	650.6	-	+	-	-
6	1.45	0.018	-	-	-	-
7	1.61	0.020	-	-	-	-
8	1.92	0.043	-	-	-	-
9	2.07	157.5	-	+++	-	-
10	1.76	0.099	-	+++	-	-

DISCUSSION

The number of samples evaluated in this study was relatively small compared to other studies that have evaluated the performance of commercially available anti-HCV antibody detection tests^{1,5,8}.

Of the 67 samples analyzed, 10 (14.9%) exhibited discordant results between the two tests evaluated in this study. For the Architect anti-HCV assay, these samples had S/CO ratios of less than 4.0 and were considered indeterminate. However, according to the Elecsys anti-HCV assay, two of these samples were positive and eight were negative. The use of the Chiron RIBA resolved the discordant in 50% of these samples, as five out of 10 tested negative by immunoblot analysis. The samples that exhibited indeterminate results from the Chiron RIBA tested positive only for the c33 protein.

According to CDC recommendations, anti-HCV antibody titer determination requires the use of a screening test with high sensitivity; additionally, reactions with low positivity should be confirmed with a recombinant immunoblot test or a polymerase chain reaction (PCR) test to detect viral RNA⁸.

Although RIBAs can be used as supplementary confirmatory tests for the detection of anti-HCV antibodies, their disadvantages include a high rate of indeterminate results⁵.

Berger et al¹ also evaluated two serological tests and obtained different results, with a 1.9% discordant between tests, and a resolvability of 63.2% for samples using an immunoblotting test with a reactivity of up to two proteins.

The RIBA indeterminate samples were not tested for viral RNA because of the insufficient remaining material necessary for analysis. However, the presence of anti-HCV antibodies does not necessarily indicate a current infection. Low antibody titers can occur during seroconversion and also when antibody titers decrease following infection resolution.

Patients who have a low risk for hepatitis C infection that have indeterminate RIBA results and a low S/CO ratio typically exhibit undetectable levels of virus when analyzed using PCR⁷.

When definitely positive and negative samples (62 samples) were compared, the two tests evaluated had a concordance of 91.9%; this result is lower than the concordance reported by Kim et al⁸. In their study, concordance among four anti-HCV antibody tests, including the Architect anti-HCV assay and the Elecsys anti-HCV assay, ranged from 94.5% to 98.1%.

Following the initial commercial release of the Architect anti-HCV assay, issues with false negative and false positive results were reported. The diluent reagent was subsequently modified, and new studies have shown improved sensitivity and specificity¹.

Echevarria et al⁹ demonstrated that the new Architect anti-HCV assay effectively detects low levels of anti-HCV antibodies, making it an effective screening test in blood banks.

With regards to the advantages and disadvantages of each test, the Elecsys anti-HCV assay exhibits several disadvantages compared to the Architect anti-HCV assay. The test reagents in the Elecsys anti-HCV Assay Kit are not ready for use, creating potential issues regarding contamination and pre-analytical error. Additionally,

calibration is required for each kit, not each lot; thus, as four tests and two controls are used for each calibration, the number of useable tests decreases from 100 to 94 tests per kit. In addition, the period of stability of the reagents in the equipment is three days, and the volume required for each sample is greater compared to the Architect anti-HCV assay.

CONCLUSIONS

According to the results obtained in this study, the Elecsys anti-HCV assay was less sensitive compared to the Architect anti-HCV assay regarding the detection of the c33 protein. On the other hand, the Architect anti-HCV assay had a greater number of indeterminate results in negative samples as confirmed using the RIBA test, suggesting lower test specificity compared to the Elecsys anti-HCV assay.

To avoid false positive results, all samples with low S/CO ratios should be confirmed with additional and/or confirmatory tests, such as immunoblotting or PCR tests.

Additional studies are needed to better assess assays that utilize chemiluminescence methodologies. Besides this, these studies should include a larger number of samples.



Comparaç o de dois testes automatizados por quimioluminesc ncia para a detec o de anticorpos contra o v rus da hepatite C

RESUMO

INTRODU O: O diagn stico correto da infec o pelo v rus da hepatite C (VHC)   de grande import ncia, j  que o tratamento   altamente dispendioso e tem s rios efeitos colaterais. Os testes para a detec o dos anticorpos contra o VHC (anti-VHC) sofreram v rias modifica es e, atualmente, os testes de terceira gera o s o amplamente utilizados por terem sensibilidade e especificidade maiores. **OBJETIVO:** O objetivo deste estudo foi comparar dois testes automatizados para a detec o do anti-VHC por quimioluminesc ncia, de terceira gera o, dispon veis comercialmente. **M TODOS:** Foram utilizadas 67 amostras da soroteca do Laborat rio Central de Sa de P blica do Estado da Bahia. Os reagentes utilizados foram os testes automatizados ARCHITECT[®] anti-HCV assay (Abbott Diagnostics, Wiesbaden, Alemanha), Elecsys[®] anti-HCV assay (Roche Diagnostics, Basileia, Su a) e o teste confirmat rio *Recombinant Immunoblot Assay* (RIBA) (Chiron RIBA VHC 3.0 SIA, Chiron Corporation, Emeryville, CA, EUA). **RESULTADOS:** Com o teste Architect anti-HCV assay foram obtidas 18 amostras positivas, 39 negativas e dez indeterminadas, enquanto que com o Elecsys anti-HCV assay, 47 amostras foram negativas e 20 positivas. N o houve amostras indeterminadas com este teste. Das dez amostras indeterminadas no Architect anti-HCV assay, cinco foram negativas pelo teste RIBA e cinco foram indeterminadas. Todas as amostras indeterminadas pelo RIBA apresentaram reatividade para a prote na c33. **CONCLUS O:** Os dois testes, quando comparados (62 amostras) entre si, tiveram uma concord ncia de 91,9%. O Elecsys anti-HCV assay parece ser menos sens vel que o Architect anti-HCV assay, em particular para a detec o da prote na c33, revelada pelo RIBA. Por outro lado, o Architect anti-HCV assay apresentou um maior n mero de resultados indeterminados em amostras negativas confirmadas pelo RIBA, o que sugere uma menor especificidade, quando comparado com o outro teste avaliado.

Palavras-chave: Hepatite C; Anticorpos Anti-Hepatite C; Testes Laboratoriais.

Comparación de dos pruebas automatizadas por quimioluminiscencia para la detección de anticuerpos contra el virus de la hepatitis C

RESUMEN

INTRODUCCIÓN: El diagnóstico correcto de la infección por el virus de la hepatitis C (VHC) es de gran importancia, una vez que su tratamiento es muy costoso y tiene serios efectos colaterales. Las pruebas para la detección de anticuerpos contra el VHC (anti-VHC) han pasado por varias modificaciones y, actualmente, las pruebas de tercera generación son ampliamente utilizadas, por tener mayor sensibilidad y especificidad. **OBJETIVO:** El objetivo de este estudio fue el de comparar dos pruebas automatizadas para la detección del anti-VHC por quimioluminiscencia, de tercera generación, disponibles comercialmente. **MÉTODOS:** Se utilizaron 67 muestras de la seroteca del Laboratório Central de Saúde Pública do Estado da Bahia (LACEN-BA). Los reactivos utilizados fueron las pruebas automatizadas ARCHITECT® anti-HCV assay (Abbott Diagnostics, Wiesbaden, Alemania), Elecsys® anti-HCV assay (Roche Diagnostics, Basilea, Suiza) y la prueba de confirmación *Recombinant Immunoblot Assay* (RIBA) (Chiron RIBA VHC 3.0 SIA, Chiron Corporation, Emeryville, CA, EUA). **RESULTADOS:** Con la prueba Architect anti-HCV assay se obtuvieron 18 muestras positivas, 39 negativas y diez indeterminadas, mientras que con Elecsys anti-HCV assay, 47 muestras fueron negativas y 20 positivas. No hubo muestras indeterminadas con esta prueba. De las diez muestras indeterminadas por Architect anti-HCV assay, cinco fueron negativas por la prueba RIBA y cinco fueron indeterminadas. Todas las muestras indeterminadas por RIBA presentaron reactividad para la proteína c33. **CONCLUSIÓN:** Cuando comparadas entre sí, las dos pruebas (62 muestras) tuvieron un 91,9% de concordancia. La prueba Elecsys anti-HCV assay parece ser menos sensible que Architect anti-VHC, en particular para la detección de la proteína c33, revelada por RIBA. Por otro lado, Architect anti-HCV assay presentó un mayor número de resultados indeterminados en muestras negativas confirmadas por RIBA, lo que sugiere una menor especificidad, cuando comparada con la otra prueba evaluada.

Palabras clave: Hepatitis C; Hepatitis C Antibodies; Laboratory Test.



REFERENCES

- Berger A, Rabenau H, Allwinn R, Doerr HW. Evaluation of the new Architect anti-VHC screening test under routine laboratory conditions. *J Clin Virol.* 2008 Oct;43(2):158-61.
- Morota K, Fujinami R, Kinukawa H, Machida T, Ohno K, Saegusa H, et al. A new sensitive and automated chemiluminescent microparticle immunoassay for quantitative determination of hepatitis C virus core antigen. *J Virol Methods.* 2009 Apr;157(1): 8-14.
- Zachary P, Ullmann M, Djeddi S, Meyer N, Wendling MJ, Schvoerer E, et al. Evaluation of three commercially available hepatitis C virus antibody detection assays under the conditions of a clinical virology laboratory. *J Clin Virol.* 2005 Nov;34(3):207-10.
- Watterson JM, Stallcup P, Escamilla D, Chernay P, Reyes A, Trevino SC. Evaluation of the ortho-clinical diagnostics vitros ECi anti-HCV test: comparison with three other methods. *J Clin Lab Anal.* 2007;21(3): 162-6.
- Kesli R, Ozdemir M, Kurtoglu MG, Baykan M, Baysal B. Evaluation and comparison of three different anti-hepatitis C virus antibody tests based on chemiluminescence and enzyme-linked immunosorbent assay methods used in the diagnosis of hepatitis C infections in Turkey. *J Int Med Res.* 2009 Sep-Oct;37(5):1420-9.
- Parise E. Diagnóstico sorológico das hepatites crônicas virais. *Roche Rev.* 2009;8:2-6.
- Vermeersch P, Van Ranst M, Lagrou K. Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. *J Clin Virol.* 2008 Aug;42(4):394-8.
- Kim S, Kim JH, Yoon S, Park YH, Kim HS. Clinical performance evaluation of four automated chemiluminescence immunoassays for hepatitis C virus antibody detection. *J Clin Microbiol.* 2008 Dec;46(12):3919-23.
- Echevarria JM, Avellón A, Jonas G, Hausmann M, Vockel A, Kapprell HP. Sensitivity of a modified version of the ARCHITECT® anti-HCV test in detecting samples with immunoblot-confirmed, low-level antibody to hepatitis C virus. *J Clin Virol.* 2006 Apr;35(4):368-72.