Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody

Pampi Majumder[1], Anup Kumar Shetty[2,*]

[1]PG Resident, [2]Associate Professor, Dept. of Microbiology, Father Muller Medical College, Mangalore, Karnataka

***Corresponding Author:**
Email: dranupshetty@gmail.com

Abstract

Detection of antibody to Hepatitis C Virus (Anti-HCV) by Enzyme linked immunosorbent assay (ELISA) method is one of the most popular method and today it's being slowly replaced by more sensitive and rapid automated analyzer chemiluminescence immunoassay (CLIA). This was an observational cross-sectional study and aim of this study was to compare ELISA and chemiluminescence immunoassay (CLIA) for the detection of anti-HCV. A total of 91 samples were tested for the detection of anti-HCV by CLIA and ELISA. Out of 91 samples, 30 (33%) were non-reactive by both CLIA and ELISA. 32(35.2%) samples were reactive by CLIA, 2(6.25%) CLIA reactive samples were found to be non-reactive by ELISA. 29 (31.87%) samples were interpreted as borderline on CLIA. By using ELISA technique as gold standard for Anti-HCV detection our results showed 96.07% specificity and 96.66% sensitivity for CLIA technique. Detection of anti-HCV by CLIA is comparable with ELISA. CLIA can help in detecting early infection compared to ELISA and is suitable in large sample volume laboratories.

Keywords: ELISA, CLIA, Anti-HCV antibody, Comparison.

Introduction

Hepatitis C virus (HCV) is a global healthcare problem. It causes progressive disease resulting in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.(1) It is a single stranded, enveloped, positive-sense RNA virus belonging to family Flaviviridae.(2) Important sources of hepatitis C virus (HCV) infection includes infected blood and its products and other body fluids. Risk factors like intravenous drug abuse, reuse of syringes, dental procedures, unsterile pricks, infected sexual partner and tattooing play an important role in transmission of HCV infection.(2) World Health Organization (WHO) estimates 71 million people globally have chronic HCV infection.(3) Diagnosis of HCV infection is mainly based on the detection of anti-HCV IgG antibodies as a screening by methods like Enzyme linked immunosorbent assay (ELISA), immunochromatography assays and positive result verified by more specific supplemental assay such as recombinant immunoblotting assay (RIBA) and HCV RNA polymerase chain reaction (PCR) viral load in clinical practices.(4)

Chemiluminescence immunoassay (CLIA) are being widely used now for screening anti-HCV antibodies, particularly in high volume clinical laboratories for detection of anti-HCV antibodies. This method is claimed to have excellent reliability, precision, random access and technical simplicity of full automation.(3) Studies done earlier have shown CLIA to have improved specificity, a greater positive predictive value and similar sensitivity compared to those of ELISA for detecting anti-HCV antibodies. ELISA and CLIA are based on different test principles. Although CLIA is gradually replacing the ELISA, there are not enough Indian published data on the comparative evaluation of CLIA with ELISA for detection of anti-HCV antibodies. Hence this study aims to compare the technical performance between ELISA and currently marketed automated CLIA in detection of Anti-HCV antibody.

Materials and Method

An observational cross sectional study was done for a period of 6 months in the Department of Microbiology. Ninety one serum samples routinely received for Anti-HCV antibody testing by CLIA between 1st, December 2016 to 31st, May 2017 were randomly selected and included in this study. Haemolysed samples and lipaemic samples were excluded from the study. Blood samples received in the laboratory were centrifuged at 3,000rpm and serum was separated. CLIA was performed on Vitros 3600 Immunodiagnostic equipment (Ortho Clinical Diagnostics, USA). The test kit used in the equipment was a third generation anti-HCV kit. Third generation assay is an in-vitro qualitative assay which detects antibody for both HCV structural and non-structural antigens (Core, E1, E2, NS3, NS4 and NS5). To run the test by CLIA, serum samples and reagents were loaded in the equipment at relevant positions. Once sample is loaded the equipment automatically performed and released the results. Incubation time of CLIA for Anti-HCV detection was 45 minutes and time to first result from the time of sample loading was 55 minutes. The sample volume used for detection of Anti-HCV by CLIA was 20 µl. After the CLIA results were available the samples were subjected to testing by third

generation commercial HCV ELISA (HCV Microlisa, J Mitra, Mumbai, India). Both CLIA and ELISA test methods were performed as per the manufacturer's instructions. For CLIA calibrators and controls were run as per manufacturer's protocol. Controls were also run for ELISA during each run as per kit protocol. By using ELISA technique as gold standard for HCV detection, sensitivity and specificity of CLIA techniques was analyzed.

Results were calculated automatically by the VITROS equipment. Signal-to-cut-off-signal ratio (S/CO) was used for interpretation of initial results. Values> 2.00 were interpreted as reactive, values of >1 and <2 were interpreted as borderline and values less than <1were interpreted as non-reactive. The samples included in the study interpreted as reactive, borderline and non-reactive by CLIA were tested further for Anti-HCV antibodies by third generation indirect ELISA. Each ELISA run included a kit positive control (PC) and a negative control (NC). ELISA was done as per manufacturer's recommendations. After the ELISA was run the readings of the optical density (OD) of microwell plates were taken on a automated spectrophotometer (Bio-Rad ELISA reader. USA). The equipment automatically validated the run and calculated the results. For a valid run the OD values of PC was >0.5, negative control(NC)<0.150. Interpretation of result was done based of cut-off value (Cut-off value= PC × 0.23). Test specimens with absorbance value less than the cut-off value were interpreted as non-reactive for Anti-HCV. Test specimens with absorbance value greater than or equal to the cut-off value were interpreted as reactive for Anti-HCV. Samples with absorbance value within 10% below the cut-off were considered suspect for the presence of anti-HCV. For statistical analysis, data was entered and analyzed by frequency, percentage in SPSS v.22 and Cohen's kappa was used to find the difference between two variables of study (CLIA and ELISA). A p value less than 0.5 was considered as significant.

Results

A total of 91 blood samples were randomly selected for a period of six months. Out of 91 samples, 69 (75.82%) samples were from male patients and 22 (24.18%) were from female patients. Among the 91 samples tested for Anti-HCV antibody on CLIA, 30 tested as non-reactive (33%), 32 samples (35%) tested as reactive and 29 (32%) samples tested as borderline. All the91 CLIA-screened samples were further analyzed by indirect 3rd generation ELISA. Among these, 30 (33%) samples screened non-reactive by CLIA, showed non-reactive result by ELISA also. Out of 32 CLIA reactive samples 30 samples showed reactive by indirect ELISA and 2 samples were found to be non-reactive, as shown in **Fig. 1**. A statistically significant association was found between ELISA and CLIA results, as shown in **Table 1.**

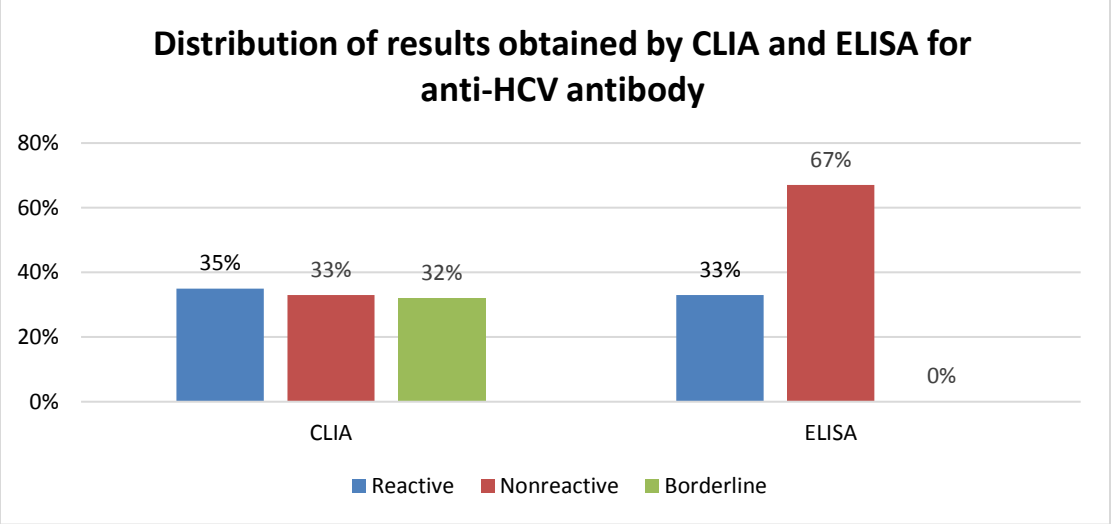

Fig. 1: Bar chart showing distribution of results obtained by CLIA and ELISA

Table 1: Distribution of reactive and non-reactive results obtained with CLIA and ELISA for Anti-HCV

HCV Screening (CLIA)	HCV ELISA		Total	p value
	Reactive	Nonreactive		κ-0.923 p value-0.0001
Reactive	30	2	32	
Nonreactive	1	49	50	
Total	31	51	82	

(κ-kappa);*p value <0.05 is significant.

The ELISA Anti-HCV assay did not detect any intermediate or suspicious (within 10% of cut-off values) results. Out of 29(31.87%)grey zone samples interpreted as borderline (cut-off value 1-2) by CLIA were retested in duplicate by indirect ELISA, and 28(96.55%) samples were found to be non-reactive and 1(3.45%) sample showed reactive result. This single reactive sample was confirmed positive by HCV RNA quantitative PCR which had viral load of 2960 IU/ml. Although HCV RNA PCR was not in the scope of this study, out of 28 borderline samples 12 (42.86%) samples were further processed for HCV RNA PCR and all 12were negative for HCV RNA. These samples were reported as negative for Anti-HCV antibody by CLIA. For remaining 16(57.14%) borderline samples PCR could not be performed. Among these 16 samples, 11(68.75%) patients had elevated liver enzymes (AST/ALT) and out of these 11 patients, 6(54.54%) patients had hepatomegaly with parenchymal liver disease, 1(9.09%) patients had fatty infiltration of liver and 1(9.09%) had splenomegaly. Therefore, in an attempt to clarify this situation, we correlated clinical findings and laboratory results and for these 16(57.14%) borderline or discordant samples repeat Anti-HCV screening by CLIA was advised after 2-4 weeks. Out of 16 samples we received only 7(43.75%) repeat samples and reported as non-reactive after retested by both CLIA and ELISA technique. But other 9(56.25%) samples we could not do repeat screening as repeat samples were not sent and reported as borderline.

Considering ELISA as gold standard sensitivity, specificity, positive predictive value and negative predictive value of CLIA shown in **Table 2 & 3** respectively.

Table 2: Sensitivity and Specificity of CLIA while ELISA using as Gold Standard

Sensitivity	96.77%
Specificity	96.07%

Table 3: Positive predictive value and negative predictive value of CLIA while using ELISA as Gold standard

Positive Predictive Value (PPV)	93.75%
Negative Predictive Value (NPV)	98%

Discussion

The commonest modes of transmission of HCV is through exposure to infected blood and body fluid. Unsafe injections have also an important role in transmission. Chronic infection may result in chronic hepatitis and hepatocellular carcinoma. Though hepatitis C is generally considered to be a curable disease, treatment is not well tolerated in some patients and early and accurate diagnosis plays a key role. Both clinical signs and laboratory findings are necessary for the diagnosis of acute HCV infection.[5] The HCV genome consists of seven functional regions- the core, the envelope, including the E1 and E2 regions, and the nonstructural region, including NS2, NS3, NS4, and NS5. For the detection of anti-HCV, third-generation tests have been widely used due to their increased sensitivity and specificity. Third-generation tests like CLIA for testing anti-HCV includes reconfigured core and NS3 antigens and an additional antigen (NS5), which reduces the time for detection of antibody to an average of 7-8 weeks after infection. The sensitivity and specificity of the tests have increased because of the addition of more antigens into the third generation tests. But false positivity rate has also increased. Hence performing confirmatory tests on samples with low sample/cut-off (S/CO) ratios will help in detecting false positive results.[5]

In our study out of 91 samples, tested for Anti-HCV antibody, 30 tested as non-reactive (33%) and 32 samples (35%) were screened as reactive on CLIA. But remaining 29 (32%) samples were interpreted as borderline based on cut-off value (S/c ratio=1-2). CLIA non-reactive samples were non-reactive by ELISA also. But out of 32 CLIA reactive samples only 30 samples showed reactive and 2 samples were found to be non-reactive by ELISA. There were no intermediate or suspicious results (within 10% of cut-off values) by 3rd generation ELISA Anti-HCV assay. 29 (31.87%) grey zone samples interpreted as borderline by CLIA were retested in duplicate by indirect ELISA according to standard protocol.[3] In ELISA 28(96.55%) samples found to be non-reactive and 1(3.45%) sample showed reactive result. This single reactive sample was further confirmed as positive by HCV RNA quantitative PCR with low viral load of 2960 IU/ml. Although HCV RNA PCR was not in the scope of this study, out of 28 borderline samples 12 (42.86%) samples were further processed for HCV-RNA PCR since we received samples for PCR, and all 12 were negative for HCV RNA. So these samples were reported as negative for Anti-HCV antibody by CLIA after confirmation by PCR. For remaining 16 (57.14%) borderline samples PCR could not be performed, since we did not receive samples for the test. We evaluated other laboratory parameters for these 16 samples, where we found 11(68.75%) patients had elevated liver enzymes (AST/ALT) and out of these 11 patients, 6(54.54%) patients had hepatomegaly with parenchymal liver disease. Only 1(9.09%) patients had fatty infiltration of liver and 1(9.09%) had splenomegaly. Therefore, in an attempt to clarify this situation, we correlated clinical findings and laboratory results and for these 16(57.14%) borderline or discordant samples repeat Anti-HCV screening by CLIA was advised after 2-4 weeks. Out of 16 samples we received only 7(43.75%) repeat samples and reported as non-reactive after retested by both CLIA and ELISA technique. But other

9(56.25%) samples we could not do repeat screening as repeat samples were not received in the laboratory. All those borderline cases were advised to repeat anti-HCV screening by CLIA after six months by clinician as per recommendation by Centers for Disease Control and Prevention (CDC) as the patients need to periodic evaluation who are on long-term hemodialysis and/or with persistently abnormal alanine aminotransferase levels (ALT).[6]

In the present study we used ELISA as gold standard and compared the results of CLIA for the screening of Anti-HCV. CLIA showed sensitivity and specificity of 96.77% and 96.07% respectively for the screening Anti-HCV in comparison with ELISA. The screening methods have limitation of false negative and false positive results. When we compared CLIA with ELISA in this study the rate of false negativity was (1/49) 2.04% and false positivity was only (2/32) 6.25%, with 93.75% PPV and 98% NPV. Therefore, based on our findings it is possible that samples with discrepant or low-positive results were frequently negative on confirmatory tests. CDC recommends RIBA confirmation for these "intermediate or borderline" samples.[6] In addition to this HCV RNA PCR is also useful test to confirm HCV infection. This study shows that the CLIA provides several advantages over ELISA, particularly useful in low risk populations, even though all low S/Co ratio samples need use of confirmatory testing. For CLIAs, specimens with a single reactive result are considered screening-test-positive and do not require retesting and can be reported as positive without further supplemental testing.[6] Studies done previously on the performance evaluation of anti-HCV CLIAs have compared the results of a CLIA with those of RIBA, HCV RNA and immunochromatography. There are no similar data available to compare with our results where CLIA and ELISA alone were compared.

False positives in HCV may be seen due to cross-reactive circulating antigens and antibodies as in cases of pregnancy, autoimmune diseases, nephrotic syndrome, Human immunodeficiency virus, Hepatitis B Virus, Herpes simplex virus infection etc. In this study among 29 borderline results one had HBV co-infection. These false positive results of Anti-HCV screening tests may prompt use of expensive confirmatory tests like HCV RNA PCR or RIBA. First time detected anti-HCV reactive in asymptomatic individuals will require further testing by confirmatory tests. So reactive tests especially with low S/CO ratios should be confirmed with confirmatory tests to avoid false positive results.[5] According to the European Union standards, anti-HCV assays were required to have 100% and >99.5% sensitivity and specificity respectively for market approval.[7] In a study of performance evaluation of anti-HCV CLIAs the sensitivity and specificity were 100% and 98% respectively which are not consistent with our study.[4] In CLIA Anti-HCV antibody screening test the clinical specificity varies from 96.5% to 98.8% which is in agreement with the present study result.[4] Comparative studies on ELISA and CLIA have confirmed that CLIA has relatively higher sensitivity, predictive value and fewer false-positive results in viral hepatitis diagnosis procedures.[6]

For diagnosing HCV infection, the increasingly sophisticated methods have a direct impact on patient management and for an efficient diagnosis of HCV infection the use of more sensitive and specific assays are essential. According to various sero-prevalence studies S/CO ratios could be used to accurately predict a positive status in conjunction with a confirmatory test.[8] Although earlier many sero-prevalence studies reported were performed using the commercially available ELISA/EIA test, in the present study we utilized VITROS anti-HCV assay (CLIA), whose performance was evaluated in some previous published studies.

CLIA has advantages of being more reliable, precise, technically simple, short turn-around time, high-speed throughout and fully automated which is a great advantage particularly in high volume hospital laboratories. Moreover, CLIAs have improved specificity and greater positive predictive value than conventional EIAs.[4] As ELISA is typically performed in microtiter plates, and it is recognized that there may be some "splashing" of sample from one well to another, which can interfere test results also. In contrast, in CLIA each test is performed in a separate reaction cell, making contamination of samples much less likely. Though in our study there was inter-sample variation or discrepant value, but considering the benefits of ease of performance and rapid turn-over time while maintaining a high concordance with ELISA make CLIA an attractive choice for routine screening for Anti-HCV antibody.

The limitations of this study was that we did not determine the specific causes of borderline results. Further studies with greater sample size and using RIBA and/or HCV RNA PCR as confirmatory tests are required to find a solution to interpret borderline cases. In developing countries as resources are scarce, use of supplemental tests like RIBA or HCV RNA PCR is not cost effective. In this context, CLIA results appear sufficiently reliable for detecting exposure to HCV without supplemental assays. It has been suggested that supplemental tests may be used only in populations with expected low prevalence, such as blood banks.[9] To define the optimal antibodies, it is necessary to evaluate other currently available assays that can be used to identify false-positive results with the objective of eliminating unnecessary supplemental testing.

In conclusion detection of anti-HCV by CLIA is comparable with ELISA. CLIA can help in detecting infection earlier compared to ELISA and is suitable in large sample volume laboratories.

References

1. Park Y, Lee J, Kim B., et al. New Automated Hepatitis C Virus Core Antigen Assay as an Alternative to Real-Time PCR for HCV RNA Quantification. J. Clin Microbiol. June 2010;48: 2253-2245.
2. Zameer M, Shazad F, Saeed M., et al. Comparison between ELISA and ICT techniques for the detection of Anti HCV antibody among blood donors. Biomedica 2016;32:4.
3. Hepatitis C [Internet]. World Health Organization. 2017 [cited 22 August 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/.
4. Kim S, Kim J, Yoon S., et al. Clinical Performance Evaluation of Four Automated Chemiluminescence Immunoassay for Hepatitis C Virus Detection. J. Clin. Microbiol. December 2008;46:3919-3923.
5. Kalem F, Yüksekkaya S, Türk D H., et al. Comparative evaluation of automated chemiluminescence tests and RIBA assay used inHCV diagnosis. Biomedical Research. April 2016;27(4):1261-1264.
6. Centers for Disease Control and Prevention. Guidelines for laboratory testing and result reporting of antibody to Hepatitis C virus (Anti-HCV). MMWR Recom Rep. 2003;52(RR-3):1-13.
7. Dufour DR, Talastas M, Fernandez MDA, et al. Low positive anti-hepatitis C virus enzyme immunoassay results: an important predictor of low likelihood of hepatitis C virus infection. Clin. Chem. 2003;49:479-486.
8. Mohammad H, Reza R, Seyed M A. Classical and Modern Approaches Used for Viral Hepatitis Diagnosis. Hepat Mon. 2014 April;14(4):17632.
9. Abdel-Hamid M, El-Daly M, El-Kafrawy S, et al. Comparison of Second- and Third-Generation Enzyme Immunoassays for Detecting Antibodies to Hepatitis C Virus. J. Clin. Microbial. May 2002;40(5):1656–1659.

How to cite this article: Majumder P, Shetty AK. Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody. Indian J Microbiol Res 2017;4(4):353-357.