Journal of Applied Pharmaceutical Research
Volume 11 Issue 3, Year of Publication 2023, Page 48 – 53
DOI: 10.18231/j.joapr.2023.11.3.48.53

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

COMPARATIVE STUDY ON ELISA, CLIA AND RAPID DIAGNOSTIC TEST IN DETECTING HCV INFECTION IN BLOOD DONOR AT A TERTIARY CARE CENTER

M. Preethi*, M. Saisudha, P. Subhashini, Resmi P. R

Article Information
Received: 26th September 2022
Revised: 29th April 2023
Accepted: 17th July 2023
Published: 31st August 2023

Keywords
ELISA, CLIA, Rapid Diagnostic Test, screening assays, HCV infection

ABSTRACT

Background: The prevalence of blood screening assays for hepatitis C infection among blood donors remains comparatively low in line with WHO guidelines, especially when compared to developing nations. Various methodologies, such as ELISA, immunochromatography assays, RIBA, HCV RNA PCR, and CLIA, are employed to detect anti-HCV IgG antibodies in all patients with HCV infection. However, there is a significant scarcity of comparative data available regarding the evaluation of HCV infection screening among CLIA, ELISA, and RDT methods in their ability to detect anti-HCV antibodies effectively. This gap in knowledge highlights the need for further research and analysis in this critical area of healthcare. In this study we evaluate the technical performance between ELISA, CLIA and RDT in detection of HCV infection. **Materials and method:** A cross-sectional study was carried out, involving 70 blood donor samples. Subsequently, the samples were subjected to screening for Anti-HCV antibodies using three different methods: RDT, CLIA, and ELISA. The results obtained from these screenings were duly recorded. **Results:** Among the 70 patients included in the study, 63 (90%) were male, and 7 (10%) were female. The following performance metrics were calculated for each method where CLIA shows 100% sensitivity, Specificity 98%, PPV 100%, NPV 98.9%, Accuracy 100%, Kappa coefficient 0.932, p-value <0.001, in case of ELISA: Sensitivity 97.6%, Specificity 99.2%, PPV 100%, NPV 97.1%, Accuracy 99%, Kappa coefficient 0.97, p-value <0.001. followed by RDT: Sensitivity 89%, Specificity 87.9%, PPV 100%, NPV 90.2%, Accuracy 96%, Kappa coefficient 0.59, p-value <0.001. These results provide valuable insights into the performance of each method in screening for HCV antibodies, with CLIA and ELISA demonstrating higher sensitivity, specificity, and overall accuracy compared to RDT. **Conclusion:** In conclusion, the study suggests that the CLIA screening method for detecting HCV infections is considered superior to both ELISA and RDT in a Tertiary care center.

*Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India

***For Correspondence:** drpreethi1290@gmail.com

INTRODUCTION

According to World Health Organization (WHO) Blood screening assay for hepatitis C infection in blood donors still remains low when compared to developing countries. Prevalence of infection and transfusion of HCV infection through blood remains high, with death mortality rate of HCV infection 78% in developing countries [1]. WHO framed many rules to ensure safe access and to provide sufficient blood supplies to patient who in need of blood through proper transfusion practices in India [2]. In developing countries when the blood screening assay test found positive for Hepatitis B, Hepatitis C, HIV, Syphilis and Malaria, the donor blood sample is discarded [3]. The blood units donated by the healthy and asymptomatic patient with screening test positive have increased risk of transmitting infection through blood and blood products [4]. Detection of anti-HCV IgG antibodies in all HCV infection patient can be identified by many methods like Enzyme linked immunosorbent assay (ELISA), immunochromatography assays and recombinant immunoblotting assay (RIBA) and HCV RNA polymerase chain reaction (PCR) and Chemiluminescence immunoassay [5]. CLIA is advanced diagnosed screening method used for screening large volume of HCV infection in blood donors, for anti-HCV antibodies detection [6]. In this method they showed good reliability, easy random assay screening, precision and technical simplicity of full automation [7]. Many previous studies show CLIA method has high specificity and positive predictive value when compared with ELISA in detecting HCV infection, due to high sensitivity and specificity value CLIA is used more frequently than ELISA in India [8]. Comparative data regarding evaluation of HCV infection screening among CLIA, ELISA and RDT in detecting anti-HCV antibodies is minimal. In this study we evaluate the technical performance between ELISA, CLIA and RDT in detection of HCV infection.

MATERIALS AND METHOD

A cross sectional study was conducted in Sree Balaji Medical College and Hospital at Department of Transfusion medicine for a period of 3 months from June 2022 to August 2022. A total of 70 blood donor samples were collected in plain vacutainers. Demographic details and consent were documented from all donors following the collection. The study design was approved by institutional ethical committee at Bharath University of Medical Sciences (Ref.No.002/SBMCH/IHEC/2022/1612). The collected samples underwent screening for HCV infection, and Anti-HCV antibody screening was conducted using three different methods: RDT, CLIA, and ELISA. The results obtained from these screenings were duly recorded for analysis and evaluation.

CLIA for HCV was conducted using the ELECTRATM HCV Ab CLIA method, which is provided by Qualpro Diagnostics, a division of Tulip Diagnostics (P) Ltd. ELISA for HCV was performed using the 3rd generation HCV Microlisa kit from J.Mitra Diagnostics. The RDT for HCV utilized the Standard Q Ultra-Dot HCV method developed by SD BIOSENSOR HEALTH CARE PVT. LTD.

The correlation between the results obtained from these different testing methods (RDT, CLIA, and ELISA) is a critical aspect of the study and may provide valuable insights into the effectiveness of each method in detecting HCV antibodies.

Inclusion Criteria:

a. Blood donors selected between 18-40 years for the study.
b. Blood samples taken for the study after informed consent from the donors

Exclusion Criteria:

a. Donors with a medical history of conditions such as Diabetes Mellitus (DM), Hypertension (HTN), and coronary artery disease (CAD) were excluded from the study
b. Donors less than 18 years and more than 40 years were excluded from the study.
c. Donors with history of trauma, burns, pregnancy and post-surgery less than 6 months were excluded from the study

STATISTICAL ANALYSIS

Validity for RDT and CLIA and ELISA was noted with sensitivity and specificity. Kappa co-efficient was calculated between the values in 3 methods. Cohen's kappa coefficient (k) was calculated using a free tool available from Graph Pad software website. A p-value <0.001 was considered as statistically significant and comparison was done among the three methods.

RESULTS

Among 70 patients 63 (90%) were male and 7 (10%) were female (table no 1). RDT, ECLIA and ELISA were done for all 70 samples irrespective of the sex of patient. HCV antibodies

screening was done with all the three methods. Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient were calculated (table no 2). CLIA showed 100%, 95%, 100%, 98.9%, 100%, 0.964 and <0.001 Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient. ELISA had 97.6%, 99.2%, 100%, 97.1%, 99%, 0.97 and <0.001 Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient. RDT had 89%, 87.9%, 100%, 90.2%, 96%, 0.59 and <0.001 respectively

Table 1: Age categorization of blood donors

Age Groups	Number of Blood Donors	%
18 – 20 years	13	15.90
21 – 30 years	12	31.40
31 – 40 years	35	50.40
Total	70	100
Mean ± SD		

Our findings demonstrated that anti-HCV, CLIA by ECiVitros showed better sensitivity than corresponding RDT (Sensitivity100% versus Sensitivity 88% respectively. Kappa co-efficient was also computed to observe to what extent the reading of two different methods (RDT and ECLIA) for anti-HCV agreed beyond which we would expect by chance alone. Strength of agreement for anti-HCV were substantial. All results were documented according to the kit manufacturers' directions. In our study CLIA showed 100%, 95%, 100%, 98.9%, 100%, 0.964 and <0.001 Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient. ELISA had 97.6%, 99.2%, 100%, 97.1%, 99%, 0.97 and <0.001 Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient. RDT had 89%, 87.9%, 100%, 90.2%, 96%, 0.59 and <0.001 respectively.

DISSCUSSION

HCV infection is transmitted through Blood and body fluids to others [9]. Chronic hepatitis and hepatocellular carcinoma is caused by chronic HCV infection [10]. HCV infection has good response early treatment. HCV infection can be detected by Clinical signs and laboratory investigations [10]. The HCV virus has core with an envelope, E1 and E2 regions with the nonstructural regions [11]. For the identification of anti-HCV antibodies many techniques and variable tests are available with increased sensitivity and specificity [12]. Third-generation tests like CLIA, ELISA, RDT 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 with a good sensitivity and specificity for the tests [13]. But false positivity rate has also increased with the entire third generation test [14].

Table 2: Enumerating the sensitivity, specificity and cohen kappa coefficient and p value for RDT, ELISA and CLIA

Tests	RDT (Anti-HCV)	CLIA (Anti-HCV)	ELISA (Anti-HCV)
True Positive	2	0	1
True Negative	64	69	68
False Positive	2	1	1
False Negative	2	0	0
Sensitivity %	89%	100%	97.6%
Specificity %	87.9%	95.6%	99.2%
PPV %	100%	100%	100%
NPV %	90.2%	98.9%	97.1%
Accuracy %	96%	100%	99%
Cohens kappa coefficient	0.59	0.964	0.97
p-value	<0.001	<0.001	<0.001

RDT method is consider one of the screening methods for HCV infection in detecting anti HCV antibodies due to faster, easy availability of antibodies and easy resource availability in developing countries like India [15]. Molecular virological techniques play a key role in diagnosis and monitoring of treatment for HCV. Because it is difficult to cultivate the virus in cell culture, molecular techniques were instrumental in first identifying HCV, making it one of the first pathogens to be identified by purely molecular methods [16]. CLIA (NAT) is considered the 'gold standard' for detecting active HCV replication [17]. HCV NAT is extremely useful in establishing the diagnosis of acute HCV infection, since RNA is detectable as early as 1 week after exposure via needle-stick or blood transfusion and at least 4-6 weeks prior to seroconversion as demonstrated in a number of transmission settings [18]. The diagnosis of HCV infection is established with antibody screening followed by NAT for HCV RNA for confirmation as well as for follow-up of patients on treatment. Viral load assessment at baseline is also critical for determining response kinetics during therapy [19]. Comparison between ELISA and CLIA for the detection of Hepatitis C virus Ab. Sensitivity of ELISA was 94.07%, while the sensitivity of CLIA was 96.66% [20]. Their results also suggest that CLIA early detected the

infection of HCV as compared to ELISA. CLIA's dynamic range is improved beyond ELISA with better linearity and discrimination of signal. While ELISA cannot read higher than 3.0 O.D [21]. Thus, requiring sample dilution, CLIA can read a dynamic range of 106 or 107 significantly increasing assay linearity and reducing overflow [22]. At the manufacturer's cutoff value of 10 AU/mL, sensitivity was 73.3% and 76.7% and specificity was 92.2% and 100% for IgM and IgG antibodies for CLIA [23].

In our study we compare the result of RDT with, CLIA and ELISA result. In the study 70 patients 63(90%) were male and 7(10%) were female. The maximum number of patients belonged to 31-40 years age group [Table/Fig-1]. RDT had 89%, 87.9%, 100%, 90.2%, 96%, 0.59 and <0.001 Sensitivity, specificity, PPV, NPV, accuracy and kappa coefficient.

In our study sensitivity 100% and specificity of 98.9% for anti-HCV screening by CLIA and ELISA performed by when compared with S.B Lin and Zheng study they had sensitivity 100% and specificity of 98.9% and coefficient of 0.964 and p value <0.001 for anti-HCV screening and CLIA performance in the result having more sensitivity like in our study. Both results were compatible with the results [24]. There was no negative result in HCV screening by CLIA. RDT showed 4 negative results in HCV screening, from this we infer that RDT has less specificity when compared to CLIA for screening anti HCV. Assay screening by RDT alone in blood donors may result in false positive result it has to be cross checked with CLIA and ELISA method.

Rao et al. [25] in his study showed high specificity, sensitivity and p value in detecting against hepatitis C antibody screening with 100%, 95.6%, 100% kappa coefficient and p value <0.001 in CLIA and ELISA which was compatible with our study in CLIA and ELISA screening method. In absolute titer values reporting there was difference observed between CLIA and ELISA in generating lower antibody titers reports and CLIA showed the best result, faster result, low turnaround time than ELISA, due to variation in the standard calibrators used in each assay. Hence both methods are reliable in detecting anti HCV titer. Among all the three CLIA as more sensitivity and specificity in screening the anti HCV antibody. It was also noticed that if cut off/OD was high for the tests by CLIA and ELISA then the results were reactive by RDT method too. In Malhotra et al, in ELISA shows high OD level in ELISA but in both RDT showed negative or nonreactive result in the study which was compatible with our study [26]. Ideal anti HCV screening method CLIA show high degree of PPV and low degree of false negative results since CLIA is consider best method in screening. It is advisable to confirm the discrepant results by other specific tests.

Now a day CLIA has advantages over other test method in screening anti HCV with high 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 and moreover, CLIAs have improved specificity and greater positive predictive value than conventional EIAs. 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. 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.

Limitations of the study

Since study duration is 3 months and the sample number are limited.

Ethical issues

The research followed the tents of the Declaration of Helsinki. The Ethics Committee of Bharath University of Medical Sciences approved this study. The institutional ethical committee at Bharath University of Medical Sciences approved all study protocols (Ref. No. 002/SBMCH/IHEC/2022/1612). Accordingly written informed consent taken from all donor participants before any intervention.

CONCLUSION

CLIA screening method for detecting HCV infections considered superior in our study when compared with ELISA and RDT in a Tertiary care center. CLIA is considered more rapid and easily usable method than ELISA. According to our study feasibility of CLIA are safe and best methods for blood screening, among blood donors in developing countries.

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
M. Preethi and Subhashini collected the data and prepared first draft. M. Sudhadha contributed in preparing and formatting the draft. Resmi P. R. contributed in planning the study, literature survey and analysing the data. All the authors contributed in proof reading the preparing the final draft.

REFERENCES
[1] Park Y, Lee JH, Kim BS, Kim DY, Han KH, Kim HS. New automated hepatitis C virus (HCV) core antigen assay as an alternative to real-time PCR for HCV RNA quantification. *J Clin Microbiol* **48**, 2253-6 (2010)

[2] Zameer M, Shazad F, Saeed M, Aziz S, Nazish, Hussain S. Comparison between ELISA and ICT techniques for the detection of Anti HCV antibody among blood donors. *Biomedica* **32(4)**, 281 – 284 (2016)

[3] Hepatitis C [Internet]. www.who.int. Available from: http://www.who.int/mediacentre/factsheets/fs164/en

[4] 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* **46**, 3919-23 (2008)

[5] Majumder P, Shetty AK. Comparison between ELISA and chemiluminescence immunoassay for the detection of Hepatitis C virus antibody. *Indian Journal of Microbiology Research* **4**, 353 – 357 (2017)

[6] Seitz WR. Immunoassay labels based on chemiluminescence and bioluminescence. *Clin Biochem* **17**, 120-5 (1984)

[7] Yu H, Raymonda JW, McMahon TM, Campagnari AA. Detection of biological threat agents by immunomagnetic microsphere-based solid phase fluorogenic- and electro-chemiluminescence. *Biosens Bioelectron* **14**, 829-40 (2000)

[8] Constantine NT, Kabat W, Zhao RY. Update on the laboratory diagnosis and monitoring of HIV infection. *Cell Res* **15**, 870-6 (2005)

[9] Sasano M, Kimura S, Maeda I, Hidaka Y. Analytical performance evaluation of the Elecsys® Cyclosporine and Elecsys® Tacrolimus assays on the cobas e411 analyzer. *Pract Lab Med* **8**, 10-7 (2017)

[10] Sommese L, Sabia C, Paolillo R, Parente D, Capuano M, Iannone C, Cavalca F, Schiano C, Vasco M, De Pascale MR, Casamassimi A, Napoli C. Screening tests for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus in blood donors: evaluation of two chemiluminescent immunoassay systems. *Scand J Infect Dis* **46**, 660-4 (2014)

[11] Jinming O, Xiaoxu H, Yangtao J, Yanan W, Hong S. A comparative study on the clinical performance of three fourth generation HIV diagnostic reagents. *Chinese Journal of Laboratory Medicine* **36(10)**, 903 – 907 (2013)

[12] Li L, Cai B, Tao C, Wang L. Performance Evaluation of CLIA for Treponema Pallidum Specific Antibodies Detection in Comparison with ELISA. *J Clin Lab Anal* **30**, 216-22 (2016)

[13] Madiyal M, Sagar S, Vishwanath S, Banerjee B, Eshwara VK, Chawla K. Comparing Assay Performance of ELISA and Chemiluminescence Immunoassay in Detecting Antibodies to Hepatitis B Surface Antigen. *J Clin Diagn Res* **10**, DC22-DC25 (2016)

[14] Olmeda MP, Balfagón P, Camacho J, Dafouz D, Fuente JDL, Murillo MA, Muñoz JL, García AF, Sanz JC, Ory FD. Comparative evaluation of assays for IgM detection of rubella and measles infections. *Enfermedades Infecciosas y Microbiología Clínica* **40(1)**, 22 – 27 (2022)

[15] Wang T, Li D, Yan K, Yuan Y, Yang T, Du X, Yan X, Tao C, Wang L. Performance evaluation of a new fourth-generation HIV Ag/Ab combination electrochemiluminescence immunoassay - evaluation of a new HIV assay. *Int J STD AIDS* **25**, 267-72 (2014)

[16] Stramer SL, Townsend RL, Foster GA, Johnson R, Weixlmann B, Dodd RY. Discordant human T-lymphotropic virus screening with Western blot confirmation: evaluation of the dual-test algorithm for US blood donations. *Transfusion* **58**, 638-40 (2018)

[17] Kiely P, Stewart Y, Castro L. Analysis of voluntary blood donors with biologic false reactivity on chemiluminescent immunoassays and implications for donor management. *Transfusion* **43**, 584-90 (2003)

[18] Malm K, Kragsbjerg E, Andersson S. Performance of Liaison XL automated immunoassay platform for blood-borne infection screening on hepatitis B, hepatitis C, HIV 1/2, HTLV 1/2 and Treponema pallidum serological markers. *Transfus Med* **25**, 101-5 (2015)

[19] Kim S, Lee JH, Choi JY, Kim JM, Kim HS. False-positive rate of a "fourth-generation" HIV antigen/antibody

combination assay in an area of low HIV prevalence. *Clin Vaccine Immunol* **17**, 1642-4 (2010)

[20] Kiely P, Wood E. Can we improve the management of blood donors with nonspecific reactivity in viral screening and confirmatory assays. *Transfus Med Rev* **19**, 58-65 (2005)

[21] Yang X, Liu YP, Zhou X, Gao C, Wang A. Investigation report of continuous improvement of clinical blood transfusion records. *Chinese Journal of Blood Transfusion* **27(9)**, 947–949 (2014)

[22] Lin SB, Zheng ZX, Zhang R. [Application and Evaluation of Chemiluminescence Immunoassay in Blood Screening]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* **27**, 569-72 (2019)

[23] Lee K, Park HD, Kang ES. Reduction of the HIV seroconversion window period and false positive rate by using ADVIA Centaur HIV antigen/antibody combo assay. *Ann Lab Med* **33**, 420-5 (2013)

[24] Rao C, Wang T, Chen Q, An J, Feng S, Tao C, Wang L. Performance evaluation of a novel automated HIV Ag/Ab chemiluminescence immunoassay. *Clin Chem Lab Med* **54**, e255-8 (2016)

[25] Malhotra, S., Marwaha, N., Saluja, K. *et al.* Improving blood safety using fourth generation HIV ELISA as the screening tool in blood banks – an Indian experience. *BMC Infect Dis* **12** (Suppl 1), P89 (2012).