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Original Research Article

Title: A Comparative Study on Chemiluminescent Immunoassay with Immunochromatography Test in the Screening Process of Hepatitis B and Hepatitis C

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A B S T R A C T

Background: Detection of HBsAg (Hepatitis B Surface Antigen) and antibody to Hepatitis C Virus (anti-HCV) are widely used in the diagnosis of viral hepatitis. Immunoassay or molecular methods can be employed for the purpose. Two such immunoassays are Immunochromatography tests (ICT) and Chemiluminescence immunoassay (CLIA) of which a comparison is donehere.

Methodology Serum from patients was screened for HBsAg and anti-HCV by CLIA method (VITROS ECiQ, Ortho Clinical Diagnostics, USA) and when positive were further tested by ICT (HEPACARD and HCV TRIDOT, J. Mitra & Co. Pvt. Ltd., New Delhi, India). In case of discordant results, enzyme linked florescent assay (ELFA) was used (VIDAS, bioMérieux, France) as the confirmatory test.

Results: It was seen that CLIA consistently gave a more accurate result compared to ICT. 73.77% of HBsAg and 67.6% of anti-HCV positive samples in the study gave positive results with both CLIA and ICT. 16.39% of HBsAg and 11.7% of anti-HCV positive samples gave negative results with ICT but positive results with CLIA and ELFA. A LU (Light Unit) range was also established for CLIA where a definite positive result can be obtained with no other confirmatory tests required. This range for HBV was 305-12800 LU and for hepatitis C was 31-9540 LU.

Conclusion: The automated CLIA test proved to be a better screening method than the traditional ICT. Reports cannot be reliably issued based on ICT results alone. Confirmatory tests need not be carried out for CLIA when LU values fall in the defined definitive positive range.

Introduction:

Hepatitis B and C are viral infectious diseases of the liver, marked by inflammation of the liver. Though in its early stages it is asymptomatic but in its later stages it can lead to various liver diseases such as liver fibrosis and ultimately cirrhosis. Hepatitis B and C are proven to be risk factors for liver cancer as well [1, 2]. About 130-170 million people worldwide are chronically infected by hepatitis C [3, 4] and about 240 million are chronic HBV carriers [5]. HBV is a member of the hepadnavirus family and consists of a relaxed circular and partially double stranded DNA molecule. The negative strand has a length of about 3.2 kb and the positive stand is about 50-100% of it. The covalently closed circular DNA is responsible for the viral infection and persistence [6, 7]. HCV is a positive stranded RNA virus from the Flaviviridae family. Its genome encodes a large polyprotein that produces various structural proteins like core, E1 and E2 proteins as well as various non-structural proteins [3,6]. Viral transmission results from exposure to infected blood or any other body fluids [8] sexual contact [9], sharing of personal items, healthcare exposure like organ transplant or intravenous drug use and vertical transmission from mother to child during child birth [10]. Diagnostic tests for hepatitis virus can be broadly classified into two: 1) Immunoassay that detect surface antigens or antibody to hepatitis virus 2) Molecular assay that detect, quantify and characterize viral genome. Examples of immunoassays include tests like enzyme immunoassay, recombinant immunoblot assay (RIBA) [11], Enzyme linked immunosorbent assay (ELISA), Immunochromatography assay (ICA) and Chemiluminescent immunoassay (CLIA).

Molecular assays include RNA/DNA Polymerase Chain Reaction and Nucleic Acid Amplification Test [12, 13]. CLIA is an automated assay with high sensitivity and good specificity and is capable of detecting multiple components of complex systems [14].

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Rapid Diagnostic Tests (RDTs) like ICTs have considerable advantages (like lower cost and skill required) in resource-limited settings over other immunoassays like ELISA or Nucleic Acid Amplification Testing (NAT) even though ICTs are often found to be lacking in sensitivity [15]. With high thorough-put automated systems like CLIA this may be mitigated somewhat with a higher signal/cut-off ratio(S/Co) [16][. The results of the CLIA method have been found to be concordant with Nucleic Acid Amplification Testing (NAT) results with some studies [17] showing greater repeatability and higher sensitivity than ELISA. CLIA has also been found to have good analytic agreement with ELISA for measuring anti-HBs antibody titers in addition to the advantages of being an automated test with low turn-around time [18]. This research work introduces a comparative study between CLIA and ICT and focuses on the various result ranges of CLIA while screening for hepatitis B and hepatitisC.

Material and methods:

This was across-sectional observational study done at Peerless hospital, Kolkata. Blood samples from all age-group patients undergoing any invasive procedure or surgery were taken and tested after taking proper consent. Already diagnosed cases of Hepatitis B and C were excluded. The data was collected between March-December 2013.

Blood samples were collected in clot vials and were centrifuged at 4000 rpm for 15 minutes to obtain the serum. Sera were checked for clots, partial clots, fibrin clots or haemolysed samples and then run on VITROS ECiQ using QNX software version 3.8.3. The VITROS ECiQ uses an immunometric immunoassay technique for HBsAg measurement. The wells of the reaction plate were coated with mouse monoclonal anti-HBs antibodies while the conjugate contains the same antibodies labelled with horseradish peroxidase (HRP) enzyme. The HBsAg in the sample reacts simultaneously with both and binds to the well. The unbound conjugate was washed away. Then a reagent containing luminol derivative was added along with substituted acetanilide that acts as the electron transfer agent. The luminol derivative produces light when oxidized and this reaction is catalysed by the HRP present in the bound conjugate. The substituted acetanilide enhanced and prolonged the light produced. This was then read by the system and expressed in terms of light units (LU). 1 LU is considered the cut off value above which the result is taken as positive. The anti-HCV assay works in a similar fashion except the wells are coated with HCV recombinant antigens and theantibody.

When positive CLIA results were obtained they were further tested by ICT. The ICT used for HBsAg testing was HEPACARD (J. Mitra& Co. Pvt. Ltd., New Delhi, India, Catalogue No. – HB010020) while anti-HCV detection done by HCV TRI-DOT (J. Mitra& Co. Pvt. Ltd., New Delhi, India; Catalogue No.- HC020050). HEPACARD is a lateral flow assay and works on the principle of antigen capture or "sandwich" principle. The reagent consists of Anti-HBsAg antibodies that are conjugated with colloidal gold. A thin line of the antibodies were also immobilized on the nitrocellulose strip. If the sample contained HBsAg it bound with the antibody-gold complex and travelled laterally through the strip until it reached the immobilized antibody line where it was trapped and formed a pink line. A control line was also formed which indicated the procedural validity of the test. HCV TRI-DOT was an immune-filtration assay where the HCV antigens were immobilized on a porous membrane. If anti-HCV antibodies were present in the sample they were trapped in the membrane after the washing step. The protein-A conjugate was then added which bound to the antibodies and gave a pinkish purple colour. A control dot was also present.

In case of discrepancy, the samples were tested by ELFA on a VIDAS instrument (bioMérieux, Marcy-L'Etoile, France), an automated immunoassay system. It involved combining a two-step enzyme immunoassay sandwich method with final fluorescence detection. The method consisted of a preliminary washing step following which the antigen (HBsAg) or antibody (anti-HCV) present in the sample bound with the antibody or antigen respectively coating the interior of the solid-phase receptacle (SPR). Additionally, the SPR acted as a pipettin device. Assay reagents were ready to use and were predispensed in the sealed reagent strips. All of the assay steps were performed automatically by the instrument. At each stage of the reaction, the reaction medium was cycled in and out of the SPR several times. Unbound sample components were washed away. In the final detection step, the substrate (4-methylumbelliferyl phosphate) was cycled in and out of the solid-phase receptacle. The conjugate enzyme catalyzes the hydrolysis of the substrate into a fluorescent product (4 -methylumbelliferone), whose fluorescence was measured at 450 nm. The intensity of the fluorescence was proportional to the concentration present in the sample. The results were analyzed and calculated automatically. A total of 95 patients showing positive result from CLIA for either HBsAg or anti-HCV were found. Out of which 61 were HBsAg positive and 34 were anti-HCV positive. All observations were tabulated andanalyzed.

Results:

A total of 95 positive sera were obtained out of which 81.9% were men and 17.89% were female. All the titer values were noted down and an interpretation of the various light unit (LU) ranges was made. Amongst the positive results obtained 73.77% of HBsAg positive cases and 67.6% of anti-HCV positive cases were also tested positive by ICT; their LU values ranging from 18.45 -12800 and 17.6 -9540 respectively as shown in Table 1. The Positive Predictive Value (PPV) of the CLIA test is given in Table2.

Test	HBsAg	Anti-HCV		
	Positive test (%) and no.of patients	Range (LU)	Positive test (%) and no.of patients	Range (LU)
CLIA	90.1 % (55)	8.41-12800	82.35% (28)	10.8-30.6
ICT	73.77 % (45)	18.45-12800	72.7 % (24)	17.6-9540
False Positive For CLIA	8.1 % (5)	1.29-5.55	20.5 % (7)	1.79-5.09

Table 1 showing positive results for both CLIA and ICT.

Test	Positive Predictive Value (HBsAg)	Positive Predictive Value (Anti-HCV)
CLIA	0.92	0.8

Table 2 showing Positive Predictive Value (PPV) for CLIA

A few discordant observations were made in ICT. About 16.39% of HBsAg positive results(LU range 6.22-304) and 11.7% of anti-HCV positive results (LU range 10.8 -32.9) showed a negative ICT result despite a positive CLIA result, the confirmatory test being ELFA.

In case of discordant values, ELFA was used to differentiate between true positive values and false positive values on CLIA test (Table 3).

8.1% HBsAg positive samples and 20.5% anti-HCV positive samples in CLIA were found to be false positive, their ranges being 1.29 -5.55 LU in case of HBsAg and 1.79 -5.09 LU in case of Anti-HCV.

The above observations were used to derive a LU range for which CLIA tests gives a definite positive result within which no other confirmative tests are required to verify a positive result. This range for HBsAg is 304-12800 LU and for anti-HCV is 32.9- 9540 LU (Table4).

Test results	HBsAg (%)	Anti-HCV (%)
True Positive values	16.39%	11.7%
False Positive values	8.1%	20.5%

Table 3 showing ELFA results for discordant results in CLIA and ICT.

Virus type	Range (LU)	Range interpretation
HBV	1.29- 304	Indeterminate range
	305- 12800	Definite range
HCV	1.79- 30.6	Indeterminate range
	31- 9540	Definite range

Table 4 showing LU Ranges for CLIA.

Performance of HbsAg ICT compared to ELFA:	
Sensitivity	97.1% (95.3 - 98.24)
Specificity	99.82% (98.88 – 100)
PPV	99.81 % (98.84 – 100)
NPV	97.18% (95.44 – 98.29)
Accuracy	98.46% (97.53 – 99.06)
Kappa	0.97 (0.95 – 0.98)

Table 5 showing performance of HBsAg ICT compared to ELFA

Discussion:

According to studies conducted in 2000 about 5- 10% of adults and upto 90% infants get chronically infected by hepatitis virus annually. Out of which about 75% falls in the Asian demographic, this calls for a serious concern. In Indonesia 4.6% of the population was found to be positive for hepatitis virus, 44% and 45% Indonesian patients with cirrhosis and HCC were HBsAg positive [13].

Studies related to comparison of immunochromatography test a CLIA is very limited but works on CLIA has been done and a close n correlation has been found with our research [19, 20, 21]. VITROS ECiQ showed 90.1% sensitivity to HBV and 82.35% sensitivity to HCV antibodies as compared with the manual immunochromatography tests. Out of all the positive tests performed about 13.6% of the tests were found to be weakly positive (LU value below 25, as determined by VITROS) and the other 86.4% was strongly positive with a LU value greater than 25, as determined by VITROS. Possible explanations for low positive values include false positive serological tests, early testing i.e. before complete seroconversion, testing during the window period, in cases of decreased immune response due to immunosuppression and diminishing antibodies due to medication [22, 23, 24]. Under these conditions other tests such as nucleic acid test, DNA-PCR etc are required to be performed to detect the presence of the virus. Confirmation of active infection is necessary when assessing individuals who undergo treatment [25, 26,27].

In comparison to VITROS the sensitivity and accuracy of immunochromatography test was recorded to be low and it even showed error results for high LU values corresponding to positive tests such as 47.9, 127, 111 etc, thus posing a question on its sensitivity, specificity and reliability. The factors attributed to such errors can be because of low diffusion rate of proteins, site blocking problem and presence of low antibody in the analyte. These are overcome by the automated CLIA which has a high sensitivity, good specificity, high throughput and detects sample of low abundance. CLIA when compared to other techniques like indirect ELISA [28, 29, 30] and enzyme immunoassay [31] with other viral parameters yielded results similar to our study. In such cases of faulty immunochromatography tests other confirmatory tests like NAT tests, RIBA tests, DNA-PCR tests should be carried out. If NAT result is found to be negative to positive screening tests then the status of infection cannot be determined. But if the screening tests are judged to be false positive no other evaluation is necessary. There are limitations to these alternative confirmatory tests as well; sometimes the RNA/DNA is not recognized during the acute phase of the infection[32]

Every test gives a certain amount of false positive results. The false positive range for CLIA is between 1-6 LU. Though the range is very narrow it cannot be ignored, with highly précised automations the range can be further reduced but not erased completely. The main cause for it being, detection of similar shaped protein as that of the antibody. All values in this range do not correspond to a false negative value, certain value were obtained which gave true positive results as well vis 3.7 in case of HBV detection and 3.10 in case of HCV detection.

In very rare cases, samples with abnormally high abundance are not detected by the machine and thus it gives a negative result with a LU value below 1 instead of giving a positive result. This kind of phenomenon is known as the Hooks effect or prozone effect [33] and is very hard to identify. The cause for it is yet to be known. In such cases the samples must be first diluted and then the tests conducted, various other parameters are also to be lookedinto.

In recent years, CLIA has gained increased attention and is now being used in various fields including life sciences, clinical diagnosis, environmental monitoring, food safety and pharmaceuticalanalysis.

Conclusion:

With its higher accuracy and faster turn-around time VITROS ECiQ was found to be a very good immunoassay for screening of HBsAg and anti-HCV antibody. An LU range was established for VITROS ECiQ that gives a definite positive result within which no other confirmatory tests are needed.

Conflict of interest:

The authors declare there are no direct and indirect conflicts of interest associated.

References:

1. Zhang JY, Dai M, Wang X, Lu WQ, Li DS, Zhang MX, Wang KJ, Dai LP, Han SG, Zhou YF, Zhuang H. A case-control study of hepatitis B and C virus infection as risk factors for hepatocellular carcinoma in Henan, China. Int J Epidemiol.1998;27(4):574-578.

2. Chen DS. Hepatitis C virus in chronic liver disease and hepatocellular carcinoma in Taiwan. Princess Takamatsu Symp. 1995;25:27-32.

3. Gerlich WH. Medical virology of hepatitis B: how it began and where we are now. Virol J. 2013;10:239. Published 2013 Jul 20 Salmonella Typhi chimaericA(2)B(5) typhoid toxin. Nature. 2013;499(7458):350-4.doi:10.1038/nature12377.

4. Scheel TK, Rice CM. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. Nat Med. 2013;19(7):837-849.

5. Yang D, Zuo C, Wang X, Meng X, Xue B, Liu N, Yu R, Qin Y, Gao Y, Wang Q, Hu J, Wang L, Zhou Z, Liu B, Tan D, Guan Y, Zhua H. Complete replication of hepatitis B virus and hepatitis C virus in a newly developed hepatoma cell line. Proc Natl AcadSci U S A.2014;111(13):E1264-E1273.

6. Tong S, Li J, Wands JR, Wen YM. Hepatitis B virus genetic variants: biological properties and clinical implications. Emerg Microbes Infect. 2013;2(3):e10

7. Li J, Ma Y, Yang W, Sun D, Zhu Q, Wang Z. Study on risk factors of hepatitis C virus infection among Han population in Henan province. Zhonghua Yu Fang Yi XueZaZhi. 2014;48(11):985-989.

8. Lam NC, Gotsch PB, Langan RC. Caring for pregnant women and newborns with hepatitis B or C. Am Fam Physician. 2010;82(10):1225-1229.

9. Busch MP. Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors?.Transfus Clin Biol. 2004;11(1):26-32.

10. Gretch, D.R, Diagnostic tests for hepatitis C. Hepatology. 1997;26:43S-47S.

11. Chen WANG, Jie WU, Chen ZONG, Jie XU, Huang-Xian JU. Chemiluminescent Immunoassay and its Applications. Chinese Journal of Analytical Chemistry.2012;40(1):3-10

12. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA [published correction appears in Vox Sang. 2005 Apr;88(3):223]. Vox Sang.2005;88(2):77-86.

13. Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Leung N et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol.2000;15(12):1356-1361.

14. Vieira GD, Ventura CG, Sousa CM. Occurrence and spatial distribution of Hepatitis C in a western Brazilian Amazon state. Arq. Gastroenterol.2014;51(4):316-319.

15. Gish RG, Gutierrez JA, Navarro-Cazarez N, et al. A simple and inexpensive point-of-care test for hepatitis B surface antigen detection: serological and molecular evaluation. J Viral Hepat. 2014;21(12):905-908. doi:10.1111/jvh.12257

16. Mahajan S, Agarwal R, Rawat V, Kumar G, Sharma MK, Gupta E. Comparative evaluation of three rapid immunochromatographic test assays with chemiluminescent microparticle immunoassay for the detection of hepatitis C virus antibody. Virus disease. 2019;30(3):373-379. doi:10.1007/s13337-019-00542-5

17. Lin SB, Zheng ZX, Zhang R. Application and Evaluation of Chemiluminescence Immunoassay in Blood Screening. Zhongguo Shi Yan Xue Ye XueZaZhi. 2019;27(2):569-572. doi:10.19746/j.cnki.issn.1009-2137.2019.02.042

18. 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 ClinDiagn Res. 2016;10(11):DC22-DC25. doi:10.7860/JCDR/2016/24108.8921

19. Bentow C, Lakos G, Rosenblum R, Bryant C, Seaman A, Mahler M. Clinical performance evaluation of a novel, automated chemiluminescent immunoassay, QUANTA Flash CTD Screen Plus. Immunol Res.2015;61(1-2):110-116.

20. Bentow C, Swart A, Wu J, et al. Clinical performance evaluation of a novel rapid response chemiluminescent immunoassay for the detection of autoantibodies to extractable nuclear antigens. Clin Chim Acta. 2013;424:141-147.

21. Ismail N, Fish GE, Smith MB. Laboratory evaluation of a fully automated chemiluminescence immunoassay for rapid detection of HBsAg, antibodies to HBsAg, and antibodies to hepatitis C virus. J Clin Microbiol.2004;42(2):610-617.

22. Sarrazin C, Teuber G, Kokka R, Rabenau H, Zeuzem S. Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. Hepatology. 2000;32(4 Pt1):818-823.

23. Takaki A, Wiese M, Maertens G, et al. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. Nat Med.2000;6(5):578-582.

24. Read SJ. Recovery efficiences on nucleic acid extraction kits as measured by quantitative LightCycler PCR. Mol Pathol.2001;54(2):86-90.

25. Krajden M. Hepatitis C virus diagnosis and testing. Can J Public Health. 2000;91Suppl1:S34-S42.

26. R. A Willson. Molecular diagnostic testing for viral hepatitis: methods and applications.

27. Marcel Dekker. Viral hepatitis: diagnosis, treatment, and prevention, vol.4.

28. Zamora BM, Hartung M, Hildebrandt G, Käsbohrer A. Detection of antibodies to S. enteritidis in broilers by means of indirect ELISA and chemiluminescent immunoassay (CLIA). J. Vet. Med B.1999;46:9-23

29. ElahianFirouz Z, Kaboosi H, Faghih Nasiri A, Tabatabaie SS, Golhasani-Keshtan F, Zaboli F. A Comparative Serological Study of Toxoplasmosis in Pregnant Women by CLIA and ELISA Methods in Chalus City Iran. Iran Red Crescent Med J.2014;16(4):e15115.

30. Feng Z, Li Z, Sui B, Xu G, Xia T. Serological diagnosis of infectious mononucleosis by chemiluminescent immunoassay using capsid antigen p18 of Epstein-Barr virus. ClinChimActa.2005;354(1-2):77-82.

31. Huh HJ, Chae SL, Cha YJ. Comparison study with enzyme immunoassay and chemiluminescence immunoassay for hepatitis B virus surface antigen detection. Korean J Lab Med.2007;27(5):355-359.

32. Beld M, Sentjens R, Rebers S, et al. Performance of the New Bayer VERSANT HCV RNA 3.0 assay for quantitation of hepatitis C virus RNA in plasma and serum: conversion to international units and comparison with the Roche COBAS Amplicor HCV Monitor, Version 2.0, assay. J Clin Microbiol.2002;40(3):788-793.

33. Gnanasegaram M, Coutts I. A case of adrenal insufficiency secondary to syphilis and difficulties in the diagnostic work-up. Australasian Journal of Dermatology. 2013;54 (1): e19-21