Biomedical Journal of Indonesia
Journal Homepage: https://bji-fk.ejournal.unsri.ac.id

Comparative Evaluation of Electrochemiluminescence and Chemiluminescence Microparticle Immunoassays for Anti-Hepatitis C Virus (HCV) Detection

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ARTICLE INFO

Keywords:
Agreement test
Anti-HCV
Hepatitis C virus
Immunoassay

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/BJI.v10i3.208

ABSTRACT

Introduction. Anti-hepatitis C virus (HCV) testing is an immunological analysis designed to identify the presence of antibodies against the HCV antigen. This investigation is typically conducted using the chemiluminescence immunoassay (CLIA) technique, which yields precise results. Current research attempts to evaluate the outcomes of the anti-HCV test utilizing the Chemiluminescence Microparticle Immunoassay (CMIA) and the Electrochemiluminescence Immunoassay (ECLIA) approaches. **Methods.** This cross-sectional study comprised 63 serum samples collected via consecutive sampling. The acquired data were subjected to statistical analysis utilizing Cohen's Kappa agreement test. **Results.** Both immunoassay methods yielded identical results, indicating four reactive samples out of 63, equating to 6.35%. The agreement test result for the anti-HCV test was κ=1.000, signifying an almost perfect level of agreement. **Conclusion.** The anti-HCV assessment utilizing CMIA and ECLIA methodologies demonstrated near-perfect agreement. This signifies that these two procedures can be employed in clinical laboratories concurrently or interchangeably for the test.

1. Introduction

Many procedures in clinical and research laboratories rely on antigen-antibody responses.[1] The rapid growth of immunobiology and immunochemistry has enabled physicians to deploy immunological laboratory tests that can help with diagnosis and patient management.[2] Development in these fields also happened at the same time as the improvement of laboratory instruments. An example of this simultaneous advancement is that in some laboratories, different examination methods have been utilized to detect same disorder,[3,4] such as in viral hepatitis detection, particularly hepatitis C virus (HCV) antibody, commonly known as anti-HCV.[5] This examination is an important parameter for screening high risk patients who may be infected by HCV through blood-borne transmission.[6]

Prior studies have investigated several methodologies for anti-HCV testing. A study in India evaluated chemiluminescence immunoassay (CLIA) and immunochromatographic test (ICT) against enzyme-linked immunosorbent assay (ELISA) as the gold standard. The two examined tests demonstrated comparable findings to ELISA testing, exhibiting a sensitivity of over 95% and a specificity surpassing 90%.[5] Another study compared the testing results of the electrochemiluminescence immunoassay (ECLIA)/Elecsys anti-HCV with those of the Enzyme-Linked Fluorescence Assay (ELFA)/Vidas anti-HCV. The study revealed a substantial overall concordance between the two tests (94%), with Vidas exhibiting greater specificity and Elecsys displaying better sensitivity. Consequently, both methodologies are appropriate for laboratory and/or blood screening operations.[7] Meanwhile, an earlier study found an elevated false seropositivity findings for anti-HCV testing among low-risk samples in Turkey, in contrast to HCV-ribonucleic acid/RNA (using polymerase chain reaction/PCR) results, with a combined value of

26.1% based on CLIA and ELISA methodologies.[8]

In the clinical pathology laboratory of our hospital (a tertiary-level hospital), there are two diagnostic instruments: the Abbott i2000, which employs the chemiluminescence microparticle immunoassay (CMIA) method, and the Cobas e601, which utilizes the ECLIA method.[9,10] Both procedures are employed for immunological assessment. Nonetheless, it remains uncertain whether the two approaches are interchangeable, as there exists a chance that they may yield divergent examination outcomes, such as in detecting anti-HCV emergence. It is essential to ascertain whether any observed difference is meaningful. Consequently, it is essential to evaluate the two methodologies to demonstrate their appropriateness.[11] This study aims to compare the findings of anti-HCV testing via chemiluminescence microparticle immunoassay and chemiluminescence immunoassay techniques, thereby elucidating the precision of both methodologies to enhance patient management.

2. Methods

This analytical observational investigation employed a cross-sectional design. Samples were consecutively recruited from the anti-HCV testing results at the Clinical Pathology Laboratory Installation of Dr. Mohammad Hoesin Hospital, Palembang, Indonesia (from June 2019 to December 2019) utilizing the Abbott i2000 (CMIA method) and Cobas e601 (ECLIA method) analyzers. The serum sample utilized in the study was first centrifuged at 4000 rpm for twenty minutes. For analysis inclusion, serum should be free from lysis, lipemia, or icterus. The clinical and laboratory standard institute (CLSI) stipulates that 40 samples are minimum threshold to perform a comparative test, followed in the current project. The evaluation results were categorized as reactive and non-reactive, with criteria specifying that the cut-off index (COI) ≥ 1.00 is considered reactive for the ECLIA method. In contrast, signal-to-cut-off signal (S/CO) ≥ 1.00 is deemed reactive for the CMIA method. No indeterminate or missing findings were utilized.

The acquired data were examined using Cohen's Kappa agreement test. The interpretation of Cohen's Kappa agreement test based on the generated κ-value is as follows: 0.00 (weak), 0.00 - 0.20 (mild), 0.21 - 0.40 (moderate), 0.41 - 0.60 (strong), 0.61 - 0.80 (substantial), 0.81 - 1.00 (near-perfect).[12] Data analysis was done utilizing the IBM SPSS Statistics for Windows software, version 25.0 (Armonk, NY: IBM Corp). The research ethics committee of Dr. Mohammad Hoesin General Hospital and Faculty of Medicine, Universitas Sriwijaya authorized this study (Approval Number: 465/kepkrsmhfkunsri/2019).

3. Results

This study utilized 63 samples for examination by semi-quantitative anti-HCV testing. Both CMIA and ECLIA techniques identified four reactive samples, constituting 6.35%. Simultaneously, most of the samples yielded non-reactive outcomes, precisely 59 samples (93.65%) in the assessment utilizing CMIA and ECLIA methodologies. The suitability test results for the anti-HCV examination using the CMIA and ECLIA methods were analyzed with Cohen's Kappa (κ), yielding a value of 1.000. This indicates an almost perfect agreement between the two methods, allowing for their interchangeable use in anti-HCV detection (Table 1).

4. Discussion

HCV, one of the most common etiological agents of acute and chronic hepatitis, is classified into eight primary genotypes and over 80 subgroups according to nucleotide variation.[13] It comprises core proteins (the initial 191 HCV amino acids), envelope glycoproteins, P7 proteins, and non-structural proteins (NSPs), which include NS2, NS3, NS4A, NS4B, NS5A, and NS5B.[14] The core protein is directly or indirectly implicated in hepatocarcinogenesis and steatosis hepatitis.[15] Meanwhile, envelope glycoproteins are crucial for facilitating entrance into host cells.[16] The anti-HCV test uses a blood plasma or serum sample to identify antibodies specific to HCV antigens, including the structural core antigen and many NSPs.[17] This procedure often utilizes enzyme immunoassays (EIAs). EIAs are frequently employed because of their numerous advantages, including ease of automation, excellent reproducibility of results, and low expenses.[18] Initially, this assay can solely identify the recombinant peptide (c100-3) corresponding to the NS4. Nevertheless, due to the protracted seroconversion duration and significant false-positive rate in low endemic locations, second-generation assays have been developed using recombinant antigens from the NSPs (NS3/c33c and NS4/5-1-1p) regions, together with a core antigen (c22-3). Further developments, which is the current iteration of this assay, termed as the third generation, identifies all HCV structures, including core antigens, NSPs (NS3 and NS4), and an NS5 epitope, greatly enhancing its sensitivity and specificity while reducing the seroconversion duration.[19] Currently, in specific high-throughput clinical laboratories, CLIA supersedes EIA for anti-HCV detection due to its superior detection agreement (since EIA's antigen composition varies among manufacturers), simplicity, complete automation, and improved positive predictive value (PPV).[17]

Table 1. Agreement test between two anti-HCV examination methods (CMIA and ECLIA)

ECLIA	CMIA		Total	κ
	Reactive	Non-reactive		
Reactive	4	0	4 (6.35%)	**1.00**
Non-reactive	0	59	59 (93.65%)	
Total	4 (6.35%)	59 (93.65%)	63	

The current recommendation published by the American Association for the Study of Liver Diseases–Infectious Diseases Society of America (ASLD-IDSA) stipulates that HCV infection determination should be assessed using an anti-HCV assay, followed by a confirmatory assessment by nucleic acid testing (NAT) following a reactive anti-HCV assay result.[20] It signifies the crucial function of anti-HCV testing to screen blood-borne diseases. In addition to detecting the progression of Hepatitis C, it also aids in screening blood intended for transfusions.[21] Several patients at elevated risk for HCV infection can be regularly monitored through this examination, including hemodialysis patients, individuals who have undergone routine blood transfusions (e.g., thalassemia), and patients with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS).[22,23]

Achieving good agreement among anti-HCV testing methods is crucial for ensuring precise results across various analyzers.[24] To assess their agreement, this study evaluated and compared two CLIAs: ECLIA and CMIA. This study determined that the methods evaluated exhibit near-perfect agreement (κ = 1.000). A prior study in India found comparable results for HCV antibody detection, with reactivity rates of 2.9% for CMIA and 2.5% for ECLIA. Both procedures exhibit high sensitivity (100%) but marginally reduced specificity: 99.02% for ECLIA and 98.62% for CMIA. Nonetheless, both procedures are susceptible to false-positive results; in the case of HCV-RNA testing via PCR, only 8 from 517 people (1.55%) exhibited an accurate positive HCV detection.[25] Simultaneously, an additional investigation comparing CLIA and ECLIA revealed a substantial agreement between these immunoassay procedures (91.9%). The Elecsys anti-HCV assay (ECLIA) seems to have lower sensitivity but greater specificity than the Architect anti-HCV (CLIA).[26] A separate study evaluated the concordance between two CLIA analyzers and ELISA as the gold standard, revealing a good level of agreement between the analyzers and the ELISA results (Cobas e 601 ROCHE, κ = 0.81; Vitrous 3600 ORTHO, κ = 0.994). Vitrous analyzers demonstrated superior sensitivity (100% vs. 95.05%) and positive predictive values (98.97% vs. 73.85%) compared to Cobas e601.[27] Furthermore, a prior study conducted in China analyzed 10,772 serum samples with CLIA and light-initiated chemiluminescence assay (LiCA), achieving a commendable overall agreement rate of 98.74%. Nevertheless, the positive agreement value of these assays was very low (37.31%), resulting in a κ=0.519. From the same study, LiCA demonstrated superior specificity, sensitivity, negative predictive value (NPV), and positive predictive value (PPV) compared to CLIA.[17]

There are several limitations in this study. First, sample size is relatively low compared to previous study, although it still managed to fulfil the minimum sample size. It can be associated with a κ = 1.000 in this study. Second, we only classified the result to reactive and non-reactive. Although it does not significantly affect the study outcome, some previous studies utilized the addition of further classification (indeterminate or unclear) to figure out their patients' characteristics. Third, we do not compare the agreement with a gold standard testing, which commonly used ELISA as also demonstrated in prior research. However, this study is the first of its kind to compare between CMIA (Abbott i2000) and ECLIA (Cobas e601) methods in Indonesia, which is still lacking the data on analyzers agreement. Moreover, specific agreement test on the tested analyzers was not available in previously available literature.

5. Conclusion

This study established no distinction between the CMIA (Abbott i2000) and ECLIA (Cobas e601) methodologies when performing anti-HCV analysis. This finding signifies that these methods may be utilized simultaneously or interchangeably in the medical laboratory for screening objectives. However, this discovery requires validation in a larger cohort and through the usage of gold standard, such as PCR testing or ELISA which could be employed in the future studies.

6. Acknowledgements

None

7. References

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