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Diagnostic Accuracy of Chemiluminescent Immunoassays Compared to Nucleic Acid Testing for Hepatitis C Screening in Blood Donors: A Systematic Review

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Abstract

Hepatitis C virus (HCV) remains a major global public health problem, requiring accurate and scalable diagnostic methods for effective screening and disease control. Nucleic acid testing (NAT) is considered the reference standard for HCV detection but is limited by high cost and technical complexity, particularly in resource-limited settings. Chemiluminescent immunoassays (CLIA) offer operational advantages and are increasingly used as alternative diagnostic tools. This systematic review was conducted following PRISMA guidelines to compare the diagnostic accuracy of CLIA with NAT for HCV detection in blood donors. A comprehensive literature search was performed in PubMed, Scopus, Web of Science, and Ebscohost for studies published between 2015 and July 2025. Eligible studies directly compared CLIA-based assays with NAT and reported sensitivity and specificity data. Study quality was assessed using the QUADAS-2 tool, and results were synthesized narratively. Four studies involving diverse populations and laboratory settings were included. CLIA-based assays demonstrated high diagnostic performance, with sensitivity ranging from 92.4% to 100% and specificity from 88.8% to 100% when compared with NAT. Several studies reported strong agreement between CLIA and molecular methods, supporting CLIA's effectiveness for large-scale screening applications. CLIA provides a reliable and cost-effective alternative to NAT for HCV screening, particularly in blood donor and resource-limited settings. However, reduced sensitivity at low viral loads and study heterogeneity remain important limitations.

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Abstrak

Virus hepatitis C (HCV) tetap menjadi masalah kesehatan masyarakat global utama, yang membutuhkan metode diagnostik yang akurat dan terukur untuk skrining dan pengendalian penyakit yang efektif. Pengujian asam nukleat (NAT) dianggap sebagai standar referensi untuk deteksi HCV tetapi terbatas oleh biaya tinggi dan kompleksitas teknis, terutama di lingkungan dengan sumber daya terbatas. *Imunoasai kemiluminesen* (CLIA) menawarkan keunggulan operasional dan semakin banyak digunakan sebagai alat diagnostik alternatif. Tinjauan sistematis ini dilakukan mengikuti pedoman PRISMA untuk membandingkan akurasi diagnostik CLIA dengan NAT untuk deteksi HCV pada donor darah. Pencarian literatur komprehensif dilakukan di PubMed, Scopus, Web of Science, dan Ebscohost untuk studi yang diterbitkan antara tahun 2015 dan Juli 2025. Studi yang memenuhi syarat secara langsung membandingkan assay berbasis CLIA dengan NAT dan melaporkan data sensitivitas dan spesifisitas. Kualitas studi dinilai menggunakan alat QUADAS-2, dan hasilnya disintesis secara naratif. Empat studi yang melibatkan beragam populasi dan pengaturan laboratorium disertakan. Pengujian berbasis CLIA menunjukkan kinerja diagnostik yang tinggi, dengan sensitivitas berkisar antara 92,4% hingga 100% dan spesifisitas dari 88,8% hingga 100% bila dibandingkan dengan NAT. Beberapa studi melaporkan kesepakatan yang kuat antara CLIA dan metode molekuler, mendukung efektivitas CLIA untuk aplikasi skrining skala besar. CLIA menyediakan alternatif yang andal dan hemat biaya untuk NAT untuk skrining HCV, khususnya di lingkungan donor darah dan sumber daya terbatas. Namun, sensitivitas yang berkurang pada kadar virus rendah dan heterogenitas studi menjadi keterbatasan penting.

INTRODUCTION

Hepatitis C virus (HCV) remains a significant global public health concern due to its high burden of chronic infection and potential progression to serious liver diseases, including cirrhosis and

hepatocellular carcinoma. The World Health Organization estimates that approximately 58 million people worldwide are living with chronic HCV infection, with around 1.5 million new infections occurring annually (Yang et al., 2019). A substantial proportion of infected individuals remain asymptomatic for years, contributing to ongoing transmission and delayed diagnosis, particularly in low- and middle-income countries.

Early and accurate diagnosis of HCV infection is essential to prevent long-term complications and to enable timely initiation of antiviral therapy. Effective screening strategies are especially critical in blood donor settings, where undetected infections pose a risk of transfusion-transmitted disease. Prompt identification of HCV allows early linkage to care, reduces viral spread, and supports global HCV elimination goals (Yang et al., 2019).

Nucleic acid testing (NAT), which directly detects HCV RNA, is widely regarded as the reference standard for confirming active infection due to its high analytical sensitivity and ability to detect early viremia (Scott & Gretch, 2007). NAT methods such as real-time polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA) are capable of identifying infection during the window period, before seroconversion occurs. However, the widespread implementation of NAT is constrained by high operational costs, specialized equipment requirements, and the need for trained personnel, limiting its accessibility in many resource-limited settings Khan et al., 2017;.

Chemiluminescent immunoassays (CLIA) have emerged as widely used alternatives for HCV screening because of their automation, high throughput, and relatively lower cost (Li et al., 2023; Salem & ElSayed, 2024). CLIA-based assays for anti-HCV antibodies or HCV core antigen have demonstrated high diagnostic performance and substantial agreement with NAT in multiple studies, while offering practical advantages for large-scale screening programs (Li et al., 2023; Yang et al., 2019). Nonetheless, CLIA assays may have limitations in distinguishing active infection from past exposure and may show reduced sensitivity during early infection or at very low viral loads, necessitating confirmatory testing in certain contexts.

However, despite the growing use of CLIA-based assays, there is no recent comprehensive review that systematically summarizes and compares the diagnostic accuracy of CLIA versus NAT specifically in blood donor populations. Therefore, this study aims to conduct a systematic review to evaluate the diagnostic performance of chemiluminescent immunoassays in comparison with nucleic acid testing for the detection of hepatitis C virus among blood donors.

METHODS

This study was conducted as a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological transparency and reproducibility.

Included studies directly compared chemiluminescent immunoassays (CLIA)

with nucleic acid testing (NAT) for the detection of hepatitis C virus (HCV) in human serum or plasma, specifically in blood donor populations. Studies were required to report extractable diagnostic accuracy data, including sensitivity and specificity, or provide sufficient raw data for their calculation. Original research articles with cross-sectional, cohort, or diagnostic accuracy designs were eligible. Reviews, editorials, conference abstracts, case reports, animal studies, and studies without direct CLIA–NAT comparison were excluded.

A comprehensive literature search was performed in PubMed, Scopus, Web of Science, and Ebscohost for articles published between 2015 and July 2025. The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to “Hepatitis C,” “chemiluminescent immunoassay,” “CLIA,” “core antigen,” “nucleic acid testing,” and “PCR.” Reference lists of relevant articles were also manually screened to identify additional eligible studies.

All retrieved records were screened independently by four reviewers using the Rayyan platform. Titles and abstracts were first assessed for relevance, followed by full-text evaluation of potentially eligible studies. Any disagreements were resolved through discussion or consultation with a third reviewer. After the screening and eligibility assessment process, four studies met the inclusion criteria and were included in the final review, as summarized in the PRISMA flow diagram.

Data extraction was performed independently by four reviewers using a standardized form. Extracted variables included author, publication year, country, study design, population characteristics, CLIA assay type, NAT reference standard, sample size, and diagnostic accuracy outcomes (sensitivity, specificity, PPV, NPV, accuracy, and agreement statistics where available). Discrepancies were resolved by consensus.

The methodological quality and risk of bias of the included studies were assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. Domains evaluated included patient selection, index test (CLIA), reference standard (NAT), and flow and timing. Quality assessment results were considered in the interpretation of the findings.

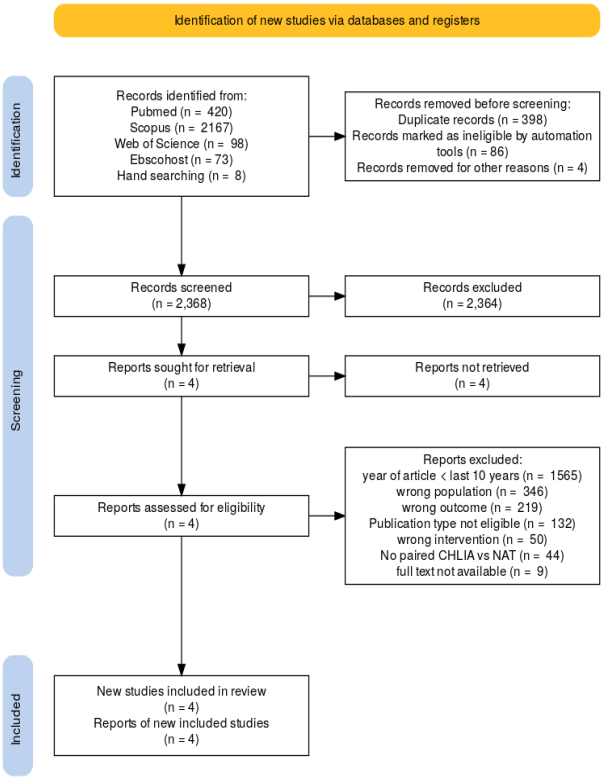

Figure 1. Diagram flow of literature search strategy for this systematic review

Table 1. Characteristics and results of the included studies

Author (Year)	Country/ Setting	Population (n)	CLIA Assay	NAT Reference Standard	Sensitivity (%)	Specificity (%)	Key Findings
Wang et al. (2017)	China; hospital-based	229 anti-HCV-positive sera	HCV core Ag CLIA (Abbott Architect)	RT-PCR	94.8	100	High specificity and strong correlation between core antigen and HCV RNA levels, supporting CLIA as a practical alternative to NAT.
Iqbal et al. (2022)	Pakistan; clinical laboratory	40 HCV Ab-positive sera	HCV core Ag CMIA (Abbott Architect)	Real-time PCR	95.4	88.8	CLIA demonstrated good diagnostic performance and may serve as a cost-effective option in resource-limited settings.
Tiwari et al., 2020)	India; blood bank	10,164 blood donors	ECLIA (VITROS 3600)	ID-NAT (TMA-based)	100	99.6	ECLIA showed excellent sensitivity and specificity, suitable for high-throughput blood donor screening.
Shahin et al. (2025)	Egypt; university blood centers	87,620 blood donors	HCV Ab CLIA (Abbott Architect i2000SR)	NAT (Cobas / Procleix)	98.3	99.8	CLIA showed near-perfect agreement with NAT, though NAT remained essential for window-period detection.

RESULTS AND DISCUSSION

Characteristics of Included Studies

The systematic search identified four eligible studies published between 2017 and 2025 that met the inclusion criteria. The studies were conducted in China, Pakistan, India, and Egypt, representing both hospital-based and large-scale blood donor screening settings. Sample sizes varied widely, ranging from 40 to 87,620 participants. All studies directly compared CLIA-based assays with nucleic acid testing (NAT) as the reference standard for HCV detection, using platforms such as RT-PCR, TMA-based ID-NAT, or fully automated NAT systems. The evaluated CLIA assays included anti-HCV antibody and HCV core antigen platforms from major manufacturers, ensuring relevance to routine laboratory practice.

Diagnostic Accuracy of CLIA Compared with NAT

Across the included studies, CLIA demonstrated consistently high diagnostic accuracy when compared with NAT. Sensitivity values ranged from 92.4% to 100%, while specificity ranged from 88.8% to 100%. Large blood donor studies reported particularly strong performance. Tiwari et al. (2020) observed 100% sensitivity and 99.6% specificity using ECLIA in more than 10,000 donors, while Shahin et al. (2025) reported sensitivity of 98.3% and specificity of 99.8% in over 87,000 donors. Smaller studies in clinical settings showed comparable sensitivity, although specificity was slightly lower in some cohorts (Wang et al., 2017; (Iqbal et al., 2022;.

Agreement with Viral Load and Molecular Methods

Several studies evaluated the relationship between CLIA results and HCV RNA levels. Wang et al. (2017) reported a strong correlation between HCV core antigen concentrations measured by CLIA and viral load quantified by RT-PCR ($r = 0.834$). This finding supports the role of antigen-based CLIA as a surrogate marker for active viremia, particularly in settings where routine viral load testing is not feasible.

Operational Findings

Operational advantages of CLIA were consistently reported. High automation, rapid turnaround time, and suitability for high-throughput screening made CLIA attractive for blood bank workflows (Shahin et al., 2025; Tiwari et al., 2020),. However, reduced sensitivity at very low viral loads and occasional false-positive results were noted, highlighting the continued importance of NAT in window-period detection and confirmatory testing (Wang et al., 2017; (Iqbal et al., 2022;.

This systematic review synthesizes evidence showing that CLIA-based assays achieve high sensitivity and specificity for HCV detection when compared with NAT across diverse blood donor and clinical populations. Diagnostic performance was consistently strong, particularly in large-scale screening settings, with substantial agreement between CLIA and molecular reference methods.

The findings align with earlier reviews and meta-analyses reporting strong concordance between HCV core antigen or antibody assays and molecular techniques (Freiman et al., 2016; Khan et al., 2017; Hadziyannis & Laras, 2018). Prior work has shown that CLIA markedly improves early detection compared with antibody-only serology and approaches NAT performance at moderate to high viral loads (Catlett et al., 2022; Tang et al., 2017). Recent evaluations of newer CLIA platforms further support these observations (Li et al., 2023; Salem & ElSayed, 2024).

CLIA offers several advantages, including automation, scalability, and lower operational costs compared with NAT. These features support its use in high-volume screening programs. Nevertheless, limitations remain. CLIA may not reliably detect very low-level viremia and cannot fully replace NAT for confirming early acute infection or monitoring antiviral therapy. False-positive results, although infrequent, may occur in low-prevalence settings (Iqbal et al., 2022; Tiwari et al., 2020).

From a clinical and public health perspective, CLIA represents a practical tool for expanding access to HCV screening, particularly in low- and middle-income countries where NAT is not universally available. Blood transfusion services may benefit from integrating CLIA into routine screening algorithms, with NAT reserved for confirmation and window-period detection. Current international guidelines continue to prioritize NAT for definitive diagnosis, but evidence supports CLIA as an effective first-line screening approach (Pawlotsky et al., 2020; Handanagic et al., 2024).

This review has limitations. The number of included studies was small, and substantial heterogeneity in study design, population characteristics, and assay platforms precluded meta-analysis. Some studies lacked detailed subgroup analyses by genotype or viral load. Publication bias cannot be excluded. Future research should focus on prospective head-to-head comparisons of next-generation CLIA platforms and NAT, particularly in early infection, low-viremia populations, and high-risk groups.

CONCLUSION

This systematic review demonstrates that chemiluminescent immunoassays (CLIA) provide high diagnostic accuracy for hepatitis C virus detection in blood donor and clinical screening settings. Across the included studies, CLIA sensitivity ranged from 92.4% to 100% and specificity from 88.8% to 100% when compared with nucleic acid testing (NAT). The operational advantages of CLIA, including automation, high throughput, and cost-effectiveness, support its use for large-scale screening, particularly in resource-limited settings.

Nevertheless, NAT remains essential for detecting window-period infections and for confirmatory testing of active HCV infection.

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