Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 25s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Evaluation of Three Diagnostic Approaches for Hepatitis C Virus Detection in Patients and Blood Donors at a Tertiary Care Hospital and Blood Center in North India

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Cite this paper as: Dr. Ankita Mittal, Dr. Ritu Priya, Dr.Anamika Rawat, Dr. Natasha Singh, Dr.Govind Mishra, Dr. Jyoti Mishra, (2025) Evaluation of Three Diagnostic Approaches for Hepatitis C Virus Detection in Patients and Blood Donors at a Tertiary Care Hospital and Blood Center in North India, *Journal of Neonatal Surgery*, 14 (25s), 681-686

1. INTRODUCTION

Hepatitis C virus (HCV) infection is a significant global health concern, with an estimated 3% worldwide prevalence, affecting over 170 million individuals at risk of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. In India, the World Health Organization (WHO) estimates 10 to 24 million active cases, with a seroprevalence ranging from 0.09% to 2.02% in the general population [3]. However, prevalence rates are markedly higher in high-risk groups such as intravenous drug users, people with HIV, hemodialysis patients, and those with high-risk sexual behavior or multiple blood transfusions, ranging from 3.5% to 44.7% [4].

Early diagnosis is challenging as most cases remain asymptomatic in initial stages. Detection primarily relies on serological assays for anti-HCV antibodies or molecular tests for HCV RNA. Serological tests, including Rapid Diagnostic Tests (RDTs) and Enzyme-Linked Immunosorbent Assays (ELISA), typically detect antibodies 7–8 weeks post-infection but may yield false negatives in immunocompromised individuals [5-6].

Diagnostic technology has progressed through successive ELISA generations, with first-generation assays offering limited sensitivity and specificity, and fourth-generation assays capable of detecting both HCV core antigen and antibodies, improving early detection [7]. In low- and middle-income countries (LMICs), RDTs are favored for their simplicity but often fall short of WHO's recommended sensitivity (≥98%) and specificity (≥97%) benchmarks [8]. Conversely, Nucleic Acid Testing (NAT), particularly real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), remains the gold standard, detecting HCV RNA as early as 1–3 weeks post-infection [9].

This study aims to evaluate the performance of RDTs and fourth-generation ELISA assays against real-time RT-PCR in detecting HCV infection among individuals in Bulandshahr, Uttar Pradesh, providing valuable data for HCV screening strategies in LMICs.

2. MATERIALS AND METHODS

Study Design and Setting

A hospital-based cross-sectional study was conducted in the Department of Pathology and Blood Centre, Kalyan Singh Government Medical College, Bulandshahr, Uttar Pradesh, India, from January 2024 to May 2025, to assess Hepatitis C Virus (HCV) monoinfection.

Inclusion Criteria

Patients of both sexes, all age groups, with risk factors for HCV (e.g., blood transfusion, injecting drug use, tattooing, hemodialysis).

Donors that came to blood bank for blood donation.

Exclusion Criteria

Individuals refusing participation.

Samples with insufficient plasma or incomplete data.

Sample Collection

Venous blood (5 mL) was collected in EDTA tubes, centrifuged at 3,000 rpm for 10 minutes to separate plasma, and stored at -20°C.

Laboratory Methods

HCV Testing: Anti-HCV antibodies were screened using third-generation ELISA. Positive samples were confirmed by RT-PCR for HCV RNA. Genotyping was performed via nested PCR (NS5B region).

HIV Testing: HIV was confirmed using rapid immunochromatographic tests and ELISA, per national guidelines.

Viral Load: HCV RNA was quantified using RT-PCR (detection limit 15 IU/mL). HIV viral load was measured with a real-time assay.

Data Collection

Demographic and clinical data (age, sex, risk factors, ART status, CD4+ counts, liver function tests) were collected via questionnaires and blood center records.

Statistical Analysis

Data were analyzed using SPSS v10.0. Categorical variables were reported as percentages with 95% confidence intervals, continuous variables as means ± SD or medians (IQR). Chi-square tests, t-tests, or Mann-Whitney U tests were used as appropriate. Odds ratios assessed risk factors. $P < 0.05$ was significant.

Limitations

The cross-sectional design limits temporal analysis. Results from a single center may not be broadly generalizable.

3. RESULTS

Out of 2000 samples tested, NAT (gold standard) detected 130 HCV-positive cases, while ELISA identified 123 and Rapid Card Test (RDT) detected 98. NAT exhibited 100% sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). ELISA showed a sensitivity of 94.6%, specificity of 99.68%, PPV of 95.9%, and NPV of 99.84%. RDT demonstrated a sensitivity of 86.2%, specificity of 100%, PPV of 100%, and NPV of 99.7%.

Age-wise distribution revealed the highest number of HCV-positive cases (55/130) in the 41–60 years age group, followed by 35 cases in the 21-40 years group, 32 cases in those above 60 years, and 8 cases in the 0–20 years category (Tables No. 1&2). Figures illustrate ELISA standard curves, plate reading in progress, real-time PCR amplification plots, assay-wise HCV detection across age groups, and comparative performance characteristics of diagnostic methods.

Table 1: HCV Detection by Different Assays

Parameter	Rapid Card Test (RDT)	ELISA	NAT (Gold Standard)
Positive Cases Detected	98	123	130
Negative Cases Detected	1888	1877	1870
Total Cases	2000	2000	2000
Sensitivity (%)	86.2	94.6	100
Specificity (%)	100	99.68	100
Positive Predictive Value (PPV) (%)	100	95.9	100
Negative Predictive Value (NPV) (%)	99.7	99.84	100

Table 2: Distribution of Positive Cases by Age Group (Among 130 NAT-Positive Cases)

Age Group (Years)	Number of HCV Positive Cases
0–20	8
21–40	35
41–60	55
>60	32
Total	130

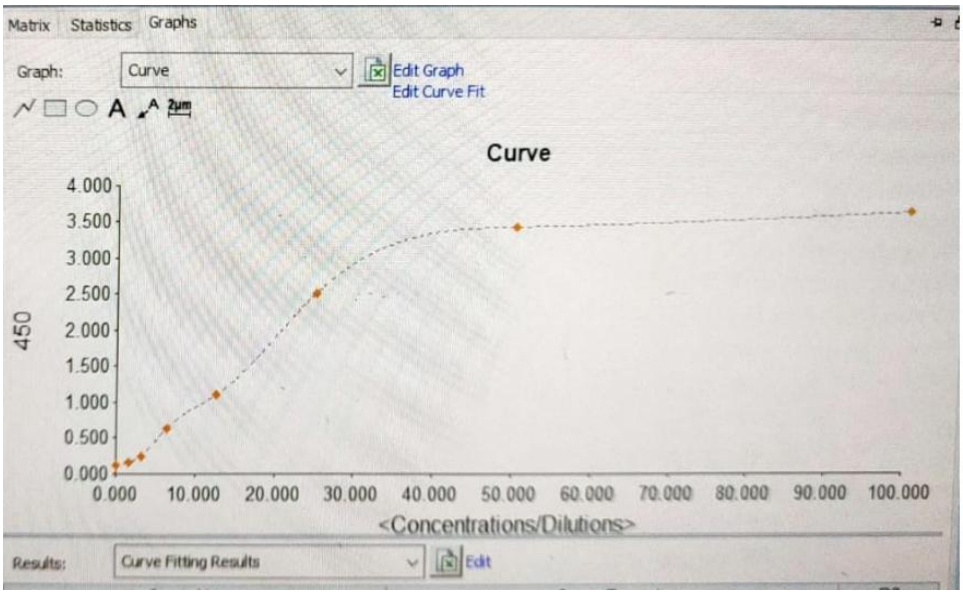

Figure 1: Standard Curve Generated from ELISA Plate Reader for Quantification of Analyte at 450 nm.

The curve demonstrates the relationship between optical density (OD) at 450 nm and varying concentrations of the analyte.

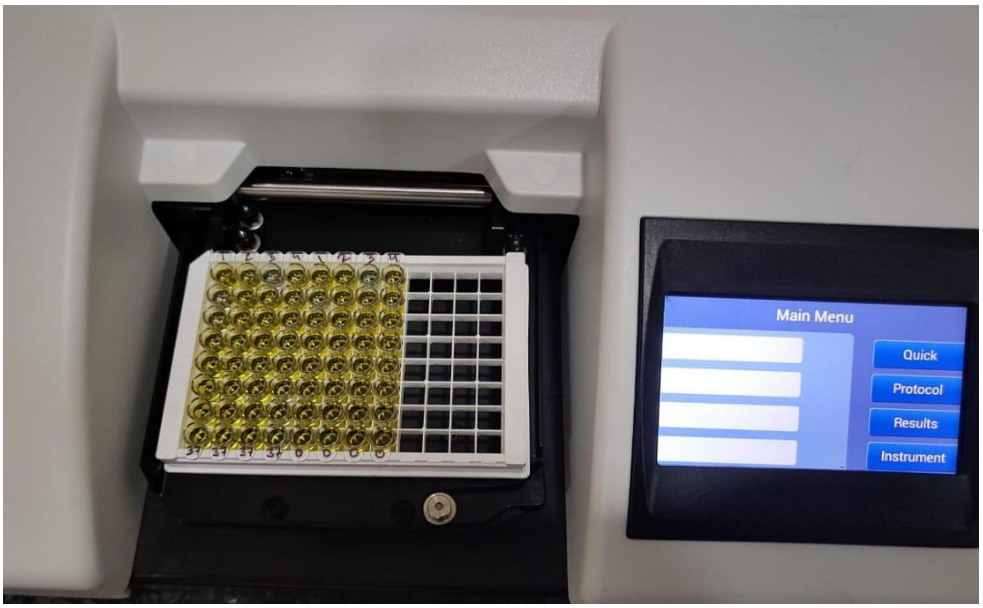

Figure 2: ELISA Plate Reading in Progress on a Microplate Reader: Image showing microtiter ELISA plate loaded in the reader, with operational menu displayed on the digital interface.

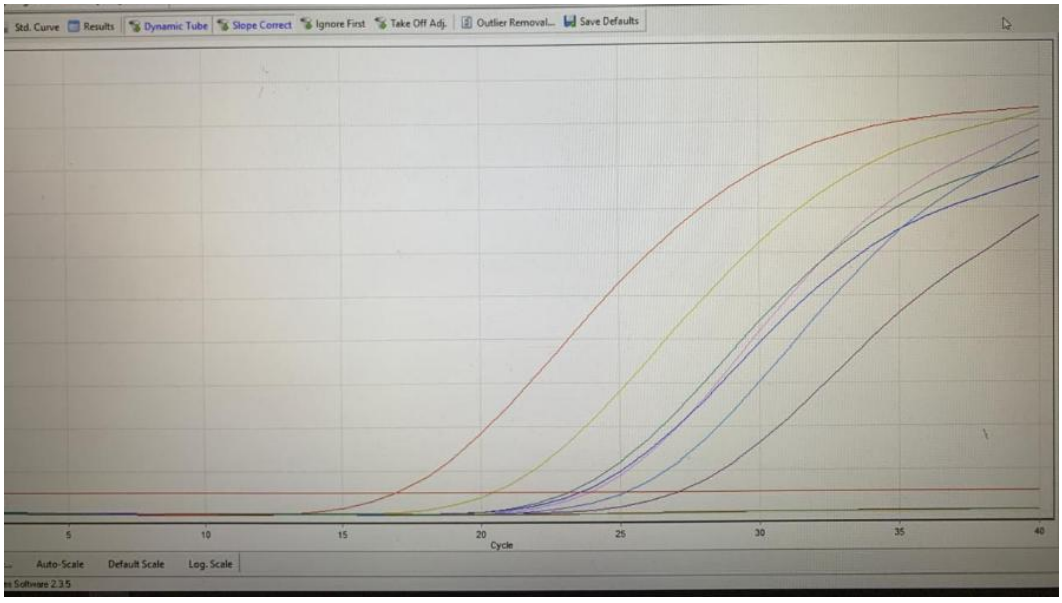

Figure 3: Amplification Plot Showing Real-Time PCR Curves for HCV Detection. Graph displaying fluorescence intensity versus cycle number for different HCV samples using a real-time PCR assay.

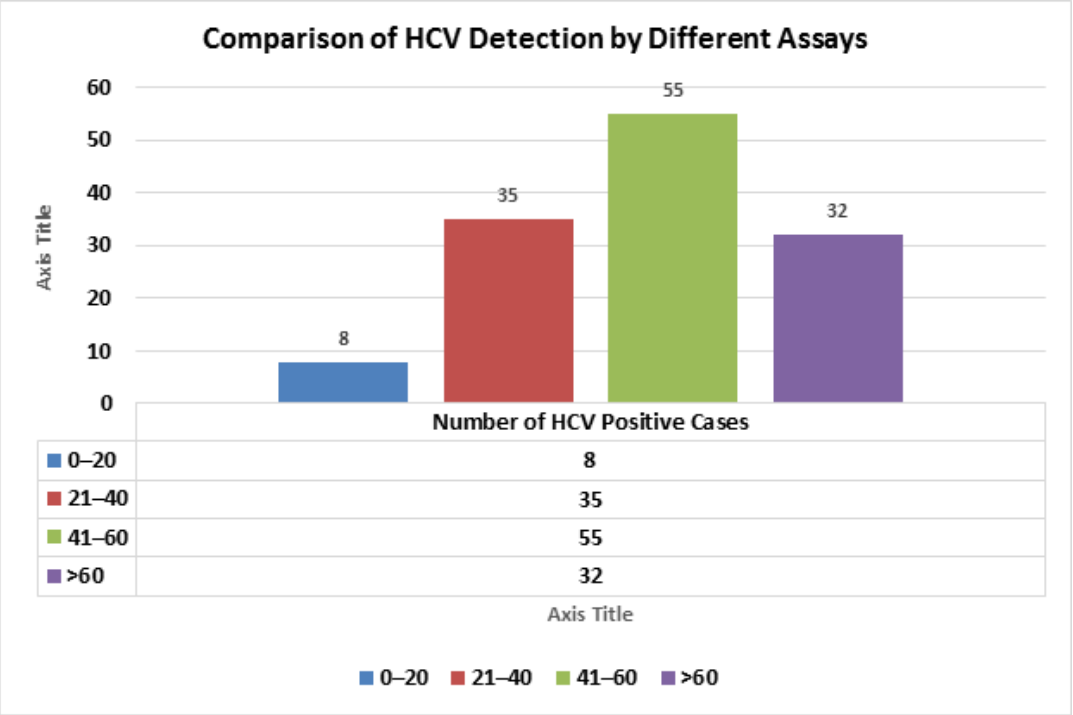

Figure 4: Comparison of HCV Detection by Different Assays Across Age Groups. Bar graph illustrating the distribution of HCV positive cases detected by various assays in different age categories.

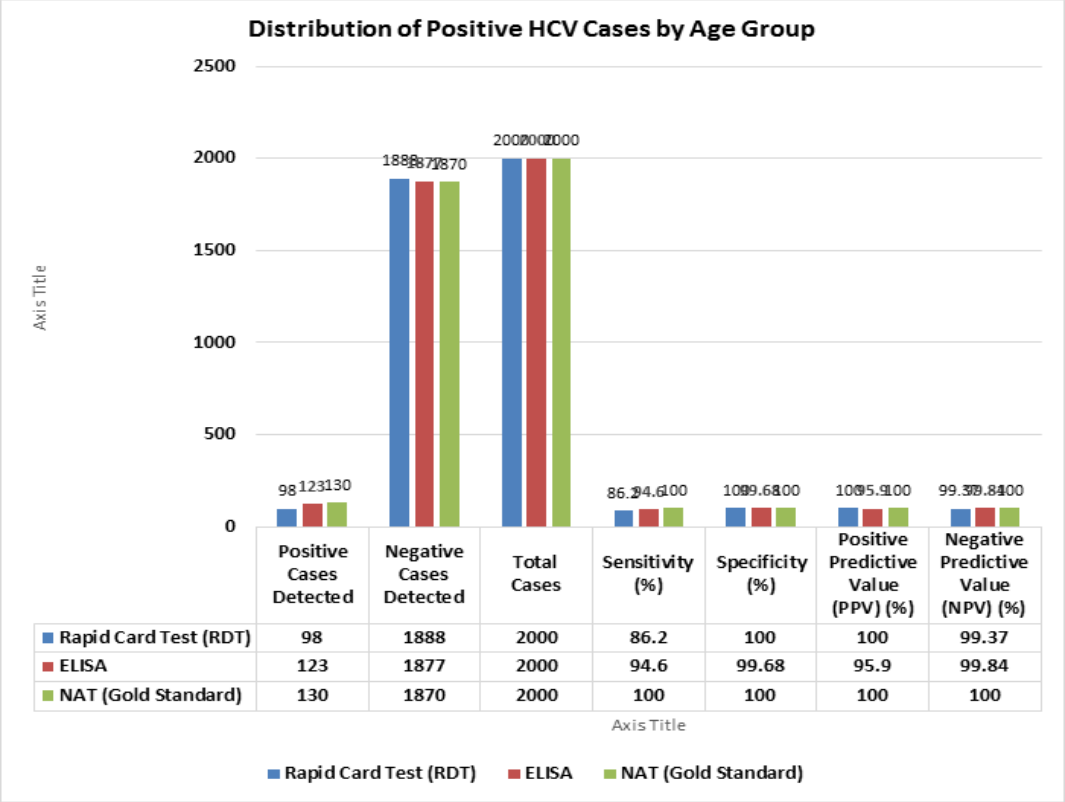

Figure 5: Distribution of Positive HCV Cases by Diagnostic Method and Performance Characteristics: Comparative bar graph depicting number of positive and negative cases detected by Rapid Card Test (RDT), ELISA, and NAT (Gold Standard), along with their sensitivity, specificity, PPV, and NPV.

4. DISCUSSION

Hepatitis C virus (HCV) remains a major global health concern, with diagnosis primarily relying on serological and molecular assays. This study compared the diagnostic performance of Rapid Diagnostic Test (RDT), fourth-generation ELISA, and Real-Time PCR (gold standard) in 2000 samples. PCR detected 130 (11.28%) positives, with ELISA identifying 123 (94.6% sensitivity) and RDT 98 (86.2% sensitivity).

The majority of participants were male (93.24%) with a mean age of 41.7 years, and intravenous drug use (IDU) was the most common risk factor (60.6%), consistent with prior studies [1,21–25]. RDT showed 100% specificity but suboptimal sensitivity (86.2%), lower than the WHO-recommended ≥98% [12], and a modest positive predictive value (79.03%). Fourth-generation ELISA outperformed RDT with 99.46% sensitivity, 98.34% specificity, and an AUC of 0.81, indicating excellent diagnostic accuracy.

These findings align with previous research [13-15] and confirm the limitations of RDT as a standalone tool. Molecular detection via real-time PCR remains essential for confirming active infection, especially in seronegative or acute cases. A combination of fourth-generation ELISA for screening and PCR for confirmation is recommended to enhance early detection and management of HCV infection.

5. CONCLUSION

This study concludes the superior diagnostic performance of fourth-generation ELISA for HCV screening, with high sensitivity and specificity. RDTs, though specific, showed limited sensitivity. Real-time PCR remains essential for confirming active infections. A combined strategy using ELISA for initial screening and PCR for confirmation is recommended to improve early detection and management, particularly in high-risk groups

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