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Comparative Analysis of Hepatitis C Virus (HCV) Screening Tests and Confirmatory PCR: Evaluation of Diagnostic Accuracy, Weak Positive Results, and Clinical Impact

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Abstract

Background: Hepatitis C virus (HCV) is a major global public health concern and a leading cause of chronic liver disease. Early and accurate diagnosis is essential to prevent disease progression and transmission. Although rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assay (ELISA) are widely used for HCV screening, polymerase chain reaction (PCR) remains the gold standard for confirming active infection.

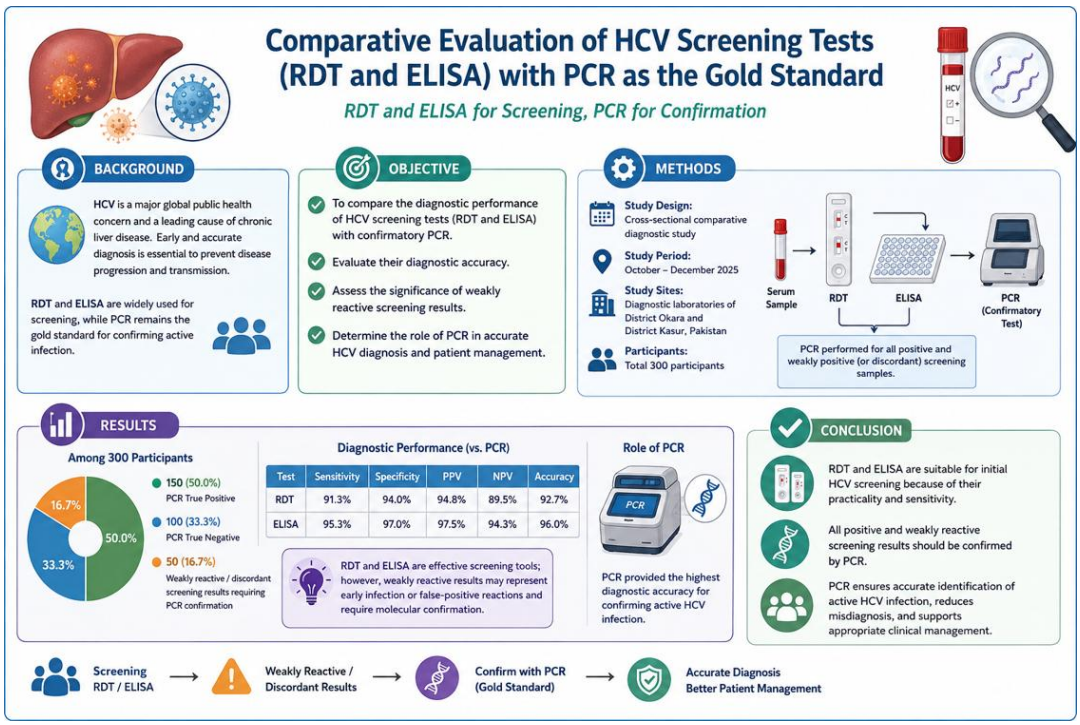
Objective: To compare the diagnostic performance of HCV screening tests (RDT and ELISA) with confirmatory PCR, evaluate their diagnostic accuracy, assess the significance of weakly reactive screening results, and determine the role of PCR in accurate HCV diagnosis and patient management.

Methods: A cross-sectional comparative diagnostic study was conducted from October to December 2025 in diagnostic laboratories of District Okara and District Kasur, Pakistan. A total of 300 participants were enrolled. Serum samples were tested using RDT and ELISA, while PCR was performed as the confirmatory reference test for positive and weakly positive screening samples. Diagnostic performance was assessed using descriptive statistics and standard diagnostic accuracy measures.

Results: Among the 300 participants, PCR confirmed 150 (50.0%) as true positive and 100 (33.3%) as true negative cases. Fifty (16.7%) participants showed weakly reactive or discordant screening results requiring PCR confirmation. The findings demonstrated that RDT and ELISA are effective screening tools; however, weakly reactive results may represent early infection or false-positive reactions and require molecular confirmation. PCR provided the highest diagnostic accuracy for confirming active HCV infection.

Conclusion: RDT and ELISA are suitable for initial HCV screening because of their practicality and sensitivity. However, all positive and weakly reactive screening results should be confirmed by PCR to accurately identify active HCV infection, reduce misdiagnosis, and support appropriate clinical management.

Graphical Abstract



Introduction

HCV (Hepatitis C virus) is a world leading cause of chronic liver disease and a worldwide threat to public health, an enveloped, positive-sense, single-stranded RNA virus capable of establishing chronic lifelong infection in hepatocytes (1). Hepatitis C infection is most likely transferred via contaminated blood, such as; the transfusion of blood that hasn't been screened properly, sharing contaminated equipment (i.e., needles, medical/dental instruments, drug-using apparatus), and from occupations working closely with the blood or other blood bodily fluids of an infected individual. In its initial phase, the infection is often unobserved, resulting in delayed diagnosis. If it is left untreated, it might cause prolonged injury and can develop into liver failure with end-stage disease such as; cirrhosis, fibrosis, and even hepatocellular carcinoma (4). Approximately 50–60 million people are currently infected in a chronic state worldwide (2). Hepatitis C is endemic in Pakistan (5,6) owing to the overuse of inadequate practices (7,8), the absence of universal screening (9,10), and a lack of adequate hygiene systems; coupled with lack of a preventive vaccine (3). Early screening of the patients to detect the infection early so it can be appropriately handled as well as prevent transmitting the infection further to others. HCV Screening in routine laboratories mostly utilizeserological tests, because they're economical, fast and are easy to work with. Anti-HCV antibodies and Hepatitis C RNA are the indicators to test positive for hepatitis C infection. In addition, rapid immunochromatographic tests/Rapid diagnostic tests ICT/RDT have been commonly employed for preliminary screening especially in blood banks but have limitations as their accuracy/precision may sometimes not be very reliable (14,15). Enzyme-linked immunosorbent assay(ELISA) is generally more sensitive/accurate than ICT/RDTs and is regularly used in screening (16); weak positive ELISA may be observed, these would require further tests for conclusive result. Poly merase Chain reaction (PCR) is capable of identifying ongoing active infection since it targets viral RNA directly. The test is highly sensitive/accurate for hepatitis C infection screening, identifying infections even prior to antibody creation (13) but it tends to be rather costly and require proper laboratory amenities. Both weak and strong positive, false-positive and negative ELISA results will require confirmation by PCR to distinguish a true infection. The purpose of this investigation is to determine the difference in efficacy between quick kits screening, ELISA, and PCR tests when diagnosing hepatitis C virus infection, enabling me to define their relative effectiveness,

investigate positive but borderline ELISA results and thereby improve our HCV diagnostic methods within clinical laboratory. Hepatitis C virus (HCV) infection is one of the world leading causes of liver chronic disease, potentially progressing to cirrhosis, hepatocellular carcinoma, death or liver failure if not timely and properly managed. Given that often this is a asymptomatic infection it is essential to early identify it. Laboratory tests to diagnosis hepatitis C virus (HCV) usually include the detection of anti-HCV antibodies through RDTs and ELISA followed by the detection of HCV RNA through PCR. The ELISA, because it is reliable and inexpensive for mass screening and also as an antibody detection test, has been traditionally selected as the screening test; although, it does not differentiate between an infection with the virus and a past exposure to it (17). Rapid tests have a lengthy period between exposure and detectable infection (13), but give result in a matter of minutes, they're suitable for blood banks and settings lacking intensive lab resources; nevertheless they can give false or border line positive results, in some cases due to lower accuracy of their sensitive or specificity (18). The PCR, being a confirmatory test due to the fact that it can prove active infection via the detection of HCV RNA in blood, offers great diagnostic accuracy, and it can identify infected persons before the development of antibodies, and thus PCR must be used to confirm positive or weakly positive antibody testing results (12). The primary aims of this research work is to explore difference in diagnosis capacity between RDT, ELISA and PCR, to identify diagnostic relevance of weak positive HCV result.

Methodology

A cross-sectional comparative diagnostic study was conducted from4 October,2025 to 2nd December, 2025 at diagnostic laboratories in District Okara and District Kasur, Pakistan. A total of 300 patients were enrolled in study to compare the diagnostic efficacy of RDT, ELISA and PCR for HCV detection. Five milliliter of venous blood sample were obtained from each patient under aseptic conditions. RDT and ELISA on Serum samples and PCR on positive or weak positive samples were performed as standard or gold test. About 150 true positive, 100 true negative and 50 weak positive sample were enrolled in study. Ethical approval was granted from the University of Okara, sampling consent was obtained from each patient. Data regarding tested patient's information was noted down on structured proforma and were entered into SPSS 26.0. Descriptive study and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy and Cohen's kappa statistics were derived, following standard reporting practice for diagnostic accuracy studies (19,20). P value was recorded at a < 0.05 as statistically significant.

Results

One hundred patients were found positive and50 patients were identified as false positive among the 300 suspected HCV patients by RDT/ELISA method (overall 16.67%) and 150 patients identified true positive and100 patients false negative for HCV after confirmed by PCR (50%) techniques. Other 50 patients were identified positive by screening but found weak positive or false negative with the PCR very low even below the detection level. The majority of these non-reactive or low positive screening tests were due to early infection or window period but minority due to false positive screening test themselves. These data indicate that though serological screening tests appear reliable for screening of HCV as a routine practice but imperfect in case of borderline results and PCR was the most accurate approach for confirmation of positive cases and should be performed whenever reactive/worth positive screening tests available.

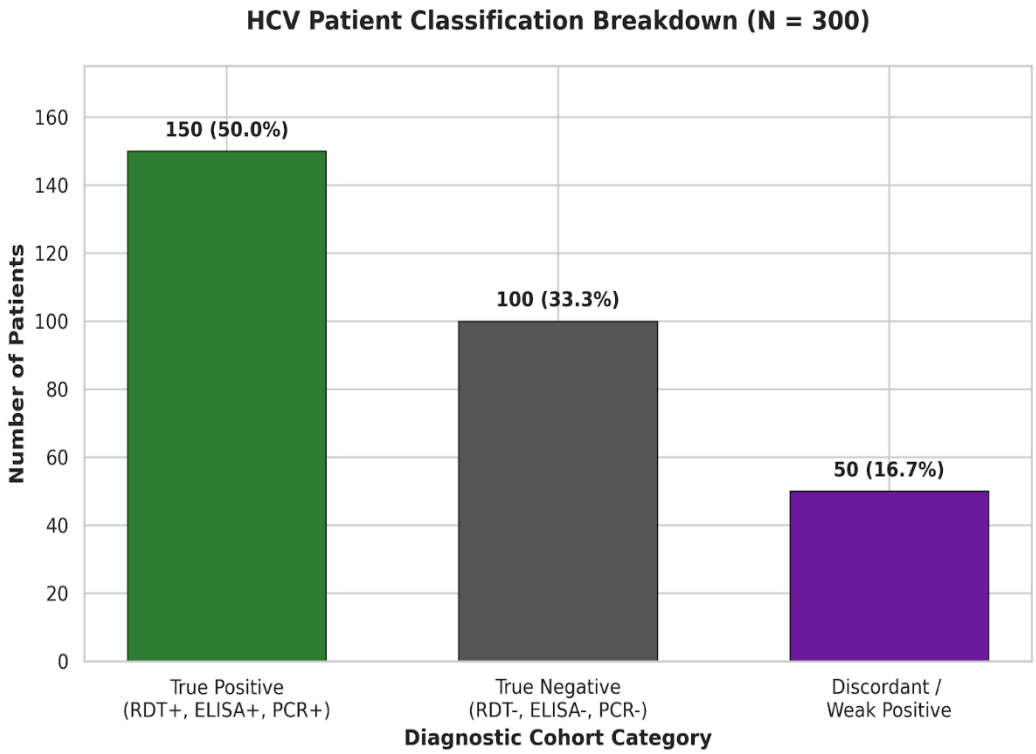


Figure 1:Distribution of HCV Screening and Confirmatory PCR Results

The above bar chart show the distribution of 300 participants according to the result of either HCV screening or the confirmatory PCR test. The results showed that 150(50.0%) of the participants were true positive, 100(33.3%)were true negative, 50(16.7%)have discordant or weak positive screening results. This result shows that the HCV screening test can correctly identify most of the cases. However in such cases there should be confirmatory test to distinguish between true positive or false positive cases.

Table 1. Distribution of HCV Screening and Confirmatory PCR Results (n = 300)

Category	**Number of Patients (n)**	**Percentage (%)**	**RDT Result**	**ELISA Result**	**PCR Result**	**Interpretation**
True Positive	150	50.00	Positive	Positive	HCV RNA Detected	Active HCV infection confirmed
True Negative	100	33.33	Negative	Negative	No HCV RNA Detected	No active HCV infection
Weak Positive/ Discordant	50	16.67	Weak Positive/ Variable	Weak Positive/ Variable	PCR required for confirmation (Positive) or (Negative)	Borderline/Indeterminate infection status
Total	**300**	**100.00**	—	—	—	—

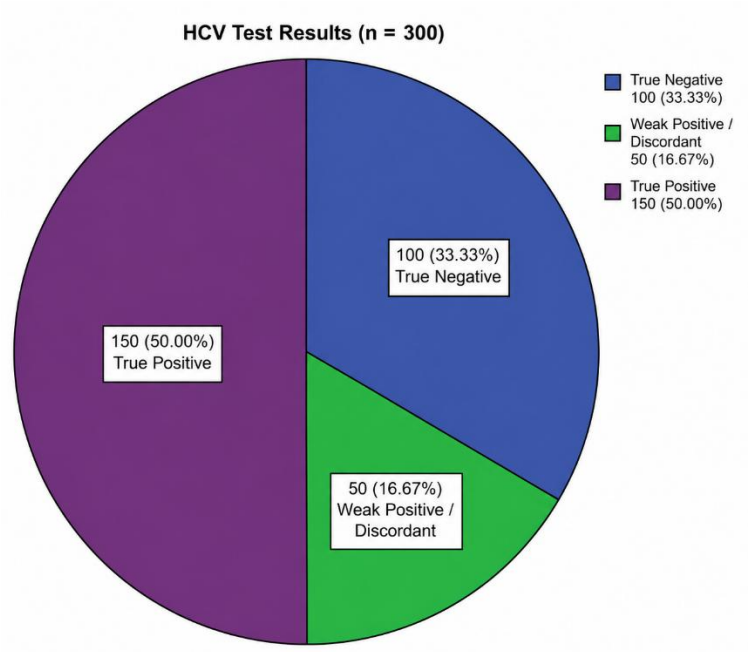


Figure 2:Distribution of HCV Test Results Among Study Participants

Pie chart showing the distribution of HCV test results among the 300 study subjects. True positive cases accounted for the biggest proportion (150, 50.0%) of the total testing population, where as true negative cases accounted for the next biggest proportion (100, 33.33%) of the total number of subjects. Weaker positive/discordant cases accounted for the smallest proportion (50, 16.67%).

DISCUSSION

The present study compared the screening performance of HCV assays (ELISA/rapid tests) to confirmatory PCR testing in 300 study participants. Results showed 50.0% of positives were confirmed as true positive by PCR while 33.3% true negative and 16.7% weak positive or weak negative on screening testing. These results show HCV screening assays are good for screening but confirmatory PCR is necessary for diagnosis. Previous studies have shown similar results and recommended antibody-based screening tests for RDTs were good at initial screening but needs to be followed by molecular confirmatory testing because it cannot discriminate between current and past infection (17). The diagnostic outcome showed a high number of true positive cases (50.0%) highlighting the high sensitivity of the ELISA and rapid screening tests to identify infected persons. Previous research has shown similar results with third-generation ELISA detection of HCV antibodies showing excellent sensitivity and usefulness for routine screening (15,16). False-positive antibody results may occur due to antibody cross-reactivity, autoimmune disease, previous cleared infection or technical reasons (18). PCR confirmation is recommended especially in low prevalence and low clinical suspicion populations (18). Furthermore, the number of true negative results was quite high (100 (33.3%) which proved the high specificity of the screening assays. False-positive reactions may occur for antibody assays but these are few and positive results must be confirmed with PCR. The weak positive/negative still represents a diagnosis dilemma in HCV screening. It may indicate early HCV infection, late convalescence, low resultant antibody titre, antibody problems or prior resolved infections. Previous work has also seen this issue and recommended confirmatory PCR testing for all borderline positive results in order to discern true infection from spurious antibody levels (14). This study reemphasizes the importance of two-step HCV diagnosis, for initial screening with ELISA or RDTs then verification of infection with PCR. As well as avoiding mis-diagnosis and treatment for false positives, confirming active infection early allows appropriate intervention, including timely initiation of curative direct-acting antiviral therapy (11).

Future Perspectives

Future research should validate the findings of the present study through large-scale, multicenter, and longitudinal investigations involving diverse populations, healthcare settings, and HCV genotypes. Incorporating quantitative viral load estimation, genotype-specific analysis, treatment monitoring, and cost-effectiveness evaluations would further strengthen diagnostic algorithms for hepatitis C virus infection. Moreover, artificial intelligence, predictive analytics, and automated decision-support systems have considerable potential to improve interpretation of borderline serological findings, optimize laboratory workflows, and facilitate rapid identification of active HCV infection while minimizing diagnostic uncertainty [28, 36, 44, 51, 52].

Future diagnostic strategies should also integrate healthcare governance, workforce sustainability, laboratory quality management, strategic risk governance, and AI-assisted professional education to enhance implementation of standardized diagnostic protocols and evidence-based clinical decision-making. Such multidisciplinary frameworks may improve healthcare delivery while ensuring sustainable laboratory practices and continuous professional development [39, 41, 46, 55, 56].

Although several recent investigations have primarily focused on food science, biotechnology, nutrition, and sustainable food production rather than infectious disease diagnostics, their emphasis on quality assurance, biosafety evaluation, advanced analytical methodologies, sustainable innovation, and translational research provides valuable methodological insights for improving laboratory diagnostics. Future HCV studies may adapt these approaches to enhance assay validation, biomarker discovery, specimen processing, and quality control systems [21–27, 34, 35, 42, 45, 57, 58].

Similarly, advances in CRISPR technology, epigenetics, nutrigenomics, multi-omics, environmental toxicology, and molecular systems biology illustrate how integrated biological datasets can improve biomarker identification and predictive modelling. Although these studies investigate different biological systems, their analytical frameworks may facilitate the discovery of novel molecular markers for early HCV detection, disease progression, therapeutic response, and precision diagnostics [31–33, 47–50].

Furthermore, interdisciplinary evidence from clinical nutrition, metabolic health, orthopaedics, dentistry, refugee health, and population-based health research highlights the growing importance of personalized medicine, biomarker-guided clinical assessment, patient-centered healthcare, and population-specific risk stratification. While these investigations are not directly related to hepatitis C diagnostics, they demonstrate advanced approaches to individualized healthcare that may inspire future HCV screening programs tailored to demographic, socioeconomic, and clinical risk profiles [29, 30, 37, 38, 53, 54]. Collectively, integrating molecular diagnostics with artificial intelligence, interdisciplinary biomedical research, translational laboratory science, and sustainable healthcare systems will facilitate the development of more accurate, accessible, and personalized diagnostic strategies, ultimately contributing to earlier HCV detection, improved patient management, and progress toward global hepatitis elimination goals.

Conclusion

In conclusion, screening tests for HCV (ELISA / rapid diagnostic testing) proved useful to identify HCV infection in the first instance and are highly sensitive. However, weak reactive and false positive screening results must be followed up with more specific PCR testing, which provides definitive diagnosis by sequencing the viral RNA. The use of screening testing followed by confirmatory PCR testing resulted in less misdiagnosis and the best outcome for patients was often achieved. It is recommended that all positive and weakly reactive screening test results are followed up by specific HCV PCR testing.

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