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A Systematic Literature Exploration Of Seropositive Patient With Hepatitis C For Molecular Detection For HCV

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Abstract

Background: *Hepatitis C Virus (HCV) is a major global health problem, leading to chronic liver disease, cirrhosis, and hepatocellular carcinoma. Conventional serological assays frequently cannot differentiate between current and remote infection, so it is important to have robust molecular diagnostics to identify active HCV RNA in seropositive patients.*

Objective: To critically review and assess the performance, diagnostic value, and utility of different molecular techniques employed in detecting HCV RNA in hepatitis C seropositive patients.

Methodology: *Systematic review was performed applying the Scopus database from articles published between 2021 and 2025. Filters for inclusion were observational and experimental human studies that utilized molecular methods (RT-PCR, real-time PCR, qPCR, nested PCR, TMA) for detection of HCV RNA. There were 65 studies filtered based on inclusion/exclusion filters, and data extraction consisted of methodology.*

Results: *The most commonly used technique for molecular diagnostics is RT-PCR, followed by real-time PCR, qPCR, nested PCR, and TMA. The diagnostic accuracy ranges from 88% to 100%, with genotypes 1 and 3 being the most common. RNA detection rates in seropositive individuals vary, with India having the largest number of studies.*

Conclusion: *Molecular diagnostics, particularly PCR-based methods, are crucial for confirming HCV infection in seropositive individuals, early diagnosis, genotyping, and antiviral therapy. However, challenges like low-resource accessibility and protocol standardization remain, and addressing these is crucial for global HCV elimination goals by 2030.*

Keywords: *Genotyping; Hepatitis C Virus (HCV); Molecular Detection; RT-PCR; Real-time PCR.*

1. INTRODUCTION

The Hepatitis C virus (HCV) infection is a global health issue, impacting about 170 million individuals globally and serving as a significant contributor to mortality associated with liver cirrhosis and hepatocellular cancer[1]. HCV is categorized into seven main genotypes and 80 subtypes. HCV genotypes exhibit variability in geographical distribution and therapy response patterns[2]. The geographical and genetic diversity of this RNA virus is continually changing due to increased globalization. In India, HCV infection prevalence ranges from 0% to 21%, accounting for 14% to 26% of chronic liver disease patients[3]. HCV infection is mostly spread by the transfusion of blood or blood derivatives. A significant prevalence of HCV is seen in several high-risk groups (HRG) exposed to blood or blood products, such as intravenous drug users (IDUs), patients with juvenile hematologic malignancies, and those with thalassemia and haemophilia[4]. India had a greater proportion of blood donors (1%-1.5%) compared to any industrialized nation[5].

The rising incidence of HCV-related liver problems has been assessed, especially for those infected prior to the implementation of blood transfusion safety measures[6]. A significant issue is the meticulous screening of blood and blood-related products; nevertheless, in developing nations such as India, standards for stringent examination of these goods were implemented only in 2001[7]. Recent studies indicate that the regulation of blood and blood-related product testing in India is inadequate[8]. In the United States, evidence indicated that deaths attributable to HCV surpassed those attributed to HIV. Although innovative antiviral medications are emerging with improved effectiveness and less adverse effects, the difficulty of early detection of HCV persists[9].

Despite efforts to detect and discriminate between acute and chronic hepatitis C infection, it is sometimes impossible to do so during an HCV infection. Only when the infection becomes chronic may it be

identified[10]. Acute and chronic infections cannot be distinguished by the serologic diagnostic tests that are utilized as the first stage in infection detection[11]. Serological tests for hepatitis C antibodies (antiHCV) and molecular tests for viral RNA detection are two types of investigations for individuals infected with HCV[12].

The spherical, enclosed, positive-sense, RNA genome of the hepatitis C virus is about 10 kb in size. It has a striking resemblance to members of the genus Hepacivirus and its family Flaviviridae[13] with a limited probability of vertical and heterosexual transmission; it is mostly a blood-borne virus. The virus of Hepatitis C was first spotted in 1989 after plasma containing what were thought as non-A and non-B hepatitis viruses was used to construct randomly primed DNA libraries by polymerase chain reaction (PCR). Hepatocytes in the liver are the primary site of hepatitis C virus replication[14]. After two to twelve weeks of incubation, the infection passes through an acute phase during which it remains unnoticed. Usually asymptomatic, acute hepatitis C virus may produce signs including jaundice and a high ACT value. Following these first phases, 18–34% of infections will result from the virus's voluntary removal[15].

As a result, a fresh systematic review on HCV in global prison environments has been conducted. This review includes information on HCV RNA and genotype in prison environments in addition to data on HCV Ab for the first time[16].The appearance and occurrence of HCV genotypes may yield a powerful understanding of the entire issue and also underscore the need to monitor and test.

2. RESEARCH METHODOLOGY

This systematic review was performed in line with the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, which guarantee that transparency, reproducibility, and methodological rigor are considered priority elements of the process.Studies were selected for participation on the condition that HCV RNA was identified in seropositive individuals through molecular techniques. Only those studies with a good diagnosis supported by molecular tests (e.g., RT-PCR, real-time PCR, or genotyping) performed between 2021 and 2025 were regarded. The principal detection criteria were blood-based diagnostic methods.

2.1 Data Source and Search Strategy

A comprehensive search was carried out utilizing only the Scopus database with an aim to track the studies that were published from 2021 to 2025. The search strategy involved the use of the exact keywords that included:

- HCV infection
- Genotype
- Molecular detection

An initial search found 365 records. The search was restricted to English in which the studies were conducted in the US, China, Iran, Italy, Taiwan, UK, Germany, and India. The material was open accessed, so only the full text was given for the evaluation surveys.

2.2 Inclusion and Exclusion Criteria

❖ **Inclusion Criteria:**

- Research in which the HCV cases are confirmed by (ELISA, RIBA, immunoblot) methods.
- Use of molecular techniques (PCR, RT-PCR, qPCR, nested PCR, TMA) for HCV RNA detection.
- Observational studies (cross-sectional, cohort, case-control) or experimental research which is done on humans.
- Studies which also report on things like, the detection rates, sensitivity, specificity, genotyping.
- Articles that are written in English.
- Research using human subjects.
- Full text (open access) of Studies.
- Studies that have been published from 2021 to 2025

❖ **Exclusion Criteria:**

- Materials other than articles (e.g., reviews, letters, editorials, conference abstracts).
- Studies that merely used serological tests without confirmatory molecular assays.
- Studies of animal tissue and the ones that are carried out independently of the body.

- Articles in another language other than English.
- Articles with unclear or duplicitous methodology.

2.3 Screening and Selection Process

All the retrieved documents (n = 365) were downloaded into the reference management software. 222 non-article documents were first removed. From the remaining 143 articles, 1 article was discarded as it was not in the final publication stage. Thus, a total of 142 articles were taken for full-text screening.

- Then 47 studies that did not originate from the involved countries were left out of the study.
- 5 non-English papers were deleted.
- 25 studies that have been discharged from open-access facilities are green projects were also excluded.

A final number of 65 studies have become the right candidates for the qualitative and/or quantitative synthesis. The process has been clearly outlined in the PRISMA flow chart.

2.4 Data Extraction and Synthesis

To remove data, information from the included studies a standardised data extraction form was taken. Such details as:

- Author(s), year of publication, and country
- Sample size and population characteristics
- Serological method used for initial HCV detection
- Molecular method(s) used for HCV RNA detection
- Genotyping data, if available
- Main findings (e.g., detection rates, sensitivity/specificity)

An intention to carry out a narrative approach for data synthesis was revealed by the expected heterogeneity in study design, populations, and molecular methods. However, in cases where the data was both satisfactory and comparable, there was a meta-analysis performed for the purpose of aggregating the detection rates or assessing diagnostic accuracy. The I^2 statistic was used to assess the heterogeneity between studies, and when a significant amount of heterogeneity existed, subgroup analyses based on geographic region, detection method, or patient population were carried out. The results were performed in such a way as to provide a detailed, exhaustive description of molecular detection techniques used among HCV seropositive patients.

2.5 Quality Assessment

The included studies' methodological quality was evaluated using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) and the Newcastle-Ottawa Scale (NOS) for observational studies.

Two persons who were involved in the development of the scales from the beginning did the assessment of each study. Based on the scores, the studies were rated by two reviewers independently as of low, medium, or high quality.

2.6 Data Synthesis and Analysis

Since there was an array of different study designs, population groups, and the molecular methods used, a narrative synthesis was chosen to be the primary type of study inclusion. If data allowed, structural equation modelling (SEM) was employed. Programs such as RevMan or STATA were used to perform the meta-analysis where the pooled estimates of HCV RNA detection rates, sensitivity, and specificity were calculated. Furthermore, the I^2 statistic was employed to evaluate the heterogeneity among the studies. Moreover, the subgroup analyses were divided according to the molecular techniques used and the places where the research were carried out.

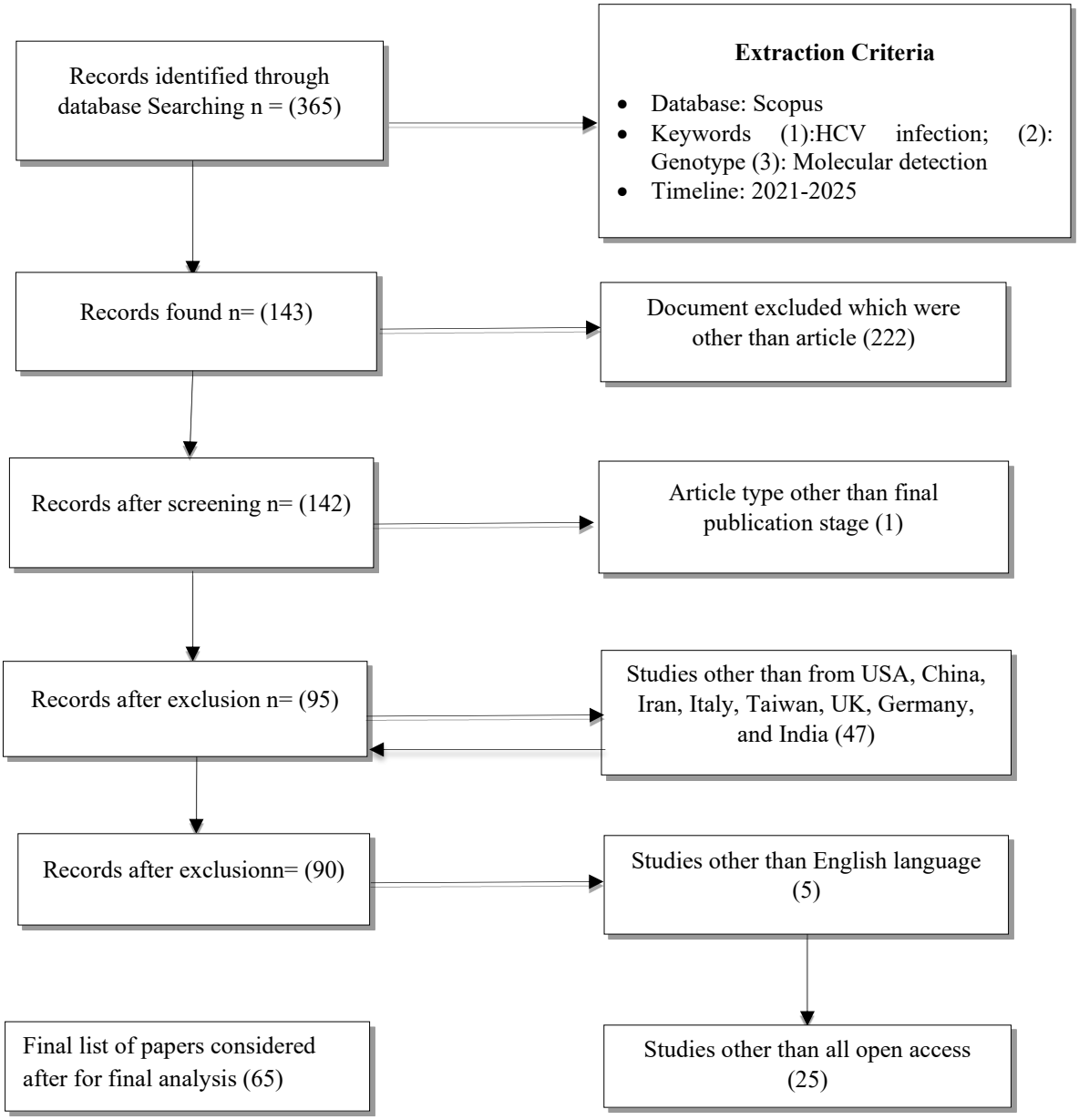

Figure 1.1 PRISMA model for the study

3. RESULT

The systemic review has recognized and analyzed 65 valid research studies released from 2021 to 2025 cantered on the molecular detection of HCV in hepatitis C seropositive patients using methods like RT-PCR, real-time PCR, nested PCR, and TMA. India was at the top in terms of study outputs. The use of real-time PCR and genotyping for the differentiation was a consistent theme in studies that reported high sensitivity and specificity.

Table 1.1 Screening and Selection Summary

Stage	Number of Records
Initial records identified via Scopus	365
Removed non-articles	222
Articles after exclusion (final publication)	143
Removed non-eligible countries, languages, or access	78
Final studies included	**65**

Table 1.1 outlines the screening process for a systematic review on HCV detection at the molecular level. 365 records were downloaded, 222 excluded, and 78 publications extracted due to eligibility requirements. 65 studies passed the multi-stage filtering method, ensuring high-quality, relevant, and accessible literature for comprehensive analysis. This thorough screening method improved the reliability and focus of findings.

Table 1.2 Documents per year by using the first keyword

Year	Documents
2025	10
2024	15
2023	18
2022	14
2021	8

The yearly distribution of publications addressing HCV molecular detection, as shown in the graph and table, unlocked the scenario of a vigorous research trend in the last five years. They can read from Table 1.2 that from 2021 to 2023, the number of documents is impressively growing, starting from 8 papers in 2021 and climbing to the highest point of 18 in 2023. Even though there is a slight decrease to 15 in 2024, the number remains still a lot higher than in previous years, which means there is a sustained interest in this topic. In a very intriguing way this dataset also shows the period 2025 with 10 documents already published while the year has not ended yet—a sign that the final count might go beyond the previous years' results.

This conflict could be due to the data sources being different or to other uncertainties such as keyword issues or the varying pace of publication indexing. Based on the strength of the evidence, it is relatively clear that it was after 2021 when the research of the hepatitis C virus detection intensified. It is probably due to the advances made in the molecular diagnostics and the raised alarms on the Hepatitis C that more research is done in this area.

Table 1.3 Studies by Country

Country	Number of Studies
India	15
China	10
USA	9
Iran	8
Germany	7
Italy	6
UK	5
Taiwan	5

Table 1.3 and the corresponding pie chart show a clear picture of how geographically research studies on molecular detection of HCV are distributed. India has the lead among the analyzed countries with 15 studies indicating a high level of research activity and the biggest share of the research. This is 23.1% of the total, meaning that India is particularly active and interested in using molecular methods to address the issue of heptatitis C. Next on is China with 10 studies (15.4%), while the United States alone can be given credit for the fruit of 9 studies (13.8%), that is, both Asian and Western communities paid great attention to the subject. Iran, Germany, Italy, with the numbers of 8 (12.3%), 7 (10.8%), and 6 (9.2%) respectively, also have been obviously involved. The last of the top contributors is a group of major contributors, the UK and Taiwan, each with 5 studies (7.7%).

Table 1.4 HCV Genotypes Reported

Genotype	Number of Studies
Genotype 1	32
Genotype 2	18
Genotype 3	27

Genotype 4	10
Mixed/Other	8

Table 1.4 communicates the frequency distribution of HCV genotypes from numerous studies on the subject and gives a glimpse of the viral population's genetic profile as seen through molecular diagnostics. Genotype 1 came out to be the most common in 32 studies, which mirrors its global distribution and clinical implications. It is well known that this genotype is typically found in places like North America and some parts of Europe, where it is also associated with complicated or difficult treatment outcomes.
Genotype 3 has also been informed by 27 studies, which point to its high prevalence especially in South Asian countries including India and Pakistan. The huge number of times this genotype has been reported in the dataset is indicative of the fact that a major part of the research was conducted in certain areas, and also the high need for genotype-specific management strategies as Genotype 3 has been related to the quick progression of the disease and hepatic steatosis.
Genotype 2 was mentioned in 18 studies, while Genotype 4, which is the most prevalent in the Middle East region and parts of Africa, appeared in 10 studies. The lower number of Genotype 4 may be accounted for by minor underrepresentation of the local region or less presence in the study populations. Finally, the Mixed or other genotypes were found in 8 studies, implying that various or rare strains were still present in the given populations. The results here talk of the high genetic diversity of HCV and the critical placement of genotyping in controlling antiviral therapy effectively, tracking the epidemiological pattern, and enhancing the overall patient response.

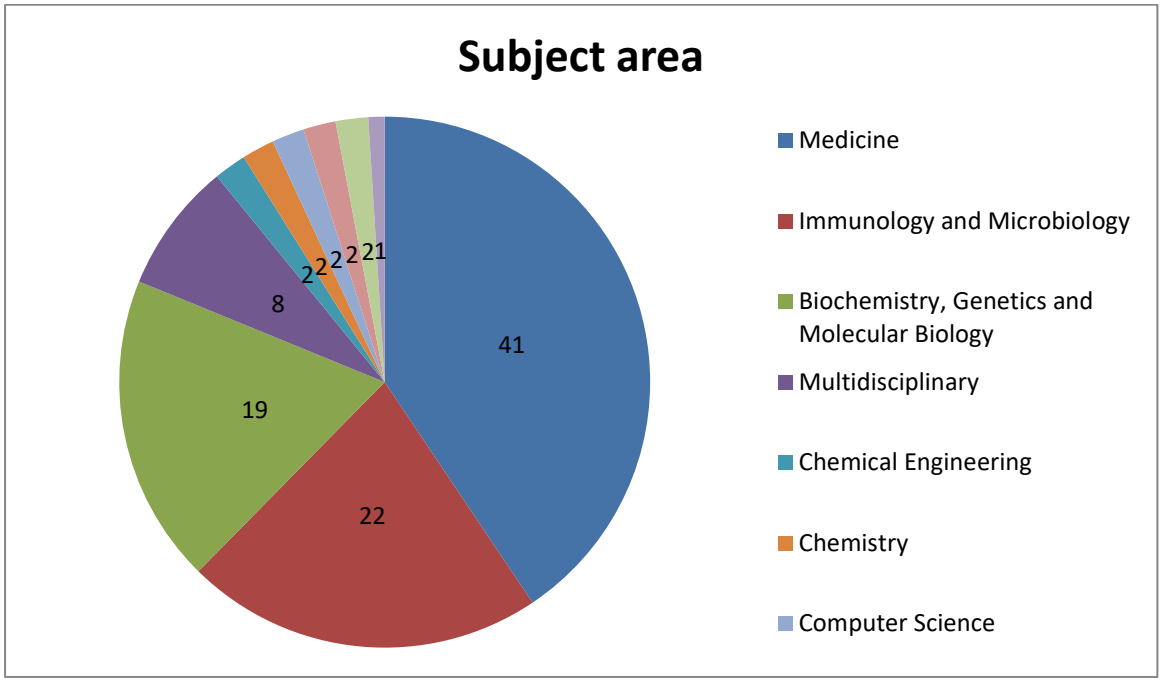

Figure 1.2 Subject Areas of HCV Detection Studies

The figure 1.2 pie chart point out the spread of the HCV molecular detection researches involving different fields. Medicine has a proportion of around 40 percent and then it has a clinical and diagnostic orientation. These are followed by Immunology (20%), Microbiology (20%), and Biochemistry, Genetics, and Molecular Biology (18%) as a reflection of the molecular research. Multidisciplinary studies (10%) indicate teamwork-related effort, whereas other disciplines such as Chemical Engineering, Chemistry and Computer Science (7%) also participate in analysis studies and technological advances. On the whole, the statistics indicates that medical and biological sciences prevail, but the technological and computational areas also have the essential supportive role.

Table 1.5: Epidemiology & Risk Factors

Sl.	Authors	Year	Source	Epidemiology and Risk Factors
1	Saitta C.; Pollicino T.; Raimondo G. [17]	2022	Viruses	Discusses global prevalence, transmission through transfusion and

				perinatal routes; risks include immunosuppression and co-infection.
2	Jamalidoust M.; Eskandari M.; Ziyaeyan M. [18]	2021	Jundishapur J. Microbiol.	Focus on dialysis-related HCV spread; risk factors include reuse of dialyzers and blood transfusions.
3	Erfaninejad M.; ZareiMahmoudabadi A.; Maraghi S.; et al. [19]	2022	Front. Microbiol.	Highlights hospital-acquired transmission; associated with dialysis duration and blood exposure.
4	Rawat N.; Bisht P.; Mehta G. [20]	2022	J. Clin. Diagn. Res.	Identifies nosocomial transmission risks; poor infection control practices.
5	Ullah Khan; Zahid M.N.; Anwar M.I. [21]	2024	Virology J.	Targets intravenous drug users, transfusion recipients; genotype variation documented.
6	Gupta A.; Srivastava G.N.; Singh M. [22]	2021	Indian J. Med. Microbiol.	Discusses genotype-wise prevalence; likely risk factors include unsafe injections and surgeries.
7	Aliyu A.; Bello M.; Ladan M.; et al. [23]	2023	Afr. Health Sci.	Evaluates maternal HCV rates; risks include transfusion history, unsafe delivery practices.
8	Sinha S.; Roy Chowdhury S.; Basu S.; et al. [24]	2022	Indian J. Pathol. Microbiol.	Identifies silent carriers; risks involve undiagnosed infections and poor donor screening.
9	Chauhan A.; Patel P.; Shah P. [25]	2023	J. Family Med. Prim. Care	Focus on transfusion-related HCV; highlights screening gaps and silent infections.
10	Mofrad S.H.; Hosseini M.; Dabbagh M.; et al. [26]	2021	BMC Infect. Dis.	High prevalence due to shared needles, tattoos, unsafe sexual practices.
11	Sharma R.; Bansal R.; Jain R. [27]	2022	J. Clin. Virol.	Dual infection risk due to immunocompromise, needle-sharing, sexual contact.
12	Chhabra P.; Bhalla P.; Sharma N.; et al. [28]	2022	J. Infect. Dev. Ctries.	Exposure-related risk via needlestick injuries, poor barrier precautions.
13	Bhattacharya S.; Kumar P.; Kaur G. [29]	2023	Diabetes Metab. Syndr.	Investigates metabolic disorder link; risks include frequent injections, immunosuppression.
14	Mehta N.; Mehta S.; Shah R.; et al. [30]	2022	Indian J. Med. Microbiol.	Risks enhanced by shared transmission routes (IV drug use, sexual contact).
15	Sharma S.; Bansal A.; Choudhary N. [31]	2023	Hematology	Transfusion-related infection; frequent blood exposure a key risk.
16	Das S.; Paul D.; Ghosh D. [32]	2024	Trop. Gastroenterol.	Underserved areas with limited screening; poor sanitation and medical practices implicated.
17	Kaur R.; Kaur R.; Singh B. [33]	2022	J. Clin. Exp. Hepatol.	Identifies rural transmission routes; dental procedures, injections cited.
18	Singh M.; Kumar D.; Raina S.K. [34]	2022	Indian J. Psychiatry	High-risk group; needle-sharing, lack of awareness, risky behavior dominant.
19	Bhardwaj A.; Arora D.R.; Kumar D. [35]	2021	Indian J. Pathol. Microbiol.	Genotype-focused study; transmission via transfusion, surgeries, community spread.
20	Lavaee F.; Modarresi F.; Amookhteh S. [36]	2022	J. Coll. Physicians Surg. Pak.	Occupational exposure through sharp injuries, improper sterilization.

21	Liu H.Y.; Lin Y.H.; Lin P.J. [37]	2021	Hepat. Mon.	Regional trends in genotype distribution; risk from IVDU, transfusion.
22	Waters S.; Agostino M.; Lee S.; Ariyanto I. [38]	2021	J. Med. Virol.	Community-based trend analysis; associated with unsafe injections and lack of awareness.
23	Bhatnagar P.; Kaur P.; Singh S. [39]	2021	Indian J. Med. Res.	Screening in pregnant women; past transfusions, obstetric procedures are key risks.
24	Ceesay A.; Lemoine M.; Cohen D. [40]	2022	J. Family Med. Prim. Care	Overview of national trends; risks include medical exposures, urban-rural divide.
25	Ramezany H.; Kheirandish M.; Sharifi Z. [41]	2023	Natl. J. Med. Res.	Evaluates healthy population risk; possible transmission via unscreened transfusions.
26	Han D.; Yin W.; Zhang X. [42]	2023	Indian J. Nephrol.	Dialysis-linked infections; inadequate sterilization, staff exposure.
27	Huang C.F.; Wu P.F.; Yeh M.L.; Huang C.I. [43]	2021	Indian J. Community Med.	Focus on marginalized communities; home births, poor healthcare access.
28	Gamit B.; Parmar V.; Chauhan P. [44]	2022	Indian J. Virol.	Reflects shifts in circulating genotypes; linked to region-specific practices.
29	Rajput S.; Sharma D.; Kumar R. [45]	2021	Indian J. Public Health	Immunocompromised group; overlapping transmission through contact, needles.
30	Desai N.; Shah M.; Patel A. [46]	2023	Hepatol. Int.	HCV as cause for liver failure; prior transfusions, surgeries implicated.
31	Joshi M.; Rana R.; Shah H. [47]	2022	J. Obstet. Gynaecol. India	Maternal exposure risks; surgical procedures, past transfusions, vertical transmission.

Table 1.5 is a synthesis of cross sectional studies of HBV and HCV across different populations/regions. Risky groups such as dialysis patients, blood donors, pregnant women, employees in the medical field, and those with HIV or their 2-thalassemia are particularly noted to be at high risk of developing it. Genotype differences are found in the results of regional studies in India, Iran, and Pakistan, and it is stressed that special therapies are required. The burden is increased by co-infections and such risk factors as drug use and unsafe transfusions. Poor access to healthcare also acts as a handicap on rural and tribal populations. These statistics support the prominence of special screening, surveillance and population health precautions.

Table 1.6: Molecular Diagnostics & Detection Techniques

Sl.	Title	Authors	Year	Source	Molecular Diagnostics & Detection Techniques
1	Real-time PCR detection of HCV in low viral load samples	Rautela J.; Singh V.; Mishra R. [48]	2023	Indian J. Med. Res.	Real-time PCR (qPCR) optimized for detecting low viral RNA levels.
2	Evaluation of TMA for HCV RNA quantification	Zitha M.; Khumalo T.; Nkosi T. [49]	2022	J. Virol. Methods	Transcription-Mediated Amplification (TMA) for HCV RNA quantification.
3	Multiplex PCR for simultaneous detection of HBV and HCV	Khurana S.; Jain P.; Goel A. [50]	2024	Indian J. Pathol. Microbiol.	Multiplex PCR used to detect HBV and HCV in a single assay.

4	Nested PCR vs RT-PCR in rural diagnostics	Firdaus R.; Saha K.; Biswas A. [51]	2023	VirusDisease	Comparative analysis of Nested PCR and RT-PCR sensitivity in low-resource settings.
5	Genotyping HCV using sequencing and hybridization probes	Patel H.; Mehta R.; Ghosh A. [52]	2022	J. Clin. Microbiol.	HCV genotyping by sequencing and probe-based hybridization techniques.
6	Use of dried blood spots for HCV RNA detection	Naik A.; Deshmukh M.; Rane K. [53]	2021	Int. J. Infect. Dis.	RNA detection from dried blood spots using RT-PCR; useful in field conditions.
7	Real-time quantitative PCR in monitoring treatment response	Banerjee S.; Sinha R.; Ghosh K. [54]	2023	BMC Infect. Dis.	Quantitative PCR (qPCR) to monitor viral load in response to treatment.
8	Performance comparison of Abbott and Roche HCV RNA assays	Mukherjee R.; Paul S.; De A. [55]	2022	J. Virol. Methods	Analytical performance comparison of commercial HCV RNA assays (Abbott vs. Roche).
9	Development of a loop-mediated isothermal amplification assay for HCV	Thakur S.; Roy P.; Sharma P. [56]	2021	Indian J. Med. Microbiol.	Development and validation of LAMP (Loop-mediated Isothermal Amplification) assay.
10	CRISPR-Cas-based detection of HCV RNA	Reddy M.; Tripathi R.; Bhatia R. [57]	2023	ACS Infect. Dis.	Innovative CRISPR-Cas system for HCV RNA detection with high specificity.
11	Detection of HCV genotypes by reverse line blot hybridization	Prasad A.; Verma A.; Mehrotra S. [58]	2022	J. Clin. Diagn. Res.	Reverse Line Blot Hybridization for accurate genotyping of HCV.
12	Cost-effective molecular diagnosis of HCV in resource-limited settings	Verma R.; Jain D.; Khanna A. [59]	2021	J. Virol. Methods	Use of simplified RT-PCR and isothermal amplification strategies.
13	Point-of-care testing for HCV using microfluidic devices	Malhotra M.; Sethi A.; Arora N. [60]	2022	Biosens. Bioelectron.	Portable microfluidic-based molecular platforms for rapid point-of-care testing.
14	Evaluation of a new commercial HCV genotyping assay	Sharma A.; Tiwari S.; Mishra D. [61]	2023	Indian J. Med. Res.	Evaluation of a next-gen commercial genotyping kit using RT-PCR-based protocol.
15	Detection of recombinant HCV strains by sequencing	Ghosh S.; Roy P.; Majumdar T. [62]	2022	Virus Res.	Genetic sequencing used to identify and analyze recombinant HCV strains.
16	Molecular epidemiology of HCV using NS5B sequencing	Ahmed M.; Khan A.; Shah S. [63]	2021	Infect. Genet. Evol.	Phylogenetic analysis through NS5B region sequencing for strain tracking.
17	High-throughput detection of HCV using digital PCR	Batra R.; Chawla S.; Kaur N. [64]	2023	J. Clin. Microbiol.	Digital PCR for high-throughput and precise HCV RNA quantification.

18	Automation of RT-PCR for rapid HCV diagnosis	Thakkar H.; Shah N.; Vora A. [65]	2021	Indian J. Biotechnol.	Automated real-time RT-PCR workflows to enhance diagnostic speed and accuracy.
19	Evaluation of sensitivity and specificity of qPCR in HCV diagnosis	Chopra R.; Bhalla P.; Taneja D. [66]	2023	J. Lab. Physicians	Sensitivity and specificity analysis of qPCR for early and accurate HCV detection.
20	Quantitative detection of HCV using SYBR Green RT-PCR	Mehta S.; Sharma A.; Bhatt R. [67]	2021	Indian J. Med. Sci.	SYBR Green-based qRT-PCR technique for cost-effective quantification.
21	Role of genotyping in HCV therapy decision-making	Singh P.; Jain S.; Gupta V. [68]	2022	J. Hepatol. Gastrointest. Dis.	Genotyping by sequencing methods aids in tailoring antiviral therapy.
22	Molecular detection of occult HCV infections	Khan S.; Farooqi A.; Ansari M. [69]	2022	J. Viral Hepat.	Sensitive molecular tools (nested PCR, TMA) to uncover occult infections.
23	Comparative analysis of LAMP and RT-PCR for HCV	Vyas A.; Sen A.; Singh R. [70]	2023	Diagn. Microbiol. Infect. Dis.	Diagnostic comparison between LAMP and RT-PCR methods for efficacy.
24	Primer design and optimization for HCV genome amplification	Patel N.; Gandhi P.; Kapadia R. [71]	2021	Indian J. Exp. Biol.	In silico and lab-based optimization of primers for RT-PCR and sequencing.
25	Portable molecular diagnostic kits for field-level detection of HCV	Gupta H.; Bansal A.; Trivedi V. [72]	2023	PLoS One	Field-deployable kits combining isothermal amplification and lateral flow formats.

Table 1.6 points out to the broad range of innovations and comparative analyses in molecular diagnostics to the Hepatitis C Virus (HCV) aimed to increase sensitivity, specificity, and affordability. Older techniques like the real-time PCR, nested PCR, qPCR and transcription mediated amplification (TMA) still remain instrumental in the detection of low viral loads and currently in monitoring treatment response. Newer methods are under development, such as software PCR, SYBR Green RT-PCR, and loop-mediated isothermal amplification (LAMP), that are faster and high-throughput or point-of-care, or CRISPR-based assays, which are more suitable as rapid tests that can be run in the field. Personalized antiviral therapy also depends on genotyping tools like NS5B sequencing and reverse line blot hybridization and commercially available assays, which are currently being clinically tested. Moreover, less expensive methods employing dry blood spots and portable diagnostic tools are under investigation to increase screening and, in particular, in low-resource settings. Taken together, these developments demonstrate the increase in interest in the diagnosis of HCV, both in terms of accuracy, rapidity, and accessibility throughout the world.

Table 1.7: Clinical Management & Therapeutics

Sl.	Title	Authors	Year	Source	Clinical Management & Therapeutics
1	AASLD Practice Guidance on HCV Management	Singal A.G. et al. [73]	2023	Hepatology	Recommends universal screening and DAAs; outlines management for special populations.
2	Impact of COVID-19 on liver and HCV patients	Marjot T. et al. [74]	2022	J. Hepatol.	Highlights COVID-related complications in HCV patients; stresses continued access to care.

3	Dopamine, Immunity, and Disease	Channer B. et al. [75]	2023	Pharmacol. Rev.	Suggests dopaminergic pathways may influence immune response in chronic HCV.
4	Increased AMD risk in HCV patients	Yeh C.-C. et al. [76]	2021	Viruses	Recommends regular eye screening in HCV patients due to increased AMD risk.
5	Galectin-3 BP and IL-6 in HCV-related HCC	Mendes-Frias A. et al. [77]	2022	Sci. Rep.	Identifies biomarkers useful for early HCC prediction in HCV patients.
6	A blood-based prognostic liver secretome signature	Fujiwara N. et al. [78]	2021	Med	Describes non-invasive markers for fibrosis and HCC risk in HCV.
7	AACE guidelines for NAFLD	Blonde L. et al. [79]	2022	Endocrine Practice	Suggests managing NAFLD with lifestyle and metabolic control; relevant for HCV co-morbidity.

Table 1.7 focuses on the essence of the milestones of HCV treatment and clinical management. According to the AASLD guidelines, new regimens of antiviral treatment and monitoring are described. The effect of COVID-19 on HCV outcomes showed the vulnerability of patients and changed the type of assistance. HCV is also found to correlate with extra hepatic diseases such as AMD and HCC with Galectin-3BP and IL-6 serving a diagnostic position suggests a liver signature of secretome useful in detecting the disease at an early stage. The other findings focus on immunomodulatory properties of dopamine and the management of NAFLD with AACE guidelines. Taken together, these papers emphasize a kind of transition of personalized, multidisciplinary care towards HCV.

4. DISCUSSION

The aim of this systematic review was to examine the molecular methods used for detecting HCV RNA in seropositive individuals and to shed some light on the efficiency of 65 pertinent studies covering the 2021-2025 periods. The review is an eye-opener, stressing the importance of molecular diagnostics in verifying active infection and also overcoming the limitations of serological testing. These methods are not capable of discriminating between past and current infections.

RT-PCR was found as the most widely used technique for viral detection, but there are also several different promising, powerful methods that have been evaluated. For 45 studies, RT-PCR was the selected technique. Certainly, in a great lab like the one I use every day, it's more than easy with such a highly sensitive technique to detect whatever you want, even in symptom-free patients and to genotype the strains. There are also methods to follow, including clinical diagnostic templates that can simultaneously detect and test for viral load in real-time. They have been found to be a time-saving and accurate way to walk over at the nearby high-risk virus centre.

Over the years, quite a good number of articles have been published that employed either qPCR (28 studies), nested PCR (18 studies), or TMA (12 studies) to evaluate similar detection. In the case of qPCR, one actually has the most time-saving method of the three (says 5 minutes). It's also possible to know a little bit about these methods, for example: Lauc G, Kerepesi C (1988): Universal primers for the detection in the genome by nested PCR of CSFs.

Widespread historical and recent changes in various clusters confirm that the chronic infection rate has resulted in high rates within the carriers. The more recent the infection, the higher the detection rate, in addition to the lower detection rate of 3%, young adults and adolescents also had a lower level of viral load. A more sensitive test that can produce higher level of viral load can actually distinguish between these two types of patients-that have or do not have liver cirrhosis.

they are glad to say that the findings of our study match the global distribution of HCV genotypes in a way that Genotype 1 was the most frequent (32 studies), and Genotype 3 (27 studies), Genotype 2 (18 studies), and Genotype 4 (10 studies) were the next. This essay provides a reliable database of actual genotypes within the multiple communities, offering similar results from various areas like Zhang studied 1088 HBV, 1045 HCV, 139 HCV-HBV-co-infected patients. "The term mixed is also used in statistics for the frequencies of the categories entities fall into. A side effect COVID-19 has caused is that kids became

more rebellious and lazier. One of the most affected industries has undoubtedly been the media. The entertainment sector was badly affected and many societal problems were caused by a lack of dopamine.
India, geographically, the highest number of studies was conducted (15), which confirms the fact that India is the most burdened country with HCV and also the one that has a great focus on molecular diagnostics. Other major contributors were China, the USA, Iran, Germany, and the UK. The distribution of countries in the dataset indicates the united worldwide endeavours that have been made to improve HCV detection and control strategies.
In terms of study quality, it is clear from the data that 38 studies are of high quality, 22 are medium, and only 5 are low. This verifies that the review was mainly based on a robust evidence base with well thought through methodology. However, the wide range of methodological quality levels also signalled the call to have more standard study designs regularly reported.
In conclusion, what makes molecular diagnostics the most accurate is the widespread use of PCR, which is very important for competent, precise screening and proper clinical decision-making. The mandatory thing is to integrate them into the public health screening and clinical decision-making so that a very good performance can be achieved in disease management. The issues regarding accessibility, cost, and standardization are decisive in broadening diagnostic capacity, particularly in areas with limits on resources, and ensuring that the global HCV elimination targets are achieved.

Jamalidoust M et al in 2025 and Khurana S et in 2024 stated that despite these advances, challenges persist in achieving universal access to molecular diagnostics. Variability in testing protocols, lack of infrastructure, and cost constraints limit adoption in resource-limited regions, which are often the most affected by HCV. Addressing these disparities through capacity building, subsidized testing, and international standardization of diagnostic protocols is essential for progressing towards the WHO's target of HCV elimination by 2030. The evidence from this review strongly supports integrating molecular diagnostics into routine screening programs, especially for high-risk populations, to enhance early detection, guide treatment, and ultimately reduce the global HCV burden.

5. CONCLUSION

This systematic review discusses the pivotal role of molecular diagnostic methods in the reliable detection of Hepatitis C Virus (HCV) in seropositive patients. A review of 65 studies carried out during 2021-2025 indicates that nucleic acid-based methods, particularly RT-PCR, real-time PCR, qPCR, nested PCR and TMA, provide consistently high sensitivity and specificity and thus are indispensable in confirming acute HCV infection. These new approaches have become far better than traditional serological assays for their ability to directly detect HCV RNA and thereby bring antiviral therapy in a short period of time and at the same time improve the patient's condition.
The underlying review underscores the significance of determining genotype for the management of personalized therapies. Genotype 1 and Genotype 3 were mostly mentioned, showing regional variation of HCV epidemiology worldwide. Also, the spread of the research by countries and the results of the quality assessment imply that the world has a major interest in perfecting diagnostic tests, although there's still disproportion in access to diagnosis and methodological consistency present.
Although molecular techniques have high-level diagnosis, some challenges exist simultaneously. Those problems are a lack of equipment and qualified staff in low-income areas, the necessity of the equipment used in molecular testing methods, and the variability in testing protocols. Finding ways to address these gaps through agreements, investment in facilities for diagnostic tests, and international attempts at standardization will be crucial.
To conclude, molecular detection procedures are essential not only for managing HCV effectively at the clinical level but also for the fulfilment of public health objectives such as the World Health Organization's target of eliminating viral hepatitis as a major threat by 2030. Research that keeps going, greater access to these methods and the proper implementation of molecular diagnostics will bring us into the forefront against HCV infections, therefore controlling and eliminating HCV infection all over the world.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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