REVIEW

Quantitative Real-Time PCR testing in the control of hepatitis B and C: Progress and challenges towards eradication by 2030

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Worldwide, chronic hepatitis B and C infections remain a significant public health challenge, causing millions of cases of liver disease globally.

The objective of this article is to highlight the need for testing and monitoring hepatitis B and C virus infections using Real-Time PCR, as well as to analyze the implementation of strategies for the eradication of hepatitis in accordance with WHO targets for 2030.

This narrative review highlights the necessity, performance, advantages, limitations, and challenges of implementing Real-Time PCR testing in clinical practice and public health policies for hepatitis B and C.

The results show that Real-Time PCR has superior sensitivity and specificity in the early detection of active infection and monitoring of viral load, facilitating optimal therapeutic management. Serological testing retains its essential role in initial screening, identifying exposure to viruses. Vulnerable groups, including hemodialysis patients, people who inject drugs, HIV-positive patients, healthcare workers, and marginalized populations, have increased prevalence and require prioritization in testing. The main limitations reported include unequal access to PCR technology and potential technical errors. Proposed strategies for improving testing include expanding access to molecular techniques, awareness campaigns, standardization of protocols, and international collaborations to support screening and treatment.

The conclusions emphasize that integrating serological testing with Real-Time PCR and focusing on vulnerable groups are crucial for achieving the objectives.

Keywords: Real-Time PCR, hepatitis, molecular diagnosis, eradication

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Introduction

Chronic viral hepatitis B and C are a major global public health problem, affecting over 300 million people and responsible for a significant number of deaths from complications such as liver cirrhosis and hepatocellular carcinoma [1]. The World Health Organization estimates that by 2030, 4.5 million premature deaths can be prevented in resource-limited countries through public health interventions such as vaccination, early diagnosis, antiviral treatment, and public education [2].

In the current context, where modern antiviral treatments offer real prospects for controlling and even eradicating viral infection, early diagnosis and effective patient monitoring have become major priorities in medical practice. [3]. Real-time PCR provides accurate detection and quantification of viral genetic material with high sensitivity and specificity, making it the gold standard. Serological testing remains important in the initial screening of vulnerable groups, but PCR is essential for confirming active infection and evaluating treatment [4].

Quantitative testing by Real Time PCR allows accurate measurement of HBV DNA and HCV RNA in the patient's blood, providing critical information for initiating treatment, evaluating virological response, and adjusting therapeutic decisions. The method offers high sensitivity (recent studies have shown detection down to 2.5 ≈IU/mL for HBV, and in the case of HCV, a sensitivity of up to 1000 copies of RNA per reaction), well-calibrated standard lines, and a wide dynamic range (up to 10^7–10^9 IU/mL), making it indispensable in clinical patient monitoring [5,6].

Other advantages of Real-Time PCR technology are high specificity (exclusive reaction with target viral sequences), reproducibility (consistent results in repeated tests, both within the same laboratory and between laboratories, with variations below 0.3 $\log_{10}$ IU/mL, essential for tracking viral dynamics over time) and WHO standardization (most commercial tests are calibrated against international standards) [7–9].

This article aims to provide a critical narrative synthesis of recent evidence from various studies, including meta-analyses, cohort studies, and clinical guidelines, highlighting the necessity, performance, advantages, limitations, and challenges of implementing Real-Time PCR testing in clinical practice and public health policies, with a focus on the impact on the WHO's goal of eliminating hepatitis B and C by 2030.

Methods

This article is a narrative review based on an extensive literature synthesis of relevant meta-analyses, cohort studies, narrative reviews, and clinical guidelines obtained from PubMed and official sources such as WHO and CDC. Un-

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like a systematic review, strict study selection criteria, data extraction protocols, and risk of bias assessments were not performed. The aim was to provide an overview of the current status, advantages, limitations, and challenges in the use of quantitative Real-Time PCR testing for hepatitis B and C management.

Indications for quantitative Real-Time PCR testing in hepatitis B and C infections are summarized in international guidelines such as EASL and AASLD. These include diagnosis confirmation, treatment initiation and monitoring, detection of antiviral resistance, and prevention of reactivation in immunocompromised patients. The detailed recommendations can be found in the cited guidelines [10,11].

The WHO, EASL, and AASLD guidelines emphasize the essential role of Real Time PCR in the diagnosis and management of hepatitis B and C, with some differences in application. For hepatitis C, all recommend testing viral RNA by PCR to confirm infection and document sustained virologic response (SVR), but WHO highlights simplified approaches, such as reflex or point-of-care tests, adapted to resource-limited settings. In hepatitis B, PCR for viral DNA is the standard for assessing disease activity and monitoring therapy; EASL and AASLD propose criteria based on specific viral DNA levels, while WHO recommends more accessible thresholds and alternatives when PCR is not available. Thus, all guidelines recognize Real Time PCR as the reference method for infection control and eradication, with differences mainly related to treatment thresholds and feasibility of implementation [12,13].

Across Central and Eastern Europe, including Romania, access to molecular testing for hepatitis B and C viruses remains constrained by financial and logistical barriers, despite clear recommendations from international guidelines. The World Health, the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases, all emphasize the essential role of quantitative Real-Time PCR in confirming diagnosis, monitoring viral load, and guiding therapeutic decisions. In practice, challenges such as the high cost of NAT assays, the centralization of laboratory facilities, administrative delays in reimbursement, and logistical issues related to sample transport continue to reduce accessibility and delay antiviral treatment initiation. To address these barriers, the guidelines recommend reflex testing, the implementation of point-of-care technologies, and the use of dried blood spot samples to simplify diagnostic pathways and minimize patient loss across the care cascade [14,15].

The most vulnerable categories requiring attention for testing

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections affect certain vulnerable groups differently, for whom testing and monitoring are essential in preventing transmission and managing the disease. These groups are often exposed to increased risks due to socio-economic conditions, limited access to healthcare services, and risky behaviors, which justifies focusing testing efforts on these populations [16]. High-risk groups that need to be prioritized for testing are hemodialysis patients, pregnant women, injecting drug users, people with other comorbidities or infections (e.g., HIV), exposed healthcare workers, and marginalized communities. Targeted testing in these groups reduces transmission and helps achieve the WHO goal of eradication by 2030 [17].

Immigrants and migrants from regions with high prevalence

In a prospective multicenter study conducted in southern Italy, 3,501 unregistered migrants and refugees were involved, of whom 97.6% agreed to be tested for hepatitis C virus (HCV) infection after a dedicated educational session. Of those tested, 4.7% were positive for anti-HCV antibodies, and 28.6% of these had active infection confirmed by the presence of viral RNA. People with active infection were generally older and had been in Italy for a longer period of time. Treatment with direct antivirals for 12 weeks, administered to 90.5% of HCV-RNA positive patients, resulted in a cure rate of 97.9%, with no adverse events reported. This integrated model, comprising education, testing, and immediate treatment, is proving effective in achieving HCV elimination targets in vulnerable populations [18].

Hemodialysis patients

The overall prevalence of hepatitis B infection in hemodialysis patients was analyzed, and an average rate of 7.32% with a 95% CI was found based on 795,000 cases measured by the presence of HBsAg (7.57%) and viral DNA (6.09%). Prevalence varies between regions, from 4.32% in North America to nearly 10% in South America. Detection of HBV DNA by PCR confirmed active infection, with high prevalences detected in HBsAg-positive patients. The predominant genotypes in some regions are D and A. The authors emphasize the need for rigorous implementation of preventive measures and continuous monitoring of dialysis patients [19].

Healthcare workers and other professionals exposed to blood

According to a study analyzing the prevalence of occupational exposure to hepatitis B and C viruses among medical staff in a military hospital with 232 employees, it was found that 20.1% of employees had been exposed in the last 12 months, and only 37.8% of incidents were reported. The study highlights the significant risks to healthcare workers and recommends strengthening education, clear reporting and testing systems, and supportive policies for the protection of employees and patients [20].

Homeless people, marginalized people, socially excluded groups

A study conducted among 534 homeless adults in Los Angeles, finding a 43% prevalence of hepatitis B (HBV) and C (HCV) virus infection, of which 72% were unaware of their infection. Lack of awareness of positive status was linked to older age, risky sexual behavior, lack of a primary care physician, and inadequate case management. The Gelberg-Andersen behavioral model explained these relationships, highlighting the need for increased screening and case management to detect and treat hepatitis among homeless individuals [21].

HIV-positive individuals

HIV-positive individuals are at increased risk of HBV and HCV coinfection, with a more severe clinical course. In an observational study of 58,239 HIV patients undergoing antiretroviral treatment, co-infection with hepatitis B and C viruses was identified in 11.5% and 6.6% of participants, respectively, and triple co-infection in 1.5%. The presence of co-infection was associated with a significant increase in mortality compared to HIV mono-infection, with an adjusted hazard ratio for death of 1.42 for HIV+HBV, 1.65 for HIV+HCV, and 2.23 for triple co-infection. Co-infections also increased the risk of antiretroviral therapy discontinuation, highlighting the need for an integrated and proactive approach to diagnosis and treatment in this vulnerable population [22].

Pregnant women

Studies have shown that the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections among pregnant women, highlighting a significant risk in low- and middle-income countries. The results show an overall prevalence of HBV of approximately 4.8% (95% confidence interval: 3.8%-5.8%) and for HCV around 1% (95% confidence interval: 0.8%-1.3%), with higher values in poorer regions. The included studies highlight that vertical (mother-to-child) transmission of HBV is influenced by the presence of specific serological markers (HBeAg), with transmission risks ranging from 12% to up to 90%, and that cesarean delivery can reduce the risk. For HCV, the risk of perinatal transmission is lower (below 5%), but increases in cases of co-infection with HIV. The meta-analysis highlights the importance of screening during pregnancy, monitoring viral status, and specific prophylactic strategies, including antiviral treatments and vaccination, to reduce transmission and maternal-fetal complications [23].

The importance of Real-Time PCR testing for hepatitis B and C

Numerous meta-analyses and recent studies published in PubMed have evaluated the diagnostic performance of Real-Time PCR tests, highlighting their advantages in monitoring response to antiviral therapy, detecting low-level viremia, and identifying asymptomatic forms of infection. High sensitivity and specificity lead to more accurate therapeutic decisions and very good results in patients [24].

The impact of viral load monitoring on therapeutic management

The implementation of rigorous quantitative monitoring of viral load by Real-Time PCR has demonstrated a reduction in the risk of disease progression by up to 55% in well-monitored cohorts, through prompt adjustment of antiviral therapy according to viral load dynamics [25].

Real-time PCR testing has also proven effective in identifying asymptomatic forms, including occult infections, which is important in preventing transmission and adapting public health strategies [26,27].

A retrospective study of a cohort of over 2,000 HBV patients showed the diversity of viral genotypes, with direct implications for response to therapy and disease progression. Molecular testing enabled the identification of genotypes and resistant mutations, informing personalized therapeutic decisions. The authors emphasize that the widespread implementation of molecular testing in hepatitis control programs allows for treatment optimization and increases the chances of eradication by adapting interventions to the specific viral profile [28].

Diagnostic performance of Real-Time PCR

An internal quantification method was developed using Real-Time PCR with the TaqMan MGB system to detect and measure the viral load of hepatitis B (HBV) and C (HCV) viruses in serum. The method uses specific primers and TaqMan probes, validated with international standards and standard plasmids. The standard curve showed a linear relationship between 19 IU/mL and 1.9 × 10^9 IU/mL, with a coefficient of determination (r^2) of 0.99. The detection limit was 190 IU/mL, and the correlation coefficient between cycle threshold values and viral concentrations was 0.983 for HBV and 0.963 for HCV. The method was shown to be accurate and sensitive for routine diagnosis and confirmation of ambiguous serological results, being particularly useful in immunocompromised patients. [29].

According to another study, a Real-Time PCR method was developed for the quantitative detection of the M204V mutation of the hepatitis B virus (HBV), a mutation that confers resistance to nucleoside antiviral inhibitors such as lamivudine. The method uses specific fluorescent primers and probes to highlight the amplification of mutated DNA, with a very good linear correlation between the Ct value and DNA concentration (R^2 = 0.996). The detection limit is 10^3 copies/mL, and the test has demonstrated 92.86% sensitivity and 100% specificity compared to direct sequencing. This method is fast, reproducible, and accurate, allowing for effective monitoring of viral resistance and providing a useful tool for early adjustment of antiviral therapy in patients with chronic hepatitis B [30].

An analysis of HCV screening in DBS (dried blood spot testing) was also performed, including 10 studies for HCV-RNA (most using Real-Time PCR). An overall sensitivity of 97.8% and specificity of 99.2% were calculated. Thus, the authors confirm the diagnostic utility of qPCR

with DBS in resource-limited countries. [31]

Comparison with serological methods

Several studies have evaluated the performance of molecular tests compared to serological tests for diagnosing HBV infection. Molecular tests based on the detection and quantification of viral DNA showed a sensitivity of over 95% and superior specificity, facilitating the diagnosis of occult infection and early stages in situations where serology is insufficient. This high level of accuracy recommends the use of molecular tests as a central method in organized screening programs, contributing significantly to the eradication of hepatitis B through the identification and comprehensive monitoring of active cases. [32].

The importance of quantifying viral load

A review of 103 studies evaluated over 16,000 patients in the indeterminate phase of HBV, showing that antiviral therapy reduces the risk of severe liver complications by approximately 40-55%, as measured by a significant decrease in viral load. Viral load assessment was predominantly performed by Real-Time PCR, with an average detection limit of 10 IU/mL, allowing treatment doses to be adjusted according to viraemia dynamics. The results underscore the importance of frequent checks to monitor the effectiveness of therapy in this group of patients [33].

Following another analysis of 18 studies involving 9,773 patients, it was reported that approximately 33.6% of patients treated with antivirals had low-level viremia (LLV), defined as a detectable viral load below 2,000 IU/mL. LLV was statistically associated with an increased risk of progression to cirrhosis and antiviral resistance, highlighting the need for regular monitoring by Real-Time PCR. The sensitivity of the PCR tests used in the studies was generally below 10 IU/mL, ensuring quantitative detection even of minimal viral loads [34].

Limitations and practical considerations

- Detection of HBV DNA in serum does not always accurately reflect the actual level of hepatic cccDNA, the viral form responsible for persistent infection. PCR may underestimate cccDNA due to the presence of replicative intermediate forms (rcDNA, RI) that interfere with specific quantification. This limitation is well documented in recent guidelines on the methodological optimization of cccDNA quantification by qPCR, which recommend strict extraction controls and experimental validation[35].
- Viral load values (HBV DNA) can vary significantly depending on the technological platform used (e.g., different kits or extraction methods), which justifies re-monitoring the patient with the same method to maintain consistency and reduce inter-platform variations [36].
- In the presence of HIV or HCV co-infections, or under immunosuppressive treatment, viral load interpretation should be performed with great caution:
  - In HBV–HCV coinfection, bidirectional suppression of viral replication has been observed, in the sense that HCV often inhibits HBV replication, leading to lower HBV DNA values compared to monoinfection. After treating HCV with DAAs, HBV reactivation may occur[37].
  - In HIV–HBV coinfection, patients experience accelerated progression of liver fibrosis and an increased risk of developing hepatocellular carcinoma (HCC) due to HIV-induced immunosuppression and chronic liver inflammation. the mechanisms involve activation of Kupffer cells and hepatic stellate cells (HSCs) via LPS and excitation of HBV-specific T cells [38].

Genetic analysis has revealed mutational variability in the RT/HBsAg region that can cause false negative PCR detection rates of up to 5-7% in some studies, particularly in geographical areas with diverse genotypes. Mutations affect PCR primer sites, which limits sensitivity for certain viral variants, with implications for diagnosis and therapy monitoring. Recommendations include periodically updating primer and probe designs to ensure the accuracy of molecular diagnoses [39].

Results

The results of multiple studies reinforce the idea that molecular testing is a fundamental pillar in the diagnosis, screening, monitoring, and management of hepatitis B and C at the population level. High sensitivity and specificity, often exceeding 95%, make molecular techniques (PCR, NAAT, genotyping, sequencing) the gold standard for identifying active, low-viral-load, or occult infections, as well as for early detection of infections before clinical signs or seroconversion occur. This technological advantage facilitates the implementation of large-scale screening and increases the likelihood of identifying residual cases, enabling early intervention and limiting transmission in the community.

Molecular testing also allows dynamic monitoring of treatment response and viral load, providing clinicians with a valuable tool for adapting antiviral therapy and rapidly detecting relapses or the development of viral resistance mutations.

Identifying viral genotypes using molecular methods contributes to personalizing the therapeutic approach, given the differences in treatment response between various subtypes, as well as to anticipating hepatic complications.

Also, alternative methods, such as dried blood spot (DBS) testing, have the potential to expand screening coverage in areas with limited laboratory access, but they have limitations in sensitivity for detecting very low viral loads, which can affect clinical decisions in post-treatment monitoring.

The use of additional biomarkers such as HBV RNA and HBcrAg is recommended in contexts of coinfection or under antiviral therapy, as they correlate more robustly

with cccDNA activity than serum HBV DNA.

However, the results also highlight significant variability between studies, reflected in sensitivity, specificity, and predictive value rates due to methodological and infrastructure differences. The technical platform used (type of PCR, methodological threshold, quality of DNA/RNA extraction), sample type (whole blood, plasma, serum), clinical context (acute versus chronic phase, monoinfection versus coinfections), and patient recruitment strategies differ significantly between studies and regions. The heterogeneity of the populations analyzed and epidemiological contexts, as well as viral bio-variability, further contribute to this variability.

A major point of discussion is the cost-benefit ratio, because although molecular testing is consistently superior to serology in terms of diagnostic performance, the costs remain significant and can be a barrier in countries with limited resources. Furthermore, the lack of uniform access to adequately equipped laboratories, underdeveloped logistics infrastructure (sample transport, storage), lack of ongoing staff training, and regional disparities can affect the quality and implementation of large-scale molecular screening, limiting the impact of eradication programs.

Selection biases, differences in defining the diagnostic gold standard, and the lack of detailed data on chronic mixed infections or complex cases of comorbidities, which are often underreported or poorly documented in studies, may underestimate or overestimate actual performance at the population level. Furthermore, in many meta-analyses, the assessment of cost-effectiveness, acceptability, sustainability, and long-term impact on public health remain partially addressed, requiring additional dedicated studies.

Discussions

In order to achieve the WHO goal of eradicating hepatitis by 2030, a structured plan for the organized implementation of molecular testing and relevant strategies is needed according to Table I:

Conclusion

In conclusion, molecular testing, implemented in an organized manner and integrated with public health strategies, offers real opportunities to reduce the incidence of hepatitis B and C, but overall effectiveness will depend on coordinated efforts to overcome the technical, logistical, financial, and educational limitations identified by current meta-analyses.

The integration of quantitative testing by Real-Time PCR into national and global strategies for the elimination of hepatitis B and C by 2030 is essential, as viral load monitoring is directly correlated with therapeutic success and the prevention of serious complications. However, technological accessibility and associated costs remain significant barriers in many regions, requiring dedicated policies and investments.

On the other hand, protocol standardization and quality assurance in laboratories are imperative to limit technical errors and variations between results. WHO guidelines and AASLD recommendations provide a clear framework for the implementation of Real-Time PCR tests, but their widespread adoption requires ongoing education and logistical support.

Authors' contribution

IP: Conceptualization, methodology, writing – original draft, writing- review & editing

Conflict of interest

The author declares that there is no conflict of interest.

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Table I. Strategic Framework for the Elimination of Viral Hepatitis at the Global Level

Area of Intervention	Detailed Actions and Strategic Measures
1. Development of the strategic and legislative framework	- prioritising the elimination of viral hepatitis as a national public health goal, in line with WHO and EU recommendations, which should provide for mandatory or recommended screening in key populations and support sustainable funding for the programme;
2. Technical standardization and unified protocols	- defining screening algorithms; - standardized technical platforms, and standardizing methods for collecting, transporting, and processing samples.
3. Development of infrastructure and human resources	- expanding the network of molecular biology laboratories to the county/regional level, equipped with modern equipment, maintenance, and strategic reagent stock; - increasing professional expertise: continuous training of staff (doctors, biologists, chemists, assistants) in molecular biology techniques, control procedures, and advanced interpretation, and implementation of a national traceability and electronic reporting system, interconnected between laboratories, clinicians, and authorities.
4. Launching screening and organized monitoring programs	- launching pilot programs in large urban centers and gradually expanding them throughout the country. Another strategy would be to integrate screening for other infections (HIV, syphilis, TB) and family medicine, family planning clinics, and community health services.
5. Education, information, and community involvement	- public information campaigns on the importance of testing, access to screening, and the benefits of early detection.
6. Evaluation, financing, and sustainability	- continuous monitoring of program performance through epidemiological, qualitative, and economic indicators; - rapid adjustment of strategies; - cost-effectiveness assessment through periodic analyses that support budget allocation and negotiations with suppliers to reduce costs; - access to European funds and public-private partnerships, such as; - development of regional centers of excellence for continuous development and research.

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