

HEPATITIS VIRUSES AND METHODS USED IN THEIR DIAGNOSIS

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Abstract

Hepatitis virus infections remain one of the leading causes of liver diseases and hepatocellular carcinoma worldwide. This article analyzes the pathogenesis and epidemiology of hepatitis A, B, C, D, and E viruses, with particular emphasis on diagnostic methods—serological tests, molecular techniques, rapid diagnostics, and screening strategies. The accuracy, limitations, and practical application of diagnostic methods are discussed. In addition, the use of modern technologies (such as nucleic acid amplification, quantification, and highly sensitive tests) in the diagnostic process is reviewed.

Keywords: Viral hepatitis, hepatitis A, B, C, D, diagnostic methods, serological tests, molecular diagnostics, screening, nucleic acid amplification, antigen/antibody markers.

Introduction

Viral hepatitis—including hepatitis A, B, C, D, and E—is a group of infectious diseases that can lead to inflammation of the liver parenchyma, fibrosis, cirrhosis, and hepatocellular carcinoma. These diseases are among the most common causes of infectious mortality worldwide and occupy a leading place in the structure of liver diseases. According to the latest data from the World Health Organization (WHO), in 2022 approximately 1.3 million people died as a result of chronic hepatitis B and C infections, which means that on average 3,500 people die every day from liver disease and liver cancer.

Epidemiological estimates indicate that in 2022, about 254 million people worldwide were living with chronic hepatitis B virus (HBV) infection, and around 50 million people were living with chronic hepatitis C virus (HCV) infection. These figures reflect not only the widespread distribution of the infection but also the fact that a large proportion of cases remain latent and asymptomatic for long periods, representing the “submerged part of the iceberg.” Chronic HBV and HCV infections are recognized as the main etiological factors for liver cirrhosis and hepatocellular carcinoma, and liver cancer currently ranks among the top three causes of cancer-related mortality worldwide.

One of the most alarming aspects of the global situation is the low coverage of diagnosis and treatment. According to the WHO Global Hepatitis Report 2024, by 2022 only 13% of people living with chronic HBV had been diagnosed, and less than 3% had started antiviral treatment. The situation with HCV is not much better: approximately 36% of the 50 million people living with chronic HCV are aware of their diagnosis, and about 20% (around 12.5 million patients) had been



treated with direct-acting antivirals by 2022. These figures indicate serious gaps in the diagnostic cascade—screening, laboratory confirmation, and linkage to care.

From a diagnostic perspective, the “heart” of the clinical problem associated with hepatitis viruses is the failure to detect the infection in a timely manner. The infection may remain asymptomatic or present with minimal nonspecific complaints for many years, leading patients to consult general practitioners or even specialists in other fields, with diagnosis often made only at the stage of cirrhosis or carcinoma. Global assessments show that in many regions most people living with chronic HBV and HCV are not tested at all, and as a result, the diagnosis of “hepatitis” is introduced into their medical history only at late stages.

The classical approach to the diagnosis of viral hepatitis is based on a combination of serological tests (antigen and antibody markers) and molecular methods (nucleic acid tests—NAT). Serological tests are relatively inexpensive and suitable for large-scale screening; for HBV, they detect HBsAg, HBeAg, and various antibodies, while for HCV they detect anti-HCV antibodies, indicating exposure to the infection. Molecular diagnostics allow detection and quantification of HBV DNA and HCV RNA, making it possible to confirm chronic infection, assess viral load, choose treatment strategies, and monitor response to therapy, and they have become the “gold standard.”

However, the broad implementation of these methods in clinical practice is not equally easy for all countries. In low- and middle-income countries, there are limitations related to the cost of serological tests and especially molecular technologies, lack of laboratory infrastructure, cold chain requirements, reagent supply, and shortage of trained personnel. For this reason, in recent years rapid tests, dried blood spot (DBS)-based diagnostics, and self-testing approaches have been actively proposed. The literature shows that detection of HBsAg and HCV antibodies using DBS has high diagnostic accuracy compared to venous blood, and this method is recommended as a promising approach to support national screening programs in resource-limited settings.

Another strategic aspect is that the WHO goal of “eliminating viral hepatitis by 2030” presupposes a substantial increase in diagnostic coverage, specifically the identification of 90% of people living with chronic HBV and HCV and the treatment of 80% of them. With current diagnostic rates of 13–36%, achieving this goal requires, first of all, revision of testing and diagnostic algorithms, wide implementation of affordable and highly sensitive methods, strengthening of laboratory systems, and comprehensive optimization of the “care cascade” that links diagnosis to treatment.

Therefore, an in-depth analysis of hepatitis viruses and the diagnostic methods used for their detection is of not only theoretical but also practical and health policy–shaping importance. Comparing the advantages and limitations of serological, molecular, rapid, and resource-adapted tests, evaluating their sensitivity and specificity, and determining the most effective combinations under real clinical and organizational conditions constitute an urgent task of our time. This article is based on this need and is aimed at analyzing the methods used in the diagnosis of hepatitis viruses based on scientific sources and available statistical data.

Research Objective

The main objective of this study is to evaluate the effectiveness of modern laboratory and instrumental methods used in the diagnosis of hepatitis viruses (A, B, C, D, E), to analyze their



sensitivity, specificity, and practical applicability, and to develop recommendations aimed at improving early detection and clinical decision-making processes.

Materials and Methods

For the preparation of this article, the literature was systematically searched in PubMed, Scopus, and Google Scholar databases. Articles published between 2015 and 2024 were analyzed using keywords such as “viral hepatitis diagnosis,” “hepatitis B serological tests,” “molecular diagnostics hepatitis C,” and “screening hepatitis B adults.”

The selected literature included original studies and recommendations from international organizations (e.g., the U.S. national public health agency, the Centers for Disease Control and Prevention). Inclusion criteria were: publications in English, diagnostic analyses, studies reporting the sensitivity and specificity of different tests, and the availability of statistical data. Data from these sources were collected, analyzed, and compared with alternative diagnostic approaches.

Results

This section presents the results of large recent studies and meta-analyses on serological, molecular, and resource-adapted diagnostic methods used for the detection of hepatitis viruses (mainly HBV, HCV, and HDV). The data are mainly derived from systematic reviews and multicenter clinical studies and are analyzed in detail in terms of sensitivity, specificity, and practical applicability.

Diagnostic performance of serological tests (HBsAg, anti-HCV, anti-HDV). For HBV, a meta-analysis including 40 studies from 23 countries and data from 23,716 participants evaluated rapid diagnostic tests (RDTs) and laboratory-based ELISA (enzyme-linked immunosorbent assay) and CLIA (chemiluminescent immunoassay) methods for HBsAg detection. When compared with reference laboratory immunoassays, HBsAg RDTs showed an overall sensitivity of 90.0% (95% confidence interval [CI]: 89.1–90.8%) and a specificity of 99.5% (95% CI: 99.4–99.5%). These findings indicate that RDTs are sufficiently reliable for mass screening and primary diagnosis in low-resource settings; however, the meta-analysis emphasized significant performance differences between brands.

Several field-based clinical studies have complemented these general results. For example, a study conducted in Rotterdam and Mozambique reported that HBsAg RDTs used on whole blood samples achieved 100% sensitivity and 100% specificity, showing complete agreement with the laboratory “gold standard.” However, such high performance was generally observed in well-equipped centers with strong quality control.

Systematic reviews and meta-analyses published in 2023–2024 on HDV serological diagnostics demonstrated that tests detecting HDV antibodies, particularly anti-HDV IgG, have very high diagnostic accuracy. The pooled sensitivity of anti-HDV IgG tests was approximately 97.4%, and the specificity was around 95.3%, indicating that these serological tests are reliable tools for HDV screening. Although IgM and “total anti-HDV” tests showed lower sensitivity (51–62%), they provide additional value in identifying active infection and acute disease.

For HCV, serological tests—especially ELISA and CLIA assays detecting anti-HCV antibodies—have been evaluated in dozens of studies. Meta-analyses comparing dried blood spot (DBS) samples with venous blood reported a laboratory sensitivity of about 95% (95% CI: 92–97%) and a



specificity of around 99% (95% CI: 98–100%). These figures confirm that anti-HCV tests are highly accurate and suitable for screening and primary diagnosis.

Summary of key meta-analytic results for serological tests

Virus/test type	Sample	Sensitivity (%)	Specificity (%)	Source
HBsAg RDT (multi-brand, meta-analysis)	Whole blood/serum	90.0	99.5	Amini A., BMC Infect Dis, 2017
HBsAg RDT (field conditions)	Whole blood	100	100	Debes J., Ann Glob Health, 2020
Anti-HDV IgG	Serum	97.4	95.3	Akuffo G., Sci Rep, 2024
Anti-HCV (laboratory, DBS vs venous)	DBS	95	99	Carty P., Rev Med Virol, 2022

These results show that, when properly selected and used with quality reagents, serological tests provide high accuracy for hepatitis virus screening. At the same time, brand variability, manufacturers, test protocols, and patient populations (e.g., HIV co-infection) can significantly influence diagnostic performance.

Results of DBS-based serological diagnostics. Due to the difficulties of venous blood collection, transport, and cold-chain maintenance in resource-limited settings, DBS technology has been extensively studied. A large epidemiological study in Cambodia compared HBV and HCV seromarkers in DBS and serum samples. Sensitivity was $\geq 90\%$ and specificity close to 100% for HBsAg, high-quality HBsAg (HBsAg-HQ), HBeAg, and anti-HBe. For anti-HBc and anti-HBs, sensitivity was approximately 70%, but overall diagnostic accuracy was 94.6% and 89.3%, respectively.

Other studies conducted in African and Asian countries also demonstrated that DBS-based HBsAg and anti-HCV tests have high accuracy compared with venous blood. For example, a study from Cameroon reported a sensitivity of 99% and specificity of 98% for DBS-based anti-HCV testing, with positive and negative predictive values around 98–99%.

A European study on HCV evaluated DBS samples from 108 HCV-positive patients and correctly identified 100 cases, yielding a sensitivity of 92.6% and a specificity of 100%. These findings indicate that DBS is a practical and cost-effective approach for HCV screening; however, some studies reported sensitivity ranging from 70% to 90%, highlighting the need for careful selection of test platforms and laboratory algorithms.

Summary of DBS-based serological test results

Virus/test type	Sensitivity (%)	Specificity (%)	Notes/source
HBsAg (DBS)	≥ 90	≈ 100	Multicenter study, Cambodia
Anti-HBc (DBS)	≈ 70	> 90	High specificity, lower sensitivity
Anti-HCV (Africa, DBS)	99	98	Kenmoe S., dissertation, Cameroon
Anti-HCV (Europe, DBS)	92.6	100	Vázquez-Morón S., Sci Rep, 2018

Molecular diagnostics (PCR, LAMP, DBS-based NAT). Nucleic acid tests (NAT), including real-time PCR, transcription-mediated amplification (TMA), LAMP, and other technologies, are central to confirming chronic HBV and HCV infection, quantifying viral load, and monitoring treatment response.



A meta-analysis evaluating the diagnostic accuracy of the LAMP method for HBV combined data from multiple regions and found a pooled sensitivity of 0.91 (95% CI: 0.89–0.92) and specificity of 0.97 (95% CI: 0.94–0.99). The relatively simple laboratory infrastructure and short reaction time make this method suitable for low-resource settings; however, risks of contamination and issues with standardization remain.

A 2022 study published in Scientific Reports showed that qualitative PCR for HBV DNA in DBS samples had a sensitivity of 75% and specificity of 100%, while quantitative PCR reliably detected viral load up to certain thresholds. Another meta-analysis reported pooled sensitivity of about 95% and specificity of 99% for DBS-based HBV DNA quantification, though sample storage conditions (cold chain, humidity) can significantly affect results.

For HCV, several major studies have focused on the detection and quantification of HCV RNA in DBS samples. A study published in Swiss Medical Weekly reported that measurement of HCV RNA using the Xpert® HCV VL test in capillary blood DBS samples closely matched results from venous plasma. Systematic reviews by Carty and colleagues also emphasized the high diagnostic accuracy of anti-HCV and HCV RNA tests using DBS, enabling combined screening and confirmatory testing while reducing the need for venous blood collection and logistical costs.

Summary of key NAT-based results

Virus/test type	Sample	Sensitivity (%)	Specificity (%)	Source
HBV LAMP (meta-analysis)	Serum/blood	91	97	Chen C., J Clin Lab Anal, 2020
HBV DNA PCR (DBS, qualitative)	DBS	75	100	Bezerra C., Sci Rep, 2022
HBV DNA PCR (DBS, meta-analysis)	DBS	95	99	Xiao Y., Cells, 2020
HCV RNA VL (DBS, Xpert)	DBS	High, strong agreement with plasma	–	Bregenzer A., Swiss Med Wkly, 2021

Point-of-care tests and emerging biomarkers (HBcAg, HCV core antigen). Beyond classical HBsAg and anti-HCV serological tests, rapid tests based on functional biomarkers have been developed in recent years. A multicenter study published in 2025 in The Lancet Gastroenterology & Hepatology evaluated a rapid test for HBV core-related antigen (HBcAg-RDT). The pooled sensitivity was 93.1% (95% CI: 90.5–95.2%), and the specificity was 94.3% (95% CI: 93.0–95.4%). These results indicate that this test can be used not only for screening but also for real clinical decision-making, such as treatment initiation and monitoring of HBV replication activity.

For HCV, HCV core antigen (HCVcAg) tests, especially in DBS samples, are being studied as simplified surrogate markers for HCV RNA. Studies from 2024 showed that while HCVcAg sensitivity is slightly lower than that of HCV RNA PCR, it can partially substitute for NAT in settings with limited laboratory infrastructure.

Discussion

The results of this study show that modern diagnostic approaches used to detect hepatitis viruses have improved substantially. Joint analysis of serological, molecular, and adaptive (resource-limited) methods confirms that there is no single universal test in current clinical practice; instead, stepwise, combined diagnostic algorithms represent the most appropriate solution.



The findings on serological tests—particularly the high specificity of HBsAg, anti-HCV, and anti-HDV assays—strengthen their role as key tools for mass screening. The fact that the specificity of HBsAg rapid tests exceeds 99% in many studies indicates a low probability of false-positive results. However, variability in sensitivity (around 85–90% for some platforms) introduces a risk of missing clinically important cases, especially in patients with low viral loads. This issue is particularly relevant during the “window period,” in low-viremia states, and in immunosuppressed individuals. Therefore, serological tests should not be viewed as stand-alone definitive diagnostic tools, but rather as one component of the screening stage.

The high sensitivity and specificity of anti-HCV tests (typically >95%) support their use as the backbone of global HCV screening strategies. A key consideration, however, is that a positive anti-HCV result reflects an immune response to past or current infection, rather than ongoing viral replication. For this reason, confirmatory testing with HCV RNA assays is emphasized in virtually all international clinical guidelines. These findings show that relying solely on serological markers is insufficient for robust clinical decision-making.

The results obtained for molecular tests (HBV DNA and HCV RNA detection) support their designation as the diagnostic “gold standard.” The high sensitivity, low limits of detection and ability to quantify viral load offered by real-time PCR and LAMP technologies for HBV enable not only confirmation of diagnosis, but also assessment of disease activity, determination of the need for treatment, and monitoring of therapeutic response. The study results show that high HBV DNA levels are strongly associated with an increased risk of liver cirrhosis and hepatocellular carcinoma, highlighting the prognostic significance of molecular testing.

At the same time, the high cost of molecular tests, the need for specialized laboratory infrastructure, and the requirement for highly trained personnel continue to limit their widespread implementation in low- and middle-income countries. As demonstrated in the results, it is precisely at this point that DBS technology emerges as an important alternative solution. Studies on HBV and HCV diagnostics using dried blood spots have shown that sensitivity and specificity are generally high. The logistical advantages of this method—including reduced reliance on cold-chain systems, easier transport, and simplified sample collection—make it particularly valuable in geographically remote areas.

However, DBS-based methods also have limitations. Sample volume, drying and storage conditions, elution protocols, and pre-analytical steps can significantly influence performance. The observed variation in sensitivity (70–90%) in different studies reflects the impact of these factors. Thus, DBS technology should not be regarded as a universal solution, but rather as a highly adaptable tool appropriate for specific contexts.

New biomarkers—particularly HBcrAg and HCV core antigen tests—are emerging as important intermediate components within the diagnostic system. The results show that HBcrAg assays have high accuracy and may serve as a “functional bridge” between HBsAg and HBV DNA in assessing HBV replication activity. This suggests the emergence of simpler, faster, and clinically useful biomarkers that may partially replace expensive and complex molecular tests in the future.

Overall, the results indicate that hepatitis virus diagnostics should rely not on a single method but on an integrated, multi-step approach. Serological tests should be viewed as screening tools; molecular tests, as confirmatory and monitoring tools; and DBS and point-of-care technologies, as



adapted solutions for hard-to-reach and resource-limited settings. Only such an integrated model can substantially increase early detection of hepatitis on a global scale, expand access to treatment, and bring the world closer to achieving the WHO 2030 elimination targets.

In this regard, our analytical findings confirm the developmental trajectory of modern hepatology and infectious disease diagnostics: the future of diagnostics lies in combinations of affordable, highly sensitive, rapid tests that are brought as close to the patient as possible.

Conclusion

The results of this study demonstrate that modern diagnostic methods used to detect hepatitis viruses possess high accuracy and significant practical value. An integrated approach that combines serological and molecular technologies has been confirmed as the most optimal strategy for early detection of infection, assessment of disease activity, and improving treatment effectiveness. The findings show that improving the quality of diagnostic processes is crucial for reducing complications and mortality related to hepatitis at the global level.

1. Serological tests (HBsAg, anti-HCV, anti-HDV) are effective tools for large-scale screening of hepatitis viruses, and their high specificity minimizes the likelihood of false-positive results.
2. Molecular diagnostic methods (HBV DNA, HCV RNA) remain the “gold standard” for determining actual infection activity and assessing viral load, and they play a decisive role in clinical decision-making.
3. Dried blood spot (DBS) technology and point-of-care tests are promising approaches for expanding diagnostic coverage in resource-limited settings, offering substantial reductions in logistical and organizational burden.
4. Rapid tests based on new biomarkers (HBcrAg, HCV core antigen) may, in the future, serve as practical alternatives to traditional complex molecular methods and create the foundation for further simplification of systems for early detection and monitoring of hepatitis.

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