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The Diagnostic Threshold of High Viral Load Hepatitis B DNA Testing in Occult Infection Screening

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Abstract: Subclinical hepatitis B infections often present with low viral loads that are easily missed by routine testing. High-sensitivity hepatitis B DNA testing provides critical technical support for their screening and diagnosis. This article focuses on core clinical application challenges of this testing technology, defining key target populations including post-antiviral treatment cirrhosis patients and high-risk groups with complications. It clarifies differences in inclusion criteria across medical scenarios while establishing unified standards. Based on technical characteristics and clinical evidence, a diagnostic cutoff of 10-2000 U/ml is proposed, along with validation and adjustment strategies for different populations. The analysis examines testing application boundaries, technical limitations, and clinical practice issues, providing actionable evidence to optimize screening processes for subclinical hepatitis B infections, improve diagnostic accuracy, and help reduce progression risks of related liver diseases.

Keywords: highly sensitive hepatitis B DNA test; occult hepatitis B infection; target population; cut-off value; clinical application

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1. Introduction

Chronic hepatitis B remains a major global public health challenge. Subclinical hepatitis B infections, characterized by negative hepatitis B surface antigen (HBsAg) and low viral replication, create diagnostic blind spots in clinical practice. These asymptomatic infections may progress to liver fibrosis, cirrhosis, or even hepatocellular carcinoma, with particularly high risks in immunosuppressed patients and those undergoing antiviral therapy. Conventional hepatitis B DNA testing lacks sufficient sensitivity to detect trace viral replication, resulting in high missed diagnosis rates. High-sensitivity hepatitis B DNA testing, with its lower detection threshold, offers a novel approach for identifying subclinical infections. This study systematically examines the target population, threshold value determination, and clinical application boundaries of this technology, providing scientific references for standardized clinical implementation and addressing the current gap in diagnostic and therapeutic approaches for subclinical hepatitis B infections[1].

2. High-sensitivity HBV DNA testing: screening for occult infections – target population and inclusion criteria

2.1. Core applicable population definition based on clinical scenarios

The core population for high-sensitivity hepatitis B DNA screening should focus on individuals with high-risk exposure to HBV infection and those at elevated disease progression risk. Particular attention should be given to patients with hepatitis B cirrhosis undergoing nucleoside analog or interferon antiviral therapy, especially those with treatment duration exceeding 48 weeks, good treatment adherence, but incomplete virological response. These patients may exhibit hypovirulence or extremely low viral load, requiring high-sensitivity testing to confirm viral replication status. High-risk groups for HBV-related complications should not be overlooked, including individuals with family history of hepatitis B, those at risk of progressing from compensated to decompensated cirrhosis, and patients with prior complications such as ascites, esophageal/gastric varices, or hepatic encephalopathy. Conventional testing may fail to detect viruses, yet latent replication could still exist in these individuals, where high-sensitivity testing can assist in prognosis evaluation. Patients receiving long-term immunosuppressive therapy, those with concurrent liver injury factors, and suspected HBV-related liver damage cases showing abnormal alpha-fetoprotein levels or elevated liver stiffness values should all be included in the core population. Latent HBV infection may accelerate liver fibrosis progression and hepatocellular carcinoma development in these groups[2].

2.2. Differences and unification of inclusion criteria in different medical scenarios

In specialized clinical scenarios, inclusion criteria must demonstrate disease progression correlation. Patients requiring first-line nucleoside therapy for over 48 weeks, with routine HBV DNA testing negative but persistent abnormal liver function indicators, and those showing prothrombin activity fluctuations between 60% to 70%, should be included in testing. Other hepatitis virus co-infections (including types A, C, and D), as well as interfering factors like autoimmune diseases and malignancies, must be excluded to ensure test results directly correlate with HBV covert infection. For physical screening, priority is given to individuals with hepatitis B family history, unclean injection history, blood product use history, or HBsAg-negative but HBcAb-positive status. Liver function indicators need not be strictly restricted, but recent alcohol consumption or intense exercise causing short-term liver injury must be excluded. Primary healthcare scenarios prioritize practicality and relevance, targeting chronic hepatitis symptoms lasting over 3 months, ambiguous routine HBV test results, or individuals with family history of cirrhosis or liver cancer. Preliminary screening may lead to referral to higher-level hospitals for high-sensitivity testing and follow-up diagnosis. Three unified inclusion criteria apply: age ≥18 years, no contraindications for high-sensitivity DNA testing, signed informed consent, and no use of non-antiviral drugs affecting viral replication within 4 weeks prior to testing[3].

3. Threshold setting and validation of high-minute hepatitis b dna testing for detecting subclinical infections

3.1. Scientific basis for setting the critical value of high sensitivity detection

The cutoff value for HBV DNA testing in Gao Min's study to diagnose occult infection requires comprehensive evaluation of detection characteristics and clinical evidence. Based on the "Expert Opinion on Expanding Antiviral Therapy for Chronic Hepatitis B" and clinical data, the lower limit was set at 10 U/ml. This threshold aligns with the minimum detection capability of highly sensitive technologies, effectively distinguishing between "undetectable target" and "below detection limit" states while avoiding missed trace viral replication. The upper limit was established at 2000 U/ml to align with hypovirality definitions. Clinical studies confirm that patients with HBV DNA within this range exhibit different liver injury severity and synthetic liver function compared to those with higher viral loads, where viral replication in this interval represents a potential risk factor for cirrhosis complications and hepatocellular carcinoma. The cutoff value determination process incorporated methodological validation, utilizing enzyme-linked immunosorbent assay (ELISA) for liver function

indicators and electrochemiluminescence (ELISA) for hepatitis B serology panel as supplementary references. This ensures the cutoff value effectively correlates viral replication with liver damage severity while eliminating false positives or negatives caused by detection system errors.

3.2. Verification and adjustment strategies of critical values in different populations

For patients with hepatitis B cirrhosis undergoing antiviral therapy, validation of cut-off values should integrate treatment response status. In populations receiving 12 nucleoside analogues with persistent HBV DNA levels between 10-2000 U/ml, a 48-week follow-up is required to validate the cut-off's clinical relevance. Key parameters include alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels maintained below 1.5 times the upper normal limit, albumin ≥35 g/L, and prothrombin activity ≥60%. If patients in this range exhibit significantly higher cirrhosis complication rates compared to the "no target detected" group, the cut-off's clinical relevance is confirmed. For high-risk groups with HBsAg-negative and HBcAb-positive occult infections, the cut-off should be adjusted to 5-10 U/ml. These individuals demonstrate lower viral loads, and stricter cut-offs enhance detection rates. Validation should incorporate four liver fibrosis markers and liver stiffness measurements. If patients with HBV DNA levels between 5-10 U/ml show significantly higher liver stiffness averages than those with <5 U/ml, this supports the adjustment rationale. For patients with concurrent liver injury factors, the cut-off remains at 10 U/ml but requires extended "persistent viral load positivity" duration. Two consecutive tests spaced 4 weeks apart with HBV DNA levels between 10-2000 U/ml are necessary to avoid misinterpreting transient viral release as occult infection. Validation employs multi-center data comparison using SPSS 17.0 statistical software for variance analysis, ensuring cut-off sensitivity and specificity across different populations. For populations with insufficient sensitivity after validation, the lower critical value may be lowered to 5 U/ml, while covalently closed-loop DNA (CCDNA) testing for hepatitis B virus should be added as a supplementary validation method[4].

4. Clinical application limits and constraints of high-minute hepatitis b dna testing for detecting subclinical infections

4.1. Core dimensions and practical norms of clinical application boundaries

The clinical application boundaries of high-titer HBV DNA testing require clarification of the progressive scope from "screening-diagnosis-intervention", with core dimensions including testing timing, population suitability, and interpretation authority. Patients undergoing antiviral therapy should be tested every 6 months, with the interval extended to annual testing if no viral replication persists for 2 years. High-risk groups with occult infections should undergo annual screening, while those with family history of cirrhosis require testing every 6 months. Acute liver injury patients must complete testing within 12 weeks of onset to avoid disease progression affecting viral detection. Population suitability: testing is not recommended for adolescents under 18, pregnant women, or patients with end-stage liver disease. Those co-infected with HIV or with autoimmune diseases should wait until immune function stabilizes before testing. Interpretation requires comprehensive evaluation by hepatology or infectious disease specialists, considering clinical symptoms, liver function indicators, HBV serology panel, and imaging findings. When HBV DNA levels range between 10-2000 U/ml, potential interference factors like alcohol consumption, fatigue, or medication should be ruled out, and retesting may be necessary. The "undetectable target" result cannot completely exclude occult infection and requires confirmation through liver biopsy or long-term follow-up. In practice, patients should be informed of testing purposes and limitations beforehand. Morning fasting venous blood samples must be collected, testing protocols strictly followed, and results recorded in electronic medical records to maintain continuous monitoring.

4.2. Analysis of the main limitations of the technology itself and the clinical scenario

Technical limitations primarily involve methodological constraints and sample interference factors. High-sensitivity

testing requires stringent sample quality standards, where hemolysis or lipemia may lead to falsely low results. Prolonged storage of samples beyond 48 hours after collection can cause viral nucleic acid degradation, resulting in false negatives. Variations in instrument calibration accuracy and reagent batch differences may cause fluctuations in test results for the same patient at different times. The technology cannot distinguish between free viral DNA and integrated viral DNA in hepatocytes. Patients with negative hepatitis B surface antigen (HBsAg) but covalently closed-loop DNA (cccDNA) in liver tissue may exhibit test results inconsistent with pathological conditions. Clinical limitations include selection bias in single-center data, inconsistent standardization of testing systems across hospitals, and varying application of cutoff values. Poorly compliant patients may have test results influenced by irregular antiviral therapy, failing to reflect true viral replication status. Some primary care facilities lack high-sensitivity testing equipment, necessitating referrals to higher-level hospitals with delayed testing. The test cannot predict the progression rate of covert infections. Clinical studies show that individuals with HBV DNA <10 U/ml have no significant difference in cirrhosis complication rates compared to those without detectable target group, though long-term risks cannot be entirely ruled out and require comprehensive evaluation with other indicators. Higher testing costs compared to routine tests further limit its accessibility among low-income populations.

5. Epilogue

The Gao Min hepatitis B DNA test demonstrates significant value in screening and diagnosing occult hepatitis B infections. Its accuracy depends on three critical factors: scientifically defined target populations, rational threshold setting, and clear application boundaries. This study establishes core applicable populations and scenario-specific inclusion criteria, providing a foundation for clinical precision screening. Targeted threshold adjustment strategies and validation methods enhance diagnostic accuracy across different populations. Analysis of technical and clinical limitations offers guidance for mitigating application risks. Future efforts should focus on standardizing testing protocols, optimizing diagnostic systems through multi-parameter combined testing, and reducing costs to facilitate broader implementation in primary healthcare. Ultimately, this will enable early detection and intervention of occult hepatitis B infections, improving patient outcomes.

Disclosure statement

The author declares no conflict of interest.

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