

The Interference Effect of Hepatitis B Virus Genotypes on the Accuracy of DNA Quantitative Detection

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Abstract: Hepatitis B virus genotype sequence variations constitute the primary determinant of DNA quantitative detection accuracy. The established correlation between genotype characteristics and detection targets reveals that genetic sequence variations, replication activity differences, and specific mutations create interference mechanisms across target recognition, amplification efficiency, and signal capture processes. This study proposes integrated strategies including detection technology optimization, multi-genotype calibration, and standardized clinical interpretation protocols. By implementing targeted optimized detection systems and standardized application protocols to mitigate genotype interference, this approach enhances detection accuracy and clinical applicability, thereby providing reliable experimental evidence for precision diagnosis and treatment of hepatitis B.

Keywords: hepatitis B virus genotype; DNA quantitative detection; interference effect

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1. Introduction

Hepatitis B is a prevalent clinical infectious disease, comparable to AIDS and tuberculosis in mortality rates. The primary cause of hepatitis B is hepatitis B virus (HBV) infection^[1]. Quantitative HBV DNA testing serves as the cornerstone for assessing viral replication, guiding treatment decisions, and predicting clinical outcomes, with its accuracy directly influencing medical decisions. As HBV genotypes have become increasingly diverse, single-genotype or limited-genotype testing systems have shown limitations in accommodating clinical practice. Sequence variations in viral genomes have become a major source of testing inaccuracies, potentially leading to misjudged viral loads, inadequate treatment, or excessive therapy.

2. The Core Foundation of Hepatitis B Virus Genotype and DNA Quantitative Detection

The rapid advancement of modern biological technologies has introduced real-time PCR-based fluorescence quantitative detection methods, which have revolutionized clinical diagnosis for hepatitis B patients^[2]. Hepatitis B virus genotypes are classified through specific genomic variations, with characteristic nucleotide differences in the core gene, S gene, and

promoter region. These variations directly influence primer-probe binding targets in DNA quantification, posing challenges to detection accuracy. As a critical tool for assessing viral replication activity, guiding clinical treatment, and predicting prognosis, DNA quantification fundamentally relies on nucleic acid amplification technology to specifically identify and quantify viral genetic fragments. Current detection systems predominantly use reference sequences of dominant genotypes. With the increasing global diversity of hepatitis B virus genotypes, the mismatch between single-genotype detection systems and clinical reality has become increasingly evident. Clarifying the correlation between genotype sequence characteristics and core detection targets forms the foundation for analyzing genotype interference effects. This understanding provides a logical starting point for exploring interference mechanisms and optimizing detection strategies, ultimately establishing a theoretical link between genotype variations and detection accuracy to prevent clinical misdiagnosis caused by conceptual ambiguities.

3. Mechanism Analysis of Accuracy in Quantitative Detection of Hepatitis B Virus Genotype Interference DNA

Nucleotide variations in core genes, pre-S/S genes, and promoter regions among different hepatitis B virus genotypes directly affect primer-probe binding in DNA quantification. When specific genotype reference designs encounter heterologous sequences, this leads to reduced primer binding efficiency, diminished probe hybridization capacity, insufficient amplification of target gene fragments, and quantification results deviating from actual viral load. Genotypic differences in viral replication regulatory regions alter viral replication activity and gene expression levels within host cells. Under identical infection conditions, varying detectable template quantities between different genotypes indirectly impact detection sensitivity. Mutations in certain genotypes may cause target sequence deletions or modifications, preventing primers from effectively recognizing target fragments or reducing amplification product-probe binding affinity, resulting in false negatives or underestimation of quantification values. These mechanisms are interconnected, forming a chain of interference across target recognition, amplification efficiency, and signal capture that compromises DNA quantification accuracy. The core issue lies in mismatch between detection system design and molecular characteristics of heterologous genotypes.

4. Optimization Strategies and Clinical Applications for Genotype-Interference-Free DNA Quantification

4.1. Improvement direction of detection technology based on genotype characteristics

The improvement of genotype-oriented detection technology requires advancing four core aspects: molecular target screening, primer/probe design, amplification system optimization, and detection platform upgrades, ensuring technical solutions align with molecular characteristics of different genotypes. In the target screening phase, bioinformatics technology is employed to compare whole-genome sequences of major global genotypes, identifying highly conserved regions shared by multiple genotypes. Priority is given to selecting stable fragments with overlapping coding/non-coding regions as detection targets, avoiding single-genotype-specific mutation regions to minimize interference caused by sequence variations at the source. The core sequences of conserved regions form the foundation for primer design. Using degenerate base substitution technology, compatible degenerate sites are introduced at critical positions to accommodate nucleotide variations across genotypes. This approach shortens primer length and optimizes base composition, enhancing binding specificity and affinity between primers and heterozygous genotype targets. Probe design incorporates nucleic acid locking modification technology to strengthen hybridization stability with target sequences, reducing probe dissociation caused by genotype variations. Through optimized combinations of fluorescent and quenching groups, detection sensitivity is significantly improved.

The optimized amplification system focuses on reaction condition adjustments, determining the optimal annealing temperature through gradient experiments to avoid insufficient amplification specificity or decreased efficiency. The buffer solution contains enhancers such as bovine serum albumin and betaine to improve amplification efficiency for high GC-content genotypes and balance amplification kinetics differences among genotypes. The upgraded detection platform enables broader clinical application of digital PCR technology, which eliminates the need for standard curves and achieves absolute quantification through single-molecule amplification. This technology demonstrates greater tolerance to genotype-induced amplification efficiency variations, making it suitable for low viral load and rare genotype sample detection.

The development of a multiplex PCR detection system involves designing primer-probe combinations targeting conserved genotypes within a single reaction system, enabling simultaneous genotyping and DNA quantification. This approach reduces sample consumption while enhancing the correlation and accuracy of test results. When developing reagent kits, validation using multi-genotype samples is essential to ensure consistent performance across common genotypes and variants, thereby avoiding genotype bias caused by design flaws. The chemiluminescence method utilizes suspended photo-activated carriers with expanded reaction areas, allowing rapid detection of hepatitis B virus variations and effectively addressing the limitations of enzyme-linked adsorption assays^[3].

4.2. Calibration method of multi-genotype compatible detection system

The calibration of multi-genotype compatibility testing systems requires establishing a comprehensive operational protocol encompassing “standard material library construction, calibration process optimization, and quality control system enhancement” to ensure comparability and accuracy across different genotypes. The standard material library should be built around high-purity natural viral particles, systematically covering major circulating genotypes and common clinical variants worldwide to align closely with actual clinical infection patterns. Each standard material undergoes rigorous multi-stage validation: genetic sequencing confirms genotype purity while eliminating cross-contamination and variant impurities; nucleic acid purification techniques remove contaminants like proteins and polysaccharides to ensure template integrity; viral titer calibration establishes precise concentration benchmarks. Additionally, a long-term stability monitoring mechanism must be implemented, regularly assessing nucleic acid integrity and concentration variations under different storage conditions, promptly phasing out degraded samples to maintain reliability. For clinical scenarios involving mixed genotype infections, standard material ratios should be designed based on epidemiological data to simulate infection proportions. This ensures mixed standard materials accurately reflect complex clinical testing scenarios, providing precise calibration references that overcome limitations of single-genotype standards in handling multi-genotype infections.

The calibration process optimization is implemented in two phases. The primary step involves genotype pre-typing-guided calibration, where genotyping technology is used to determine genotype categories before clinical sample testing. Corresponding specific calibration curves are then selected based on genotype types. For highly variable genotypes, dedicated calibration parameters are applied, while common genotypes utilize universal calibration curves to ensure genotype-matching calibration. The construction of universal calibration curves requires analyzing amplification efficiency of multi-genotype standards and calculating genotype-specific amplification efficiency correction coefficients. These coefficients are integrated into the universal calibration curve algorithm, enabling a single curve to accommodate multiple genotypes and simplifying testing procedures. The laboratory’s internal quality control system incorporates multi-genotype quality control materials with high, medium, and low viral load gradients. Regular validation of testing system stability is conducted. When test results for a specific genotype quality control exceed permissible error ranges, issues such as primer/probe degradation or reaction condition drift must be promptly investigated, necessitating recalibration. A genotype detection interference early-warning mechanism is established, automatically prompting genotype re-verification and calibration parameter adjustments when test results significantly deviate from clinical phenotypes.

Cross-laboratory calibration comparisons are conducted through inter-laboratory quality assessment activities. Authoritative institutions distribute blind samples containing multiple genotypes, which are then quantitatively analyzed using each laboratory’s proprietary detection systems. Through aggregated analysis of results, deviations in genotype

detection across different systems are identified, leading to the development of a unified calibration and correction protocol. Standardized testing procedures are promoted, establishing uniform protocols for sample processing, nucleic acid extraction, amplification reactions, and result interpretation. This approach effectively reduces genotype-related detection discrepancies caused by inter-laboratory operational variations.

4.3. Interpretation of clinical test results combined with genotype

To ensure accurate interpretation of genotype-related clinical test results, a standardized workflow integrating “genotyping-quantification-clinical correlation” should be established. This requires clarifying operational protocols, refining interpretation criteria, and strengthening clinical integration to guarantee test results effectively guide clinical practice. The clinical testing protocol specifies optimal timing for hepatitis B virus genotype analysis: initial diagnosis should include simultaneous genotype identification and DNA quantification, with periodic retesting during treatment to detect genotype variations or mixed infections. For genotyping, the preferred method combines precise nested PCR with sequencing technology, which simultaneously identifies genotype categories and key mutation sites, providing molecular evidence for quantitative interpretation. Primary healthcare institutions should adopt rapid genotyping techniques like nucleic acid hybridization to ensure timely availability of genotype results.

The interpretation of quantitative results involves establishing genotype-specific correction standards. Correction coefficients are determined based on genotype-dependent interference characteristics of the detection system. When a particular genotype tends to cause underestimation of quantitative results, the original results are adjusted using these coefficients. The reliability level of results is annotated by considering the genotype compatibility of the detection method. Special attention should be paid to exceptional cases: For mixed genotype infection samples, the relative proportions of each genotype must be specified, indicating that the quantitative result reflects total viral load. For samples with borderline detection results, genotype characteristics should be evaluated to determine whether false negatives or false positives are caused by interference. If necessary, the specific genotype test kit should be replaced for retesting. Clinical reports should present standardized information, clearly listing genotype subtypes, detection methods, calibration references, and correction status of quantitative results. Interpretation notes should be added to explain potential impacts of the genotype on detection accuracy and clinical reference value, preventing clinicians from relying solely on quantitative values for conclusions. Different HBV genotypes exhibit distinct clinical features and epidemiological patterns, which correlate with drug treatment efficacy and liver lesion severity, influencing disease progression and prognosis^[4].

Clinical decision-making should strengthen the correlation between test results and treatment plans, adjusting strategies based on genotype characteristics and corrected quantitative results. When a genotype shows low sensitivity to specific antiviral drugs and quantitative results indicate active viral replication, treatment plans should be promptly modified. For patients with genotype interference causing fluctuating quantitative results, comprehensive evaluations should integrate liver function tests, hepatic histopathology, and other indicators to prevent treatment under- or over-therapy due to quantitative deviations. Enhanced collaboration between clinicians and laboratory personnel is crucial, with lab technicians providing genotype-related testing consultations and clinicians offering timely feedback on treatment responses and result alignment. This collaborative approach optimizes interpretation standards. Regular training programs should be implemented to improve healthcare professionals' understanding of genotype interference effects, enabling them to master result correction methods and clinical application logic, ensuring standardized protocols are effectively implemented.

5. Epilogue

This study systematically analyzes the core mechanisms of quantitative HBV genotype interference DNA detection, establishing a comprehensive optimization pathway encompassing technical refinement, system calibration, and clinical interpretation. By creating a closed-loop process from molecular design to clinical application, it effectively resolves genotype-specific detection compatibility challenges, significantly enhancing test reliability and providing precise data

support for hepatitis B diagnosis and treatment. Future efforts should focus on continuously improving the multi-genotype compatibility standardization system, advancing clinical translation, and promoting the precision and standardization of hepatitis B testing and treatment, thereby strengthening safeguards for hepatitis B prevention and control.

Disclosure statement

The author declares no conflict of interest.

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