qPCR Methodology for Quantifying Latent Hepatitis B Virus: First Step to Finding a Cure

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Hepatitis B virus (HBV) infects 300 million people worldwide, causing 1.3 million annual deaths. Current treatments can suppress viral replication but fail to eliminate covalently closed circular DNA (cccDNA), a stable form of viral DNA that persists in liver cells and can reactivate infection. Accurate quantification of cccDNA is essential for evaluating new therapies. Southern blotting can directly visualize cccDNA, but it is labor-intensive and lacks sensitivity. Quantitative PCR (qPCR) is more sensitive but cannot reliably distinguish cccDNA from relaxed circular DNA (rcDNA) and other replicative intermediates. Enzymatic pretreatments such as plasmid-safe DNase or exonuclease digestion have been used to improve specificity but may incompletely digest rcDNA or degrade cccDNA. My study evaluates a novel commercial bisulfite (BS)-based cccDNA PCR assay and combines it with existing nuclease-pretreatment qPCR methods to optimize its selectivity. Bisulfite conversion disrupts rcDNA structure while allowing selective amplification of cccDNA across the nick region. The assay showed a detection range of 10^2–108 copies per reaction. Multiple pretreatments were tested using Hirt DNA from HBV-infected hepatocyte-derived cells. Results showed that previous methods produced variable DR1/DR2 ratios as high as 1.73, suggesting significant amplification of nicked DNA forms. In contrast, my Exo V + BS-cccDNA assay yielded a consistent DR1/DR2 ratio of 1.10. That represents a greater than 7.3x increase in selectivity for the detection of closed circular DNA. This study establishes a new benchmark for viral quantification. This optimized strategy provides the essential tool for clinical trials to validate the clearance of persistent cccDNA in the search for an HBV cure.

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