

THE DESIGN AND APPLICATION OF A REAL-TIME PCR ASSAY TO ASSESS rcDNA AND cccDNA PRODUCED BY HBV DURING INFECTION

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Chronic hepatitis B virus (HBV) infection is endemic to sub-Saharan Africa, and despite the availability of anti-viral agents, there is currently no cure. This double stranded DNA virus is hepatotropic, and active viral replication results in two genomic equivalents, the relaxed circular DNA (rcDNA) and covalently closed circular DNA (cccDNA). The virion encapsulated rcDNA contains a partially synthesised positive DNA strand and a gap region within the negative strand. After infection of hepatocytes, the rcDNA is repaired in the nucleus to form cccDNA. An important objective of HBV therapy is the elimination of cccDNA, as its persistence within hepatocytes has been attributed to chronic HBV infection. Therefore a reliable assay for this replication intermediate is crucial. The objective of this study was to develop a method based on real-time PCR to detect and quantify HBV cccDNA. PCR primers which flank the rcDNA gap were designed to amplify cccDNA whilst primers flanking the pre-S1 region quantify total HBV DNA. Viral DNA was extracted from HepG2.2.15 cells, along with serum and livers from HBV transgenic mice. According to this assay, cccDNA was readily detectable in transgenic mouse livers, but was present at low concentrations in serum samples. The intrahepatic HBV DNA profile of transgenic mice was found to be 40% cccDNA to 60% rcDNA. In HepG2.2.15 cells, only 2% of HBV DNA was cccDNA whilst the majority was in the form of rcDNA. These results were validated using non-radioactive Southern blot hybridisation. Additionally, it was

established that although RNAi-based effecters inhibit HBV replication, established cccDNA pools were not eliminated. Real-time PCR provides a convenient platform for HBV cccDNA detection as it allows for the rapid simultaneous amplification and quantification of a specific DNA target through either non-specific or specific DNA detection chemistries. In conclusion, this HBV qPCR assay should enable improved monitoring of patients' responses to antiviral therapy.