HBV DNA Real Time Quantification according to ARNS 12187 project

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Abstract

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Using the Arrow extractor (NorDiag, Biotrin International, Ireland), DNA was extracted from 240 µl of plasma, pre-treated with10 µl of proteinase K, using the Arrow Viral NA extraction kit, according to the manufacturer's instructions during 45 min. Template DNA was eluted into 60 µl of kit elution buffer. Besides clinical samples, quantification standard (Acrometrix HBV Panel, Acrometrix, Menica, CA, USA) was also extracted for each run with the same protocol and1:10 diluted (from 50,000,000IU/mL to50IU/mL). For amplification, we used a primers/probe set designed under the auspices of "Agence Nationale de Recherches sur le SIDA et les hépatites virales" (ANRS 12187 project) and targeting a conserved region in the HBVS gene (nucleotide (nt) positions, 379–426). All runs were performed in a 50-µl volume containing DNA extract (10 µl), Master Mix (Platinum UGD, USA) (25 µl), pure water (HyClone Pure Water, Thermo Fisher Scientific, Waltham, MAUSA)(12.5 µl), forward primHBV1 (5'-GTGTCTGCGGCGTT TTATCA-3') and reverse primHBV2 (5'-AGGCATAGCAGCAGGAT GAA-3') primers at 10 µM (1µl each) and probe (5'FAM-TGCGGCGTTTTATCAT-MGB3') at 5µM (0.5 µl). Each reaction consisted of: 2min at50°C and 10min at95°C; followed by 50 cycles of 15sec at 95°C and 1 min at 60°C each. The lower limit of quantification (LLOQ) of our technique was 100IU/mL and the lower limit of detection (LLOD) was 50IU/mL.

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