HBV genotyping of S and C genes.

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Abstract

HBV genotyping was performed by sequencing and phylogenetic analyses of the surface (S) and core (C) fragments. Briefly, HBV DNA was first extracted from 400 µL of plasma and eluted in 100 µL of pure water, using the QIAamp Viral DNA Mini Kit (QIAGEN, Courtaboeuf, France) followed by semi-nested PCR amplification of the S (930 bp) and C (1010 bp) gene fragments using MP *Taq* Core Kits 25 (MP Biomedical Diagnostic, Europe). The S fragment amplification was performed as described elsewhere.

(Hu X, et *al*., 2000 [PMID: 10677515] ; Makuwa M et *al*., 2006 [PMID: 16847965]; Olinger CM et *al*., 2006 [PMID: 16603517])

The first round was performed using primers sets 58P (5'-CCT GCT GGT GGC TCC AGT TC-3') and 979 (5'-ATT GGA AAG TAT GTC AAA GAA TTG TGG GTC TTT TG-3'). The 50 µL final reaction mixture contained 31.4 µL of RNase DNase Free water, 5 µL of buffer 10X with $MgCl_2$ (25 mM), 0.4 µL of dNTPs (25 mM), 1.5 µL of each primer (10 µM), 0.2 µL of *Taq* polymerase (5 U/µL) and 10 µL of extracted DNA. The second round PCR used the 58P/Mc2r (5'-TGGAAGTTGGGGATCATTGCC-3') primer on a 50 µL final reaction mixture containing 36.4 µL of RNase DNase Free water, 5 µL of buffer 10X with $MgCl_2$ (25 mM), 0.4 µL of dNTPs (25 mM), 1.5 µL of each primer (10 µM), 0.2 µL of *Taq* polymerase (5 U/µL), and 5 µL of first round PCR product. The PCR program was the same for the first and second round PCRs including denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s and elongation at 72°C for 1 min, followed by final elongation at 72°C for 5 min. For the C fragment amplification, the couplet of primers BCP1F (5'-GCA TGG AGA CCA CCG TGA AC-3') / 2853N (5'-TCA CCA TAT TCT TGG GAA CA-3') was used for the first round and the couplet BCP2F (5'-CAT AAG AGG ACT CTT GGA CT-3') / 2853N for the second round. The amplification conditions were the same as for the S fragment.

The PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Courtaboeuf, France) and submitted for sequencing at Macrogen Inc (Meibergdreef, Netherlands).

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