

Supplementary Materials

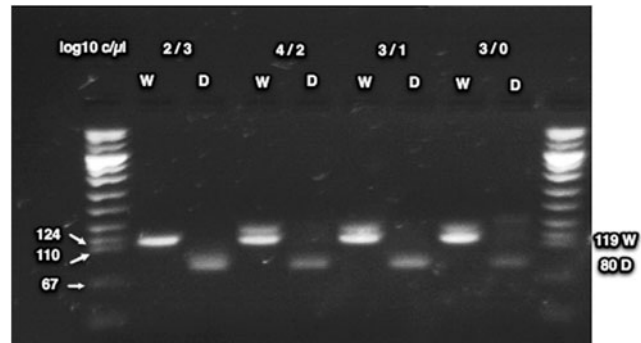
Specific detection of the patient integrase gene

Differential nested polymerase chain reaction (PCR) with primers specific for the deleted or the wild-type (WT) region. The first PCR was done using the sense (F567: 5'-GGGGATTGGGGGATACAGTG-3') and antisense (R674-712: 5'-GTCCTTTCCAAATGGGGTCTCT-3') primers (designed from the consensus integrase obtained by population sequencing of the patient virus), giving a 146-bp or 107-bp fragment for the WT and deleted gene, respectively. PCR conditions were 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 54°C, 30 s at 72°C, and a final extension of 10 min at 72°C, in a final volume of 50 μ l, containing 1.5 mM MgCl₂ and 0.5 μ M of each primer. The Titan one tube RT PCR kit (Roche Diagnostics) was used for RNA samples and the AmpliTaq kit (Applied Biosystems) for DNA samples.

Nested PCR was performed with the AmpliTaq kit and 0.5 μ M of specific primers. For the deleted sequence the primers were 5'-TACAGTGCAGGGGAAAGAAAGAATAA TA-3' (F580) for the sense strand and 5'-GGGTCTCTGC TGTCCCTGAT-3' (D660) for the antisense strand. A hemi-nested PCR was performed for the WT sequence with the first PCR sense primer (F567) and an internal antisense primer (W686: 5'-TCCCTGTAATAGACCCGAAAAT-3'). The conditions were as for the first PCR but with an annealing temperature of 54°C for the WT-specific and 60°C for the deleted-specific primers. Amplicon sizes were 119 bp for the WT fragment and 80 bp for the deleted fragment.

The sensitivity was evaluated using plasmids obtained after cloning of the amplified patient's full integrase gene with Gateway recombination technology (Invitrogen, Life Technology). The assay is able to detect one copy of the deleted gene in 10³ copies of the WT gene (Supplementary Fig. S1).

Detection and quantification of the deleted gene by real time PCR with specific probes. To increase the detection threshold, a 15 cycle preamplification was done with the primers described above (F567 and R674-712). Real time



SUPPLEMENTARY FIG. S1. Plasmids containing the sequence of the WT and deleted integrase of the patient were mixed in variable proportions, as indicated at the top of the figure in logarithmic values, and amplified using specific primers as indicated in the Supplementary Methods section. PCR products were run on a 3% agarose gel. The band sizes of the molecular weight marker are indicated on the *left* and those of the PCR products on the *right*. W, WT product; D, deleted product; c, copies.

PCR was performed with the Rotor Gene Multiplex PCR kit (Qiagen), with 0.4 μ M of each primer (F580 and R674-712) and 0.2 μ M of each probe (W-F: 5'-FAM-TTCCCTGTAA-TAGACCCGAAAATTTTGA-DAB-3' for the WT and D-H: 5'-HEX-CTGTCCCTGTTGTAGTTC-BHQ1-3' for the deleted sequence). The product was amplified in a Rotor Gene instrument by 5' at 95°C followed by 50 cycles of 10 s at 95°C and 15 s at 60°C.

A standard curve was obtained from mixtures of the patient's integrase WT and deleted clones in an increasing proportion (0, 1%, 10%, 50%, 90%, 99%, and 100%) at 100 copies/ μ l. Each mixture was amplified with the two probes and a curve was drawn for WT from the WT probe cycle threshold (C_t) and for the deleted variant from the deleted-specific probe C_t, according to their respective amounts in the mixture.