

Genotyping of Hepatitis C Virus by Sequence Analysis of the Amplicon from the Roche Cobas AmpliPrep/Cobas TaqMan Viral Load Assay[▽]

Hepatitis C virus (HCV), a positive single-stranded RNA virus, is one of the major causes of end-stage liver disease worldwide (5). The nucleotide sequence of the HCV genome is an important predictor of response to antiviral therapy (6). Genotypic analysis is used together with measurement of viral load (VL) to help in the management of patients with HCV infection (1). The 5' untranslated region (UTR) is often used for monitoring VL because it is less variable than other regions of the genome and consequently less likely to suffer PCR failures due to sequence variation at the primer binding sites (3, 6, 8). Although this region is not ideal for determination of the genotype, it is often used for this purpose because of the availability of the template after a VL assay and also because the genotypic information needed for clinical use can be obtained from it (2, 4, 9, 10).

Our initial attempt to sequence the HCV 5' UTR using the reverse transcription-PCR (RT-PCR) amplicon from the recently introduced Roche Diagnostics (Basel, Switzerland) Cobas AmpliPrep/Cobas TaqMan HCV test (HCV-T) (8) employed a set of sequencing primers originally described by Gargiulo et al. (2) for analysis of the RT-PCR amplicon from the Roche Cobas AmpliPrep HCV monitor test (HCV-M). This attempt failed because the sequence obtained was often inconsistent with the genome of HCV (data not shown). We suspected that this failure was due to the presence of an abundant amplicon from the internal quantitation standard (QS). The QS is a small nucleic acid that is added to the specimen in the VL assay as an internal control. It is amplified using the same primers used to amplify the HCV target but contains a different internal sequence so that it can be detected using a TaqMan probe with a different fluorophore than the one used to detect the HCV amplicon. To troubleshoot this problem, we sequenced the amplicon from a clinical specimen that was negative for HCV, using the sequencing primers KY78 and KY80 (2), to obtain the sequence of the QS amplicon that is generated by the Roche HCV-T. As shown in Fig. 1, the primers designed by Gargiulo et al. (2) to sequence the amplicon from the HCV-M assay (labeled MF and MR in Fig. 1) are no longer specific for HCV, but hybridize equally well to the QS. Presumably the QS in the HCV-T assay contains additional nucleotides from the HCV genome compared with the QS from the HCV-M assay. We designed new sequencing primers to regain specificity for HCV and avoid sequencing the QS to obtain an acceptable HCV sequence (data not shown). The upstream primer is 5'-AGCCATGGCGTTAGTATGAG-3', and the downstream primer is 5'-GCAGTACCACAAGGCCCTTTC-3'. As illustrated in Fig. 1, the upstream primer from Gargiulo et al. (MF) is moved downstream by 3 bp to produce the TF primer, and the downstream primer (MR) is moved upstream by 3 bp to produce the TR primer. Both of the present primers have three nucleotides at the 3' end to create specificity for the HCV 5'-UTR sequence and avoid amplification of the QS but still avoid the variable regions that are needed for determination of the genotype (9). To obtain as complete a sequence in both directions as possible, we have been using the Applied Biosystems (Foster City, CA) BigDye Terminator v1.1 cycle sequencing system because it gives a readable sequence close to the primer. This system provides

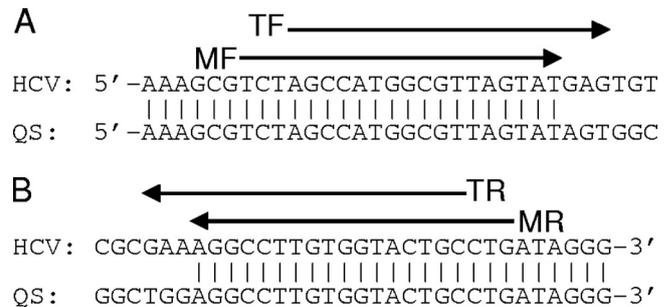


FIG. 1. Selection of sequencing primers for the HCV-T VL amplicon. In each panel, the top line of the sequence is from HCV and the bottom line of the sequence is from the HCV-T QS. Positions that are the same are connected by a vertical line. All sequences are written 5' to 3' with respect to the viral genome. Panel A shows the upstream part of the amplicon (nucleotides 72 to 103 in accession no. NC_004102), and panel B shows the downstream part of the amplicon (nucleotides 270 to 301). The positions of the primers are indicated by horizontal arrows as follows: MF and MR for the upstream and downstream primers, respectively, from Gargiulo et al. (2) for sequencing the amplicon from the HCV-M VL assay; and TF and TR for the upstream and downstream primers, respectively, for sequencing primers that are useful with the amplicon from the HCV-T VL assay.

sequence information in both directions from nucleotides -241 to -70 (101 to 272 in accession no. NC_004102) in the HCV genome. This region, although useful for determination of the genotype, has limitations in certain circumstances, as published previously (2, 4, 7, 10).

Using the methods described above, we genotyped 63 clinical specimens positive for HCV and compared these results with those obtained from the Hologic Third Wave Technologies (Madison, WI) HCV genotyping analyte-specific reagents. The reported genotypes were fully concordant for 45 type 1, 8 type 2, 7 type 3, and 1 type 4. The two discrepancies included one specimen reported as a type 5 genotype by the Hologic assay, which looked more similar to a type 1 by sequence analysis, and one specimen reported as a mixed type 1 and 2 genotype by Hologic, which was not uniquely typeable by sequence analysis because of several positions with overlapping nucleotides. In addition, our results were fully concordant for genotypes 1, 2, 3, 4, 5, and 6 using the Teragenix HCV genotype performance panel (SeraCare Life Sciences, Milford, MA). We conclude that the amplicon generated by the Roche HCV-T VL assay is adequate for sequence analysis to genotype HCV using the sequencing primers provided above.

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