Specific detection of Hepatitis C virus in clinical samples

using a novel simplification strategy

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Human Genetic Signatures

Methods

HCV RNA samples were separated from Acrometrix (OptiQual HCV high positive controls), or SB diagnostics (HCV RNA finely panel of 64 strains, and worldwide HCV genotype panel RNA HCV 4) and purified with the QIAGEN Viral RNA purification kit according to the manufacturer’s protocol (21). Blinded clinical samples were obtained from a local hospital and purified as above. Samples were treated with sodium bisulfite and 10% of the converted HCV RNA samples were directly treated with SuperScript II reverse transcriptase (Invitrogen) or ScpT reverse transcriptase (Biolynx) random primers. One third of the cDNA was then subjected to other variants or real-time PCR amplification with primers (and probe) specific for converted HCV RNA. End-point PCR was performed in 25μl reactions using 1.5X Promega master mix and 100ng of base and reverse primers and cycled at 56°C, 3 min, 36°C, 10 sec, 54°C, 1 min 45°C, 1 min 45°C in a Taqman PCR thermal cycler. Real-time PCR was performed in 25μl reactions using 0.4Ha Juntaeed master mix, 50ng of forward and reverse primers, DNA MgCl2, and 4500×FAM-labelled probe and cycled at 56°C, 3 min, 56°C, 10 sec, 53°C, 30 sec, 45°C in a Corbett RotorGene. One third of the PCR products was electrophoresed on a 2% precast gel for sizing.

Results

Figure 1: A schematic diagram of the blind clinical samples used in our study. Using our simplified HCV PCR assay shown in Figure 3. Using this assay we see to detect HCV RNA from 1 652 550 IU down to 386 IU/ml.

Figure 2: Full length window of the amplified 18 bases of converted HCV RNA samples. The assay can simultaneously detect all major genotypes of HCV including 1a, 1b, 2a, 2b, 3a, 3b, 4a, 5a, 6a, 6b, 6c, 6d, 7a, 7b, 7c, 8a, 8b, 8c, 9a, 9b, 9c, 9d, 10, 11 and 12.

Figure 3: A dynamic range of concentrations from 3.9 to 1 652 350 IU/ml, was purified from Acrometrix OptiQual HCV high positive control, bisulfite converted, reverse transcribed and amplified with primers and probe specific for converted HCV RNA. The legend indicates the amount of HCV RNA equivalent in IU/mL. The O indicates a serum negative control which has been subject to a 10 fold serial dilution measurement.

Discussion & Conclusions

We have developed a completely novel assay for detecting all strains of HCV based on simplification of the RNA by sodium bisulphite. Simplification of RNA has not been achieved previously using simplification. Our assay is a quick and easy to perform and results in a sensitively broad range of HCV concentrations ranging from 1 652 550 IU/ml down to 386 IU/ml.

Detection is currently based on endpoint or real-time PCR using primers specific for bisulfite converted HCV RNA. The HGS HCV assay can simultaneously detect all major genotypes of HCV including 1a, 1b, 2a, 2b, 3a, 3b, 4a, 5a, 6a, 6b, 6c, 6d, 7a, 7b, 7c, 8a, 8b, 8c, 9a, 9b, 9c, 9d, 10, 11 and 12.

Figure 4: Selection of the results from the blinded clinical samples assayed for HCV RNA by either the Bayer Venky HCV 3.0 or Ortho AMPLICOR HCV 2.0 and the HGS HCV assay.

Blot analysis shows that the samples had deteriorated over the storage period. The final sample was positive by our test but negative by the Roche or Bayer test at least one year prior to our testing. However, we showed that the final sample was not positive by our test but negative by the Ortho test. It is likely that we had to use the detection control and the samples had deteriorated over the storage period. The final sample was positive by our test but negative by the Roche or Bayer. Using real-time PCR to confirm the finding, but this may represent another case of improved sensitivity of our assay.

Figure 5: Comparison of the results from the blinded clinical samples assayed for HCV RNA by either the Bayer Venky HCV 3.0 or Ortho AMPLICOR HCV 2.0 and the HGS HCV assay.

No. of samples 138

Blinded clinical samples

Table 1: Comparison of the results from the blinded clinical samples assayed for HCV RNA by either the Bayer Venky HCV 3.0 or Ortho AMPLICOR HCV 2.0 and the HGS HCV assay.

1a 1b 2a 2b 3a 3b 4a 5a 6a 6b 6c 6d 7a 7b 7c 8a 8b 8c 9a 9b 9c 9d 10 11 12

In order to ensure that our assay was able to detect all common HCV genotypes, we evaluated it’s performance, linearity and genotyping panels. Finally the assay was assessed on a variety of viral RNA over a very broad range of concentrations.

The HGS HCV assay was used to assess a blinded panel of 138 clinical samples and was shown to be positive by the Roche or Bayer kit on 126/138 (91%) samples. The HGS assay performed better than the Roche or Bayer kit HCV RNA detection assay, and the Ortho COBAS AMPLICOR HCV II test with 126/138 (91%) agreement. Of the 12 samples that were not in agreement one of the strains had been positive by one of the other tests but was negative by our test. The other ten samples were positive by our test and was shown to contain HCV by sequencing. Thus, we believe that this illustrates that our test is more sensitive than the other tests. Two of the other three samples were positive by our test but not by sequencing. It was shown to be positive by the Roche or Bayer test at least one year prior to our testing. However, we had shown that the final sample was positive by our test but negative by the Ortho test. It is likely that we had to use the detection control and the samples had deteriorated over the storage period. The final sample was positive by our test but negative by the Roche or Bayer. Using real-time PCR to confirm the finding, but this may represent another case of improved sensitivity of our assay.