

## **QUANTITATIVE DETECTION OF HEPATITIS E VIRUS (HEV) FROM INFECTED STOOL SAMPLES BY REAL-TIME FLUOROGENIC RT-PCR**

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Hepatitis E virus is a positive strand RNA virus that is transmitted by the fecal-oral route and causes an acute self-limited or fulminant liver disease. The HEV genomic sequence data show that the virus has two major geographically distinct strains, Asian and Mexican. The pathogenesis of hepatitis E has been difficult to characterize, because of the lack of an *In vitro* culture system. In this study, a sensitive 3'-noncoding based fluorogenic RT-PCR was developed to detect HEV of different geographic origins. A fluorogenic dye labeled oligonucleotide, HEV-EP2 was used as internal hybridization probe and reporter in this 5'-3' nuclease assay. Combination of HEV-EU2/EL4 (sense primer, EU2 and anti-sense primer, EL4) flanking primer pair and HEV-EP2 probe mixture can be used to detect HEV SAR55 strain and other HEV strains of Asian origin. The optimal condition of the HEV RT-PCR was systematically worked out by evaluating various primers/probe concentrations and ratio, annealing and extension temperature for Taq DNA polymerase. The quantitative HEV assay developed from this study has a dynamic range of 10-10<sup>6</sup> HEV genomic equivalent (GE) copies with coefficient of regression of 0.98 or greater. The highly efficient HEV-specific fluorogenic RT-PCR assay and its GE standard developed in this study can be used to quantitatively diagnose active HEV infection and to improve *In vitro* infection systems for HEV propagation.

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