EVALUATION OF A COMMERCIAL REAL-TIME PCR KIT FOR DETECTION OF HEPATITIS E VIRUS

Karel Boissinot, Étienne Delangre, David Beaulieu, Christian Renaud*
Centre Hospitalier Universitaire Sainte-Justine, Université de Montréal, Montréal, Québec, Canada.

Background

- Hepatitis E virus (HEV) is responsible for acute hepatitis in developing countries, however, it can also cause chronic hepatitis and cirrhosis in transplant patients under immunosuppression. Recent studies have also identified HEV in blood products destined for transfusion. There is currently no FDA approved HEV molecular diagnostic tests but there is a clinical need for detection in transplant patient and high-risk transfusion recipients. Furthermore, modulation of immunosuppressive medication could help clearance while minimizing risk by having quantitative monitoring of viral load.

Methods

- Evaluation of the Altona Diagnostics RealStar™ HEV RT-PCR Kit 1.0 was performed with the addition of a standard curve, also from Altona Diagnostics. Quantitative detection of HEV was performed using the World Health Organization viral control available from Paul Ehrlich Institute (HEV genotype 3a) as a spike into negative clinical samples composed of hepatic biopsies, stool, serum, plasma and whole blood. Samples preparation was performed using Total RNA extraction kits and Maxwell 16 automatic extractors from Promega. Enogenous internal controls were added after lysis but before nucleic acids extraction. Amplifications were performed on ABI 7500 thermocyclers. All analyses were performed in Sainte-Justine University Hospital Center in Montréal, Québec, Canada.

- We decided to include the WHO viral control as an external control in each run.

Objectives

- Evaluate the performance of the Altona Diagnostics RealStar™ HEV RT-PCR Kit 1.0. Determine which sample types are compatible with the kit and routine use.

Altona RealStar HEV Kit Description

- Spiked Concentration (IU/µl) Detected Samples Mean Ct SD CV% Quantification
  - 30 16/16 30.79 0.56 1.83 100.20
  - 3 16/16 33.95 0.61 1.79 12.83
  - 1.5 15/16 36.33 1.31 3.60 3.56
  - 0.65 14/16 39.52 2.07 5.23 0.52

- Spiked Concentration (IU/µl) Detected Samples Mean Ct SD CV% Quantification

Results

- Table 1: Performance in Plasma

- Table 2: Performance in Serum

- Table 3: Performance in Hepatic Biopsy, Stool and Whole Blood

- Table 4: Precision of the method

Summary

- The Altona Diagnostics RealStar™ HEV RT-PCR Kit 1.0 was evaluated over a broad range of concentration with emphasis towards the lower limit of detection.

- We determined a LOD of 0.58 IU/µl (95% CI 0.35-1.7) using probit analysis.

- No inhibition in plasma and serum was detected.

- Variable inhibition in liver biopsies

- We decided to include the WHO standard as an external control in each run.

Conclusion

- Realstar™ HEV RT-PCR Kit 1.0 plus standard curve allows quantitative detection of hepatitis E virus in serum, plasma and hepatic biopsies.

- However, for hepatic biopsies, partial inhibition is possible and variable from sample to sample while stool and whole blood sample resulted in total inhibition.

- Plasma has been selected for routine testing of HEV.

- Availability of a rapid quantitative molecular test will increase our understanding of this disease and inform us on the prevalence of the virus in transplant patients from Québec.