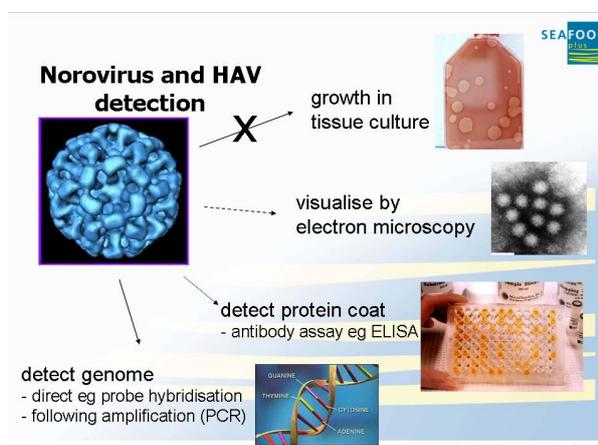
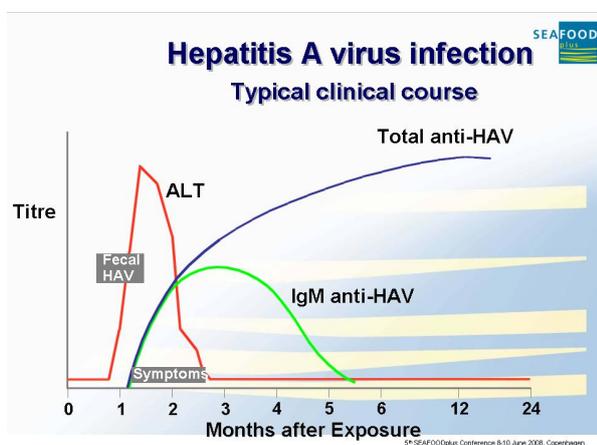


## New validated rapid methods for detection of viruses in bivalve molluscs

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Norovirus (NoV) is the most commonly identified cause of outbreaks and sporadic cases of acute gastroenteritis in human. Hepatitis A virus (HAV) infection is the leading cause of acute viral hepatitis throughout the world. Within the REFHEPA project, the development of sensitive reliable techniques for the accurate quantification of NoV and HAV in shellfish was developed as it is required to ensure the safety of these products. A dramatic improvement in diagnostic virology comes from the emergence of real-time RT-PCR, which makes use of fluorescent probes and enables not only qualitative determination but also quantitative assays. Assays for the detection of NoV genogroups I and II must overcome the difficulties due to NoV genetic diversity. Molecular data have demonstrated relatively conserved regions at the 3' -end of ORF1 (polymerase) and the 5' -end of ORF2 (capsid) regions of NoV, being the ORF1/ORF2 junction employed as a target for real-time amplification. Real time procedures based on the amplification of a fragment of the highly conserved 5' non-coding region (5'NCR) have also been successfully developed for HAV quantitative detection in shellfish.

Specific and sensitive Taqman real-time reverse transcription-PCR assays for the detection and discrimination between the two NoV genogroups have been designed. Consensus primers and probes have been selected for both genogroups and PCR conditions have been optimized for sensitivity and specificity. Conditions then have been compared and adapted to be compatible with the real-time assay designed for HAV.

To avoid any false negative result due to inhibitors, NoV and HAV RNA internal controls have been constructed and titrated. Each sample then is analyzed for the different NoV genogroups as well HAV and also co-amplified with each internal control to evaluate the RT-PCR efficiency. Quantification may be then estimated based on the standard curve done for each genogroup.

Standard reagents, such as the MC0 Mengo virus strain and ssRNA internal controls have been employed as controls of nucleic acids extraction and RT-PCR, respectively. Quality control and quality assurance issues have been implemented through the use of standardized molecular procedures that may enable its inclusion in regulatory standards for viruses in molluscan bivalves.

Validation of the assay was performed with shellfish bioaccumulated with GI & GII NoV reference strains at several NoV concentrations. The assay was also successfully applied to naturally contaminated samples.