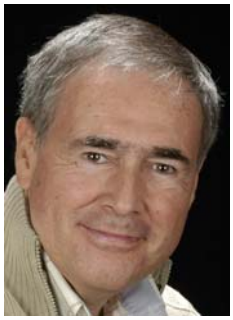
Standardised real-time RT-PCR assay for Norovirus and Hepatitis A virus detection



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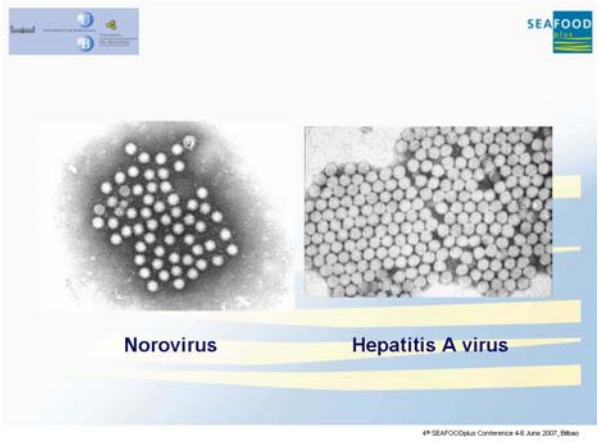

The objective of the project REFHEPA within SEAFOODplus is the development of sensitive, standardised methods for the detection of hepatitis A virus (HAV) and norovirus (NoV) in bivalve molluscan shellfish to the point at which they could be used successfully in a routine diagnostic context to increase the safety of shellfish delivered to the consumer. Methods cited in the literature are diverse, complex, poorly standardised and restricted to a few specialist laboratories. It seems obvious that quality control and quality assurance issues must be solved, as well as simplification and automatation, of molecular procedures before they could be adopted by routine monitoring laboratories.

NoV are the most commonly identified cause of outbreaks and sporadic cases of acute gastroenteritis for all class age. HAV infection is the leading cause of acute viral hepatitis throughout the world. During epidemics high numbers of viral particles are rejected into the environment, making possible contamination of different type of food. Both NoV and HAV infections are propagated via the fecal-oral route being the person-to-person contact the most common mode of transmission. Shellfish grown and harvested from waters receiving urban contaminants is a cause of outbreaks of gastroenteritis and infectious hepatitis. However, due to technical limitations, it remains still unknown the role of lightly contaminated shellfish in the burden of sporadic cases.

The development of sensitive reliable techniques for the accurate quantification of NoV and HAV in shellfish 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, and particularly, quantitative diagnostic assays. Assays for the detection of NoV genogroups I and II must overcome the difficulties due to NoV genetic diversity. 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 assays designed for HAV.

Standard reagents, such as the MC_0 mengo virus strain and the 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.