Title Detection and quantification by real-time RT-PCR of hepatitis A virus from inoculated tap waters, salad vegetables, and soft fruits: Characterization of the method performances

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Abstract

Water, salad vegetables and fruits exposed to fecal contamination may cause outbreaks of hepatitis A. A protocol of viral concentration by filtration on electronegative membrane filter and a protocol based on a viral elution in Tris–glycine buffer, pH 9.5 with concentration by polyethylene glycol precipitation were associated with real-time, reverse transcriptase-PCR to detect hepatitis A virus (HAV) artificially inoculated in 2 l of tap water, or on 25 g of fruits or salad vegetables. These methods were characterized by an intra-laboratory study using the international standard ISO 16140 on five types of tap water, six types of fruit and five types of salad vegetable. Linear regression models describing the quantitative reactions were good fits to data, and the variances of results were constant in the whole range of viral concentrations tested, which was from about 1.7 to 5.7 log plaque-forming units (PFU) per 2 l of tap water, from about 2.0 to 4.5 log PFU/25 g of fruits, and from 1.5 to 3.5 log PFU/25 g of salad vegetables. Fractions of inoculated viruses recovered were estimated to be about 20% for tap water, about 16% for salad vegetables, and about 7% for fruits. The probability of detecting positive samples was 50% (the critical level of detection) when 2 l samples of tap water were inoculated with 0.7 log PFU of HAV, 25 g samples of iceberg lettuce were inoculated with 1.0 log PFU of HAV, and 25 g samples of fresh and frozen raspberries were inoculated with 1.0 and 1.5 log PFU of HAV, respectively.