

Topic: Food and environmental virology

IMPLEMENTATION OF AN ISO-COMPLIANT METHOD FOR THE SURVEILLANCE OF HAV AND NOROVIRUSES IN WATER FOR HUMAN CONSUMPTION AND SURFACE

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Aim of the study: The viral food-borne diseases are one of most relevant problem. Infact, approximately 70% of acute gastroenteritis cases and 10% of traveler's diarrhea have a viral origin, with Hepatitis A virus (HAV) and Noroviruses being among the main causes [1]. This study, followed the ISO 17025 [2], has the aim to accreditation for the method used to monitor and surveil Hepatitis A virus, Norovirus GI, and GII in bottled water, in human consumption water and surface swab, using qualitative detection methods according to ISO 15216-2:2019 [3].

Methods: The experiments consist of an artificially contaminating matrix of drinking water and swab with quantified viral suspensions of HAV, Norovirus GI, and Norovirus GII, as well as the Mengovirus, used as a control. Samples are extracted using commercial kits following the manufacturer's instructions, and amplified by RT Real-Time PCR using primers and probes recommended by ISO 15216-2:2019 [3].

Results and conclusions: The experiments consist of three steps, according to the requirements in the "EURL Guidance document for verification of ISO 15216-2:2019" [4]. The aim of Step 1 is to check for natural contamination of the sample, potential PCR inhibition, and extraction efficiency. With Step 2 we value the matrix effect associated with the sample. In Step 3, we calculate the LOD50 and sensitivity, using six serial 5-fold dilutions (1:5) to cover different contamination levels (high, medium, and low), starting from approximately 10,000 copies/sample. The LOD50, expressed in copies/ml for water and in copies/cm² for swab, is 0.065 and 2,099 for HAV, 0.024 and 0,431 for Norovirus GI and 0.018 and 1,952 for Norovirus GII respectively. Sensitivity for all contamination levels cover the 100% both for water and for swabs. The results satisfy the requirements concerning i) extraction inhibition, ii) extraction efficiency, and iii) LOD50 as specified in the "EURL Guidance document for verification of ISO 15216-2:2019". Thanks by this results the laboratory successfully passed the accreditation body's surveillance audit and the method is used in the routine laboratory analysis for detectin of HAV, Norovirus GI, and GII in official samples.

1. Fernandez-Cassi X, Martínez-Puchol S, Silva-Sales M, Cornejo T, Bartolome R, Bofill-Mas S, Girones R. Unveiling Viruses Associated with Gastroenteritis Using a Metagenomics Approach. *Viruses*. 2020 Dec 13;12(12):1432. doi: 10.3390/v12121432. PMID: 33322135; PMCID: PMC7764520.
2. UNI EN ISO 17025:2018 Requisiti generali per la competenza dei laboratori di prova e taratura
3. UNI EN ISO 15216-2:2019 – Microbiology of the food chain – Horizontal method for determination of Hepatitis A virus and Norovirus using real-time RT-PCR – Part 2: Method for detection.
4. EURL Guidance document for verification of ISO 15216-2 :2019