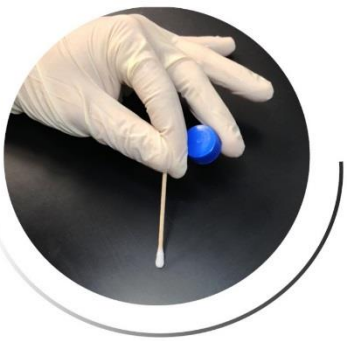


WHATfinder

Norovirus and Hepatitis A Real-Time PCR kits

According to ISO/TS 15216

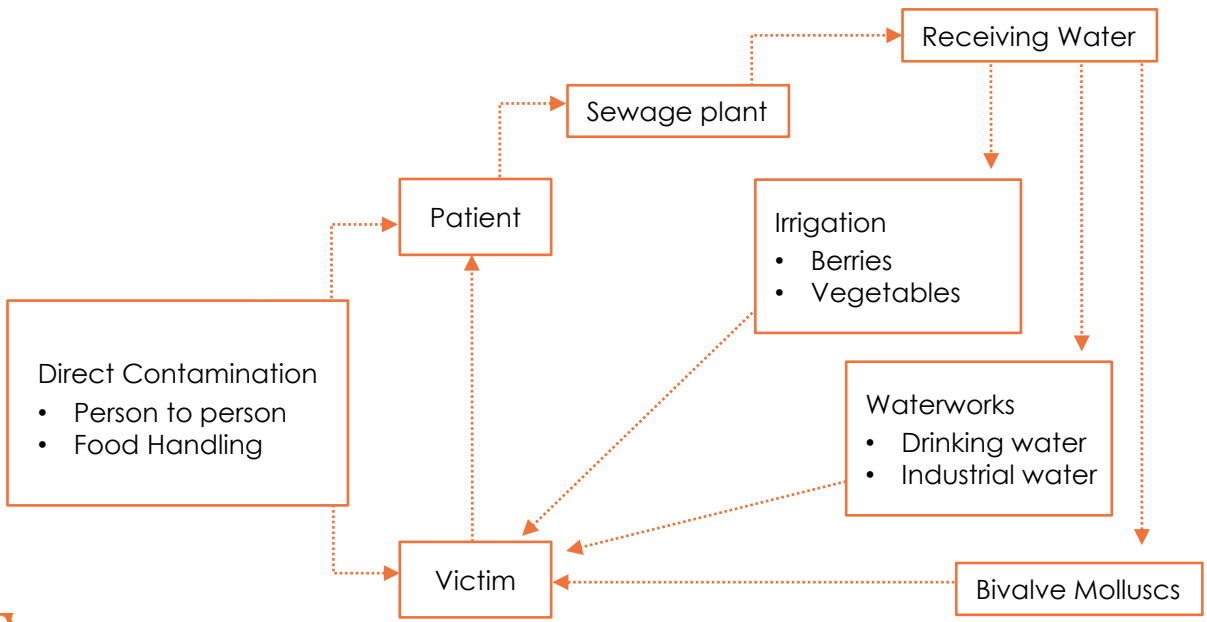
GENERON
Food Safety in our Genes

Foodborne viruses are a priority hazard for FAO and WHO since 2008

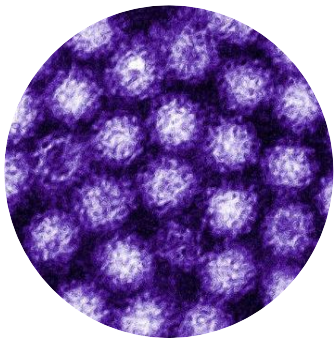
The control of viruses is an area of increasing importance for the food industry. A recent report by the UK FSA states that norovirus poses the highest burden to society out of 13 foodborne pathogens including Campylobacter and Salmonella.

Hepatitis A virus (HAV) and Norovirus (NoV) are important agents of food-borne human viral illness. They are transmitted by the faecal-oral route, hence infecting their host after ingestion, followed by invasion of cells in the epithelial lining of the gut, and subsequent shedding into sewage. It's intuitive that food-borne transmission can occur by food contamination, by infected food handlers or by food contamination during the production process.

Diagram 1 – Pathways of transmission for HAV and NoV



Foodborne viruses' control presents many technical hurdles



Bacteria-based control processes can not address the risks posed by viral hazards

In the past, the risk of contamination by HAV and NoV was indirectly assessed monitoring fecal contamination by testing for levels of E. coli above a specified limit. However, the use of indicator microorganisms of fecal pollution is not a reliable means of determining the extent of HAV and NoV because of the higher persistence and survival of viruses in food, as well as greater variability in virulence, infectivity rates, and host response

No routine methods exist to culture these viruses from food

Vegetables, berries and shellfish represents a major cause of outbreak also because they are perishable and so consumed raw and in few hours after harvesting. This hampers the possibility of a strict quality control.

Since 2013, ISO 15216 is the standard method of detection

qPCR is to date the only published method offering the possibility of direct detection of HAV and NoV in environmental samples and foods. Its high sensitivity and accuracy enables the detection of as little as 10 virus copies.

Availability of surrogate viruses similar to HAV and NoV but can be easily cultured are fundamental

Surrogate viruses like feline Calicivirus (FCV) or bacteriophage MS2 can be easily cultured and used to give an indication of how the pathogens behave when they are subjected to control measures such as heat processes, acidification, changes to water activity, and disinfecting systems. Moreover, surrogates can be used, instead of the target virus, to validate virus control analytical methods.

Generon developed a portfolio of kits to provide the customers with a turn-key solution to test the presence of these pathogens according to ISO/CEN indications. The portfolio includes all the reagents and controls to execute the ISO 15216 detection protocol

Availability of surrogate viruses similar to HAV and NoV but can be easily cultured are fundamental

Losses of target virus can occur at several stages during sample virus extraction and RNA extraction. Hence, ISO 15216 includes a thorough process control to monitor the efficacy of virions recovery. Samples must be spiked prior to processing with a defined amount of a *Caliciviridae* surrogate virus; the level of recovery of the process control virus is then determined for each sample. Moreover, in order to control for RT-PCR inhibition in individual samples, an external control must be added to an aliquot of sample RNA and tested using the RT-PCR method (see diagram 2).

ISO 15216 workflow

Virus extraction and concentration

Swab surface up to 100 cm^2 into 500 µL PBS buffer volume.

Add 25 g sample to 40 ml TGBE (with pectinase for soft fruit), elute viruses by shaking and filter eluate. Precipitate using PEG/NaCl, resuspend pellet in 500 µL PBS buffer volume. For soft fruit further clarify using chloroform and butanol mixture

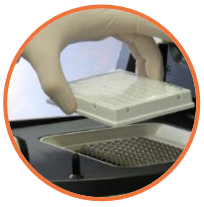
Filter concentrate 0.3 to 5 litres through a positively charged membrane, elute in TGBE buffer, adjust to pH 7, concentrate to 500 µL using a centrifugal filter device

Chop 2 grams of excised digestive gland, add an equal volume of proteinase K solution, incubate at 37°C and 60°C then clarify by centrifugation

RNA extraction

Extract nucleic acid from 500 µL sample using lysis with guanidine isothyocianate and silica binding matrix, or an alternative suitable method. Elute RNA in 100 µL volume

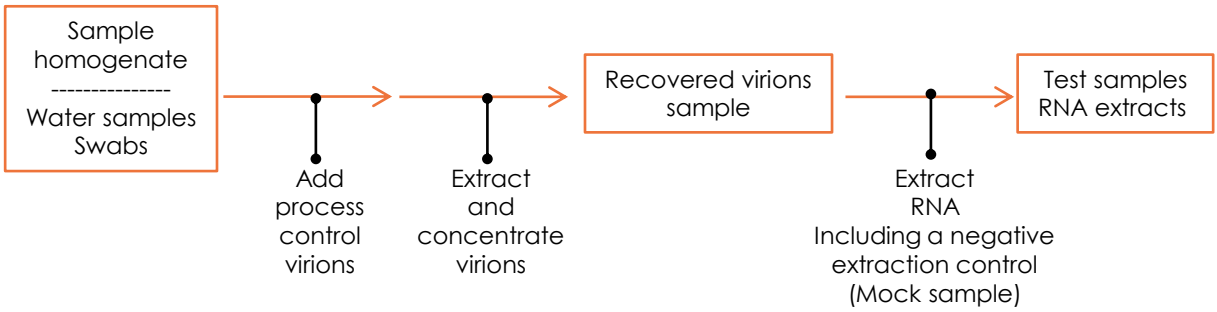
Real-Time PCR detection and results interpretation



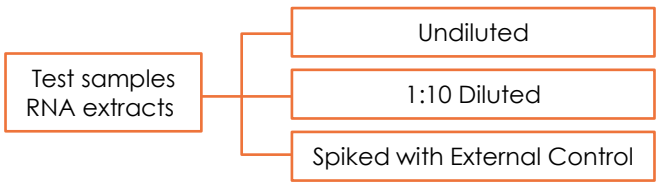
One step real-time RT-PCR, RNA (diluted and diluted) assayed for each target (HAV, NoV-I and II). Controls for extraction efficiency (process control virus assay), amplification efficiency and standard curves for quantification included

Diagram 2 – Experimental set-up for qualitative and quantitative analysis

A. Unknown test samples RNA extracts preparation

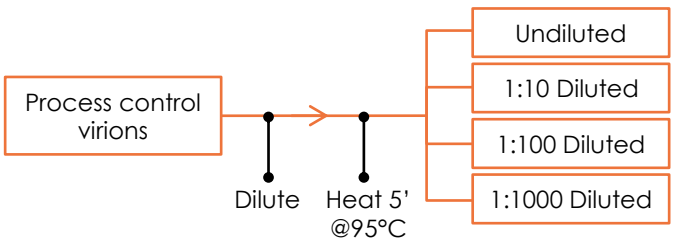


B. Real-Time PCR **"unknown samples"** preparation



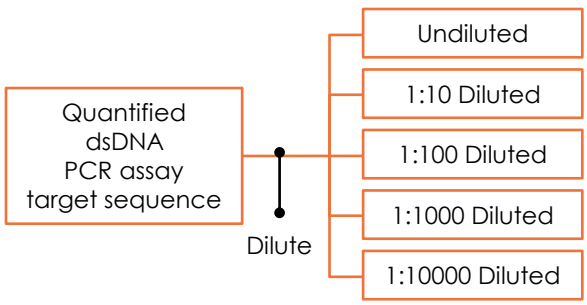
To control for RT-PCR inhibition in individual samples, external control RNA (EC RNA), carrying the target sequence of interest, must be added to an aliquot of sample RNA and tested in parallel. Comparison of the results of this with the results of EC RNA in the absence of sample RNA highlights the level of RT-PCR inhibition in each sample tested.

C. Real-Time PCR **"process control"** samples preparation for setting "recovery standard curve"



Losses of target virus can occur at several stages during sample virus extraction and RNA extraction. Hence, samples are spiked prior to processing with a defined amount of a process control virus. The recovery of the process control virus is determined for each sample referencing the C_q value for the process control virus assay from the test sample RNA well (undiluted or 10^{-1}) to the process control virus RNA standard curve. If extraction efficiency is <1 % sample results are not valid and may need to be retested.

D. "**Quantification standard**" preparation for setting "virus genomic copies standard curve"



For quantification of a target virus, results shall be related to a standard of known concentration. A dilution series of quantified dsDNA plasmid carrying the target sequence of interest can be used to produce a standard curve in template copies/µl. Reference to the standard curve enables quantification of the sample in detectable virus genome copies/µl. This step must be done only in case a quantification of HAV and/or NoV according to ISO-15216-1 is requested.

A. Singleplex Real-Time PCR kit according to ISO 15216 Protocol for Hepatitis A and Norovirus I and II detection

- PVW08A-50 WHATfinder HAV ID assay - 50 Tests
- PVW09A-50 WHATfinder Norovirus Type I ID assay - 50 Tests
- PVW10A-50 WHATfinder Norovirus Type II ID assay - 50 Tests

Each kit contains: One-step Reverse transcriptase + DNA Polymerase mix; primers and probes mix for the detection of the target; positive control; target external control; negative control (water). According to ISO 15216 Each kit must be coupled with an appropriate one-step mastermix without IPC and to a process control (Cat.# PVW05A-50)

- PVW08R WHATfinder DigiCount HAV Quantification Standard - 1 Vial
- PVW09R WHATfinder DigiCount Norovirus Type I Quantification Standard – 1 Vial
- PVW10R WHATfinder DigiCount Norovirus Type II Quantification Standard – 1 Vial

Each vial contains 120 µl of a plasmid inserted with the specific sequence of the amplified target as indicated in ISO 15216. The amplification of a dilution range from this quantification standard generates a standard curve, thus allowing for the quantification of a determined target in contaminated samples. The quantification standard is not a reference material.

B. Multiplex Real-Time PCR kits based on ISO 15216 sequences for Hepatitis A and Norovirus I and II

Each kit contains: One-step Reverse transcriptase + DNA Polymerase mix; primers and probes mix for the detection of the targets; positive control; negative control (water). Each kit must be coupled with an appropriate process control (Cat.# PVW05A-50)

- PVW02A-50 WHATfinder 2-plex HAV + NoV-I + NoV-II / IAC detection Kit – 50 Tests

  Detects internal amplification control (Cy5) and all the viruses in a single well (FAM channel) without distinguishing the virus detected.

- PVW03A-50 WHATfinder 3-plex HAV / NoV-I + NoV-II / IAC detection kit – 50 Tests

  Detects HAV (FAM channel), NoV without distinguishing the serotype detected (HEX channel) and internal amplification control (Cy5) in a single well.

- PVW04A-50 WHATfinder 4-plex HAV / NoV-I / NoV-II / IAC detection kit – 50 Tests

  Detects HAV (FAM channel), NoV-I(HEX channel), NoV-II (Texas-Red channel) and internal amplification control (Cy5) in a single well distinguishing the virus detected.

- PVW07A-50 WHATfinder 3-plex NoV-I / NoV-II / IAC detection Kit – 50 Tests

  Detects NoV-I (FAM channel), NoV-II (HEX channel) and internal amplification control (Cy5) in a single well.

- PVW18A-50 WHATfinder 2-plex HAV / IAC detection kit – 50 Tests

  Detects HAV (FAM channel) and internal amplification control (HEX) in a single well.

- PVW19A-50 WHATfinder 2-plex NoV-I / IAC detection kit - 50 Tests

  Detects NoV-I (FAM channel), internal amplification control (HEX) in a single well.

- PVW20A-50 WHATfinder 2-plex NoV-II / IAC detection kit – 50 Tests

  Detects NoV-II (FAM channel), internal amplification control (HEX) in a single well.

C. Process control Kit according to ISO 15216 Protocol

- PVW05A-50 WHATfinder Recovery Efficiency Kit (according to ISO15216 Protocol) - 50 Tests

Each kit contains: One-step Reverse transcriptase + DNA Polymerase mix, Primers and probes, Feline Calicivirus virions, Negative control.

D. Surrogate virus detection

- PMB19A-50 VETfinder Real-Time PCR kit for validation of viral detection experiments (FCV) – 50 Tests
- PMB27P VETfinder Real-Time PCR kit for validation of viral detection experiments (MS2 Phage) – 50 Tests
- IL_IC289970 Intype IC-RNA - Kit per il controllo di processo nell'estrazione del RNA - 1ml

Each kit detects the target virus and a synthetic extraction control (Intype IC-RNA from INDICAL). The kit includes: a 2-plex oligo mix deteting MS2 phage (FAM) and Intype (HEX) - a prime quality one-step RTqPCR mastermix - a titrated FCV/MS2 phage solution to perform the study of spike/recovery.

E. Extraction kits

- EXD199 Spin-column based kit for viral DNA/RNA extraction - 50 Tests

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