

A critical review of methods for distinguishing infectious and non-infectious norovirus

Area of research interest: [Foodborne pathogens](#)

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Background

The conclusion of the Second Study of Infectious Intestinal Disease in the Community (IID2 Study) confirmed a high burden of intestinal viral disease in the UK, particularly that caused by norovirus. Person-to-person spread is a key transmission route for norovirus, although food contaminated at source, or by infected food handlers, is also frequently associated with outbreaks of disease and probably sporadic cases. There are significant gaps in our knowledge of how norovirus is able to survive and remain infectious in food, and on surfaces in kitchens.

Current detection methods for norovirus use reverse transcriptase polymerase chain reaction (RT-PCR) to detect and estimate the titre of norovirus present in food or environmental samples. Enzyme-linked immunosorbent assay (ELISA) methods are also available but are considered to be less sensitive. While the molecular methods are able to detect and in some cases quantify the amount of norovirus RNA present, they do not provide information on whether or not the detected virus is capable of causing human infection or the degree of any degradation/damage to the RNA or capsid.

It is not possible to culture norovirus and other viruses (feline calicivirus, murine norovirus) tend to be used as surrogates for norovirus in in vitro studies. In the absence of human volunteer studies to assess infectivity, other approaches need to be explored to assist the interpretation of the findings from molecular-based testing of food and or the environment. It has been suggested that assessing the condition of the norovirus capsid might provide an indication of the degree of protection for norovirus RNA detected by RT-PCR. However, the applicability and practicality of this approach for food and environmental samples is likely to require further investigation.

Research Approach

This study helped to identify potential approaches to assist with the interpretation of norovirus RT-PCR results. The work involved a short critical literature review to identify:

- the methods currently used to detect norovirus in food, the environment and clinical samples
- the methods in the pipeline or on the horizon
- the methods or approaches that could be capable of distinguishing infectious and non-infectious norovirus
- the methods or approaches capable of assessing the integrity of norovirus capsid or RNA as a surrogate for infectivity

This review considered all detection and quantification methods, including molecular techniques. Each was critically assessed for their respective strengths, weaknesses and their capacity to distinguish between infectious and non-infectious norovirus particles. The levels of norovirus detected in food by RT-PCR are lower than those in clinical samples and this was taken into account in assessing the different approaches and options. Each approach identified was assessed for the feasibility of their use in routine laboratories and applicability to testing in real food systems. The review also identified research/knowledge gaps in this area and made recommendations for future work.

Results

Comparison of data in this review was complicated by the wide range of different viruses and experimental conditions used in different studies. In particular, experimental conditions need to be controlled carefully to allow comparison of studies. Particular attention should be paid to important variables including treatment type and conditions, virus concentration, matrix composition, enzyme activity, and potential RT-qPCR (Reverse Transcription Polymerase Chain Reaction) inhibition. Nevertheless, this review has identified gaps in our knowledge regarding the detection of infectious human norovirus and are listed below:

- There remains an absence of a suitable culture system for infectious human norovirus.
- Defining an 'infectious' virus is complicated and there is no standard as to what constitutes infectious human norovirus. However, it is clear that RT-qPCR methods alone cannot currently distinguish infective and non-infective virus.
- Current RT-qPCR methods used to detect infectious human norovirus rely on the generation and detection of a fluorescent signal without secondary confirmation of product identity. Improved characterisation of RT-qPCR products by direct sequencing or electrophoresis would increase confidence in the validity of current test results.
- It is known that infectious human norovirus are resilient and can persist and retain infectivity in the environment. It is also possible that the products of degraded virus particles, viral RNA and ribonucleoprotein complexes (RNPs), could persist in the environment leading to false positive identification of infectious human norovirus. The occurrence of RNPs in the environment is not known as this has not been investigated.
- RT-qPCR signals may be obtained from the products of degraded virus particles, i.e. RNA and RNPs, as well as from intact particles. Sample pre-treatment can allow differentiation of these RT-qPCR signals. Further research is required to differentiate these signals reliably in relation to infectious particles and whether these signals could be applied to the draft European CEN method used to detect hNoVs in foods and the environment. This CEN method is currently unable to differentiate between infectious and non-infectious human norovirus particles.

Published Papers

1. Knight, Angus; Li, Dan; Uyttendaele, Mieke; Jaykus, Lee-Ann (2012), A critical review of methods for detecting human noroviruses and protecting their infectivity, *Critical Reviews in Microbiology*, (online publication) doi:10.3109/1040841X.2012.709820

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