Analytical Methods for Virus Detection in Water and Food

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Abstract

Potential ways to address the issues that relate to the techniques for analyzing food and environmental samples for the presence of enteric viruses are discussed. It is not the authors' remit to produce or recommend standard or reference methods but to address specific issues in the analytical procedures. Foods of primary importance are bivalve molluscs, particularly, oysters, clams, and mussels; salad crops such as lettuce, green onions and other greens; and soft fruits such as raspberries and strawberries. All types of water, not only drinking water but also recreational water (fresh, marine, and swimming pool), river water (irrigation water), raw and treated sewage are potential vehicles for virus transmission. Well over 100 different enteric viruses could be food or water contaminants; however, with few exceptions, most well-characterized foodborne or waterborne viral outbreaks are restricted to hepatitis A virus (HAV) and calicivirus, essentially norovirus (NoV). Target viruses for analytical methods include, in addition to NoV and HAV, hepatitis E virus (HEV), enteroviruses (e.g., poliovirus), adenovirus, rotavirus, astrovirus, and any other relevant virus likely to be transmitted by food or water. A survey of the currently available methods for detection of viruses in food and environmental matrices was conducted, gathering information on protocols for extraction of viruses from various matrices and on the various specific detection techniques for each virus type.

Keywords Enteric viruses – Gastroenteritis – Hepatitis – Detection – Concentration

Introduction

The transmission of viruses through consumption of or contact with contaminated water and food is well recognized. Transmission of viruses associated with the consumption of contaminated bivalve shellfish, particularly, oysters which are eaten uncooked, is regularly reported (Koopmans and Duizer 2004; Widdowson et al. 2005). Other foods, including raspberries (Cotterelle et al. 2005; Le Guyader et al. 2004; Gaulin et al. 1999; Korsager et al. 2005; Hedlund et al. 2000; Falkenhorst et al. 2005; Hjertqvist et al. 2006; Fell et al. 2007; Ponka et al. 1999) and salads (Vivancos et al. 2009), have caused outbreaks after being contaminated by polluted water or virus-infected food handlers. Polluted water, both drinking water and recreational water, have been shown to have transmitted viruses (Cannon et al. 1991; Lawson et al. 1991; Maunula et al. 2005). Because many people may consume a batch of food or come into contact with the contaminated material, outbreaks involving large numbers infected people are common. The outbreaks of viral gastroenteritis are known to be mainly caused by norovirus (NoV) and outbreaks of viral hepatitis are caused by Hepatitis A virus (HAV) (Ramsay and Upton 1989; Reid and Robinson 1987; Calder et al. 2003; Niu et al. 1992; Hutin et al. 1999) and, in the case of water, more rarely, Hepatitis E virus (HEV) (Jothikumar et al. 1993).

In all the above cases, it is important to have effective tools with which to analyze the food or water matrix for its viral content. The following sections address the issues of what and how to take samples of food and water, how to release the virus from each matrix type, nonmolecular virus detection, nucleic acid extraction methods, and molecular detection techniques.

Sampling for Viruses Associated with Food

When sampling procedures for food/fresh produce are considered, the questions which arise are as follows: (i) is a specific weight of a particular fruit or vegetable representative, (ii) is one item, e.g. tomato, per crate, representative, (iii) is one leaf of a lettuce or cabbage representative, and if so, which leaf (iv) should the food item be analyzed whole or chopped, and (v) will a single sample suffice or should the test be repeated in triplicate?

In order to assess the real role of food in virus transmission, cost-effective standardized or comparable methods need to be developed for application in reference laboratories. The infectious dose for viruses, such as HAV and NoV, is estimated to be about 10–100 infectious particles; therefore, although the viral load on fresh produce, minimally processed, and ready-to-eat foods may be low, it may still be a source of infection and illness. Data on shellfish is still scarce, but some publications reported NoV concentrations ranging from 10^2 to 10^4 copies per gram of digestive tissues (Nishida et al. 2007; Le Guyader et al. 2003, 2006a, 2008, 2009). HAV has recently been quantified in naturally contaminated shellfish samples, showing titers ranging from 10^3 to 10^5 HAV genomes per gram of clam (Costafreda et al. 2006; Pintó et al. 2009), and it has also

been reported in titers of 0.2–224 infectious particles per 100 g shellfish meat (Williams and Fout 1992).

Therefore, the methods for detection of viruses on food samples must have a high level of analytical sensitivity and specificity. With regard to sample size, it should represent the whole batch or crop, and that the US Environmental Protection Agency (US EPA), the International Organization for Standardization (ISO), and the national quality assurance (QA) regulations must be followed. There is, however, very little information in the literature, as well as in the US EPA and the European Committee for Standardization or Comité Européen de Normalisation (CEN)/ISO guidelines, regarding sampling procedures for the viral analysis of food. References to sampling for microbiology usually refer to the statistical representativeness of the samples, and economic and logistical considerations usually limit the number, type, and location of samples to be taken (Murray et al. 2002). Sampling for virological analyses of food will not necessarily follow the bacterial approach since the low level of contamination and the complexity and cost of assays are greater. The US EPA Manual of Methods for Virology (EPA, U.S. Environmental Protection Agency, Cincinnati 1984) only addresses the virological examination of water, with no mention of the virological examination of food. The Health Protection Agency (HPA) of UK has a standard operating procedure (SOP) for the preparation of samples for the microbiological examination of food samples (BS 5763). In this procedure, a 25 g sample of food, which is homogenized, is used, but this SOP only relates to subsequent bacteriological analyses where the results are reported as colony forming unit per gram (CFU/g) or milliliter (ml). Many of the published or methods under development for the detection of viruses on fruit and vegetables use a 10–100 g sample size (a detailed overview of these methods have been described by Croci and co-authors (2008); however, there is no mention of how many 10–100 g samples need to be taken in from a crate, field, or truckload of the particular food matrix to have a statistically representative sample. Obviously, sampling procedures vary according to food matrix type and must have to take the quantity of sample, seasonality, rainfall, and probable amount of contamination or pollution into account.

From the literature, it is also not clear whether the food sample should be analyzed as a whole or chopped. As most viral contamination would be from external sources during spraying or irrigation, a critical factor influencing the decision to analyze vegetables, whole or chopped, would be whether the claims that viruses can enter plants through root damage are substantiated or not. It appears that internal contamination of the leaves of tomato plants and green onions can occur (Oron et al. 1995; Chancellor et al. 2006) and that the internal contamination is of a much lower level than external contamination (Carter 2005; Urbanucci et al. 2009).

The detection of viruses inside a plant crop could potentially be an indicator of higher levels of external contamination and would be significant as these viruses would not be removed or inactivated by washing or ultraviolet irradiation (Carter 2005). Sampling of foods implicated in an outbreak of viral disease would be focused on the particular batches consumed.

Sampling for Viruses in Water

With regard to the virological analysis of water, a similar question arises as to those encountered with the virological analysis of food, namely, “What is a representative sample?” When sampling procedures for irrigation and washing water are considered the questions which come up are: (i) will a specific volume of water be representative, and (ii) will the water quality influence the sampling procedure?

Microbes pose the most significant waterborne health risk (Carter 2005) with waterborne diseases being misdiagnosed or underdiagnosed (Meinhardt 2006). Contamination of surface water with enteric viruses through disposal of human waste is a concern for public health, especially if these surface waters are used for recreational water, irrigation water, and the production of drinking water (Rutjes et al. 2005). The surveillance of irrigation water and water for washing the fresh produce is therefore essential to facilitate correct management procedures for the protection of fruit and vegetable growers and the health of farm workers and the consumers. In order to monitor the virological quality of water, an efficient combination of techniques has to be applied for the optimal recovery and detection of the low titres of viruses present in water (Gilgen et al. 1997; Soule et al. 2000).

Sampling and analytical procedures for the virological analysis of water are well documented (EPA, U.S. Environmental Protection Agency, Cincinnati 1984; Gerba 1987; Hurst and Reynolds 2002); American Public Health Association, American Water Works Association, Water Environment Federation 2005; Wyn-Jones 2007. A variety of techniques have been described for the recovery of viruses from water—each with their own advantages and disadvantages as the physicochemical quality of the water, including but not limited to the pH, conductivity, turbidity, presence of particulate matter, and organic acids, can all affect the efficiency of recovery of viruses (Richards et al. 2004). Viral recovery and concentration techniques include ultrafiltration (Soule et al. 2000; Divizia et al. 1989a; b; Garin et al. 1994), adsorption–elution using filters or membranes (Gilgen et al. 1997; Passagot et al. 1985; Senouci et al. 1996), glass wool (Vilaginès et al. 1993; Vilaginès et al. 1997) or glass powder (Gajardo et al. 1991; Menut et al. 1993), two-phase separation with polymers (Schwab et al. 1993), flocculation (Nasser et al. 1995; Backer 2002), and the use of monolithic chromatographic columns (Branovic et al. 2003; Kramberger et al. 2004; Kovac et al. 2009; Gutierrez-Aguirre et al. 2009). The use of the glass wool adsorption–elution procedure for the recovery of enteric viruses from large volumes of water has proven to be a cost-effective method and has successfully been applied for the routine recovery of human enteric viruses from large volumes of water in the South African setting (Taylor et al. 2001; Van Heerden et al. 2004, 2005; Vivier et al. 2001, 2002, 2004; Van Zyl et al. 2004, 2006; Venter 2004). This method can be adapted for the in-line recovery of viruses from water (Grabow et al. 1996) which circumvents transporting of large volumes of potentially highly polluted water great distances to a central laboratory which would be expensive and a potential health hazard.

It is important to acknowledge that no single method may universally be recognized as superior: efficiency, constancy of performance, robustness, cost, and complexity are all factors to be considered for each method and performance characteristics must be continually monitored.

Sampling Aerosols and Surfaces

There is concern over the potential transmission of viruses into the food chain through aerosols and from surface contamination. The crucial issues are sample collection and preparation for different virus detection techniques: different methods have been developed based on the attachment properties to surfaces of airborne particles (Verreault et al. 2008).

The most used air samplers are based on impact on solid surfaces, impingement, and filtration: all of them have been successfully used for virus detection, but have advantages and disadvantages.

Impact Samplers

An air flow with a fixed speed is directed to impact on a solid surface, generally an agar medium (Booth et al. 2005). Some equipment (Andersen sampler) has multiple stages and can sample separately particles with different size. After sampling, virus can be eluted from the solid medium, purified and prepared for subsequent virus analysis. This sampling method is easy, but dehydration or impact trauma can affect the virus survival; flow rate and sampling duration are critical.

Impinger Samplers

The air is forced to flow through a narrow orifice to make bubbles in a liquid medium (Pyankov et al. 2007). After sampling the medium can be concentrated or directly decontaminated, purified, and analyzed. The recovery efficiency of this method is high because it avoids dehydration but flow rate and the composition of the collection fluid are again critical for virus recovery (Hogan et al. 2005; Hermann et al. 2006).

Filter Samplers

The air passes through a filter and airborne particles are retained as a function of their aerodynamic size and surface properties, such as electrostatic charge. For aerosol sampling, membranes with 1–3 μm pore size can retain droplets with an aerodynamic size <500 nm more efficiently than other samplers (Verreault et al. 2008). Viruses in an aerosol are associated with particles and can be collected. The membrane material can be polytetrafluoroethylene (PTFE), cellulose, polycarbonate, or gelatine (Burton et al. 2007). The last one is easier for viral sampling, because it can be directly dissolved in an appropriate liquid medium. This sampling method is easy to use, but the flow rate,

the sampling duration, and the membrane composition have to be strictly controlled to avoid dehydration.

Further methods for the analysis of viruses in aerosols include cyclone (Alexandersen et al. 2002) or electrostatic precipitators (Moore et al. 1979), and in the last years, the fear of bioterrorism stimulated the study of new methodologies (like mass spectrometry) (Johnston 2000) that are able to identify pathogens in air. However, their application to the routine environmental analysis is still far in the future and will require very large result data bases from many environmental samples.

To better understand the fate of virus dispersed through air, surface monitoring should be also performed, because of the settling of droplets with greater size. Surface sampling has its major indication in health care settings (Carducci et al. 2002) and in food production (Scherer et al. 2009) to assess not only viral contamination but also efficiency and correct application of disinfection procedures. To this aim, a definite surface area (i.e., 10 cm^2) is swabbed, then the swab is eluted and the eluate is processed as a liquid sample. For biomolecular tests, some swabs can be submitted directly for nucleic acid extraction. Alternative methods are contact plates that can be eluted.

Virus Release from Food Matrices

The food matrix and the route of contamination involved, determines the way of virus release prior to nucleic acid extraction. Viral particles need to be extracted from homogenized tissues in case of intrinsic contamination (i.e., oysters tissues, pig liver) or eluted from the surface of the food item (i.e., contamination by irrigation water or food handling).

Shellfish are filter feeders and concentrate enteric viruses from their environment while feeding. The majority of accumulated virus is found in the pancreatic tissue, also called the digestive diverticula. Mechanical entrapment and ionic bonding are among the mechanisms that have been suggested to explain observed differences in accumulation of different viruses and among different oyster species (Burkhardt and Calci 2000; Di Girolamo et al. 1977). Another potential mechanism for the uptake and concentration of viruses in shellfish has been proposed based upon the observation of specific binding of a NoV genogroup I to shellfish tissues (Le Guyader et al. 2006b).

Several efficient methods are now available for shellfish analysis; for example, Atmar et al. (1995), proposed the dissection of digestive tissues for virus extraction. Testing the stomach and digestive gland for virus presented several advantages in comparison with testing whole shellfish: less time-consuming procedure, increased test sensitivity, and decreased sample-associated interference with reverse transcription polymerase chain reaction (RT-PCR) (Atmar et al. 1995). Since the initial description of analyzing only digestive tissues, a number of variations have been published, and most have analyzed the same weight (1.5–2 g) of digestive tissues. Viruses are eluted using various buffers

(e.g., chloroform-butanol or glycine) before concentration by polyethylene glycol (PEG) or ultracentrifugation (Nishida et al. 2007; Schwab et al. 2001; Fukuda et al. 2008; Milne et al. 2007). Direct lysis of virus particles is used more and more frequently. For example, proteinase K or Trizol and lysis of shellfish tissues using Zirconia beads and a denaturing buffer have all been used for virus elution (Jothikumar et al. 2005; Lodder-Verschoor et al. 2005).

Methods that have currently been developed and optimized for virus detection from fruit and salad vegetables focus on elution of the virus from the surface (Croci et al. 2008). A number of washing procedures and buffer systems have been described for the recovery of viruses from fruits and vegetables. The average recovery rates vary depending on the food matrix and virus (Croci et al. 2008). Dubois et al. (2007) described a protocol that included the rinsing of fruit and vegetable surfaces with a buffer of pH 9.5, supplemented with 100 mM Tris, 50 mM glycine, and 1% beef extract, a protein- and nucleic acid-rich substance. This buffer ruptures the electrostatic and hydrophobic interactions between fruit or vegetable surfaces and virus. In the case of soft fruits, pectinase has to be added to prevent the formation of a gelatinous substance (Rzezutka et al. 2005; Butot et al. 2007).

Some authors have reported the presence of viral particles trapped inside vegetables taken up intracellularly through the roots (Oron et al. 1995; Chancellor et al. 2006; Carter 2005; Urbanucci et al. 2009). This mechanism warrants further examination, and if confirmed, it will change future approaches for the detection of viruses from vegetables.

Virus concentrations on food are likely to be low, indicating that the virus which is present in the relatively large volumes of elution buffer, needs to be concentrated prior to detection. The choice of virus concentration method is dependent on the food matrix and eluant. Frequently used concentration methods include precipitation by PEG, ultrafiltration, and ultracentrifugation.

Immunological methods have also been applied to concentrate virus in food (Bidawid et al. 2000; Kobayashi et al. 2004; Shan et al. 2005; Tian and Mandrell 2006). However, NoV immunoconcentration is unlikely to be adapted widely for NoV detection due to the difficulties in obtaining antibodies and its variability at the capsid level.

Nucleic Acid Extraction and Purification

Following virus elution or concentration, a variety of subsequent nucleic acid extraction and purification protocols may be employed. Recently, a number of methods using kits have been published. A wide variety of commercial kits has been applied for nucleic acid purification, offering reliability, reproducibility, and they are quite easy to use. Most of these kits are based on guanidinium lysis and then the capture of nucleic acids on a column or bead of silica (commonly called Boom's method (1990)). Although most perform well, differences can be found depending on the virus and/or matrix analyzed

(Rutjes et al. 2005; Hourfar et al. 2005; Knepp et al. 2003; Kok et al. 2000; Le Guyader et al. 2009; Burgener et al. 2003). In the last years, automated nucleic acid extraction platforms have been developed by commercial companies, which have been shown to be suitable for the analysis of virus in water samples (Rutjes et al. 2005; Perelle et al. 2009).

Virus Detection

Virus detection is mainly based on two principles: the detection of infectious viruses by propagation in cell culture or the detection of the viral genomes by molecular amplification techniques such as PCR or RT-PCR. Detection by cell culture is mainly based on the formation of cytopathic effects, with the quantification of the viruses by plaque assay, with the most probable number or tissue culture infectious dose 50 ($TCID_{50}$). Virus typing may be done by immunofluorescence or neutralization assays. However, efficient cell culture systems are not available for all viruses, and others grow slowly or do not produce a cytopathic effect.

In the last decade, real-time PCR assays have revolutionized nucleic acid detection by the high speed, sensitivity, reproducibility, and minimization of contamination. These methods are widely used in the field of food virology and are continuously evolving. For instance, Sanchez et al. (2007) summarized published real-time RT-PCR methods for HAV detection in food, and since then, several new methods have become available (Houde et al. 2007; Casas et al. 2007). It is essential that the specificity, the range of viruses detectable, and the sensitivity of real-time RT-PCR assays are demonstrated. All these points are interconnected and depend mostly on the target sequences for primers and probe. The selected targets must guarantee an absolute specificity and must reach equilibrium between high sensitivity, broad reactivity, and reliability of quantification.

Real-time RT-PCR procedures enable not only the qualitative but also the quantitative detection, which opens the possibility of quantitative hazard risk assessment analysis critical for several public health actions or food ban regulations. Quantification can also be performed as most probable number by conventional PCR (Rutjes et al. 2005, 2006a). Qualitative real-time PCR producing a positive or negative result is most appropriate when testing matrices that are unlikely to be contaminated with virus as it is least expensive and straightforward. Quantitative real-time PCR is required when a sample, such as shellfish, is likely to contain viruses and the degree of contamination needs to be ascertained.

Molecular assays by conventional PCR, i.e., gel-based, remain useful, as larger volumes of sample can be tested, larger PCR products can be obtained, and it is less expensive. The alternative molecular technique nucleic acid sequence-based amplification (NASBA) was shown to be less prone to environmental PCR inhibitors present in large volumes of surface water samples (Rutjes et al. 2006b).

Although the detection of enteric viruses in food is mainly done by molecular techniques, there are several limitations. The method is prone to inhibition, favoring false negative results and demonstrating the need for proper quality control (QC). Several ways have been described to overcome this inhibition, such as the analysis of samples dilutions, smaller sample sizes, adaptation of the PCR by, e.g., the addition of Tween, BSA, or commercial reagents (Rutjes et al. 2005; Butot et al. 2007; Al-Soud and Radstrom 2001).

One of the major limitations of PCR is its inability to differentiate between infectious and noninfectious viruses. Various approaches to overcome this limitation have been evaluated. Of them, integrated systems based on the molecular detection of viruses after cell culture infection are the most promising techniques (Pintó et al. 1994; 1995; Reynolds et al. 2001); a detailed overview of these approaches can be found elsewhere (Rodriguez et al. 2009). The integrate cell culture (ICC)-PCR assay is based on a selective enumeration of infectious viruses in combination with a rapid molecular detection, circumventing long incubation periods for cytopathic effect formation. Such ICC-PCR assays have been successfully utilized for the detection of several enteric viruses in environmental samples (Reynolds et al. 2001). Other alternatives, such as a protease and RNase pretreatment, have successfully been used to differentiate between infectious and noninfectious virus (Nuanualsuwan and Cliver 2002, 2003; Lamhoujeb et al. 2008; Topping et al. 2009), although Baert and collaborators (2008a) did not find correlation for murine NoV.

Quality Controls

One of the most critical challenges is the implementation of novel molecular-based methods for the detection of enteric viruses in the routine food analytical laboratories. However, obstacles that influence routine virus detection in foods include the low efficiency of concentration and nucleic acid extraction procedures and the presence of inhibitors to the molecular reactions. It seems obvious that harmonization of the molecular techniques, as well as addressing QA/QC (quality assurance/quality control) issues is required before adoption of the procedures by routine monitoring laboratories. QA/QC measures include the use of positive and negative controls, thus tracing any false negative or false positive result, respectively. Most false negatives are consequence of inefficient virus and/or nucleic acid extraction and of inhibition of the RT reaction. Most false positives result from cross-contamination.

The first dilemma is to choose between an actual internal control and an added external control for the extraction procedure. For the diagnosis of an RNA virus, the use of an internal control based on the detection of the expression of a housekeeping gene, ideally containing introns, through the amplification of its messenger RNA (mRNA) in the target tissues is a clear first choice. However, this is an unrealistic approach for its

Table 1 Terms and definitions in standardized molecular detection assays for virus detection in food

Process control virus	A virus added to the sample portion at the earliest opportunity prior to virus extraction to control for extraction efficiency
Process control virus RNA	RNA released from the process control virus in order to produce a standard curve data for the estimation of extraction efficiency
Negative RNA extraction control	Control free of target RNA carried through all steps of the RNA extraction and detection procedure to monitor any cross-contamination events
Negative process control	Control free of target RNA carried through all steps of the virus extraction, RNA extraction, and detection procedure to monitor any cross-contamination events
Hydrolysis probe	An oligonucleotide probe labeled with a fluorescent reporter and quencher at the 5′ and 3′ ends, respectively. Hydrolysis of the probe during real-time PCR due to the 5′–3′ exonuclease activity of Taq DNA polymerase results in an increase in measurable fluorescence from the reporter
Negative RT-PCR control	An aliquot of highly pure water used as template in a real-time RT-PCR reaction to control for contamination in the real-time RT-PCR reagents
External control RNA	Reference RNA that can serve as target for the real-time PCR assay of relevance, e.g., run-off transcripts from a plasmid carrying a copy of the target gene, which is added to an aliquot of sample RNA in a defined amount to serve as a control for amplification in a separate reaction
Cq value	Quantification cycle; the PCR cycle at which the target is quantified in a given real-time PCR reaction. This corresponds to the point at which reaction fluorescence rises above a threshold level
Theoretical limit of detection (tLOD)	A level that constitutes the smallest quantity of target that can, in theory, be detected. This corresponds to one genome copy per volume of RNA tested in the target assay, but will vary according to the test matrix and the quantity of starting material
Practical limit of detection (pLOD)	The lowest concentration of target in a test sample that can be reproducibly detected (95% confidence interval), as demonstrated by a collaborative trial or other validation (Annex L)
Limit of quantification (LOQ)	The lowest concentration of target in a test sample that can be quantitatively determined with acceptable level of precision and accuracy, as demonstrated by a collaborative trial or another

application in food virology, which involves an increasingly heterogeneous selection of food matrices. For instance, in shellfish only, a pair of primers to amplify an mRNA for a specific hepatopancreas transcribed gene would be required for each species. It is impossible to apply this for the range of foodstuffs susceptible to be assayed for viruses,

which leads to compromise in the use of an external control, applicable to all matrices under assay. Table 1 depicts the complete list of terms and definitions in standardized molecular detection assays for virus detection in food (Hoorfar et al. 2003, 2004; Rodríguez-Lázaro et al. 2007; Costafreda et al. 2006; Hoorfar and Cook 2003; Rodriguez-Lazaro et al. 2004, 2005).

One of the most important issues is the control of nucleic acid extraction efficiency. Recently, the use of a nonpathogenic viruses, mengovirus MC0 (Mattison et al. 2009) and feline calicivirus (Butot et al. 2008; Cannon et al. 2006; Hewitt and Greening 2004; Pintó et al. 2009), have been proposed as process control, although the latter has been reported to be an inappropriate surrogate for NoV in acid conditions (Pintó et al. 2009). Quantitative standardized procedures presently enable to perform quantitative microbial risk assessment (QMRA) in food samples (Gassilloud et al. 2003; Arnal et al. 1998).

Several authors have reported that the number of infectious viruses did not correlate with the number of genomes detected by real-time RT-PCR in water samples (Baert et al. 2008a, b; Butot et al. 2008, 2009; Hewitt and Greening 2004). This is more evident when water or food undergoes a removal/inactivation process.

It is a matter of debate whether the detection of enteric viruses in food or water by PCR or real-time PCR should be considered a safety issue and confirmation of a public health risk. However, one can argue that if viruses were found, even if not infectious, it would be an indication that the food or water is contaminated and that viruses were present in the food or water at some point.

Summary

The analysis of food and water matrices for the detection of viruses is now well established to the extent that European Standards are in draft:

– Microbiology of food and animal feeding stuffs—Horizontal method for detection of hepatitis A virus and norovirus in food using real-time RT-PCR—Part 1: Method for quantitative determination
– Microbiology of food and animal feeding stuffs—Horizontal method for detection of hepatitis A virus and norovirus in food using real-time RT-PCR—Part 2: Method for qualitative detection

Validation studies are expected to be undertaken for each of the process stages before the standard is confirmed. This QA will ensure that the highest level of QA is achieved. Developmental studies on matrices not covered by the standard will continue to be required to reach consensus on the optimum techniques necessary to ensure effective systems.

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