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New approaches in microbial pathogen detection

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DOI: 10.1533/9780857098740.3.202

Abstract: Viruses are common causes of foodborne outbreaks. Viral diseases have low fatality rates but transmission to humans via food is important due to the high probability of consuming fecally contaminated food or water because of poor food handling. Because of the low infectious doses of some foodborne viruses, there is a need for standardization and the development of new sensitive methods for detecting viruses. The focus is on molecular and non-molecular approaches, and emerging methods for the detection of foodborne viruses. The detection of noroviruses, hepatitis A and E viruses, rotaviruses and adenoviruses will be discussed. The chapter will conclude with insights into future research directions.

Key words: foodborne outbreak, virus, detection, food safety, nanotechnology.

11.1 Introduction

An estimated 40 million illnesses each year are caused by foodborne pathogens, including 5.2 million (13%) due to bacteria, 2.5 million (7%) due to parasites and 30.9 million (80%) due to viruses (Mead et al., 1999). In the last decade, the occurrence of gastroenteritis in humans as a result of consumption of foods contaminated by viruses has increased (WHO, 2000). The transmission of viruses has been predominantly associated with the consumption of shellfish, mainly, raw oysters (Koopmans and Duizer, 2004; Widdowson et al., 2005), which have been contaminated by polluted water or virus-infected food handlers (Bosch et al., 2011). Common symptoms of viral gastroenteritis include vomiting and diarrhea (FAO/WHO, 2008). Foodborne viruses can be divided into three categories based on disease symptoms: those that cause gastroenteritis (noroviruses, rotaviruses and adenoviruses), those that cause fecal-orally transmitted hepatitis (hepatitis A and E viruses), and those that cause other illnesses after they migrate to other...
organs, such as the central nervous system or the liver (enteroviruses). Of these, noroviruses and the hepatitis A virus have been recognized as the most important human foodborne pathogens in terms of the number of outbreaks reported and the number of people affected in the world (Koopmans and Duizer, 2004; Cook and Rzezutka, 2006; Verhoef et al., 2008). Even though these viruses are the major cause of foodborne outbreaks, there is still an urgent need for standardization and the validation of detection methods at the national and international levels (FAO/WHO, 2008). In this chapter, an overview of existing detection methods for foodborne viruses are presented including non-molecular and molecular approaches as well as promising emerging methods for virus detection. The detection of noroviruses, hepatitis A and E viruses, rotaviruses and adenoviruses will be discussed as these viral agents have a high potential for foodborne outbreaks. The rest of this section briefly gives the essential characteristic features of these viruses.

Noroviruses (NVs) comprise a genus in the family Caliciviridae. They are mostly linked to non-bacterial gastroenteritis in humans and are estimated to cause 93% of the food-related outbreaks of gastroenteritis in the United States (Widdowson et al., 2005; Fankhauser et al., 2002). An NV is a small round virus, 27 to 35 nm in diameter, with a single-stranded, positive-sense, polyadenylated RNA genome of 7400 to 7500 nucleotides (Atmar and Estes, 2001). These small viruses show high genomic diversity and antigenic variation within five genogroups (GI, GII, GIII, GIV and GV) based on the genome sequence of the RNA-dependent RNA polymerase (RdRp) and the capsid regions (Vinjé et al., 2004).

Hepatoviruses (or hepatitis A viruses or HAVs) comprise a genus in the family Picornaviridae with a diameter in the range between 27 and 32 nm. They were first identified by electron microscopy in 1973 (Lemon and Robertson, 1993; Koopmans et al., 2002). They are small, non-enveloped, spherical viruses with a single (positive-) stranded RNA genome of approximately 7.5 kb in length (Koopmans et al., 2002). Each year, approximately 1.4 million people become ill with HAV costing $1.5–3.0 billion worldwide (WHO, 2000).

The hepatitis E virus (HEV), one of the major causes of viral hepatitis other than HAV, causes infection with a high rate of mortality particularly in pregnant women (Widen et al., 2011; Ahn et al., 2005). A HEV, similar to a HAV, is a small, non-enveloped, positive sense, single-stranded RNA virus, reclassified in the genus Hepevirus of the family Hepeviridae although previously classified as a member of Caliciviridae family (Berke and Matson, 2000; Gyarmati et al., 2007). The primary source of infection is the consumption of fecally contaminated drinking water in developing countries (Koopmans et al., 2002). Based on geographical origin, all isolated HEV strains can be identified into four genotypes as Asian/African, Chinese, Mexican or US/European (Emerson and Purcell, 2001). Among these genotypes, genotypes 1 and 2 only infect humans, while genotypes 3 and 4 appear to infect other hosts, particularly pigs, and are associated with zoonotic transmission (Gyarmati et al., 2007).

Rotaviruses comprise a genus in the family Reoviridae and are one of the major causes of acute diarrhea in infants and young children with high morbidity.
and mortality, especially in developing countries (Kapikian et al., 1996). These round, non-enveloped viruses are estimated to cause the death of more than 600,000 children worldwide every year and infect almost all children under five years of age (Pineda et al., 2009; Gutierrez-Aguirre et al., 2008).

Adenoviruses are non-enveloped viruses with double-stranded DNA (Vasickova et al., 2005). Human adenoviruses are the only human enteric DNA viruses and are often detected in association with other human enteroviruses or hepatitis A viruses (Rigotto et al., 2005). Outbreaks have caused gastroenteritis in children (Vasickova et al., 2005). Adenovirus types 40 and 41 cause gastroenteritis when transmitted through a fecal–oral cycle (Koopmans et al., 2002).

11.2 Detection methods

HAVs and other enteric viruses may be found in large numbers in clinical samples (≥10^6 virus particles per gram of stool); however, they are usually found in much lower numbers in food, e.g. 0.2–224 particles per 100 g shellfish meat (Sanchez et al., 2007). The infectious dose of HAVs and NVs is estimated to be as low as 10–100 infectious viral particles even though the ingestion of thousands of cells is required for bacterial infection to occur with the same probability (Sair et al., 2002; Gerba, 2006; Guevremont et al., 2006). Unlike bacterial pathogens, viruses cannot multiply in foods, making the traditional food microbiological techniques of cultural enrichment and selective plating inapplicable (D’Souza and Jaykus, 2006). Therefore, methods with high reliability and sensitivity are required for viral detection. In the sections below we discuss some of the current methods.

11.2.1 General approaches

Conventional assay systems to detect enteric viruses in clinical specimens cannot be directly used for food (Rodriguez-Lazaro et al., 2007). In general, electron microscopy, tissue cultures and immunological and molecular methods are used to detect viruses in food. Viruses were diagnosed historically by scanning a stool suspension under an electron microscope (EM) (Koopmans and Duizer, 2004). Many of the small round viruses, including HAVs, astroviruses, noroviruses, sapovirus and parvoviruses, were first discovered through the use of EM (Greening, 2006). EM is fairly insensitive, labor intensive and requires a minimum of 10^6 virus particles per milliliter of sample for detection in patient fecal samples, thus, using this method, detecting viruses at low levels in contaminated food, water and environmental samples is not possible (Koopmans and Duizer, 2004; Seymour and Appleton, 2001).

Detection by cell culture depends on cytopathic effects, and virus quantification is performed by plaque assay, the most probable number or 50% tissue culture infectious dose (TCID_{50}) (Bosch et al., 2011). Cell-culture-based assay can differentiate between infectious and non-infectious viruses; nevertheless it is limited and not practical, mainly due to the lack of sensitivity, the long analysis...
time and the lack of susceptible cell lines for many epidemiologically important enteric viruses (Casas and Sunen, 2001; Verhoef et al., 2008). Even though these assays are commonly used to enumerate levels of viable polioviruses and adenoviruses, they are inadequate for the detection of the two most important foodborne viruses, HAVs and NVs, since neither of these replicate or express themselves efficiently in cell cultures (Goyal, 2006; Jiang et al., 2004; Koopmans and Duizer, 2004). Thus, HAVs and NVs have been detected conventionally using EM and enzyme-linked immunosorbent assay (ELISA) but even these methods are insensitive, lengthy and expensive (Morales-Rayas et al., 2010).

Non-culture-based detection methods, such as immunoassays, have been developed to detect viruses over the years (Lees, 2000). Although immunoassays, such as ELISA, have been used to detect viruses in water and HAVs in shellfish, reports are very limited and not always successful (Lees, 2000). The limited success of this approach is probably due to the lack of sensitivity of the immunoassay and like EM requires a thousand or more virus particles for a positive result (Kogawa et al., 1996). Therefore, new approaches have focused on molecular methods as these techniques for detecting enteric viruses are faster and more sensitive compared to infectivity tests performed with in vitro cell cultures or with immunological methods, even though molecular methods cannot discriminate between infectious and non-infectious particles (Green and Lewis, 1999; Morales-Rayas et al., 2010).

11.2.2 Molecular approaches
Several molecular methods using nucleic acid amplification have been developed for virus detection in food (Jean et al., 2003). In recent years, polymerase chain reaction (PCR)-based methods in particular, have become the gold standard for virus detection in food due to their high sensitivity, specificity and potential to detect even a single virus particle (Bosch et al., 2011; Martinez-Martinez et al., 2011; Richards et al., 2003; Cook and Rzezutka, 2006). Selected examples with detection limits are listed in Table 11.1.

Reverse transcription PCR (RT-PCR)
Reverse transcription PCR (RT-PCR), a modified form of PCR that allows the amplification of viral RNA, is currently the most sensitive and widely used method for foodborne virus detection (Casas and Sunen, 2001; Morales-Rayas et al., 2010). However, the application of this technique for routine analysis of food matrices is elaborate due to the need for sample concentration and the presence of residual food-related PCR inhibitors (Sair et al., 2002). Since only low numbers of viruses are present in food, inhibition is a more serious issue (Morales-Rayas et al., 2010). Therefore, several methods have been developed to concentrate and purify viruses and remove inhibitors from food samples before RT-PCR (Dubois et al., 2002; Croci et al., 2008).

The sample preparation procedures for detecting viruses in food typically involve one or more of the following: (i) elution of the virus particles from the
| Type of virus | Method of detection | Detection limit | Samples tested | References |
|--------------|---------------------|-----------------|----------------|------------|
| Norovirus    | TaqMan qRT-PCR      | 0.01 PDU        | Clinical       | Lamhoujeb et al., 2009 |
|             | Real-time NASBA     | 0.01 PDU        |                |            |
|             | RT-PCR              | 1 RT PCRU/25 g  | Green onion    | Guervremont et al., 2006 |
|             | RT-PCR              | 1–10 PCRU/mL    | Ham            | Kim et al., 2008b |
|             | Nested PCR          |                 |                |            |
| Hepatitis A  | Duplex qRT-PCR      | 10 PFU/1.5 L    | Bottled water  | Blaise-Boisseau et al., 2010 |
|             |                     | 100 PFU/1.5 L   | Tap water      |            |
|             |                     | 50 PFU/25 g     | Fresh raspberries |            |
|             |                     | 100 PFU/25 g    | Frozen raspberries |            |
|             | TaqMan RT-PCR       | 14 PFU/g        | Tomato sauces  | Love et al., 2008 |
|             |                     | 33 PFU/g        | Blended strawberries |            |
|             | Nested RT-PCR       | 1 TCID$_{50}$/10 g | Mollusks |            |
| Hepatitis E  | TaqMan qRT-PCR      | 1.2 PID$_{50}$ | Water | Croci et al., 1999 |
| Rotavirus    | qRT-PCR             | 125 PFU/g       | Oyster         | Jothikumar et al., 2006 |
|             | NASBA-ELISA         | 0.2 PFU         | Water          | Kittigul et al., 2008 |
|             |                     | 15 PFU          | Sewage effluent | Jean et al., 2002a, 2002b |
| Adenovirus   | Nested PCR          | 1.2 PFU/g       | Oysters        | Rigotto et al., 2005 |
|             | Conventional PCR    | 1.2 × 10$^2$ PFU/g |            |            |
|             | ICC-PCR             | 1.2 × 10$^2$ PFU/g |            |            |
|             | Nested mPCR         | 1 copy of adenovirus DNA/PCR reaction | Sewage | Formiga-Cruz et al., 2005 |

Note: PFU: plaque-forming unit; PCRU: RT-PCR amplifiable unit; TCID$_{50}$: 50% tissue culture infective dose; PID: pig infectious dose.
food using a variety of buffers and solutions including solutions of glycine and sodium chloride, borate and beef extract, saline and beef extract, and beef extract alone; (ii) extraction with an organic solvent, most commonly with Freon to remove insoluble or poorly soluble organic compounds in the water; (iii) concentration of the viruses using sedimentation by antibody or ligand capture, flocculation, ultra-centrifugation or precipitation (commonly polyethylene glycol precipitation); and (iv) extraction of viral nucleic acids (there are two main approaches using phenol: chloroform extraction and guanidinium isothiocyanate extraction) (Cook and Rzezutka, 2006; Goyal, 2006; Rodriguez-Lazaro et al., 2007). Various strategies have been proposed to improve the performance of each step over the years.

There are several commercial kits for nucleic acid purification, which are reliable, produce reproducible results and are easy to use. Most of these kits are based on guanidinium lysis and the capture of nucleic acids on a column or bead of silica (Bosch et al., 2011). However, sample preparation methods still require improvement to isolate viral particles from diverse food matrices without decreasing the sensitivity of the molecular method used for detection (Morales-Rayas et al., 2010).

The sensitivity and specificity of RT-PCR assays depends mainly on primer selection (Atmar and Estes, 2001). The major obstacle in NV detection with PCR arises from the very high genomic diversity of NV since new variants continue to evolve constantly (Widen et al., 2011). Therefore, it is difficult to select a single or even a small number of probes that can detect all possible NV variants (Atmar and Estes, 2001). Although ORF1 of the RdRp gene has been targeted in most of the assays (Nakayama et al., 1996; Jiang et al., 1999), the ORF1-ORF2 region has also been shown to be well conserved and is used in several assays (Katayama et al., 2002; Hohne and Schreier, 2004; Jothikumar et al., 2005b). One of the first enteric viruses detected by RT-PCR was HAV (Jansen et al., 1990). The VP1 capsid region was previously commonly targeted by primers in HAV detection; however, nowadays the 5′ non-coding region is highly preferred for targeting. It has similar performance as VP1, approximately 1 RNA copy per reaction (Sanchez et al., 2007). For HEV detection, various specific sets of primers have been developed to amplify conserved regions within ORF1, ORF2 and ORF3 (Enouf et al., 2006). Most of the RT-PCR assays developed for rotaviruses target the structural genes VP4, VP6 and VP7 (Atmar, 2006). The hexon gene in adenoviruses is most commonly used as the target in PCR assays; it has been shown to be reactive in all adenovirus species (Jothikumar et al., 2005a; Atmar, 2006). More recently, a FRET-based real-time assay, which amplifies the adenovirus fiber gene, was described. It showed slightly better performance in terms of detection limits of AdV40 and AdV41 compared to TaqMan assays (Jothikumar et al., 2005a).

The major limitation of RT-PCR is its inability to distinguish between infectious and non-infectious viruses (Richards, 1999). Integrated cell culture PCR (ICC-PCR) and ICC/strand-specific RT-PCR have been proposed to compensate for this problem (Atmar, 2006; Jiang et al., 2004). ICC/strand-specific RT-PCR is a
combination of cell culture and molecular biology-based methods, which requires initial propagation of infectious virus particles in a cell culture and the detection of a negative-strand RNA replicative intermediate as an indicator of viral replication (Jiang et al., 2004). The limitations of RT-PCR were eliminated in environmental samples by increasing the equivalent sample volume and thereby reducing the effects of inhibitory compounds (Reynolds et al., 1996). ICC-PCR and ICC/strand-specific RT-PCR assays targeting the VP3 genes, which code for a major HAV capsid protein, have been developed to detect viruses in water (Jiang et al., 2004). The ICC/strand-specific RT-PCR used in this study was demonstrated to be a novel, rapid, sensitive and reliable method, since it can detect infectious HAVs at inoculation level of 100TCID$_{50}$ per flask within four days in water samples.

Even though RT-PCR is a rapid and sensitive method and can detect viruses that are difficult or impossible to culture (Casas and Sunen, 2001), several different types of RT-PCR have been developed to improve the specificity and sensitivity of the standard method for foodborne virus detection such as nested RT-PCR (Love et al., 2008; Croci et al., 1999) and multiplex RT-PCR (Rosenfield and Jaykus, 1999; Formiga-Cruz et al., 2005; Coelho et al., 2003).

**Nested PCR**

In nested PCR, two different primer pairs are used successively to amplify a target sequence (Haqqi et al., 1988). Nested PCR was developed to ensure detection specificity, to minimize false-positive results and to enhance the amplification signal (Rigotto et al., 2005). It has been widely used in the performance evaluation and verification of different PCR-based methods as well as viral extraction, concentration and purification (Kim et al., 2008a, 2008b; Di Pinto et al., 2003; Jothikumar et al., 2005b).

The superior sensitivity of nested PCR over other methods has been demonstrated in several studies (Croci et al., 1999; Rigotto et al., 2005; Love et al., 2008). Nested PCR gives a more sensitive and specific identification of HAV at concentrations as low as 1 TCID$_{50}$/10 g of mollusk compared to 10$^3$–10$^4$TCID/10 g of mollusk after one round of PCR (Croci et al., 1999). It had a higher level of sensitivity in shellfish compared to conventional PCR and ICC-PCR when detecting adenoviruses (Rigotto et al., 2005) (Table 11.1). Recently, TaqMan RT-PCR has been used to detect HAV RNA from artificially inoculated tomato sauce and blended strawberries (Love et al., 2008). The lower limits of HAV detection were reported as 14 PFU/g (plaque-forming units per gram) of tomato sauce and 33 PFU/g of blended strawberries at initial seeding levels. Moreover, the nested RT-PCR was not inhibited by undiluted final RNA extracts of tomato sauce or blended strawberries unlike TaqMan RT-PCR.

The sensitivity of standard RT-PCR was further increased when combined with semi-nested or nested PCR by using an aliquot of the product from the primary RT-PCR as a template for the second round of amplification (O’Connell, 2002; Abad et al., 1997). Nested multiplex real-time PCR (mRT-PCR) has also been developed to provide a highly sensitive, rapid and cost-efficient approach for HAV,
adenovirus and enterovirus detection in urban sewage and shellfish (Formiga-Cruz et al., 2005). This method was able to detect as little as one copy of adenovirus DNA, and ten copies of both enterovirus and HAV RNA, which was shown to be similar to the previously determined sensitivities of monoplex PCR with 1–10 viral particles for adenoviruses, and 5–10 viral particles for enteroviruses both in sewage and shellfish samples (Formiga-Cruz et al., 2005). Most recently, RT nested PCR targeting the VP7 gene of rotaviruses in naturally contaminated oyster samples was shown to give the highest sensitivity and the lowest detection limit of 125 PFU/g of oyster with acid adsorption–alkaline elution (Kittigul et al., 2008).

**Multiplex PCR**

In multiplex PCR, two or more primer sets are used simultaneously in the amplification of different target sequences in a single tube (Chamberlain et al., 1988). Thus, this method could be used for the detection of more than one virus in a single reaction tube (Rosenfield and Jaykus, 1999; Coelho et al., 2003; Beuret, 2004). A multiplex reverse transcription polymerase chain reaction (mRT-PCR) method has been described for the simultaneous detection of the human enteroviruses, HAV and NV (Rosenfield and Jaykus, 1999). Detection limits lower than 1 infectious unit (poliovirus type 1 (PV1) and HAV) or RT-PCR-amplifiable unit (NV) for all viruses were obtained by the multiplex method. In a similar vein, mRT-PCR has been developed to concentrate and purify HAV, PV1 and simian rotaviruses (RV-SA11) simultaneously from experimentally seeded oysters (Coelho et al., 2003). However, this method could not detect the three viruses simultaneously when tested on experimentally contaminated raw oysters. This was attributed to the low concentration of viral RNA present in the oyster extract as a result of an ineffective extraction method.

**Quantitative real-time PCR**

Quantitative real-time PCR (qRT-PCR) is used to amplify and quantify simultaneously a targeted DNA molecule by using DNA-binding fluorophores or, commonly, by specific fluorescently labeled oligoprobes (Atmar, 2006). In recent years, qRT-PCR has been widely used in food virology as the most promising nucleic acid detection method, since it offers several advantages over conventional RT-PCR, including high sensitivity, the possibility of simultaneous amplification, detection and quantification of the target nucleic acids in a single step, and with minimum risk of carry-over contamination through the use of a closed system (Mackay et al., 2002; Bosch et al., 2011; Houde et al., 2007). Sensitive and specific detection with real-time PCR is achieved using novel fluorescent technology probes (Espy et al., 2006). In qPCR assays, three types of fluorescently labeled target-specific probes have been used most often: TaqMan probes, molecular beacons and fluorescence resonance energy transfer (FRET) hybridization probes (Sanchez et al., 2007). These detection methods all depend on the transfer of light energy between two adjacent dye molecules, a process known as fluorescence resonance energy transfer (Espy et al., 2006).
TaqMan-based assays have been widely used to detect HAVs using qPCR in recent years (Sanchez et al., 2007). These assays combine a specific linear dual-labeled oligoprobe in the TaqMan master mix to eliminate the need for post amplification steps and also offer the opportunity of multiplexing amplification reactions (Houde et al., 2007). Several studies targeting the 5’ non-coding region (NCR) have been performed with TaqMan qRT-PCR to detect HAVs (Costa-Mattioli et al., 2002; El Galil et al., 2005; Jothikumar et al., 2005b, Costafreda et al., 2006). The detection limits ranged from one to five copies per reaction. In NV detection, real-time TaqMan-PCR targeting in the well conserved ORF1-ORF2 region has been developed (Hohne and Schreier, 2004; Jothikumar et al., 2005b). These TaqMan RT-PCR assays were able to detect as few as 100 genomic equivalents of different NV strains, including subtypes of GI and GII, rapidly, sensitively and reliably. A TaqMan RT-PCR assay targeting a conserved region in ORF3 has also been developed to detect HEVs in clinical and environmental samples (Jothikumar et al., 2006). This assay was shown to be sensitive and specific for detecting HEV genotypes 1–4 with the detection limit as few as four genome equivalent copies of HEV plasmid DNA and as low as 0.12 50% pig infectious dose (PID50) of swine HEV. Moreover, the detection of different concentrations of swine HEVs (120–1.2 PID50) in a surface water concentrate was performed successfully.

Molecular beacons (MBs) are single-stranded fluorescent probes and have a stem-loop structure that is labeled both with a fluorescent dye and a universal quencher at the 5’ and 3’ ends, respectively (El Galil et al., 2005). MBs undergo a fluorogenic conformational change upon binding to their target, which allows the progress of the reaction to be followed in real-time PCR (El Galil et al., 2004; Valdivia-Granda et al., 2005). A qRT-PCR based on the amplification of 5’-NCR was used to detect genome copies of HAVs using TaqMan and MB probes in clinical and shellfish samples (Costafreda et al., 2006). MB had a lower sensitivity and reproducibility compared to TaqMan probes, which was able to detect as little as 0.05 infectious unit and 10 copies of a single-stranded RNA (ssRNA) synthetic transcript.

Two FRET hybridization probes, made from DNA, are used: one with a fluorescent dye on the 3’ end and the other with an acceptor dye on the 5’ end. They are intended to anneal next to each other in a head-to-tail configuration on the PCR product (Espy et al., 2006). These probes are also referred to as LightCycler probes and are commercially available (Espy et al., 2006; Sanchez et al., 2006). A commercial qRT-PCR assay, the LightCycler HAV quantification kit (Roche Diagnostics), coupled with immunomagnetic separation (IMS) pretreatment, has been shown to be sensitive and specific in the detection of HAVs in fresh produce (Shan et al., 2005). IMS is based on the isolation of an antigen from the sample with a monoclonal antibody against HAV (anti-HAV 1009) combined with streptavidin-coated magnetic beads to recover low levels of viruses and to remove PCR inhibitors. In this assay, 5’ NCR was chosen as the highly conserved target region and a detection limit as low as 1 PFU was obtained. In a similar study, two commercial qRT-PCR HAV assays, the LightCycler HAV
quantiﬁcation kit (Roche Diagnostics) and the RealArt HAV LC RT PCR kit (artus GmbH), were compared in terms of precision, accuracy, linearity and detection limits (Sanchez et al., 2006). The results showed that both kits were suitable for detecting and quantifying HAVs; however, the Roche kit had a slightly better detection limit with the capability of differentiating between different HAV strains and it was also able to detect HAVs in spiked water and food samples.

Several commercial kits for detecting and quantifying NVs have been developed due to the high incidence of NV outbreaks (Butot et al., 2010). The NV qRT-PCR Kit (AnDiaTec GmbH and Co. KG, Kornwestheim, Germany) and the NV Type I and Type II kits (Generon S.r.l., Castelnuovo, Italy) were evaluated and compared with the assay designed by the CEN/TC/WG6/TAG4 research group in the speciﬁc detection and quantiﬁcation of 59 NV samples, including different subtypes of NV genogroups I and II (Butot et al., 2010). The commercial kits failed to detect the vast majority of NV strains, showing poor performance.

The challenges associated with the detection of foodborne viruses, such as PCR inhibitors and low virus concentrations in foods, affect the efﬁciency of real-time assay adversely, therefore, for process control (PC) an internal ampliﬁcation control (IAC), which is extracted and ampliﬁed with the target sequence, is crucial in the evaluation of PCR and to prevent false negatives (Di Pasquale et al., 2010). A real-time PCR IAC has been developed recently for the simultaneous detection of GI and GII NVs, which may also reduce the cost of the assay (Stals et al., 2009). Likewise, the use of non-pathogenic viruses, such as the mutant mengovirus MC0 strain, the MS2 bacteriophage and feline calicivirus (FCV), as sample process controls has been proposed in detecting HAVs in different food matrices (e.g. shellﬁsh, raspberries and strawberries) (Costafreda et al., 2006; Blaise-Boisseau et al., 2010; Di Pasquale et al., 2010). In these studies, no loss of HAV detection sensitivity was observed after the addition of controls.

**Nucleic acid sequence-based ampliﬁcation (NASBA)**

NASBA is an alternative approach to PCR-based molecular methods. In this method, an RNA template is ampliﬁed under isothermal conditions using three enzymes (avian myeloblastosis virus reverse transcriptase, RNase H and T7 RNA polymerase) in the reaction tube (Compton, 1991). NASBA is particularly suitable for detecting RNA viruses since the direct ampliﬁcation of RNA targets is possible without a separate reverse transcription step (Jean et al., 2001, 2004). It has also been shown to be less susceptible to environmental PCR inhibitors (Rutjes et al., 2006). Even though the ampliﬁcation power and the sensitivity of NASBA assays are comparable or even better than that of RT-PCR (Jean et al., 2001), NASBA assays have been used in relatively few studies for detecting enteric viruses compared to RT-PCR.

NASBA assays have been multiplexed or coupled to RT-PCR and ELISA assays to achieve lower detection limits, high sensitivity and speciﬁcity in virus detection (Jean et al., 2002a, 2002b, 2003, 2004). Ampliﬁcation of viral RNA from HAVs and human rotaviruses with selected primers in the multiplex NASBA mixture had detection limits of 40 and 400PFU/ml for rotaviruses and HAVs,
respectively (Jean et al., 2002b). In this study, highly conserved regions in rotavirus gene 9 and in the HAV VP2 gene encoding a major capsid protein were targeted for amplification. Accordingly, multiplex NASBA has been used to detect HAVs, GI and GII noroviruses from representative ready-to-eat foods (Jean et al., 2004). All three viruses were detected in the food matrix simultaneously through targeting relatively conserved genomic regions for each of these, with detection limits ranging from $2 \times 10^2$ to $2 \times 10^3$ PFU/9 cm$^2$. These results show that NASBA is a promising alternative to RT-PCR as it offers rapid and simultaneous detection in a single reaction tube.

A semi-quantitative form of real-time NASBA estimated a viral load in less than half an hour (Patterson et al., 2006). Molecular beacons can be used with NASBA coupled with RNA amplification to produce a specific fluorescent signal, which can be monitored in real time. The measurable fluorescence is directly proportional to the concentration of the target sequence (Leone et al., 1998). More recently, real-time NASBA using a MB probe has been demonstrated to be a sensitive and specific assay for NV detection in clinical and environmental samples (Lamhoujeb et al., 2009). Molecular methods, despite being sensitive and specific, cannot differentiate between infectious and non-infectious viruses. Hence, an enzymatic treatment followed by molecular beacon NASBA targeting of the highly conserved ORF1-ORF2 junction has been developed to distinguish infectious from non-infectious NVs in ready-to-eat food (Lamhoujeb et al., 2008). The proposed enzymatic pretreatment utilized proteinase K and RNase at the same time to digest non-infectious virus particles (Nuanualsuwan and Cliver, 2002).

11.3 Emerging methods

In general, current detection methods have poor sensitivity and selectivity at low virus concentrations. In the main, PCR-based methods have been used to overcome the challenges associated with virus detection; however, these methods also have limitations in terms of complexity in sample preparation and amplification. Thus, the following section is an overview of emerging detection methods.

11.3.1 Spectroscopic approaches

Spectroscopic techniques to detect and identify viral infections are promising owing to their sensitivity, speed, cost and simplicity (Erukhimovitch et al., 2011).

*Surface enhanced Raman spectroscopy*

Surface enhanced Raman spectroscopy (SERS) and electrochemical impedance spectroscopy are the most commonly used spectroscopic approaches in virus detection.

Even though Raman spectroscopy has been used previously to characterize virus structures, it lacks sensitivity due to the extremely small cross section of
Raman scattering, which is about 12–14 orders of magnitude less than fluorescence cross sections (Porter et al., 2008; Shanmuk et al., 2006; Kneipp et al., 2002). With the help of metallic nanostructures, SERS amplifies low-level Raman signals within highly localized optical fields on metallic surfaces. It overcomes the limitations of conventional Raman spectroscopy because of the electromagnetic field or chemical enhancement (Kneipp et al., 2002). SERS spectral fingerprints have been used to discriminate between different types of viruses (Fan et al., 2010; Shanmuk et al., 2006). Recently, several food and waterborne viruses, namely noroviruses, adenoviruses, parvoviruses, rotaviruses, coronaviruses, paramyxoviruses and herpesviruses, were detected and identified using a gold substrate (Fan et al., 2010). Viruses with or without an envelope were differentiated using multivariate statistical analyses (SIMCA) with more than 95% classification accuracy. For SERS, the detection limit was a titer of $10^5$, demonstrating promise for the rapid detection and identification of viruses in food and water samples.

In addition to discriminating between different virus types, SERS has also been used to detect different strains of a single virus type (Tripp et al., 2008). Using silver nanorod arrays fabricated by oblique angle deposition (OAD), SERS was able to detect trace levels of DNA viruses (adenoviruses) and RNA viruses (rhinoviruses and human immunodeficiency viruses (HIVs)) in real time. Moreover, it was able to discriminate between respiratory viruses, virus strains and viruses with gene deletions in biological media (Shanmukh et al., 2006). Further studies indicated that SERS spectra could be used to differentiate between respiratory syncytial virus (RSV) strains and detect viruses with gene deletions using partial least squares (Shanmukh et al., 2008). In a similar study, SERS-active silver nanorod arrays prepared by OAD detected and differentiated between the molecular fingerprints of several important human pathogens, including RSV, HIV and rotavirus (Driskell et al., 2008). SERS also showed high sensitivity and specificity in the identification and classification of rotavirus strains (Driskell et al., 2010). Even though the spectra were similar for each strain, the relative intensities were different (Fig. 11.1). Besides being determined as rotavirus positive or rotavirus negative, samples could be classified by the difference in spectral shapes.

Recently, tip-enhanced Raman spectroscopy (TERS), a combination of optical spectroscopy with SERS, was used to obtain a representative virus spectrum in the identification of different virus strains of avipoxvirus and adeno-associated virus (Hermann et al., 2011). In recent years, SERS substrates and probes have been developed to detect viral genes from HIVs, West Nile viruses and RSVs (Liang et al., 2007; Malvadkar et al., 2010; Zhang et al., 2011a). These studies indicate that the specificity, speed and sensitivity may make SERS-based virus detection a competitive alternative to current detection methods used for food matrices.

Electrochemical impedance spectroscopy
Electrochemical impedance spectroscopy (EIS) monitors the electrical response of a system when a periodic, small amplitude AC signal is applied (Hassen et al.,
EIS has been used to detect several viruses including the influenza virus, the rabies virus, the hepatitis B virus and HIV (Kukol et al., 2008; Hassen et al., 2008, 2011; Hnaien et al., 2008). Many types of biosensor are based on EIS (Hassen et al., 2008). Recently, the influenza A virus was detected using EIS with an antibody-neutravidin-thiol structure immobilized on the surface of an Au electrode in solutions of phosphate buffer saline with large amounts of non-target protein, which showed the detection sensitivity and selectivity (Hassen et al., 2011). The detection limit was as low as 8 ng/ml, which shows the efficiency of this approach for virus detection. A biosensor based on EIS has been used to detect the label-free viral DNA hybridization of avian influenza virus (Kukol et al., 2008). Even though EIS has not been used for foodborne virus detection, it is a promising approach in terms of sensitivity, selectivity and response.

### 11.3.2 Immunoassays

Immunoassays are analytical methods that produce a sensitive, selective and measurable response based on highly specific antibody and antigen interactions.
Until recently, ELISA and enzyme immunoassays were widely used in foodborne virus detection. Even though these methods are reliable, they are time-consuming and labor intensive. An immunoassay using microsphere technology can overcome the limitations associated with traditional ELISA (Go et al., 2008). The well-known microsphere assay system, the xMap system (Luminex Corp., Austin, TX), combines three well-established technologies: bioassays, solution phase microspheres and flow cytometry (Go et al., 2008). A liquid suspension array consisting of unique color-coded microsphere polystyrene beads is coupled to antigens and antibody reactions, and the emissions are then measured by a flow-based detector (Deregt et al., 2006). Microsphere immunoassays offer several advantages, including accuracy, high sensitivity, specificity, reproducibility, high-throughput sample analysis and multiplexing capability, over traditional ELISAs (Go et al., 2008). In particular, the multiplexing capability enables the detection of a multiplex analyte in a single reaction tube based on individually identifiable, fluorescently coded sets of polystyrene microbeads (Binnicker et al., 2011; Khan et al., 2006). In the last decade, a number of microsphere-based immunoassays have been described for the antigen and antibody detection of several viruses including HIV (Bellisario et al., 2001), non-human primate viruses (Khan et al., 2006), avian influenza virus (Deregt et al., 2006), West Nile virus (Johnson et al., 2007), Epstein–Barr virus (Binnicker et al., 2008) and hepatitis C virus (Fonseca et al., 2011).

Immuno-PCR (IPCR) is a method similar to ELISA. Reporter DNA is used instead of an enzyme in IPCR, which may have a $10^2$ to $10^5$ increase in sensitivity as a result of the amplification of the reporter DNA (Deng et al., 2011b). More recently, this method has been used in rapid screening for trace levels of avian influenza viruses (Deng et al., 2011b), Newcastle disease viruses (Deng et al., 2011a), RSVs (Perez et al., 2011) and foot and mouth disease viruses (Ding et al., 2011). IPCR had an approximately 1000-fold improvement over conventional ELISA, and a 100-fold enhancement over RT-PCR. The detection limit was as low as $10^{-4}$ EID$_{50}$ (50% egg infective dose) for the H5 subtype avian influenza virus (Deng et al., 2011b).

### 11.3.3 Microelectromechanical systems and microfluidics

Microelectromechanical systems (MEMSs) can act as transducers for sensing and actuation in various engineering applications. They can be used to integrate micron-sized mechanical parts with electronics and they can be batch fabricated in large quantities (Gau et al., 2001). MEMS-based and microfluidic-based biosensing approaches have received considerable interest in recent years owing to their advantages over conventional methods including low cost and sample volume, portability, disposability, parallel processing and automation (Wang et al., 2011). More recently, a MEMS biosensor has been developed to detect hepatitis A and hepatitis C viruses (HCVs) in serum using dynamic-mode microcantilevers without any labels or preamplification (Timurdogan et al., 2011). Electroplated nickel MEMS cantilevers functionalized with HAV or HCV
antibodies were exposed to either HAV antigens (Case 1 and Case 3) or HCV antigens (Case 2 and Case 4), in increasing concentrations in an undiluted serum (Fig. 11.2). The minimum detection limit concentration was 0.1 ng/ml for both HAVs and HCVs, which is comparable with labeled sensing detection methods such as ELISA. Moreover, it was shown that the dynamic range of this biosensor was in excess of 1000:1 for the specific type of hepatitis antibody used.

MEMS technology enables PCR using microfluidics and consequently the synthesis of complementary DNA (cDNA) on microfluidic devices (Li et al., 2011c). This microfluidic-based PCR method has several advantages including lower thermal capacitance giving rapid thermal cycling, reduced analysis times, low consumption of sample and reagent, portability and the potential for high automation and integration of various analytical procedures (Li et al., 2011b). Microfluidic-based RT-PCR has been developed to detect foodborne viruses (Li et al., 2011b, 2011c). An integrated microfluidic system for continuous-flow RT-PCR reactions with online fluorescence detection has been developed for the rapid identification of NVs and rotaviruses; the limit of detection (LOD) is

Fig. 11.2 Hepatitis detection measurement results using two biosensor chips. Measurements were taken at different concentrations ranging from 0.04 to 100 ng/ml for negative and positive controls. Different HAV and HCV concentrations were introduced into fetal bovine serum. Taken from Timurdogan et al. (2011) with permission.
6.4 × 10^4 copies per μl using a one-step RT-PCR process (Li et al., 2011b). This restricted LOD was mainly attributed to the inhibition effect of the channel surface. Detection of the amplified products was carried out online using fluorescence microscopy with SybrGreen I. This method did not require the time-consuming and labor-intensive agarose gel electrophoresis and ethidium bromide staining and had much faster reaction times compared to conventional RT-PCR.

11.3.4 Nanostructures
Spherical (quantum dots) and linear particles (nanowires, nanotubes or nanorods) with specific optical, electrical, mechanical, thermal and magnetic properties can be fabricated by combining different metals, semiconductors and carbon (Valdivia-Granda et al., 2005). Nanoparticles (NPs) can be used to provide additional functional properties, including signal enhancement or purification, in virus detection (Fournier-Wirth and Coste, 2010).

Quantum dots (QDs), clusters of a few hundred to a few thousand atoms, are synthesized from metallic materials such as gold, silver or cobalt and semiconductor materials such as cadmium sulfite, cadmium selenide and cadmium telluride (Valdivia-Granda et al., 2005). QDs have often been used to label biomolecules owing to their outstanding properties such as negligible photobleaching, fairly high quantum yield, stability, narrow emission spectrum and broad excitation spectrum (Zhang et al., 2011b). These particles have been conjugated to antibodies and nucleic acids and used as a label in the detection of several viruses including RSV (Agrawal et al., 2005), porcine reproductive virus (Stringer et al., 2008), cauliflower mosaic virus (Huang et al., 2009), Newcastle disease virus and avian virus arthritis virus (Wang et al., 2010) and the Epstein–Barr virus (Chen et al., 2010); however, QDs have never been used to detect foodborne viruses.

Carbon nanotubes (CNTs) are widely used in novel nanostructures and devices due to their large surface area per unit mass and excellent mechanical and electrical properties (Bhattacharya et al., 2011). Moreover, the functionalization of CNTs through the alteration of the surface chemistry increases their potential for use as biosensing markers (Valdivia-Granda et al., 2005). Using the surface functionalization feature, CNTs can be used to immobilize antibodies or nucleic acid that target a type of virus. The process can be monitored using a change in the mechanical or electrical property of the CNTs (Bhattacharya et al., 2011). This concept has been recently used to detect hepatitis C viruses (Dastagir et al., 2007), avian influenza viruses (Zhu et al., 2009; Tam et al., 2009) and swine influenza viruses (Lee et al., 2011) other than foodborne viruses. As CNT-based biosensors are easy to produce, have reproducible results and are inexpensive, and since they have better sensitivity and time responses than current techniques, they are very promising for detecting viruses.

One other promising approach for detecting biomolecules is the use of a semiconducting nanowire where the conductance is proportional to the viral load. The change in conductance is in response to binding between the target and the probe, which is attached to the nanowire (Patolsky et al., 2004; Valdivia-Granda...
et al., 2005). Nanowires act as a capture agent on the sensor surface and selectively bind target biomolecules much like CNTs (Ishikawa et al., 2009). Nanowires have several attractive features for the real-time detection of a single virus with high selectivity (Valdivia-Granda et al., 2005). Silicon nanowires have been used in label-free field effect transistor (FET)-based biosensors to detect influenza A viruses (Zheng et al., 2005) and dengue viruses (Zhang et al., 2010). The results showed that silicon nanowire-based sensors are more sensitive and have a more rapid response compared to traditional methods. Recently, an alternative nanomaterial to silicon nanowire, a metal oxide nanowire, has been used to detect a protein related to severe acute respiratory syndrome (SARS) at a subnanomolar concentration in a background of 44 μM bovine serum albumin (Ishikawa et al., 2009).

11.4 Future trends

Research into the detection of foodborne viruses has grown in recent years due to the high incidence of outbreaks. Currently, immunological and PCR-based methods are commonly used to detect viruses in food samples. Despite their reliability, most of these methods have limitations in terms of speed and sensitivity owing to low viral concentrations and inhibitory substances present in food. Even though methods for concentrating and purifying viruses in food samples have been widely investigated and developed, the inhibitory substances can remain and cause false-negative results. Therefore, new detection methods that are rapid and sensitive are necessary for direct detection in food samples.

Some of the approaches described for detecting viruses are relatively new and some are still in their infancy. It is expected that electrochemical-based detection techniques will become more prominent, while spectroscopic and microfluidic assays will be developed in parallel. It is anticipated that research using microfluidics will focus on combining the pretreatment of a viral sample and multiplex detection into a biochip. Thus, the microfluidic approach could be a promising platform for rapid detection of viruses. Additionally, the conjugation of antibodies, antigens and nucleic acids with quantum dots, nanowires and carbon nanotubes offers several advantages over current detection methods in terms of sensitivity and speed. These systems may also be used for the label-free detection of very low concentration of viral particles, or even for detecting a single virus without amplification. These approaches can be further improved with the advent of novel nanostructures. Even though most of these approaches have not been used to detect foodborne viruses, all of them are promising and can complement the existing methods.

11.5 References

ABAD, F., PINTO, R., VILLENA, C., GAJARDO, R. and BOSCH, A. 1997. Astrovirus survival in drinking water. Applied and Environmental Microbiology, 63, 3119–3122.
AGRAWAL, A., TRIPP, R., ANDERSON, L. and NIE, S. 2005. Real-time detection of virus particles and viral protein expression with two-color nanoparticle probes. *Journal of Virology*, 79, 8625–8628.

AHN, J., KANG, S., LEE, D., SHIN, S. and YOO, H. 2005. Identification of novel human hepatitis E virus (HEV) isolates and determination of the seroprevalence of HEV in Korea. *Journal of Clinical Microbiology*, 43, 3042–3048.

ATMAR, R. L. 2006. Molecular methods of virus detection in foods. In: Goyal, S. M. (ed.) *Viruses in Foods*. New York, USA: Springer.

ATMAR, R. and ESTES, M. 2001. Diagnosis of noncultivatable gastroenteritis viruses, the human calciviruses. *Clinical Microbiology Reviews*, 14, 15.

BELLISARIO, R., COLINAS, R. and PASS, K. 2001. Simultaneous measurement of antibodies to three HIV-1 antigens in newborn dried blood-spot specimens using a multiplexed microsphere-based immunoassay. *Early Human Development*, 64, 21–25.

BERKE, T. and MATSON, D. 2000. Reclassification of the Caliciviridae into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. *Archives of Virology*, 145, 1421–1436.

BEURET, C. 2004. Simultaneous detection of enteric viruses by multiplex real-time RT-PCR. *Journal of Virological Methods*, 115, 1–8.

BHATTACHARYA, M., HONG, S., LEE, D., CUI, T. and GOYAL, S. 2011. Carbon nanotube based sensors for the detection of viruses. *Sensors and Actuators B – Chemical*, 155, 67–74.

BINNICKER, M., JESPERSEN, D., HARRING, J., ROLLINS, L. and BEITO, E. 2008. Evaluation of a multiplex flow immunoassay for detection of Epstein–Barr virus-specific antibodies. *Clinical and Vaccine Immunology*, 15, 1410–1413.

BINNICKER, M., JESPERSEN, D. and ROLLINS, L. 2011. Evaluation of the Bio-Rad BioPlex measles, mumps, rubella, and Varicella-Zoster virus IgG multiplex bead immunoassay. *Clinical and Vaccine Immunology*, 18, 1524–1526.

BLAISE-BOISSEAU, S., HENNECHART-COLLETTE, C., GUILLLIER, L. and PERELLE, S. 2010. Duplex real-time qRT-PCR for the detection of hepatitis A virus in water and raspberries using the MS2 bacteriophage as a process control. *Journal of Virological Methods*, 166, 48–53.

BOSCHI, A., SANCHEZ, G., ABBASZADEGAN, M., CARDUCCI, A., GUIX, S. et al. 2011. Analytical methods for virus detection in water and food. *Food Analytical Methods*, 4, 4–12.

BUTOT, S., LE GUYADER, F., KROL, J., PUTALLAZ, T., AMOROSO, R. et al. 2010. Evaluation of various real-time RT-PCR assays for the detection and quantitation of human norovirus. *Journal of Virological Methods*, 167, 90–94.

CASAS, N. and SUNEN, E. 2001. Detection of enterovirus and hepatitis A virus RNA in mussels (Mytilus spp.) by reverse transcriptase-polymerase chain reaction. *Journal of Applied Microbiology*, 90, 89–95.

CHAMBERLAIN, J., GIBBS, R., RANIER, J., NGUYEN, P. and CASKEY, C. 1988. Deletion screening of the Dusshene muscular-dystrophy locus via multiplex DNA amplification. *Nucleic Acids Research*, 16, 11141–11156.

CHEN, L., QI, Z., CHEN, R., LI, Y. and LIU, S. 2010. Sensitive detection of Epstein–Barr virus-derived latent membrane protein 1 based on CdTe quantum dots-capped silica nanoparticle labels. *Clinica Chimica Acta*, 411, 1969–1975.

COELHO, C., VINATEA, C., HEINERT, A., SIMOES, C. and BARARDI, C. 2003. Comparison between specific and multiplex reverse transcription-polymerase chain reaction for detection of hepatitis A virus, poliovirus and rotavirus in experimentally seeded oysters. *Memorias Do Instituto Oswaldo Cruz*, 98, 465–468.

COMPTON, J. 1991. Nucleic acid sequence based amplification. *Nature*, 350, 91–92.

COOK, N. and RZEZUTKA, A. 2006. Hepatitis viruses. In: Motarjemi, Y. and Adams, M. (eds.) *Emerging Foodborne Pathogens*. Woodhead Publishing.

COSTA-MATTIOLI, M., MONPOEHO, S., NICAND, E., ALEMAN, M., BILLAUDEL, S. et al. 2002. Quantification and duration of viraemia during hepatitis A infection as determined by real-time RT-PCR. *Journal of Viral Hepatitis*, 9, 101–106.
CROCI, L., DE MEDICI, D., MORACE, G., FOIRE, A., SCALFARO, C. et al. 1999. Detection of hepatitis A virus in shellfish by nested reverse transcription-PCR. International Journal of Food Microbiology, 48, 67–71.

DARGAGIR, T., FORZANI, E., ZHANG, R., AMLANI, I., NAGAHARA, L. et al. 2007. Electrical detection of hepatitis C virus RNA on single wall carbon nanotube-effect field transistors. Analyst, 132, 738–740.

DENG, M., LONG, L., XIAO, X., WU, Z., ZHANG, F., et al. 2011a. Immuno-PCR for one step detection of H5N1 avian influenza virus and Newcastle disease virus using magnetic gold particles as carriers. Veterinary Immunology and Immunopathology, 141, 183–189.

DENG, M., XIAO, X., ZHANG, Y., WU, X., ZHU, L. et al. 2011b. A highly sensitive immuno-PCR assay for detection of H5N1 avian influenza virus. Molecular Biology Reports, 38, 1941–1948.

DEREGT, D., FURUKAWA-STOFFER, T., TOKARYK, K., PASICK, J., HUGHES, K. et al. 2006. A microsphere immunoaassay for detection of antibodies to avian influenza virus. Journal of Virological Methods, 137, 88–94.

DI PASQUALE, S., PANICONI, M., DE MEDICI, D., SUFFREDINI, E. and CROCI, L. 2010. Duplex real time PCR for the detection of hepatitis A virus in shellfish using feline calicivirus as a process control. Journal of Virological Methods, 163, 96–100.

DI PINTO, A. FORTE, V., TANTILLO, G., TERIO, V. and BUONAVOGLIA, C. 2003. Detection of hepatitis A virus in shellfish (Mytilus galloprovincialis) with RT-PCR. Journal of Food Protection, 66, 1681–1685.

DING, Y., LIU, Y., ZHOU, J., CHEN, H., WEI, G. et al. 2011. A highly sensitive detection for foot-and-mouth disease virus by gold nanoparticle improved immuno-PCR. Virology Journal, 8.

DRISKELL, J., SHANMUKH, S., LIU, Y., HENNIGAN, S., JONES, L. et al. 2008. Infectious agent detection with SERS-active silver nanorod arrays prepared by oblique angle deposition. IEEE Sensors Journal, 8, 863–870.

DRISKELL, J., ZHU, Y., KIRKWOOD, C., ZHAO, Y., DLUHY, R. et al. 2010. Rapid and sensitive detection of rotavirus molecular signatures using surface enhanced Raman spectroscopy. Plos One, 5, e10222.

D’SOUAZA, D. H. and JAYKUS, L. A. 2006. Molecular approaches for the detection of foodborne viral pathogens. In: Maurer, J. (ed.) PCR Methods in Foods. Food Microbiology and Food Safety Series. New York, USA: Springer.

DUBOIS, E., AGIER, C., TRAORE, O., HENNECHART, C., MERLE, G. et al. 2002. Modified concentration method for the detection of enteric viruses on fruits and vegetables by reverse transcriptase-polymerase chain reaction or cell culture. Journal of Food Protection, 65, 1962–1969.

EL GALIL, K., EL SOKKARY, M., KHEIRA, S., SALAZAR, A., YATES, M. et al. 2004. Combined immunomagnetic separation-molecular beacon-reverse transcription-PCR assay for detection of hepatitis A virus from environmental samples. Applied and Environmental Microbiology, 70, 4371–4374.

EL GALIL, K., EL SOKKARY, M., KHEIRA, S., SALAZAR, A., YATES, M. et al. 2005. Real-time nucleic acid sequence-based amplification assay for detection of hepatitis A virus. Applied and Environmental Microbiology, 71, 7113–7116.

EMERSON, S. and PURCELL, R. 2001. Recombinant vaccines for hepatitis E. Trends in Molecular Medicine, 7, 462–466.
genotypes of hepatitis E virus in clinical specimens. *Journal of Medical Virology, 78*, 1076–1082.

ERUKHIMOVITCH, V., BOGOMOLNY, E., HULEHIL, M. and HULEHIL, M. 2011. Infrared spectral changes identified during different stages of herpes viruses infection in vitro. *Analyst, 136*, 2818–2824.

ESPY, M., UHL, J., SLOAN, L., BUCKWALTER, S., JONES, M. et al. 2006. Real-time PCR in clinical microbiology: Applications for a routine laboratory testing. *Clinical Microbiology Reviews, 19*, 165–256.

FAN, C., HU, Z., RILEY, L., PURDY, G., MUSTAPHA, A. et al. 2010. Detecting food- and waterborne viruses by surface-enhanced Raman spectroscopy. *Journal of Food Science, 75*, M302–M307.

FANKHAUSER, R. L., MONROE, S. S., NOEL, J. S., HUMPHREY, C.D., BRESSE, J. S. et al. 2002. Epidemiologic and molecular trends of ‘Norwalk-like viruses’ associated with outbreaks of gastroenteritis in the United States. *Journal of Infectious Diseases, 186*, 1–7.

FAO/WHO 2008. Viruses in food: Scientific advice to support risk management activities. Meeting report. *Microbiological Risk Assessment Series*. Rome.

FONSECA, B., MARQUES, C., NASCIMENTO, L., MELLO, M., SILVA, L. et al. 2011. Development of a multiplex bead-based assay for detection of hepatitis C virus. *Clinical and Vaccine Immunology, 18*, 802–806.

FORMIGA-CRUZ, M., HUNDESA, A., CLEMENTE-CASARES, P., ALBINANA-GIMENEZ, N., ALLARD, A. et al. 2005. Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage. *Journal of Virological Methods, 125*, 111–118.

FOURNIER-WIRTH, C. and COSTE, J. 2010. Nanotechnologies for pathogen detection: Future alternatives? *Biologicals, 38*, 9–13.

GAU, J., LAN, E., DUNN, B., HO, C. and WOO, J. 2001. A MEMS based amperometric detector for *E. coli* bacteria using self-assembled monolayers. *Biosensors and Bioelectronics, 16*, 745–755.

GERBA, C. P. 2006. Food virology: Past, present, and future. In: Goyal, S. M. (ed.) *Viruses in Foods*. New York, USA: Springer.

GO, Y., WONG, S., BRANSCUM, A., DEMAREST, V., SHUCK, K. et al. 2008. Development of a fluorescent-microsphere immunoassay for detection of antibodies specific to equine arteritis virus and comparison with the virus neutralization test. *Clinical and Vaccine Immunology, 15*, 76–87.

GOYAL, S. M. 2006. Methods of virus detection in foods. In: Goyal, S. M. (ed.) *Viruses in Foods*. New York, USA: Springer.

GREEN, D. and LEWIS, G. 1999. Comparative detection of enteric viruses in wastewaters, sediments and oysters by reverse transcription PCR and cell culture. *Water Research, 33*, 1195–1200.

GREENING, G. E. 2006. Molecular methods of virus detection in foods. In: Goyal, S. M. (ed.) *Viruses in Foods*. New York, USA: Springer.

GUEVREMONT, E., BRASSARD, J., HOUGE, A., SIMARD, C. and TROTTIER, Y. 2006. Development of an extraction and concentration procedure and comparison of RT-PCR primer systems for the detection of hepatitis A virus and norovirus GII in green onions. *Journal of Virological Methods, 134*, 130–135.

GUTIERREZ-AGUIRRE, I., STEYER, A., BOBEN, J., GRUDEIN, K., POLJSAK-PRIJATELJ, M. et al. 2008. Sensitive detection of multiple rotavirus genotypes with a single reverse transcription-real-time quantitative PCR assay. *Journal of Clinical Microbiology, 46*, 2547–2554.

GYARMATI, P., MOHAMMED, N., NORDER, H., BLOMBERG, J., BELAK, S. et al. 2007. Universal detection of hepatitis E virus by two real-time PCR assays: TaqMan (R) and primer-probe energy transfer. *Journal of Virological Methods, 146*, 226–235.

HAQQI, T., SARKAR, G., DAVID, C. and SOMMER, S. 1988. Specific amplification with PCR of a refractory segment of genomic DNA. *Nucleic Acids Research, 16*, 11844.
HASSEN, W., CHAIX, C., ABDELGHANI, A., BESSUEILLE, F., LEONARD, D. et al. 2008. An impedimetric DNA sensor based on functionalized magnetic nanoparticles for HIV and HBV detection. Sensors and Actuators B – Chemical, 134, 755–760.

HASSEN, W., DUPLAN, V., FROST, E. and DUBOWSKI, J. 2011. Quantitation of influenza A virus in the presence of extraneous protein using electrochemical impedance spectroscopy. Electrochimica Acta, 56, 8325–8328.

HERMANN, P., HERMELINK, A., LAUSCH, V., HOLLAND, G., MOLLER, L. et al. 2011. Evaluation of tip-enhanced Raman spectroscopy for characterizing different virus strains. Analyst, 136, 1148–1152.

HNAIEN, M., DIOUANI, M., HELALI, S., HAFIAD, I., HASSEN, W. et al. 2008. Immobilization of specific antibody on SAM functionalized gold electrode for rabies virus detection by electrochemical impedance spectroscopy. Biochemical Engineering Journal, 39, 443–449.

HOHNE, M. and SCHREIER, E. 2004. Detection and characterization of norovirus outbreaks in Germany: Application of a one-tube RT-PCR using a fluorogenic real-time detection system. Journal of Medical Virology, 72, 312–319.

HOUDE, A., GUEVREMONT, E., POITRAS, E., LEBLANC, D., WARD, P. et al. 2007. Comparative evaluation of new TaqMan real-time assays for the detection of hepatitis A virus. Journal of Virological Methods, 140, 80–89.

HUANG, D., LIU, H., ZHANG, B., JIAO, K. and FU, X. 2009. Highly sensitive electrochemical detection of sequence-specific DNA of 35S promoter of cauliflower mosaic virus gene using CdSe quantum dots and gold nanoparticles. Microchimica Acta, 165, 243–248.

ISHIKAWA, F., CHANG, H., CURRELI, M., LIAO, H., OLSON, C. et al. 2009. Label-free, electrical detection of the SARS virus N-protein with nanowire biosensors utilizing antibody mimics as capture probes. ACS Nano, 3, 1219–1224.

JANSEN, R., SIEGL, G. and LEMON, S. 1990. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. Proceedings of the National Academy of Sciences of the United States of America, 87, 2867–2871.

JEAN, J., BLAIS, B., DARVEAU, A. and FLISS, I. 2001. Detection of hepatitis A virus by the nucleic acid sequence-based amplification technique and comparison with reverse transcription-PCR. Applied and Environmental Microbiology, 67, 5593–5600.

JEAN, J., BLAIS, B., DARVEAU, A. and FLISS, I. 2002a. Rapid detection of human rotavirus using colorimetric nucleic acid sequence-based amplification (NASBA)-enzyme-linked immunosorbent assay in sewage treatment effluent. FEMS Microbiology Letters, 210, 143–147.

JEAN, J., BLAIS, B., DARVEAU, A. and FLISS, I. 2002b. Simultaneous detection and identification of hepatitis A virus and rotavirus by multiplex nucleic acid sequence-based amplification (NASBA) and microtiter plate hybridization system. Journal of Virological Methods, 105, 123–132.

JEAN, J., D’SOUZA, D. and JAYKUS, L. 2003. Transcriptional enhancement of RT-PCR for rapid and sensitive detection of noroviruses. FEMS Microbiology Letters, 226, 339–345.

JEAN, J., D’SOUZA, D. and JAYKUS, L. 2004. Multiplex nucleic acid sequence-based amplification for simultaneous detection of several enteric viruses in model ready-to-eat foods. Applied and Environmental Microbiology, 70, 6603–6610.

JIANG, X., HUANG, P., ZHONG, W., FARKAS, T., CUBITT, D. et al. 1999. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. Journal of Virological Methods, 83, 145–154.

JIANG, Y., LIAO, G., ZHAO, W., SUN, M., QIAN, Y. et al. 2004. Detection of infectious hepatitis A virus by integrated cell culture/strand-specific reverse transcriptase-polymerase chain reaction. Journal of Applied Microbiology, 97, 1105–1112.

JOHNSON, A., CRESHIER, R., COSENTINO, G., MASRI, H., MOCK, V. et al. 2007. Validation of a microsphere-based immunoassay for detection of anti-West Nile virus and anti-St. Louis encephalitis virus immunoglobulin M antibodies. Clinical and Vaccine Immunology, 14, 1084–1093.

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JOYHIKUMAR, N., CROMEANS, T., HILL, V., LU, X., SOBYSE, M. et al. 2005a. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. *Applied and Environmental Microbiology*, 71, 3131–3136.

JOYHIKUMAR, N., LOWTHER, J., HENSILWOOD, K., LEES, D., HILL, V. et al. 2005b. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. *Applied and Environmental Microbiology*, 71, 1870–1875.

JOYHIKUMAR, N., CROMEANS, T., ROBERTSON, B., MENG, X. and HILL, V. 2006. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of Virological Methods*, 131, 65–71.

KAPIKIAN, A., HOSHIYNO, Y., CHANOCK, R. and PETERSCHAEL, I. 1996. Efficacy of a quadrivalent rhesus rotavirus-based human rotavirus vaccine aimed at preventing severe rotavirus diarrhea in infants and young children. *Journal of Infectious Diseases*, 174, S65–S72.

KATAYAMA, K., SHIRATO-HORIKOSHI, H., KOJIMA, S., KAGEYAMA, T., OKA, T. et al. 2002. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. *Virology*, 299, 225–239.

KHAN, I., MENDOZA, S., YEE, J., DEANE, M., VENKATESWARAN, K. et al. 2006. Simultaneous detection of antibodies to six nonhuman-primate viruses by multiplex microbead immunoassay. *Clinical and Vaccine Immunology*, 13, 45–52.

KIM, D., KIM, S., Kwon, K., Lee, J. and Oh, M. 2008a. Detection of hepatitis A virus from oyster by nested PCR using efficient extraction and concentration method. *Journal of Microbiology*, 46, 436–440.

KIM, S., KIM, D., Kwon, K., Hwang, I. and Oh, M. 2008b. Detection of norovirus in contaminated ham by reverse transcriptase-PCR and nested PCR. *Food Science and Biotechnology*, 17, 651–654.

KITITIGUL, L., POMBUBPA, K., RATTANATHAM, T., DIRAPHAT, P., UTRARACHKIJ, F. et al. 2008. Development of a method for concentrating and detecting rotavirus in oysters. *International Journal of Food Microbiology*, 122, 204–210.

KNEIPP, K., KNEIPP, H., ITZKAN, I., DASARI, R. and FELD, M. 2002. Surface-enhanced Raman scattering and biophysics. *Journal of Physics – Condensed Matter*, 14, R597–R624.

KOGAWA, K., NAKATA, S., UKAIE, S., ADACHI, N., NUMATA, K. et al. 1996. Dot blot hybridization with a cDNA probe derived from the human calicivirus Sapporo 1982 strain. *Archives of Virology*, 141, 1949–1959.

KOOPMANS, M. and Duizer, E. 2004. Foodborne viruses: An emerging problem. *International Journal of Food Microbiology*, 90, 23–41.

KOOPMANS, M., VON BONSDORFF, C., VINJE, J., DE MEDICI, D. and MONROE, S. 2002. Foodborne viruses. *FEMS Microbiology Reviews*, 26, 187–205.

KUKOL, A., LI, P., ESTRELA, P., KO-FERRIGNO, P. and MIGLIOARIO, P. 2008. Label-free electrical detection of DNA hybridization for the example of influenza virus gene sequences. *Analytical Biochemistry*, 374, 143–153.

LAMHOUJEB, S., FLISS, I., NGAZOA, S. and JEAN, J. 2008. Evaluation of the persistence of infectious human noroviruses on food surfaces by using real-time nucleic acid sequence-based amplification. *Applied and Environmental Microbiology*, 74, 3349–3355.

LAMHOUJEB, S., CHAREST, H., FLISS, I., NGAZOA, S. and JEAN, J. 2009. Real-time molecular beacon NASBA for rapid and sensitive detection of norovirus GI in clinical samples. *Canadian Journal of Microbiology*, 55, 1375–1380.

LEE, D., CHANDER, Y., GOYAL, S. and CUJ, T. 2011. Carbon nanotube electric immunoassay for the detection of swine influenza virus H1N1. *Biosensors and Bioelectronics*, 26, 3482–3487.

LEE, D. 2000. Viruses and bivalve shellfish. *International Journal of Food Microbiology*, 59, 81–116.

LEMON, S. and ROBERTSON, B. 1993. Current perspectives in the virology and molecular biology of hepatitis A virus. *Seminars in Virology*, 4, 285–295.
LEONE, G., VAN GEMEN, B. and SCHOEN, C. D. 1998. Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA. Nucleic Acids Research, 26, 2150–2155.

LI, F., MEI, L., LI, Y., ZHAO, K., CHEN, H. et al. 2011a. Facile fabrication of magnetic gold electrode for magnetic beads-based electrochemical immunoassay: Application to the diagnosis of Japanese encephalitis virus. Biosensors and Bioelectronics, 26, 4253–4256.

LI, Y., ZHANG, C. and XING, D. 2011b. Fast identification of foodborne pathogenic viruses using continuous-flow reverse transcription-PCR with fluorescence detection. Microfluidics and Nanofluidics, 10, 367–380.

LI, Y., ZHANG, C. and XING, D. 2011c. Integrated microfluidic reverse transcription-polymerase chain reaction for rapid detection of food- or waterborne pathogenic rotavirus. Analytical Biochemistry, 415, 87–96.

LIANG, Y., GONG, J., HUANG, Y., ZHENG, Y., JIANG, J. et al. 2007. Biocompatible core-shell nanoparticle-based surface-enhanced Raman scattering probes for detection of DNA related to HIV gene using silica-coated magnetic nanoparticles as separation tools. Talanta, 72, 443–449.

LOVE, D., CASTEEL, M., MESCHE, J. and SOBSEY, M. 2008. Methods for recovery of hepatitis A virus (HAV) and other viruses from processed foods and detection of HAV by nested RT-PCR and TaqMan RT-PCR. International Journal of Food Microbiology, 126, 221–226.

MACKAY, I., ARDEN, K. and NITSCHE, A. 2002. Real-time PCR in virology. Nucleic Acids Research, 30, 1292–1305.

MALVADKAR, N., DEMIREL, G., POSS, M., JAVED, A., DRESSICK, W. et al. 2010. Fabrication and use of electroless plated polymer surface-enhanced Raman spectroscopy substrates for viral gene detection. Journal of Physical Chemistry C, 114, 10730–10738.

MARTINEZ-MARTINEZ, M., DIEZ-VALCARCE, M., HERNANDEZ, M. and RODRIGUEZ-LAZARO, D. 2011. Design and application of nucleic acid standards for quantitative detection of enteric viruses by real-time PCR. Food and Environmental Virology, 3, 92–98.

MEAD, P., SLUTSKER, L., DIETZ, V., MCCAIG, L., BRESEE, J. et al. 1999. Food-related illness and death in the United States. Emerging Infectious Diseases, 5, 607–625.

MORALES-RAYAS, R., WOLFFS, P. and GRIFFITHS, M. 2010. Simultaneous separation and detection of hepatitis A virus and norovirus in produce. International Journal of Food Microbiology, 139, 48–55.

NAKAYAMA, M., UEDA, Y., KAWAMOTO, H., HANJUN, Y., SAITO, K. et al. 1996. Detection and sequencing of Norwalk-like viruses from stool samples in Japan using reverse transcription-polymerase chain reaction amplification. Microbiology and Immunology, 40, 317–320.

NUANUALSUWAN, S. and CLIVER, D. 2002. Pretreatment to avoid positive RT-PCR results with inactivated viruses. Journal of Virological Methods, 104, 217–225.

O’CONNELL, J. 2002. RT-PCR Protocols. Totowa, NJ: Humana Press.

PATOLSKY, F., ZHENG, G., HAYDEN, O., LAKADAMYALI, M., ZHUANG, X. et al. 2004. Electrical detection of single viruses. Proceedings of the National Academy of Sciences of the United States of America, 101, 14017–14022.

PATTERSON, S., SMITH, M., CASPER, E., HUFFMAN, D., STARK, L. et al. 2006. A nucleic acid sequence-based amplification assay for real-time detection of norovirus genogroup II. Journal of Applied Microbiology, 101, 956–963.

PEREZ, J., VARGIS, E., RUSS, P., HASELTON, F. and WRIGHT, D. 2011. Detection of respiratory syncytial virus using nanoparticle amplified immuno-polymerase chain reaction. Analytical Biochemistry, 410, 141–148.

PINEDA, M., CHAN, L., KUHLENSCHMIDT, T., CHOI, C., KUHLENSCHMIDT, M. et al. 2009. Rapid specific and label-free detection of porcine rotavirus using photonic crystal biosensors. IEEE Sensors Journal, 9, 470–477.

PORTER, M., LIPERT, R., SIPERKO, L., WANG, G. and NARAYANANA, R. 2008. SERS as a bioassay platform: Fundamentals, design, and applications. Chemical Society Reviews, 37, 1001–1011.
REYNOLDS, K., GERBA, C. and PEPPER, I. 1996. Detection of infectious enteroviruses by an integrated cell culture PCR procedure. *Applied and Environmental Microbiology*, 62, 1424–1427.

RICHARDS, G. 1999. Limitations of molecular biological techniques for assessing the virological safety of foods. *Journal of Food Protection*, 62, 691–697.

RICHARDS, A., LOPMAN, B., GUNN, A., CURRY, A., ELLIS, D. *et al.* 2003. Evaluation of a commercial ELISA for detecting Norwalk-like virus antigen in faeces. *Journal of Clinical Virology*, 26, 109–115.

RIGOTTO, C., SINERO, T., SIMOES, C. and BARARDI, C. 2005. Detection of adenoviruses in shellfish by means of conventional-PCR, nested-PCR, and integrated cell culture PCR (ICC/PCR). *Water Research*, 39, 297–304.

RODRIGUEZ-LAZARIO, D., LOMBARD, B., SMITH, H., RZEZUTKA, A., D’AGOSTINO, M. *et al.* 2007. Trends in analytical methodology in food safety and quality: Monitoring microorganisms and genetically modified organisms. *Trends in Food Science and Technology*, 18, 306–319.

ROSENFIELD, S. and JAYKUS, L. 1999. A multiplex reverse transcription polymerase chain reaction method for the detection of foodborne viruses. *Journal of Food Protection*, 62, 1210–1214.

RUTIES, S., HAROLD, H. J. L., VAN DEN BERG, H., LODDER, W. J. and DE RODA HUSMAN, A. M. 2006. Real-time detection of noroviruses in surface water by use of a broadly reactive nucleic acid sequence-based amplification assay. *Applied and Environmental Microbiology*, 72, 5349–5358.

SAIR, A., D’SOUZA, D., MOE, C. and JAYKUS, L. 2002. Improved detection of human enteric viruses in foods by RT-PCR. *Journal of Virological Methods*, 100, 57–69.

SANCHEZ, G., POPULAIRE, S., BUTOT, S., PUTALLAZ, T. and JOOSTEN, H. 2006. Detection and differentiation of human hepatitis A strains by commercial quantitative real-time RT-PCR tests. *Journal of Virological Methods*, 132, 160–165.

SANCHEZ, G., BOSCH, A. and PINTO, R. M. 2007. Hepatitis A virus detection in food: Current and future prospects. *Letters in Applied Microbiology*, 45, 1–5.

SEYMOUR, I. and APPLETON, H. 2001. Foodborne viruses and fresh produce. *Journal of Applied Microbiology*, 91, 759–773.

SHAN, X., WOLFFS, P. and GRIFFITHS, M. 2005. Rapid and quantitative detection of hepatitis A virus from green onion and strawberry rinses by use of real-time reverse transcription-PCR. *Applied and Environmental Microbiology*, 71, 5624–5626.

SHANMUKH, S., JONES, L., DRISKELL, J., ZHAO, Y., DLUHY, R. *et al.* 2006. Rapid and sensitive detection of respiratory virus molecular signatures using a silver nanorod array SERS substrate. *Nano Letters*, 6, 2630–2636.

SHANMUKH, S., JONES, L., ZHAO, Y., DRISKELL, J., TRIPP, R. *et al.* 2008. Identification and classification of respiratory syncytial virus (RSV) strains by surface-enhanced Raman spectroscopy and multivariate statistical techniques. *Analytical and Bioanalytical Chemistry*, 390, 1551–1555.

STALS, A., BAERT, L., BOTTELDOORN, N., WERBROUCK, H., HERMAN, L. *et al.* 2009. Multiplex real-time RT-PCR for simultaneous detection of GI/GII noroviruses and murine norovirus 1. *Journal of Virological Methods*, 161, 247–253.

STRINGER, R., SCHOMMER, S., HOEHN, D. and GRANT, S. 2008. Development of an optical biosensor using gold nanoparticles and quantum dots for the detection of porcine reproductive and respiratory syndrome virus. *Sensors and Actuators B – Chemical*, 134, 427–431.

TAM, P., HIEU, V., CHIEN, N., LE, A. and TUAN, M. 2009. DNA sensor development based on multi-wall carbon nanotubes for label-free influenza virus (type A) detection. *Journal of Immunological Methods*, 350, 118–124.

TIMURDOGAN, E., ALACA, B., KAVAKLI, I. and UREY, H. 2011. MEMS biosensor for detection of hepatitis A and C viruses in serum. *Biosensors and Bioelectronics*, 28, 189–194.

TRIPP, R., DLUHY, R. and ZHAO, Y. 2008. Novel nanostructures for SERS biosensing. *Nano Today*, 3, 31–37.
VALDIVIA-GRANDA, W., KEATING, C., KANN, M., BERESFORD, R., KELLEY, S. et al. 2005. Detection of encephalic and hemorrhagic viruses: Integration of micro- and nano-fabrication with computational tools. *2005 International Conference on MEMS, NANO and Smart Systems, Proceedings*, 411–417.

VASICKOVA, P., DVORSKA, L., LORENCOVA, A. and PAVLIK, I. 2005. Viruses as a cause of foodborne diseases: A review of the literature. *Veterinarni Medicina*, 50, 89–104.

VERHOEF, L., BOXMAN, I. L. and KOOPMANS, M. 2008. Viruses transmitted through the food chain: A review of the latest developments. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 78, 3–15.

VINJE, J., HAMILDIJA, R. A. and SOBSEY, M.D. 2004. Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. *Journal of Virological Methods*, 116, 109–117.

WANG, G., XIE, P., XIAO, C., YUAN, P. and SU, X. 2010. Magnetic fluorescent composite nanoparticles for the fluoroimmunoassays of Newcastle disease virus and avian virus arthritis virus. *Journal of Fluorescence*, 20, 499–506.

WANG, C., LIEN, K., HUNG, L., LEI, H. and LEE, G. 2011. An integrated microfluidic system for diagnosis and multiple subtyping of influenza virus. *2011 IEEE 24th International Conference on Micro Electro Mechanical Systems (MEMS)*, 841–844.

WHO 2000. Hepatitis A. *WHO Recommended Surveillance Standards*, 55–59.

WIDDOWSON, M., SULKA, A., BULENS, S., BEARD, R., CHAVES, S. et al. 2005. Norovirus and foodborne disease, United States, 1991–2000. *Emerging Infectious Diseases*, 11, 95–102.

WIDEN, F., VAGSHOLM, I., BELAK, S. and MURADRASOLI, S. 2011. Achievement V – Methods for breaking the transmission of pathogens along the food chain: Detection of viruses in food. *Trends in Food Science and Technology*, 22, S49–S57.

ZHANG, G., ZHANG, L., HUANG, M., LUO, Z., TAY, G. et al. 2010. Silicon nanowire biosensor for highly sensitive and rapid detection of dengue virus. *Sensors and Actuators B – Chemical*, 146, 138–144.

ZHANG, H., HARPSHER, M., PARK, H. and JOHNSON, P. 2011a. Surface-enhanced Raman scattering detection of DNA derived from the West Nile virus genome using magnetic capture of Raman-active gold nanoparticles. *Analytical Chemistry*, 83, 254–260.

ZHANG, H., LIU, L., LI, C., FU, H., CHEN, Y. et al. 2011b. Multi-enzyme-nanoparticles amplification for sensitive virus genotyping in microfluidic microbeads array using Au nanoparticle probes and quantum dots as labels. *Biosensors and Bioelectronics*, 29, 89–96.

ZHENG, G., PATOLSKY, F., LIEBER, C., SEAL, S., BARATON, M. et al. 2005. Multiplexed electrical detection of single viruses. *Semiconductor Materials for Sensing*, 828, 79–84.

ZHU, X., AI, S., CHEN, Q., YIN, H. and XU, J. 2009. Label-free electrochemical detection of avian influenza virus genotype utilizing multi-walled carbon nanotubes-cobalt phthalocyanine-PAMAM nanocomposite modified glassy carbon electrode. *Electrochemistry Communications*, 11, 1543–1546.