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Chapter 3

Nucleic Acid Detection of Major Foodborne Viral Pathogens: Human Noroviruses and Hepatitis A Virus

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Abstract

Human noroviruses (hNoVs) and hepatitis A virus (HAV) are the leading cause of foodborne viral illness, and they exact a considerable economic and health toll worldwide. The detection of viral contamination in foods requires highly sensitive and accurate methods, due to the intrinsically low amounts of viruses and the complexity of the sample matrices. In recent years, a wide variety of nucleic acid-based molecular methods have been developed for the detection of hNoVs and HAV, displaying superior sensitivity, specificity, and speed. This chapter aims to provide a summary of the application of the molecular methods for the detection of the two important foodborne viruses.

Keywords: Human noroviruses, hepatitis A virus, foodborne viruses, nucleic acid-based method, detection

1. Introduction

Enteric viruses have been increasingly recognized as the leading causative pathogens in foodborne disease outbreak, causing 66.6% of foodborne illnesses in the United States, compared with 14.2% and 9.7% for *campylobacter* and *salmonella*, respectively [1]. A multitude of foodborne viral pathogens include (but are not limited to) human noroviruses (hNoVs), rotavirus, hepatitis A virus (HAV), hepatitis E virus, astrovirus, aichivirus, sapovirus, parvovirus, enterovirus, and adenovirus [2]. Foodborne viruses are transmitted not only through contaminated food and water, but also in combination with close contact with infected individuals, aerosol contamination of projectile vomit, or through contamination of environmental surfaces. Potentially fecal-contaminated food, such as bivalve molluscan shellfish harvested in polluted water areas, fresh produce irrigated with contaminated water or harvested by an infected worker, and ready-to-eat foods prepared by an infected food handler

are a means of infection [3—5]. Of all the foodborne viruses, hNoVs and HAV are the most important foodborne viral pathogens with regard to the severity of the associated illnesses and frequent occurrence worldwide [2]. Both hNoVs and HAV display high environmental stability on contaminated objects, are abundantly excreted in human feces (e.g., exceeding 10^7 viral particles per gram of stool), and have a low infectious dose (1 to 100 infectious viral particles) [2], all of which contribute to the ease of transmission of the viruses within a community. It is commonly noted that one of the most efficient ways to prevent and control the foodborne viral infections is to implement a reliable surveillance system using rapid, sensitive, and precise diagnostics to identify the associated pathogens. Human NoVs do not replicate in cell culture. Wild-type HAV strains are not readily cultivated *in vitro* and the detection is impaired by their slow and inefficient growth in cell culture and lack of apparent cytopathic effect. Cell culture-based systems for determining virus infectivity are currently not available for hNoVs and wild-type HAV. Traditional diagnosis of these foodborne viral pathogens has been reliant on electron microscopy and immunological tests, but these methods lack sufficient sensitivity. While they may be useful for the detection of the viruses in clinical specimens that contain high amounts of viruses, for foods, which harbor potentially small quantities of viruses and may yet cause illness, it is not feasible to use these traditional laboratory methods to detect the viruses. This has led to the development of new, more sensitive and robust detection methods. In recent years, the majority of newly developed detection approaches are nucleic acid-oriented. Nucleic acid-based molecular methods have demonstrated a large improvement in speed, sensitivity, and accuracy of the detection of hNoV and HAV, bringing new insights into the etiology and diagnosis of foodborne viral disease. This chapter will touch upon a number of nucleic acid-based methods that have been developed and applied to detect the two epidemiologically important foodborne viruses.

2. Key notes from norovirus and hepatitis A virus

2.1. Norovirus genome and molecular diversity

Although "winter-vomiting disease" was described in 1929 [6], the responsible viral agent was not discovered until 1972 by Kapikian [7] from fecal materials derived from an outbreak of gastroenteritis among elementary school children in 1968 in Norwalk, Ohio. The virus was named Norwalk virus and was designated as the prototype strain for the group of viruses now called Noroviruses; in the literature, they were previously referred to as small round-structured viruses (SRSVs) by their surface morphology or Norwalk-like viruses. The Norwalk agent was the first enteric virus identified that specifically caused acute gastroenteritis in humans. Successful cloning and sequencing of the NoV genomes have led to progress in understanding viral genome organization and classification. Noroviruses are a member of the family *Caliciviridae* whose name is derived from the Greek word *Calyx* for cup [8]. The viral genome is composed of a single strand of polyadenylated positive-sense, non-enveloped RNA size of approximately 7.6 kb. The linear RNA is organized into three open reading frames (ORFs), designated ORF1, ORF2, and ORF3. ORF1 encodes a 194-kDa protein that is cleaved by the viral cysteine protease into six non-structural proteins including p48, NTPase, p22, Vpg,

protease, and RNA-dependent RNA polymerase. ORF2 codes for the major capsid protein VP1, which folds in two major domains: a shell (S) and a protruding (P) domain. The P domain is comprised of P1 and P2 subdomains. P2 is a highly variable region that is thought to be involved in the binding of the histo-blood group antigens (HBGAs), which are regarded as receptors and host-susceptibility factors for human infection [8, 9].

According to the literature, NoVs have been genetically segregated into at least five genogroups (GI, GII, GIII, GIV, and GV) based on the complete amino acid sequences of the major capsid protein of 164 NoV strains [10]. New genogroups consisting of canine NoVs have also been proposed [11-12]. GI, GII, and GIV are known to cause gastroenteritis in humans. GI and GII contain the majority of human strains, with GV viruses regarded as uncommon human pathogens. GIII viruses were identified in cow, while GV viruses infect mice. Due to their enormous genetic diversity, the viruses within genogroups can be further classified into genetic clusters or genotypes that are defined as containing 14—44% VP1 amino acid sequence difference, where strains have 0—14% difference [10]. Accordingly, there are currently nine recognized genogroup I clusters and 22 genogroup II clusters. Despite their great molecular diversity, Genogroup II, genotype 4 (GII.4) variants have been responsible for the majority of outbreaks and cases in recent years, particularly those associated with person-to-person transmission [13—14]. The genotype GII.4 was first identified to predominate in outbreaks of gastroenteritis in the mid-1990s in countries on five continents [15], and new emerging variants have continued to evolve since then and have become the etiological agents for each of the four global gastroenteritis epidemics [16]. Although the majority of reported NoV outbreaks and cases are derived from person-to-person transmission, it is estimated that approximately14% of them are attributed to food, and 37% of the foodborne outbreaks are caused by mixtures of GII.4 and other genotypes, 10% by all genotype GII.4, and 27% by all other single genotypes [17].

2.2. HAV genome and genotypes

HAV, first identified in 1973 by electron microscopy, is the most common cause of infectious hepatitis with annually causing about 1.4 million clinical cases and 200 million asymptomatic carriers worldwide [18]. HAV is one of the most frequent causes of foodborne viral infection. In the United States, it is estimated that approximately 270,000 people become annually infected with hepatitis A, and most of the infection cases are not reported to health authorities [19]. Epidemics associated with contaminated food or water can occur involving hundreds of thousands of people, such as the epidemic in Shanghai, China in 1988 affected almost 300,000 people due to the consumption of HAV-contaminated clams [20]. Like other enteric viruses, HAV is resilient to environmental stressors. The virus is able to retain infectivity in acidic environments below pH3, and after refrigeration and freezing. HAV is a non-enveloped positive single-stranded RNA virus with a genome of approximately 7.5 kb in length. The virus is classified within the genus *Hepatovirus* of the family of the *Picornaviridae* [21]. The viral genome consists of (i) a 5′-untranslated region (5′-UTR) of about 735 nucleotides; (ii) a single open reading frame (ORF) that is organized into three functional regions termed P1, P2, and P3; (iii) 3′-untranslated region (3′-UTR) with a polyadenylated A tail [22]. The P region encodes

the viral capsid polypeptides VP1—VP4, and the P2 and P3 regions encode the non-structural protein. The 5′-UTR is the most conserved region of the genome and therefore is favored for primer design in polymerase chain reaction (PCR) to detect most genotypes. HAV displays a high level of antigenic conservation throughout the viral genome. An immunological study identified the existence of a single human serotype of HAV [23], but ample genetic diversity still exists to classify HAV into six genotypes based upon differences of a 186-bp nucleotide sequence in the VP1—P2A junction region [24—26]. Genotype I, II, and III are associated with human infection, while genotype IV, V, and VI are found in simians [27]. A genotype VII, designated SLF88, was proposed in an earlier study [24], but further analysis of the complete genome and capsid region of additional strains indicated that the genotype VII should be reclassified as genotype IIB [25, 26]. Genotype I and III can be each further divided into subgenotypes IA, IB, IIIA, and IIIB. Genotype I that comprises 80% human HAV strains studied is remarkably prevalent around the globe; subgenotype IA is more common than IB [24]. Since genotype I predominates worldwide, genotyping alone is rarely used to determine the source of a chain of HAV transmissions or outbreaks. Genotype III includes most of the remaining human HAV strains. Genotype II contains two subgenotypes: IIA and IIB.

3. Nucleic acid-based detection

3.1. Direct nucleic acid probe hybridization

Direct nucleic acid probe hybridization was the first molecular technique developed for the detection of suite of enteric viral pathogens. This technique can be used in diagnostics in several major formats: solid-phase, solution or liquid-phase, and *in situ* hybridization. In hybridization assays, oligonucleotide probes (single-stranded RNA or cDNA) that are complementary to the target genomic sequence of interest were labeled with signal reporters, which include radioactive molecules, chemiluminescence, or fluorescent agents. After hybridization, the probe signal from the reporter can be visualized via radioactivity, fluorescence, or color development. Detection of the probe signal indicates the presences of nucleotide sequences of interest that have high sequence similarities to the probe. HAV has been detected using these techniques such as dot blot hybridization [28—30] and *in situ* hybridization [31]. Dot blot hybridization assays were also used for detection of Norwalk viruses in 55 stool specimens from human volunteers with 27 samples tested positive [32]. A potential advantage of the direct hybridization technique is the low cost of the assay and decreased risk of cross-contamination [33]. However, the disadvantage is that the detection sensitivity is often low (approximately 10^4 virus particles) [28], thus limiting its practical application in detecting low numbers of viruses in clinical specimens, food, and environmental samples.

3.2. Nucleic acid amplification

Despite a number of reports describing the use of the direct probe hybridization technique, new molecular detection methods that incorporate the amplification of target nucleic acids are

now being developed and predominantly used for the detection of foodborne viral pathogens in samples of different origins. Nucleic acid amplification offers an edge over direct probe hybridization by enhancing the detection sensitivity through amplifying target nucleic acids extracted from samples.

3.2.1. Reverse transcription-PCR

Since the first demonstration of the PCR process in 1985[34], this technology has been widely used for the detection of foodborne pathogens. As RNA cannot be directly used as a template for PCR amplification, reverse transcription-PCR (RT-PCR) is employed for the amplification of viral RNA. RT-PCR can generally be carried out either in a one-step or two-step format. One-step RT-PCR combines the first-strand cDNA synthesis reaction (reverse transcription) and PCR amplification in a single tube, minimizing reaction setup and risk of carryover contamination. Alternatively, the two-step RT-PCR starts with the reverse transcription of either total RNA or poly (A) RNA into cDNA using a combination of sequence-specific primers, oligo (dT) or random primers in the presence of reverse transcriptase. The resulting cDNA then serves as a template for the initiation of PCR amplification in a separate tube. Separation of reverse transcription and PCR processes allows greater flexibility when choosing primers and polymerase than the one-step RT-PCR system, which allows for the use of sequence-specific primers only.

The first RT-PCR assays for detecting NoVs were described within two years of the successful cloning and sequencing of the Norwalk virus genome in the early 1990s [32, 35]. Application of this technology has allowed the detection of NoVs from samples of different origins and has generated a great deal of sequence information on various NoV strains. The sensitivity and specificity of RT-PCR assays are strongly associated with primer design. RT-PCR tests to detect NoVs are challenged by the high molecular diversity of the viruses since new variant strains continue to evolve incessantly [36]. It is difficult to select a single oligonucleotide primer set with sufficient sensitivity and specificity to detect all the NoV strains [33]. Different primer sets targeting multiple regions of the viral genome have been designed and evaluated in RT-PCR assays. A highly conserved RNA-dependent RNA polymerase region has been favored for primer design and amplification ([37—39]. Other regions such as capsid region, 2C helicase and ORF3 regions have also been targeted for amplification [40—43]. In addition, RT-PCR assays using different primer combinations in nested (two primers) or semi-nested (one primer) format have been performed to increase the likelihood of NoV detection [44, 45]. It has been reported that higher detection sensitivity (10 to 1,000 times more sensitive than single round RT-PCR) has been achieved by implementation of this strategy [44].

HAV was one of the first enteric viruses for which RT-PCR assays have been developed [46]. In contrast to NoVs, many human HAV strains across different genotypes can be amplified using a single pair of primers targeting genes coded for structural proteins, e.g., VP1—2A and VP3—VP1 junction regions [47, 48]. 5′-untranslated region (5'UTR) primers were also used in RT-PCR assays to detect HAV from clinical and environmental samples [48].

After amplifying a target of interest, post-amplification analysis is necessary to interpret the results. The simplest method is to run the amplified products on ethidium bromide- or SYBR Green dye-stained agarose gels. A band that is of the expected size to that of the positive control and/or molecular weight markers is considered a positive result. However, this method does not provide additional reassurance as to the specificity of the amplification. Analysis of the RT-PCR products by restriction fragment length polymorphism (RFLP) could discriminate genetic variants of HAV of different origins; this resolution has been enhanced by combining with the results of single-strand confirmation polymorphism (SSCP) analysis [49]. PCR-SSCP has been considered as a rapid and cost-effective approach to examining genetic diversities among hNoV strains [50] as well as HAV [51—53]. Hybridization assays such as dot blot and Southern blot have been used to identify and confirm NoV- and HAV-specific amplicons [32, 54—57]. The reverse line blot hybridization method was utilized to detect NoV RT-PCR products and to genotype the virus strains [58]. DNA microarray technology has been used to analyze amplified products for detecting and genotyping hNOVs and HAV discussed in the section below. Direct sequencing of amplified products provides detailed molecular information not only for confirming the specificity of the amplicons but also for classifying or subtyping virus strains [59].

3.2.2. Quantitative real time RT-PCR (RT-qPCR)

A RT-qPCR system combines amplification of target nucleic acids with amplicon detection in the same reaction tube, eliminating the necessity of further post-amplification analysis. RT-qPCR is currently the method of choice for the detection of hNoVs and HAV in many molecular diagnostic laboratories. This method has become the gold standard for quantification of viral load based on a reference standard curve. Two principle approaches are commonly utilized for the detection of enteric viruses in RT-qPCR assays: DNA-binding fluorogenic dyes and sequence-specific oligonucleotide probes. Selected application of these approaches for detection of NoV and HAV is outlined in Tables 1 and 2.

SYBR Green is commonly used as a dye for the quantification of double-stranded DNA in qPCR methodology. The binding of SYBR Green is non-specific; it binds indiscriminately to any double-stranded DNA including non-specific amplification and primer-dimers. To distinguish virus-specific amplified products from non-specific primer-dimers, a melting curve analysis is generally used as the virus-specific products have a higher dissociation temperature. A number of SYBR Green-based RT-qPCR assays have been developed for the detection of hNoVs by targeting different genomic regions such as the capsid region [60, 61] and RNA polymerase [61—64]. SYBR Green-based RT-qPCR employing 5′—UTR region primers was used to detect as low as 5 Tissue Culture Infectious Dose 50% ($TCID_{50}$) per gram in seeded oyster samples [65]. Using SYBR Green RT-qPCR with VP3—VP1 junction primers, HAV could be detected in all eight tested ocean water samples with viral loads varying from 90 to 3523 HAV copies/L near the mouth of Tijuana River, and 347 to 2656 copies/L near the Imperial Beach pier in San Diego [66].

Approach	Genogroup	Target Region	Lmit of Detection	Detection/ Quantitation	Sample tested	References
SYBR Green	Not reported	RNA polymerase	Not reported	D	Stool	[62]
	GI	Capsid	1 RT-PCR unit	Q	Stool	[60]
	Not reported	RNA polymerase	Up to 5 logs	D	Stool	[63]
	GI/GII	Capsid in GI/ RNA polymerase in GII	25,000 RNA copies/gram	D	Stool	[61]
	Not reported	RNA polymerase	6 log GII cDNA copies/100ml	Q	Enviromental water	[64]
TaqMan probe	GI/GII	ORF1-ORF2 junction	2.0x10∧4RNA	Q	Stool	[67]
	GI/GII/GIV	ORF1-ORF2 junction	<10GII, <100GI RNA copies	Q	Stool/water	[70]
	GI/GII	Capsid	50 GII copies, 500 GI copies	Q	Stool	[68]
	GI/GII/GIV	ORF1-ORF2 junction	16.9GI, 6.3GII, 43GIV copies	Q	Stool, vomitus, anal swab	[73]
	GI/GII	ORF1-ORF2 junction	23GI, 33GII copies	D	Shellfish & springwater	[83]
	GI/GII	Capsid	3~7 RT-PCR unit	Q	Strawberry	[72]
Hybridization probe	GI/GII	ORF1-ORF2	50 copies	D	Stool	[79]
	GI/GII	ORF1-ORF2	1~10GI, 1~100GII RNA copies	D	Stool	[78]

Table 1. Selected RT-qPCR assays for detection of human noroviruses

There are two major groups of sequence-specific oligonucleotide probes: hydrolysis (e.g., TaqMan) probes and hybridization probes (e.g., molecular beacons and fluorescence resonance energy transfer probes); both groups are homologous to the internal region of amplified products. TaqMan probes have been frequently used in RT-qPCR for detecting hNOVs [67—73]. While RNA polymerase and capsid genes are the primary targets for amplification, within the NoV genomes, a junction of ORF1—ORF2—polymerase—capsid has been demonstrated to be the most highly conserved region that can serve as an effective target for amplification.

Approach	Genotype	Target Region	Lmit of Detection	Detection/ Quantitation	Sample tested	References
SYBR Green	Not reported	VP3-VP1	2-4 log copies/l	D	Ocean water	[66]
	Not reported	5′-UTR	5 $TCID_{50}$/g	Q	Oyster	[65]
TaqMan probe	IA, IB, IIA, IIIA	5′-UTR, VP1-2A, VP3, VP1, VP2-VP3	50 copies for IIB, 500 for IA, IB, IIA and IIIB	Q	Stool, serum	[77]
	IA, IB	5′-UTR	10 copies or 0.05	Q	Shellfish, stool	[75]
	IA, IB, IIA, IIB, IIIA	5′-UTR	40 copies or 0.5	D	Cell culture	[74]
	IB	5′-UTR	491 copies	Q	Shellfish, springwater	[83]
	IB	5′-UTR	0.2 PFU, 63PFU	D	Cell culture, green onion	[76]
Molecular beacons	IB	5′-UTR	20 PFU	D	Seeded groundwater	[80]

Table 2. Selected RT-qPCR assays of detection of hepatitis A virus

Kageyama and colleagues were the first to describe the junction-targeting RT-qPCR assay [67]. Their studies showed a better detection rate in 80 of 81 (99%) stool samples positive by electron microscopy, compared to conventional RT-PCR assays that detected 77% when targeting the polymerase and 83% when targeting the capsid N/S regions, respectively, in the same panel of stool specimens [67]. In an effort to design assays capable of detecting all genogroups of these highly diverse viruses, this ORF1—ORF2 junction region has become the most widely used for primer and probe design in RT-qPCR tests. A TaqMan RT-qPCR assay using a probe for the 5′-UTR of HAV genome was able to detect 40 copies of RNA transcripts and 0.5 infectious units (IU) in cell culture strains and clinical fecal specimens, respectively [74]. Constafreda et al. (2006) also developed a TaqMan RT-qPCR method targeting 5′-UTR for quantitative detection of HAV from clinical specimens (stool and serum) and shellfish samples, and the detection limit was 0.05 IU or 10 copies of single-stranded RNA transcripts [75]. Nested RT-PCR assays combining conventional PCR, nested PCR and qPCR have been used to detect as low as 0.02 plaque forming units (PFU) of HAV from cell culture and 63 PFU from green onions [76]. Six subtype-specific RT-qPCR assays using hydrolysis probes were developed for HAV detection and subtyping [77], with limit of detection at 50 genome copies/assay for subtype IIB, 500 genome copies/assay for IA, IB, IIA, and IIIB, and 5,000 genome copies/assay for IIIA. Thirty-five clinical stool and serum specimens were tested with this method. Only a single discrepant result was observed for a serum specimen provided as IB subtype by VP1/2A region sequencing and identified as IA by the subtype-specific RT-qPCR assays.

Hybridization probes including molecular beacons and fluorescence resonance energy transfer (FRET) probes have been used in RT-qPCR assays for detecting NoVs [78, 79]. Abd El Galil et al. showed that a molecular beacon RT-qPCR assay targeting the HAV highly conserved 5′-UTR region could detect as lower as 20 PFU HAV in seeded groundwater samples in combination with immunomagnetic separation [80]. Molecular beacon probes have also been used in another target amplification method: nucleic acid sequence-based amplification described below.

3.2.3. Multiplex PCR

Multiplex PCR-based methods are designed to simultaneously amplify more than one target nucleic acids using different sets of primers in a single reaction. Primers of different targets should not be complementary to each target and can work efficiently at the same annealing temperature during PCR.

A multiplex RT-PCR method was developed for simultaneous detection of HAV, norovirus, and poliovirus type (PV1) using three different sets of primers with detection limits of ≤ 1 infectious unit (HAV and PV1) and 1 RT-PCR unit (NoV) [81]. Noroviruses (GI and GII) and HAV from different food matrices such as lettuce, strawberry, green onions, and bivalve mollusks were detected using a multiplex RT-qPCR assay [82, 83]. A multiplex RT-qPCR for simultaneous detection and quantification of GI, GII, and GIV noroviruses was recently reported [84]. Several multiplex nucleic acid diagnostic platforms for simultaneous detection of a range of pathogenic enteric pathogens including hNoVs are now commercially available [85]. Although multiplex PCR allows rapid and cost-effective detection of several targets (viruses) in a single reaction, different targets may compete with each other for resources such that a highly abundant target would get more chances to be amplified, thus preventing less abundant ones from getting detected. In addition, multiplex PCR tend to have decreased sensitivity as compared to standard single PCR.

3.2.4. Digital PCR

Digital PCR has recently been described as a novel approach to nucleic acid detection and quantification. It is a different method of absolute quantification relative to conventional quantitative PCR, because it directly counts the number of target nucleic acid molecules rather than relying on reference standards. This improvement is achieved by partitioning sample amplification reactions into tens to thousands of picoliter-scale compartments on microfluidic chips or microdroplets so that each mini-reaction contains zero or one copy of the target nucleic acid molecule. A comparative study of digital RT-PCR (RT-dPCR) and RT-qPCR for the detection and quantification of HAV and NoVs in lettuce and water samples was recently reported [86]. This RT-dPCR assay showed that the sensitivity was either comparable or slightly (around 1 $\log_{10}$) decreased to that of RT-qPCR for detecting viral RNA and cDNA of HAV and NoV, but the viral recoveries were found to be significantly higher than that of RT-qPCR for NoV GI and HAV in water, and for NoV GII and HAV in lettuce. In addition, this RT-dPCR was more tolerant to inhibitory substances present in lettuce.

3.2.5. Nucleic Acid Sequence-Based Amplification (NASBA)

Nucleic acid sequence-based amplification is an isothermal target amplification process for amplifying RNA. A NASBA reaction consists of reverse transcriptase, T7 RNA polymerase, and RNase-H with two target sequence-specific oligonucleotide primers. A NoV NASBA assay showed equivalent analytical sensitivities with RT-PCR using the NoV GII primer sets described by Kageyama et al [67] but provided less consistent signals [87]. A molecular beacon real-time NASBA method was developed to detect NoV GII from environmental samples, with 88% sensitivity compared to conventional RT-PCR [88]. This NASBA technology has been applied to detect as little as 0.4 ng of HAV RNA/ml using primers targeting the VP1 and VP2 capsid genes [89]. A multiplex NASBA and microtiter plate hybridization system was developed to simultaneously detect HAV and rotavirus where 400 PFU/ml HAV were detected with reduced time and cost compared to monoplex system [90]. Using established primer pairs, multiplex NASBA assays were developed for simultaneous detection of HAV and NoV GI and GII in spiked ready-to-eat foods. All three viruses were simultaneously detected at initial inoculum levels of 10(0) to 10(2) RT-PCR units [91].

3.2.6. Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP is a one-step non-PCR nucleic acid amplification that is performed at a constant temperature between 60 and 65°C. Unlike NASBA, it requires only two enzymes instead of three, namely, reverse transcriptase and DNA polymerase. A genogroup-specific RT-LAMP assay has been developed using 9 and 13 specially designed primers containing mixed bases for genogroup I (GI) and II (GII), respectively, and showed the limits of detection between 10^2 and 10^3 copies/tube for GI and GII. Compared to conventional RT-PCR, the clinical sensitivity and specificity of the RT-LAMP were 100% and 94% for GI, and 100% and 100% for GII, respectively [92]. Commercial loopamp NoV GI and GII detection kits were evaluated using 510 clinical fecal specimens; the sensitivity of GI (83.3%) was less than that of GII (97.4%) with regard to genogroup-specific RT-qPCR [93]. A single tube, real-time HAV RT-LAMP assay using seven primer sets was applied to identify three different subgenotypes of HVA (IA, IB, and IIIB) with detection limits of 0.4—0.8 focus forming units *per* reaction [94].

3.3. Nucleic acid amplification coupled with DNA microarray detection

DNA microarray technology consists of numerous individual target-probe hybridization reactions that are performed in a single assay. The intrinsic ability of this technology to simultaneously analyze thousands of specific DNA sequences presents a significant advantage in parallel identification of a broad spectrum of microbial pathogens. Additionally, a DNA microarray composed of well-designed probes has the potential to discover novel viruses or pathogens not well-represented in the current sequence database. Not surprisingly, this technology, coupled with virus-specific monoplex or multiplex RT- PCR amplification, has enabled sensitive detection and identification of a number of enteric viruses including hNoVs and HAV from clinical specimens, environmental samples, and virus-infected cell cultures [95—100]. However, unbiased amplification with virus-specific PCR is often complicated by the existence of enormous genetic diversities in foodborne viruses. It would be advantageous to develop amplification approaches that do not rely on specific pathogen sequence information,

yet can produce sufficient target nucleic acids from minute amounts of starting materials of viral, bacterial, plant, and animal origins for microarray analysis [101]. Recently, a sequence-independent isothermal RNA amplification approach has been developed to amplify various enteric viral nucleic acids of hNoVs, HAV, and coxsackievirus for microarray analysis [102]. Utility of this microarray platform and amplification strategy allows not only discerning genotypic information on hNoVs but also detection of mixed viral agents (hNoVs and HAV) present in the same fecal specimen [103]. Microarray detection of random-primed PCR products from a range of gastrointestinal viruses including Norwalk virus was reported recently [104].

3.4. Detection of infectious viruses

As mentioned above, there are no efficient cell culture systems available for hNoV propagation, and wild-type HAV strains are difficult to grow *in vitro*. In absence of effective culture-based infectivity assays, development of rapid and sensitive molecular methods for reliably detecting infectious vial particles to determine virus infectivity is a key issue for the application of food risk management. Integrity of the virus capsid and its genome are essential for virus infectivity; both have been targeted for the development of methods for predicting virus infectivity. Nuanualsuwan and Cliver (2002) described a method in their effort to correlate RT-PCR data with virus infectivity [105]. In their study, HAV, vaccine poliovirus, and feline calicivirus were inactivated by ultraviolet light, hypochlorite, or heating at 72°C. They observed that the inactivated viruses, which were treated with proteinase K and ribonuclease before RT-PCR, did not yield positive amplicons [106]. Integrated RT-qPCR approaches have been used to discriminate the infectivity status of NoVs based on the assumption that infectious virus particles would more efficiently bind to the appropriate receptors than non-infectious viruses [106, 107]. Long-range RT-qPCR has been used to test the integrity of the NoV genome following 72°C heat treatment [108]. Recent studies on the use of nucleic acid intercalating dyes such as ethidium monoazide (EMA) and propidium monoazide (PMA) in conjunction with RT-PCR or RT-qPCR to distinguish between infectious and non-infectious enteric viruses including hNoVs and HAV have been reported [109—111]. However, PMA RT-PCR could not differentiate infectious Norwalk virus from non-infectious Norwalk virus, although it was able to differentiate selectively between infectious and noninfectious murine NoV, coming to the conclusion that PMA RT-PCR can be used to detect intact, potentially infectious viruses only under specified conditions [108, 110]. A real-time NASBA combined with enzymatic treatment of proteinase K and RNase has been developed to discriminate the infectious from the heat-inactivated hNoVs [112].

4. Concluding remarks

Over the past few years, nucleic acid-based molecular methods have been developed, refined, and used for the detection of hNoVs and HAV that are most commonly associated with the transmission of foodborne viral disease. In particular, RT-qPCR has emerged as a preferred method for the detection and quantification of the viruses due to its high sensitivity, reproducibility, speed, and minimization of risk of carry-over contamination. Recent advances in

high-throughput next-generation sequencing (NGS) technologies have opened new avenues for genomic research and diagnostic applications. It is expected that utilization of NGS in viral metagenomics and whole genome sequencing will highly improve the opportunities for identifying viruses of different origins including those that are too divergent to be detected by PCR or other molecular approaches. However, the identification of hNoVs and HAV in vast ranges of food matrices is still a demanding task that is largely attributed to some factors such as intrinsically low quantities of contaminated viruses and broad chemical composition of food, which may inhibit the activity of enzymes used in the molecular detection. This requires a meticulous investigation into sample preparation procedures to obtain acceptable recovery of viral RNA for downstream analysis. Nucleic acid-based molecular methods have a disadvantage in that the majority of the reported assays for detection of foodborne virus contamination do not have standardized protocols, and can vary from laboratory to laboratory. This could be due to several reasons, such as different approaches required for preparation of viral nucleic acids from different test matrices, and a lack of multi-laboratory validation of promising procedures. There is a need for harmonized standards and quality control of the reagents used. Moreover, in many cases, molecular methodology has focused on merely detecting the presence of viral nucleic acids that is not necessarily associated with the detection of infectious particles, although some studies stated above have shown promising differentiating results. Research on developing new methods to accurately determine the virus infectivity is eventually needed to gauge health risk.

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References

[1] Mead PS, Slutsker L, Dietz V et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607—625.

[2] FAO/WHO [Food and Agriculture Organization of United Nations/World Health Organization]. (2007). Viruses in food: scientific advice to support risk management activities: Meeting Report.Microbiological Risk Assessment Series No. 13. Bilthoven, the Netherlands.

[3] Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. Clin Microbiol Rev. 2006;19:63—79.

[4] Montano-Remacha C, Ricotta L, Alfonsi V et al. Hepatitis A outbreak in Italy, 2013: a matched case-control study. Euro Surveill. 2014;18:19(37).

[5] Hall Aj, Eisenbart VG, Etingue Al et al. Epidemiology of foodborne norovirus outbreaks, United States, 2001-2008. Emerg Infect Dis. 2012;18:10.

[6] Zahorsky J. Hyperemesis biemis or the winter vomiting disease. Arch Pediatr. 1929;46:391—395

[7] Kapikian AZ, Wyatt RG, Dolin R, et al. Visualisation by immune electron microscopy of a 27 nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol. 1972;10:1075—1081.

[8] Glass RI, Parashar UD, and Este MK. Norovirus Gastroenteritis. N Eng J Med 2009;361:1776—1785.

[9] Huang P, Farkas T, Marionneau S, et al. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. J Infect Dis. 2003 188:19—31.

[10] Zheng DP, Ando T, Fankhauser RL et al. Norovirus classification and proposed strain nomenclature. Virology. 2006;346:312—323.

[11] Martella V, Lorusso E, Decaro N et al. Detection and molecular characterization of a canine norovirus. Emerg Infect Dis. 2008;14:1306–1308.

[12] Tse H, Lau SKP, Chan WM et al. Complete genome sequences of novel canine noroviruses in Hong Kong. J Virol. 2012,86:9531–9532.

[13] Matthews JE, Dickey BW, Miller RD et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. Epidemiol Infect. 2012;140:1161—1172.

[14] Vega E, Barclay L, Gregoricus N et al. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol. 2014;52:147—155.

[15] Noel JS, Fankhauser RL, Ando T et al. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. J Infect Dis. 1999;179:1334—1344.

[16] Siebenga JJ, Vennema H, Renchens B et al. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J of Virol. 2007; 81:9932—9941.

[17] Verhoef L, Hewitt J, Barclay L et al. Norovirus genotype profiles associated with foodborne transmission, 1999-2012. Emerg Infect Dis. 2015; 21:592—599.

[18] World Health Organization. Health Topics: Hepatitis. 2014. Geneva, Switzerland. Available from: http://www.who.int/topics/hepatitis/en/.

[19] Armstrong GL, Bell BP. Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. Pediatrics. 2002;109:839—845.

[20] Halliday ML, Kang LY, Zhou TK et al. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shangihai, China. J Infect Dis. 1991;164:852—859.

[21] Minor PD. Picornaviridae, classification and nomenclature of viruses. The Fifth Report of the International Committee on Taxonomy of Viruses. Arch Virol. 1991;Suppl. 2:320—326.

[22] Rueckert PR & Wimmer E. Systematic nomenclature of picornavirus protein. J of Virol. 1984; 50: 957—959.

[23] Lemon SM, Binn LN. Antigenic relatedness of two strains of hepatitis A virus determined by cross-neutralization. Infect Immun. 1983;42:418—420.

[24] Robertson B H, Jansen RW, Khanna B et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J Gen Virol.1992; 73:1365—1377.

[25] Costa-Mattioli M, Di Napoli A, Ferre´ V et al. Genetic variability of hepatitis A virus. J Gen Virol 2003;84:3191—3201.

[26] Lu L, Ching KZ, Salete de Paula V et al. Characterization of the complete genome sequence of genotype II hepatitis A virus (CF53/Berne isolate). J Gen Virol. 2001; 85:2943—2952.

[27] Nainan OV, Margolis HS, Robertson BH et al. Sequence analysis of a new hepatitis A virus naturally infecting cynomolgus macaques (Macaca fascicularis). J Gen Virol. 1991;72:1685—1689.

[28] Jiang X, Estes MK, Metcalf TG et al. Detection of hepatitis A virus in seeded estuarine samples by hybridization with cDNA probes. Appl Environ Microbiol. 1986; 52:711—717.

[29] Ticehurst JR, Feinstone SM, Chestnut T, Tassopoulos NC, Popper H, Purcell RH. Detection of hepatitis A virus by extractionof viral RNA and molecular hybridization. J Clin Microbiol. 1987;25:1822—1929.

[30] Jiang X, Estes MK, Metcalf TG. Detection of hepatitis A virus by hybridization with single-stranded RNA probes. Appl Environ Microbiol. 1987;53:2487—2495.

[31] Jiang X, Estes MK, Metcalf TG. In situ hybridization for quantitative assay of infectious hepatitis A virus. J Clin Microbiol. 1989;27:874—879.

[32] Jiang X, Wang J, Graham DY, Estes MK. Detection of Norwalk virus in stool by polymerase chain reaction. J Clin Microbiol. 1992;30:2529—2534.

[33] Atmar RL and Estes MK. Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. Clin Microbiol Rev. 2001;14:15—37.

[34] Saiki R, Scharf S, Faloona F et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 1985;230:1350—1354.

[35] De Leon R, Matsui SM, Baric RS et al. Detection of Norwalk virus in stool specimens by reverse transcriptase-polymerase chain reaction and nonradioactive oligoprobes. J Clin Microbiol. 1992;30:3151—3157.

[36] Boon D, Mahar JE, Abente EJ, Kirkwood CD, Purcell RH, Kapikian AZ, Green KY, Bok K. Comparative evolution of GII.3 and GII.4 norovirus over a 31-year period. J Virol. 2011; 85:8656—8666.

[37] Ando T, Monroe SS, Gentsch JR et al. Detection and differentiation of antigenically distinct small round structured viruses (Norwalk-like viruses) by reverse transcription-PCR and Southern hybridization. J Clin Microbiol. 1995;33:64–71.

[38] Green J, Gallimore CI, Norcott JP et al. Broadly reactive reverse transcriptase polymerase chain reaction inthe diagnosis of SRSV-associated gastroenteritis. J Med Virol. 1995;47:392—398.

[39] Le Guyader F, Estes MK, Hardy ME et al. Evaluation of a degenerate primer for the PCR detection of human caliciviruses. Arch Virol. 1996;141:2225—2235.

[40] Green SM, Lambden PR, Caul EO and Clarke IN. Capsid sequence diversity in small round structured viruses from recent UK outbreaks of gastroenteritis. J Med Virol. 1997;52:14—19.

[41] Matsui SM., Kim JP, Greenberg HB et al. The isolation and characterization of a Norwalk virus-specific cDNA. J Clin Investig. 1991;87:1456—1461.

[42] Wang J, Jiang X, Madore HP et al. Sequence diversity of small round structured viruses. J Virol. 1994;68:5982—5990.

[43] Moe CL, Gentsch J, Grohmann G et al. Application of PCR to detect Norwalk virus in fecal specimens from outbreaks of gastroenteritis. J Clin Microbiol. 1994;32:642—648.

[44] Green J, Henshilwood K, Gallimore CI, Brown DW, Lees DN.A nested reverse transcriptase PCR assay for detection of small round-structured viruses in environmentally contaminated molluscan shellfish. Appl Environ Microbiol. 1998;64:858—863.

[45] Stene-Johansen K, Grinde B. Sensitive detection of human Caliciviridae by RT-PCR. J Med Virol. 1996;50:207—213.

[46] Jansen RW, Siegl G, Lemon SM. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. Proc Natl Acad Sci U S A. 1990;87:2867—2871.

[47] Hutin YJF, Pool V, Cramer EH et al. A multistate foodborne outbreak of hepatitis A. N Engl J Med. 1999;340:595—602.

[48] Pina S, Buti M, Jardi R et al. Genetic analysis of hepatitis A virus strains recovered from the environment and from patients with acute hepatitis. J Gen Virol. 2001;82:2955—2963.

[49] Goswami BB, Burkhardt W 3rd, Cebula TA. Identification of genetic variants of hepatitis A virus. J Virol Methods. 1997;65:95—103.

[50] Sasaki Y, Kai A, Hayashi Y, Shinkai T et al. Multiple viral infections and genomic divergence among noroviruses during an outbreak of acute gastroenteritis. J Clin Microbiol. 2006;44:790—797.

[51] Sulbaran Y, Gutierrez CR, Marquez B et al. Hepatitis A virus genetic diversity in Venezuela: exclusive circulation of subgenotype IA and evidence of quasispecies distribution in the isolates. J Med Virol. 2010;82:1829—1834.

[52] Mackiewicz V, Roque-Afonso AM, Marchadier E etal. Rapid investigation of hepatitis A virus outbreak by single strand conformation polymorphismanalysis. J Med Virol. 2005;76:271—278.

[53] Fujiwara K, Yokosuka O, Ehata T et al. PCR-SSCP analysis of 5'-nontranslated region of hepatitis A viral RNA: comparison with clinicopathological features of hepatitis A. Dig Dis Sci. 2000;45:2422—2427.

[54] Willcocks MM, Silcock JG, Carter MJ. Detection of Norwalk virus in the UK by the polymerase chain reaction. FEMS Microbiol Lett. 1993;112:7—12.

[55] Kohn MA, Farley TA, Ando T et al. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters. Implications for maintaining safe oyster beds. JAMA 1995;273:466—471.

[56] Goswami BB, Koch WH, Cebula TA. Detection of hepatitis A virus in Mercenaria mercenaria by coupled reverse transcription and polymerase chain reaction. Appl Environ Microbiol. 1993;59:2765—2770.

[57] Nainan OV, Cromeans TL and Margolis HS. Sequence-specific, single-primer amplification and detection of PCR products for identification of hepatitis viruses. J Virol Methods.1996;61:127—134.

[58] Vinjé J & Koopmans MP. Simultaneous detection and genotyping of "Norwalk-like viruses" by oligonucleotide array in a reverse line blot hybridization format. J Clin Microbiol. 2000;38:2595—2601.

[59] Robertson BH, Khanna B, Nainan OV, Margolis HS. Epidemiologic patterns of wild-type hepatitis A virus determined by genetic variation. J Infect Dis. 1991;163:286—292.

[60] Richards GP, Watson MA, Kingsley DH. A SYBR green, real-time RT-PCR method to detect and quantitate Norwalk virus in stools. J Virol Methods. 2004;116:63–70.

[61] Pang X, Lee B, Chui L, Preiksaitis JK,Monroe SS.. Evaluation and validation of real-time reverse transcription-PCR assay using the LightCycler system for detection and quantitation of norovirus. J Clin Microbiol.2004; 42:4679–4685.

[62] Miller I, Gunson R, Carman WF. Norwalk like virus by light cycler PCR. J Clin Virol. 2002;25:231–232.

[63] Schmid M, Oehme R, Schalasta G, Brockmann S, Kimmig P; Enders G. Fast detection of noroviruses using a real-time PCR assay and automated sample preparation. BMC Infect Dis 2004;4:15.

[64] Laverick MA, Wyn-Jones AP, Carter MJ. Quantitative RT-PCR for the enumeration of noroviruses (Norwalk-like viruses) in water and sewage. Lett Appl Microbiol. 2004;39:127—136.

[65] Casas N, Amarita F, de Marañón IM. Evaluation of an extracting method for the detection of Hepatitis A virus in shellfish by SYBR-Green real-time RT-PCR. Int J Food Microbiol. 2007;120:179—185.

[66] Brooks HA, Gersberg RM, Dhar AK. Detection and quantification of hepatitis A virus in seawater via real-time RT-PCR. J Virol Methods. 2005;127:109—118.

[67] Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. Broadly reactive and highlysensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol. 2003;41:1548—1557.

[68] Hohne M, Schreier E. Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using afluorogenic real-time detection system. J Med Virol. 2004;72:312—319.

[69] Dreier J, Stormer M, Made D, Burkhardt S, Kleesiek K. Enhanced reverse transcription-PCR assay for detection of norovirus genogroup I. J Clin Microbiol. 2006; 44:2714—2720.

[70] Trujillo AA, McCaustland KA, Zheng DP, Hadley LA, Vaughn G, Adams SM, Ando T, Glass RI, Monroe SS. Use of TaqMan real-time reverse transcription-PCR for rapid

detection, quantification, and typing of norovirus. J Clin Microbiol. 2006; 44:1405—1412.

[71] Scipioni A, Bourgot I, Mauroy A, Ziant D, Saegerman C, Daube G,Thiry E. Detection and quantification of human and bovine noroviruses by a TaqMan RT-PCR assay with a control for inhibition. Mol Cell Probes. 2008;22:215—222.

[72] Park Y, Cho YH, Jee Y, Ko G. Immunomagnetic separation combined with real-time reverse transcriptase PCR assays for detection of norovirus in contaminated food. Appl Environ Microbiol. 2008;74:4226—4230.

[73] Yan Y, Wang HH, Gao L et al. A one-step multiplex real-time RT-PCR assay for rapid and simultaneous detection of human norovirus genogroup I II and IV. J Virol Methods. 2013;189: 277—282.

[74] Jothikumar N, Cromeans TL, Sobsey MD, Robertson BH. Development andevaluation of a broadly reactive TaqMan assay for rapid detection of hepatitis A virus. Appl Environ Microbiol. 2005;71,3359—3363.

[75] Costafreda MI, Bosch A, Pintó RM. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. Appl Environ Microbiol. 2006;72:3846-55.

[76] Hu Y and ArsoV I. Nested real-time PCR for hepatitis A detection. Lett Appl Microbiol. 2009 ;49:615—619.

[77] Coudray-Meunier C, Fraisse A, Mokhtari C, Martin-Latil S, Roque-Afonso AM, Perelle S. Hepatitis A virus subgenotyping based on RT-qPCR assays. BMC Microbiol. 2014;14:296.

[78] Mohamed N, Belak S, Hedlund KO, Blomberg J. Experience from the development of a diagnostic single tube real-time PCR for human caliciviruses, norovirus genogroups I and II. J Virol Methods. 2006;132:69—76.

[79] Hymas W, Atkinson A, Stevenson J, Hillyard D. Use of modified oligonucleotides to compensate for sequence polymorphisms in the real-time detection of norovirus. J Virol Methods. 2007; 142:10—14.

[80] Abd El Galil KH, El Sokkary MA, Kheira SM et al. Combined immunomagnetic separation-molecular beacon-reverse transcription-PCR assay for detection of hepatitis A virus from environmental samples. Appl Environ Microbiol. 2004;70:4371—4374.

[81] Rosenfield SI, Jaykus LA. A multiplex reverse transcription polymerase chain reaction method for the detection of foodborne viruses. J Food Prot. 1999;10:1210—1214.

[82] Morales-Rayas R, Wolffs PF, Griffiths MW. Simultaneous separation and detection of hepatitis A virus and norovirus in produce. Int J Food Microbiol. 2010;139:48—55.

[83] Fuentes C, Guix S, Perez-Rodriguez FJ et al. Standardized multiplex one-step qRT-PCR for hepatitis A virus, norovirus GI and GII quantification in bivalve mollusks and water. Food Microbiol. 2014;40:55-63.

[84] Farkas T, Singh A, Le Guyader FS et al. Multiplex real-time RT-PCR for the simultaneous detection and quantification of GI, GII and GIV noroviruses. J Virol Methods. 2015;223:109—114.

[85] Chen H and Hu Y. Molecular diagnostic methods for detection and characterization of human noroviruses. 2015 submitted.

[86] Coudray-Meunier C, Fraisse A, Martin-Latil S, Guillier L, Delannoy S, Fach P, Perelle S. A comparative study of digital RT-PCR and RT-qPCR for quantification of Hepatitis A virus and Norovirus in lettuce and water samples. Int J Food Microbiol. 2015;201:17—26.

[87] Houde A, Leblanc D, Poitras E, et al. Comparative evaluation of RT-PCR, nucleic acid sequence-based amplification (NASBA) and real-time RT-PCR for detection of noroviruses in faecal material. J Virol Methods. 2006 ;135:167—172.

[88] Patterson SS, Smith MW, Casper ET et al. A nucleic acid sequence-based amplification assay for real-time detection of norovirus genogroup II. J Appl Microbiol. 2006;101:956—963.

[89] Jean J, Blais B, Darveau A, Fliss I. Detection of hepatitis A virus by the nucleic acid sequence-based amplification technique and comparison with reverse transcription-PCR. Appl Environ Microbiol. 2001;67:5593—5600.

[90] Jean J, Blais B, Darveau A, Fliss I. Simultaneous detection and identification of hepatitis A virus and rotavirus by multiplex nucleic acid sequence-based amplification (NASBA) and microtiter plate hybridization system. J Virol Methods. 2002;105:123—132.

[91] Jean J, D'Souza DH, Jaykus LA. Multiplex nucleic acid sequence-based amplification for simultaneous detection of several enteric viruses in model ready-to-eat foods. Appl Environ Microbiol. 2004;70:6603—6610.

[92] Fukuda S, Takao S, Kuwayama M et al. Rapid detection of norovirus from fecal specimens by real-time reverse transcription-loop-mediated isothermal amplification assay. J Clin Microbiol. 2006;44:1376—1381.

[93] Iturriza-Gómara M, Xerry J, Gallimore CI et al. Evaluation of the Loopamp (loop-mediated isothermal amplification) kit for detecting NorovirusRNA in faecal samples. J Clin Virol. 2008 ;42:389—393.

[94] Yoneyama T, Kiyohara T, Shimasaki N et al. Rapid and real-time detection of hepatitis A virus by reverse transcription loop-mediated isothermal amplification assay. J Virol Methods. 2007;145:162—168.

[95] Jääskeläinen AJ, Maunula L. Applicability of microarray technique for the detection of noro- and astroviruses. J Virol Methods. 2006;136:210—216.

[96] Pagotto F, Corneau N, Mattison K, Bidawid S. Development of a DNA microarray for the simultaneous detection and genotyping of noroviruses. J Food Prot. 2008;71:1434—1441.

[97] Ayodeji M, Kulka M, Jackson SA et al. A microarray based approach for the identification of common foodborne viruses. Open Virol J. 2009;3:7—20.

[98] Chen H, Mammel M, Kulka M, Patel I, Jackson S, Goswami BB. Detection and identification of common food-borne viruses with a tiling microarray. Open Virol J. 2011;5:52—59.

[99] Brinkman NE, Fout GS. Development and evaluation of a generic tag array to detect and genotype noroviruses in water. J Virol Methods. 2009;156:8—18.

[100] Kim JM, Kim SY, Park YB, Kim HJ, Min BS, Cho JC, Yang JM, Cho YH, Ko G. Simultaneous detection of major enteric viruses using a combimatrix microarray. J Microbiol. 2012;50:970—977.

[101] Chen H and Huang S (2012). DNA microarray technology for the detection of foodborne viral pathogens. In: Yan X, Juneja VK, Fratamico PM, Smith JL (eds) Omics, microbial modeling, and technologies in foodborne pathogens. DEStech Publications, Lancaster, pp 579—602.

[102] Chen H, Chen X, Hu Y, Yan H. Reproducibility, fidelity, and discriminant validity of linear RNA amplification for microarray-based identification of major human enteric viruses. Appl Microbiol Biotechnol. 2013;97:4129—4139.

[103] Hu Y, Yan H, Mammel M and Chen H. Sequence-independent amplification coupled with DNA microarray analysis for detection and genotyping of noroviruses. AMB Express. 2015;5:69.

[104] Martínez MA, Soto-Del Río Mde L, Gutiérrez RM et al. DNA microarray for detection of gastrointestinal viruses. J Clin Microbiol. 2015;53:136—145.

[105] Nuanualsuwan S and Cliver DO. Pretreatment to avoid positive RT-PCR results with inactivated viruses. J Virol Methods. 2002;104: 217—225.

[106] Li D, Baert L, Van Coillie E, Uyttendaele M. Critical studies on binding-based RT-PCR detection of infectious Noroviruses. J Virol Methods. 2011;177:153—159.

[107] Dancho BA, Chen H, Kingsley DH. Discrimination between infectious and non-infectious human norovirus using porcine gastric mucin. Int J Food Microbiol. 2012;155:222—226.

[108] Wolf S, Rivera-Aban M, Greening GG. Long-range reverse transcription as a useful tool to assess the genomic integrity of norovirus. Food Environ Virol. 2009;1:129—136.

[109] Parshionikar SU, Cashdollar J, Fout GS. Use of propidium monoazide in reveerse transcriptase PCR to distinguish between infectious and noninfectious enteric viruses in water samples. Appl Envirom Microbiol. 2010;76:4318—4326.

[110] Sánchez G, Elizaquível P, Aznar R. Discrimination of infectious hepatitis A viruses by propidium monoazide real-time RT-PCR. Food Environ Virol. 2012;4:21—25.

[111] Karim MR, Fout GS, Johnson CH, White KM, Parshionikar SU. Propidium monoazide reverse transcriptase PCR and RT-qPCR for detecting infectious enterovirus and norovirus. J Virol Methods. 2015;219:51—61.

[112] Lamhoujeb S, Fliss I, Ngazoa SE, Jean J. Evaluation of the persistence of infectious human noroviruses on food surfaces by using real-time nucleic acid sequence-based amplification. Appl Environ Microbiol. 2008;74:3349—3355.

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