Food Research International 77 (2015) 320–325

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Comparison of process control viruses for use in extraction and detection of human norovirus from food matrices

Jennifer Gentry-Shields *[,1], Lee-Ann Jaykus

Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC 27695, United States

ARTICLE INFO

Article history:
Received 15 March 2015
Received in revised form 10 May 2015
Accepted 12 May 2015
Available online 13 May 2015

Keywords:
Norovirus
Process control virus
RT-qPCR
Processing efficiency

ABSTRACT

Although RT-qPCR is a powerful tool for human norovirus (HuNoV) detection, low virus concentrations in potentially large sample volumes necessitate the use of inefficient sample processing step(s) prior to detection. Process control viruses (PCVs) are used to monitor the efficiency of these virus concentration steps. This study compared five PCVs [Mengovirus (Mengo), murine norovirus (MNV-1), MS2 coliphage, Tulane virus, and turnip crinkle virus (TCV)] to two HuNoV strains for recovery during the steps of elution, polyethylene glycol precipitation (PEG), and RNA extraction from select foods (lettuce and sliced deli ham). Results demonstrate high recovery efficiencies of HuNoV GI.6 and GII.4 using the methods described in this study: combined (sequential) losses during processing from sliced deli ham and lettuce were <1 $\log_{10}$ genome equivalent copies (GEC). When considering the processing steps separately, HuNoV loss was negligible after elution, and low after PEG precipitation (mean 0.5 $\log_{10}$ GEC) and RNA extraction (mean 0.1 $\log_{10}$ GEC). The virus that least mimicked the behavior of HuNoV during sample processing was MNV-1. Of the viruses tested, a commercial mengovirus strain gave recovery efficiencies closest to HuNoV, showing combined losses from sliced deli ham and lettuce of <1 $\log_{10}$ GEC and ~1 $\log_{10}$ GEC, respectively. All PCVs do not behave equivalently and validation of their performance is recommended before their routine use on an application-by-application basis.

1. Introduction

Human noroviruses (HuNoVs) are the leading cause of foodborne disease in the United States (Scallan et al., 2011) and perhaps worldwide (Glass, Parashar, & Estes, 2009). Despite their public health significance, routine detection of HuNoV in food and environmental samples has been historically difficult to quantify, in part due to the potentially low densities of the virus in foods and the lack of a cell culture system. Molecular amplification methods, specifically reverse transcription quantitative PCR (RT-qPCR), offer powerful tools for rapid detection of HuNoV in complex matrices. In addition to avoiding the need for cultivation, RT-qPCR can save both time and expenses and has the potential to be sensitive, inexpensive, quantitative, and amenable to automation. However, low virus concentrations in potentially large sample volumes necessitate the use of labor intensive and potentially inefficient concentration step(s) prior to detection (Knight, Li, Uyttendaele, & Jaykus, 2013). Loss during these processing procedures can result in an underestimation of HuNoV load or false negative results.

To ensure accurate quantitation and interpretation of molecular data for HuNoV in various food matrices, controls are needed to determine the efficiency of various processing steps, including virus recovery, concentration, RNA extraction, and RT-PCR. Nucleic acid controls have been developed to quantify the RT-qPCR efficiency of HuNoV (Gregory, Webster, Griffith, & Stewart, 2011; Hata et al., 2011; Lee et al., 2011; Liu et al., 2013; Stals et al., 2009), as well as to quantify the recovery efficiency of viral nucleic acid during extraction procedures (da Silva et al., 2007; Hata et al., 2011; Mormann, Dabisch, & Becker, 2010). A few studies have attempted to quantify the recovery efficiencies of concentration procedures utilizing process control viruses (PCVs). For example, mengovirus has been utilized as a PCV for HuNoV in wastewater (da Silva et al., 2007) and in shellfish (Le Guyader et al., 2009), and MS2 has been used to monitor recovery of HuNoV from experimentally contaminated foods (Mormann et al., 2010). However, at present, there are no published studies evaluating potential PCVs for their similarity to HuNoV during processing; yet proven controls are imperative for enhancing the detection and accurate quantitation of HuNoV from complex sample matrices.

An ideal PCV for HuNoV would be similar morphologically and physiochemically to HuNoV, i.e., a non-enveloped positive-sense, single stranded RNA virus; sufficiently genetically distinct from HuNoV; and

Abbreviations: HuNoV, human norovirus; PCV, process control virus; MNV-1, murine norovirus; TCV, turnip crinkle virus; PEG, polyethylene glycol; GEC, genome equivalent copies; RT, room temperature; RT-qPCR, real-time quantitative polymerase chain reaction; BSA, bovine serum albumin; TCID50, median tissue culture infectious dose; CPE, cytopathic effect; PBS, phosphate buffered saline; FBS, fetal bovine serum.

* Corresponding author at: 8700 Mason-Montgomery Road, Mason, OH 45040, United States.
E-mail address: shields.jg@pg.com (J. Gentry-Shields).
[1] Present address: Global Microbiology Capability Organization, Procter & Gamble, Mason, OH 45040, United States.

http://dx.doi.org/10.1016/j.foodres.2015.05.027
0963-9969/

not normally expected to occur naturally in the foodstuffs being analyzed (Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR — Part 1: Method for quantification, 2013). Unfortunately, no one PCV is ideal. The goal of this study was to evaluate five viruses for use as PCVs when recovering and detecting HuNoV in foods: Mengovirus (Mengo), murine norovirus (MNV-1), MS2 coliphage, Tulane virus, and turnip crinkle virus (TCV). All viruses are icosahedral, non-enveloped, positive-sense single-stranded RNA viruses with similar size, genome, and isoelectric point to HuNoV (Table 1). Mengo is a member of the Picorniviridae family and a commonly used PCV (da Silva et al., 2007; Le Guyader et al., 2009). MS2 is a virus in the family Leviviridae that infects the bacterium *Escherichia coli* and other members of the Enterobacteriaceae. MNV-1 and Tulane are culturable HuNoV surrogates in the family Caliciviridae utilized in HuNoV inactivation and persistence studies (Cromeans et al., 2014), and MNV-1 has been used as a PCV for hepatitis A virus (Coudray, Merle, Martin-Latil, Guillier, & Perelle, 2013; Martin-Latil, Hennechart-Collette, Guillier, & Perelle, 2012). TCV is a plant pathogen in the family Tombusviridae first isolated from turnip and limited in spread to the UK and Yugoslavia (http://www.dpvweb.net/dpv/showdpv.php?dpvno=109). These potential PCVs were evaluated against HuNoV GI.6, a commonly implicated foodborne outbreak strain, and GII.4, the most common cause of HuNoV outbreaks, during virus recovery (elution), concentration, and RNA extraction procedures commonly used for foods (Baert, Uyttendaele, & Debevere, 2008; Fraisse et al., 2011; Microbiology of food and animal feed — Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR — Part 1: Method for quantification, 2013; Park, Kim, & Ko, 2010; Summa, Bonsdorff, von, & Maunula, 2012).

2. Methods

2.1. Viruses and virus propagation

HuNoV strains GI.6 and GII.4 were obtained as clinical stool specimens from HuNoV outbreaks (provided by S. R. Greene NCPH, Raleigh, NC). Stool samples were suspended 20% in phosphate buffered saline (PBS, pH 7.4) and estimated to have a threshold cycle (CT) of 25 and 21, respectively (personal communication, S. R. Green), equivalent to a titer of 7.8×10^7 (GI.6) and 2.7×10^7 (GII.4) genome equivalent copies (GEC)/ml by RT-qPCR (Section 2.3.1).

Mengovirus strain MC_0, an avirulent strain lacking the poly(C) tract, was purchased from American Type Culture Collection (ATCC VR-1597) with a concentration of 6.2×10^8 PFU/ml. Due to a shortage by the manufacturer, additional mengo was obtained as a part of an RNA extraction control kit (developed and manufactured by CEERAM SAS and distributed by Life Technologies™) with a concentration of 1.6×10^8 GEC/ml.

MS2 coliphage (ATCC 15597-B1) was purchased from ATCC (Manassas, VA). The virus stock was quantified using the Single Agar Layer method 1602 (USEPA, 2001) using *E. coli* F+ cells purchased from ATCC (15597) and cultured in tryptic soy broth (Fisher Scientific, Waltham, MA). Stock titer used in experiments was 7.7×10^7 PFU/ml.

Table 1
Human norovirus and potential process control virus size, genome length, and isoelectric point.

Virus	Particle size (nm. diam.)	Genome length (kb)	Isoelectric point (pI)
Human norovirus (HuNoV)	38–40	7.6	5.5–6.0
Mengovirus (Mengo)	30	8.4	Unknown
Murine norovirus (MNV-1)	28–35	7.4	4.8
MS2 coliphage	27	3.6	3.9
Tulane virus	40	6.7	10
Turnip crinkle virus (TCV)	28–35	4.0	5.0–6.0

MNV-1 was obtained from the laboratory of Dr. Howard Virgin (Washington University, St. Louis, MO). The virus stock was propagated in RAW 264.7 cells, a mouse monocyte macrophage line purchased from ATCC (TIB-71), and the cells were cultured in high-glucose Dulbecco's modified Eagle medium (DMEM, Life Technologies, Carlsbad, CA) supplemented with 10% low endotoxin fetal bovine serum (FBS, HyClone, Logan, UT) at 37 °C under a 5% CO_2 atmosphere as described previously (Tung, Macinga, Arbogast, & Jaykus, 2013). MNV-1 stock titers were 1.3×10^6 PFU/ml.

A purified stock of TCV was obtained from the laboratory of Dr. Steve Lommel (North Carolina State University, Raleigh, NC). Virus concentration was determined using spectroscopy as described previously (Gentry-Shields & Stewart, 2013) at 2.0×10^{15} virus particles/ml.

Tulane virus was obtained from the laboratory of Dr. Jason Jiang (Cincinnati Children's Hospital, Cincinnati, OH) and cultivated in LLC-MK2 cells (Farkas, Sestak, Wei, & Jiang, 2008), a rhesus monkey kidney epithelial line, that were purchased from ATCC (CCL-7). The cells were cultured in M199 medium (Mediatech Inc., Manassas, VA) supplemented with 10% fetal bovine serum (FBS, Cambrex Bio Science Walkersville Inc., Walkersville, MD) and 1% Penicillin–streptomycin (Life Technologies) at 37 °C under a 5% CO_2 atmosphere. Virus titer was determined by the 50% cell culture infectious dose ($TCID_{50}$) assay. Briefly, LLC-MK2 cells were grown on 48-well cell culture plates (BD, Franklin Lakes, NJ) until 90% confluent (~24 h). Eluates were serially diluted 10^{-1} to 10^{-6} in M199 media. Each well (6 per dilution) was inoculated with 50 μl of diluted sample. The inoculated plates were incubated at 37 °C in 5% CO_2 for 1 h with periodic rocking, followed by the addition of 1 ml maintenance media (M199 media supplemented with 2% FBS and 1% Pen-strep). Starting 24 h after inoculation, the wells were visually examined for cytopathic effect (CPE) using an inverted microscope (Nikon Inc. Melville, NY). CPE was generally fully developed 5 days post-inoculation. The stock titer was calculated using the Reed–Muench Calculator Spreadsheet (http://www.med.yale.edu/micropath/pdf/infectivity%20calculator.xls) to be 1.3×10^7 $TCID_{50}$/ml.

The concentrations of all viruses in genome equivalent copies (GEC) were determined as described previously (Gentry-Shields & Stewart, 2013). All viruses were stored at −80 °C.

2.2. Experimental procedures

All five potential PCVs were evaluated against HuNoV GI.6 and GII.4 for the steps of virus recovery (elution), concentration, and RNA extraction. These processes are described below.

2.2.1. Elution

Fifteen g of romaine lettuce or 25 g of sliced deli ham were seeded with 20 μl of each virus as several small drops across the food sample (total inoculum concentration ranging from 7–10 $\log_{10}$ GEC) and dried for 30 min at room temperature (RT). The virus-seeded food sample was placed in a polypropylene bag containing a filter compartment (Nasco Whirl-Pak, Fort Atkinson, WI) and soaked with 25 ml elution buffer (0.04 M glycine, 0.15 M NaCl, 0.01 M NaOH [all from Fisher Scientific], pH 9.0). The sample was stomached for 1 min at 230 rpm using a stomacher (Seward, Davie, FL). The rinse fluid was removed via the filter compartment of the bag to a 50-ml centrifuge tube and adjusted to pH 7.0 ± 0.5 with 0.1 M HCl. To quantify virus recovery during elution, un-inoculated samples were processed in the same manner as above then this set of control samples was inoculated with the same quantity of virus after the elution step. All samples were stored at −20 °C before polyethylene glycol (PEG) precipitation and chloroform:butanol purification, RNA extraction, and RT-qPCR (described below). Elution recovery was quantified as the difference in virus quantity detected by RT-qPCR from samples spiked pre-elution to samples spiked post-elution. For each virus spike concentration, experiments were performed in triplicate with three independent samples processed per replicate.