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Comparison of viral elution-concentration methods for recovering noroviruses from deli meats

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ABSTRACT

This study aimed to assess viral elution-concentration methods for recovering noroviruses from deli meats. Spiking experiments were conducted to evaluate the recovery success rates and recovery efficiencies of human norovirus (NoV) GI and GII and murine norovirus 1 (MNV-1) using polyethylene glycol (PEG 6000) precipitation, skimmed milk flocculation (SMF), TRIzol® reagent, and a combination of PEG/TRIzol® and SMF/TRIzol® methods. Molecular analysis using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) revealed TRIzol® as the best method to be used for viral recovery from ham with medium recovery rates of 37.6% for NoV GI and 50.1% for NoV GII. Viral recovery from turkey meat showed medium recovery rates of 14.4% for NoV GI and 8.9% for NoV GII. For MNV-1, the rates varied from 0.5% to 80.8% not only according to the matrix but also with the associated virus and its inoculum (NoV GI or GII). The monitoring of commercial samples obtained in the Great Metropolitan region of Rio de Janeiro in order to demonstrate the occurrence of NoV GI and GII contamination in both matrices was also performed in 60 samples. NoV GI or GII were not detected in any samples, while MNV-1 used as the sample process control viruswas successfully recovered in 100% of samples.

1. Introduction

Viruses are a great concern for food safety, since they can be introduced into the production chain from the pre-harvest environment at the farm, at the sprouting facility, during preparation via infected food handlers, or from cross-contamination in restaurant/food establishments (Hall et al., 2012; Wei and Kniel, 2010).

From several enteric viruses that infect susceptible individuals by the fecal-oral route through contaminated food/water or aerosolized vomitus or fomites, norovirus (NoV) deserves mention due to its important epidemiology and association to foodborne outbreaks (Baert et al., 2009; Daniels et al., 2000; Hall et al., 2014; Painter et al., 2013; Verhoef et al., 2010).

NoV belongs to the genus *Norovirus, Caliciviridae* family (Green et al., 2000). It is a group of genetically and antigenically diverse RNA genome viruses that can be classified into seven genogroups (GI-GVII) (Vinjé, 2015), of which GI, GII, and GIV contain human strains. The GII, genotype 4 (GII.4) strain is most commonly detected in association with disease (Han et al., 2015). A great diversity of genotypes has been associated with foodborne outbreaks (Kroneman et al., 2008; Stals et al., 2012) that commonly occur in closed settings such as restaurants, hotels, day care centers, schools, nursing homes, cruise ships, swimming pools, hospitals, and military installations, because these are crowded locations in which common foods are oftenconsumed (Hall et al., 2011).

Transmission of NoV is facilitated by several factors that include the low presumed 50% infectious dose (ID_{50}) of 18 segregated infectious particles, the fecalshedding of high amounts of virus particles excreted (up to 10^{10} virus particles per gram feces), the high environmental stability, as well as the occurrence of asymptomatic infections (Atmar et al., 2008; Baert et al., 2009; Jeong et al., 2013; Lee et al., 2007; Nicolay et al., 2011; Ozawa et al., 2007; Phillips et al., 2010; Teunis et al., 2008).

Several food matrices have been implicated in viral outbreaks, including fruits and vegetables, sliced deli meats, shellfish, and hand prepared foods such as sandwiches and salads (Daniels et al., 2000; Koopmans et al., 2003; Rosenblum et al., 1990). This great diversity represents a challenge for viral recovery methodologies mainly for deli meats that are matrices composed of fat/protein-based foods (Baert et al., 2008).

Methods for virus extraction based on physical chemical characteristic of viruses have been developed with the objective to remove inhibitors and concentrate the virus before RNA extraction andreverse transcriptase polymerase chain reaction (RT-PCR) analysis (Bartsch et al., 2016; Martín-Díaz and Lucena, 2018).In this context, the aim of

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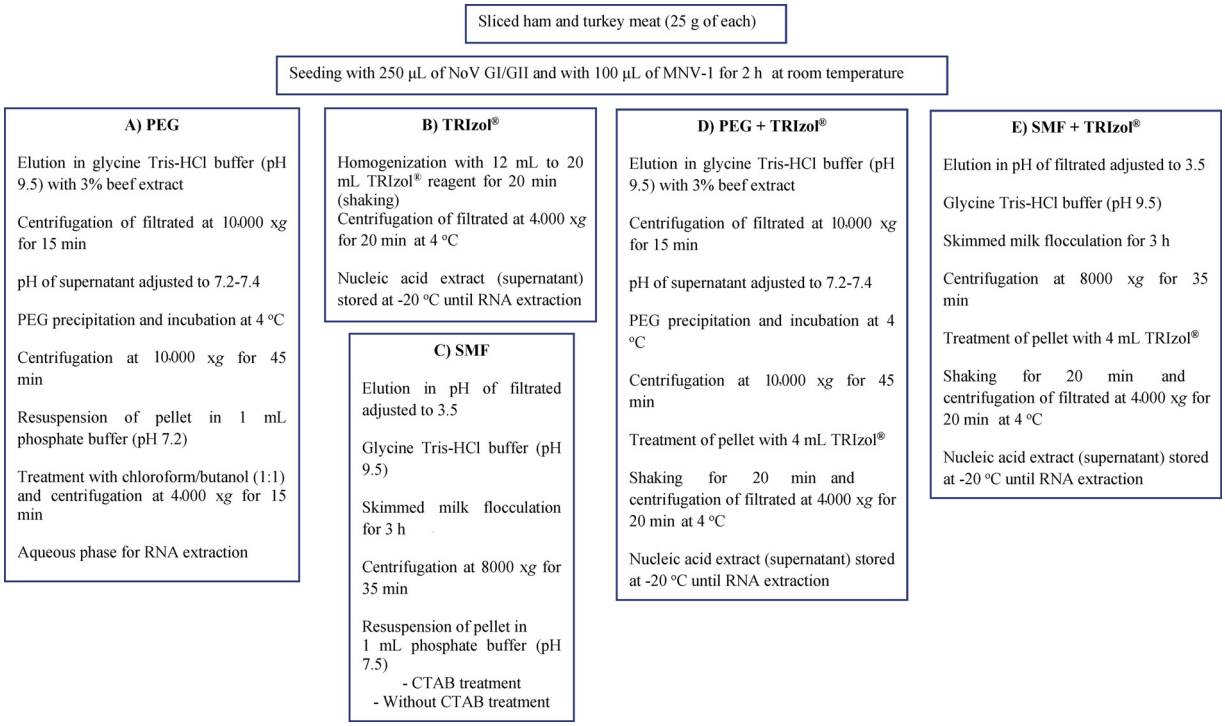

Fig. 1. Flowchart of viral elution-concentration methods used for MNV-1 + NoV GI and MNV-1 + NoV GII (A, B and C), and MNV-1 + NoV GI + NoV GII (D and E) recovery from deli meat samples.

this study was to assess some of those methods such as TRIzol® reagent, polyethylene glycol (PEG) precipitation, skimmed milk flocculation (SMF), and the association of the last two with TRIzol® for recovering human NoV GI and GII and murine norovirus 1 (MNV-1) from deli meats such as ham and turkey meat based on recovery success rate and recovery efficiency.

2. Materials and methods

2.1. Food samples and virus strains

Ham and turkey meat samples were obtained at local food stores in the Greater Metropolitan region of the state of Rio de Janeiro, Brazil.

The NoV strains (NoV GI and NoV GII) were obtained from different and separated diluted fecal samples of a 10%–20% suspension in Tris/HCl/Ca^{+2} buffer (pH 7.2–7.3). Each fecal sample contained NoV GI.1 and GII.4 separately, belonging to the Regional Reference Gastroenteritis Laboratory collection at the Oswaldo Cruz Institute, Rio de Janeiro-RJ, Brazil.

MNV-1 used as the sample process control virus (SPCV) was kindly provided by Dr. Herbert W. Virgin from Washington University School of Medicine and propagated in RAW 264.7 cells (a macrophage-like Abelson leukemia virus-transformed cell line derived from BALB/c mice), according to de Abreu Corrêa and Miagostovich (2013).

2.2. Spiking experiments

In order to assess the recovery success rate and efficiency of NoV GI, GII and MNV-1, spiking experiments were carried out in triplicate for each method (PEG, SMF and TRIzol®) and matrix (ham and turkey meat) as follow: MNV-1 + NoV GI and MNV-1 + NoV GII. For combined methods (PEG/TRIzol® and SMF/TRIzol®),MNV-1, NoV GI and GII were spiked simultaneously for each matrix.

Twenty-five grams of each food matrix were artificially contaminated with 250 µL of the 10-fold dilution of a fecal sample of NoV GI and/or GII (corresponding with around 10^4 genomic copies) and with 100 µL of MNV-1 virus lysate (corresponding with 10^6 genomic copies). To assess multi-step methods as the combinations PEG/TRIzol® and SMF/TRIzol®, NoV GI and GII and MNV-1 were spiked simultaneously. Non-contaminated food samples, only diluted with phosphate-buffered saline (PBS 1x), were also processed as negative controls in all experiments. After incubation for nearly 2 h at room temperature, food samples were placed in a 400-mL polypropylene bag with filter compartment (Nasco®, Fort Atkinson, Wisconsin, USA) and proceed to the following different elution-concentration methods.

2.3. Elution-concentration methods

2.3.1. Polyethylene glycol (PEG 6000) precipitation and combination of PEG/TRizol® method

This method was adapted from a previous one described by Dubois et al. (2002). Briefly, samples were washed with 75 mL of elution buffer (0.1 M Tris-HCl, 3% beef extract, BBL™, Le Pont de Claix, France; 0.05 M glycine, Bio-Rad, Hercules, CA, USA, pH 9.5) on a shaking platform for 20 min. The filtrate was taken and centrifuged (10,000 × *g*, 15 min, 4 °C).

After this, the pH of the supernatant was adjusted between 7.2 and 7.4 with 6 N HCl (Sigma-Aldrich, St. Louis, USA) and 37.5 mL of a mixture of PEG 6000 (Fluka, Buchs, Germany) at 10% wt/vol and 0.3 M NaCl (Sigma-Aldrich, St. Louis, USA) was added. After the addition of PEG 6000 to the samples and shaken on a platform overnight (4 °C), these samples were centrifuged (10,000 × *g*, 45 min, 4 °C), and 1 mL of PBS 1x was added. The aqueous phase was treated with one volume of chloroform/butanol (1:1 vol/vol) and centrifuged once (4000 × *g*, 15 min, 4 °C).

To combination of PEG/TRizol® method,after the step of shaking on a platform overnight (4 °C), samples were centrifuged (10,000 × *g*, 45 min, 4 °C) and subsequently the pellet was treated with 4 mL TRizol®. After shaking for 20 min, the samples were centrifuged again (4000 × *g*, 20 min, 4 °C) and supernatant recovered (Fig. 1).