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Sampling methods for recovery of human enteric viruses from environmental surfaces

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ABSTRACT

Acute gastroenteritis causes the second highest infectious disease burden worldwide. Human enteric viruses have been identified as leading causative agents of acute gastroenteritis as well as foodborne illnesses in the U.S. and are generally transmitted by fecal-oral contamination. There is growing evidence of transmission occurring via contaminated fomite including food contact surfaces. Additionally, human enteric viruses have been shown to remain infectious on fomites over prolonged periods of time. To better understand viral persistence, there is a need for more studies to investigate this phenomenon. Therefore, optimization of surface sampling methods is essential to aid in understanding environmental contamination to ensure proper preventative measures are being applied. In general, surface sampling studies are limited and highly variable among recovery efficiencies and research parameters used (e.g., virus type/density, surface type, elution buffers, tools). This review aims to discuss the various factors impacting surface sampling of viruses from fomites and to explore how researchers could move towards a more sensitive and standard sampling method.

1. Introduction

Acute gastroenteritis causes the second highest infectious disease burden worldwide with an estimated 1.45 million deaths per year (Ahmed et al., 2014). In the United States alone, acute gastroenteritis causes 178.8 million illnesses, 473,832 hospitalizations, and 5072 deaths (Scallan et al., 2011). There are approximately 31 major pathogenic agents known to cause acute gastroenteritis and/or foodborne illness including human enteric viruses such as astrovirus, rotavirus, hepatitis A virus (HAV), and human norovirus (hNoV) (Scallan et al., 2011). The most common enteric viruses that cause foodborne illnesses are hNoVs and HAV (Cliver, 1997; Koopmans and Duizer, 2004).

Generally, viral acute gastroenteritis is transmitted through food and water contamination, contaminated environmental surfaces, direct person-to-person contact, and other unknown sources (Wikswo et al., 2015). Furthermore, enteric viruses are spread by fecal-oral contamination, and there is growing evidence of viral transmission occurring through contaminated fomites in a variety of ways and settings including food preparation environments (Boone and Gerba, 2007; Rzezutka and Cook, 2004). Enteric viruses have been shown to maintain infectivity on fomites over prolonged periods of time (Escudero et al., 2012). For instance, seminal research by Kiseleva (1968) reported on the survival of echovirus, coxsackievirus, and poliovirus on representative surfaces (painted wood, glass, cotton fabric) in households and showed that these viruses maintained infectivity for two to more than 12 days. Human norovirus survival for up to 12 days has also been reported on carpets subject to vomiting episodes after an initial outbreak in a hospital ward (Cheesbrough et al., 1997). There are some studies focusing on the role of fomites and environmental contamination in the transmission of enteric viruses however this specific route of transmission is difficult to determine during outbreaks (Rzezutka and Cook, 2004).

To better understand the role of environmental surface transmission during outbreaks due to human enteric viruses, the persistence of viruses on various surface types must be investigated. To do this, a surface sampling method must be applied for recovery of viruses. For instance, understanding the persistence of human enteric viruses on inanimate fomite surfaces in relation to various environmental conditions could provide insight on ways to limit and prevent virus transmission and subsequent outbreaks. However, studies on surface sampling techniques are typically limited to swabs for application in environmental sampling during foodborne outbreaks or for investigation of baseline virus prevalence. As a result, information is lacking on evaluating tools used in laboratory sampling studies for the optimal recovery of viruses. Thus, this review aims to: (1) discuss and compare evaluations of surface sampling methods for optimal recovery of human enteric viruses from inanimate fomite surfaces and (2) explore how researchers could move towards one standard methodology for surface

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sampling of human enteric viruses and their surrogates.

2. Background

The most common foodborne viruses are categorized based on the type of disease they cause: (1) gastroenteritis (e.g. rotavirus, hNoV, Aichi virus A, coronavirus, and others), (2) enterically transmitted hepatitis viruses (e.g. hepatitis E and A), and (3) viruses that replicate in the human gut then migrate to other organs to cause disease (e.g. poliovirus) (Koopmans and Duizer, 2004). Enteric viruses are typically spread by vomiting or shedding into the stool and have a greater chance of transmission the longer the virus is able to survive outside the host. This survival is impacted by various environmental conditions such as pH, moisture, and temperature (Koopmans and Duizer, 2004; Rzezutka and Cook, 2004).

2.1. Enteric virus transmission due to environmental surface contamination

As indicated previously, enteric viruses have been shown to maintain infectivity on surfaces over prolonged periods. Human noroviruses have been detected on a variety of surfaces including cellular phones, public phones, televisions, chairs, keyboards, microwave ovens, bathroom light switches, various handles and knobs of kitchen and bathroom items, bed frames, and chairs (Boxman et al., 2011; Gallimore et al., 2006, 2008). Boxman et al. (2011) reported year round prevalence of hNoVs on environmental surfaces of catering facilities even without a recently reported outbreak of acute gastroenteritis. The authors reported that hNoV was recovered from 61.1% of catering settings with recent outbreaks in contrast to only 4.2% of catering settings without a recent outbreak. Elderly homes and pension/hotels catering company types had the highest prevalence of positive swab samples for hNoVs (Boxman et al., 2011). Moreover, multiple studies have shown institutional settings such as cafeterias and long-term facilities are more likely to have hNoVs on surfaces compared to food service settings (Boxman et al., 2011; Hall et al., 2014; Verhoef et al., 2013).

2.2. Current standard methods for surface sampling and analysis

For environmental surface sampling, the International Organization of Standardization (2017) recommends swabbing with a sterile cotton swab presoaked in PBS followed by RNA extraction and reverse transcription, real time PCR (RT-qPCR) analysis for HAV and hNoV sampling and detection on nonporous FCS. In the U.S., there is not a standardized method available. However, the Centers for Disease Control and Prevention (CDC, 2012) does recommend the use of swabs for obtaining norovirus from environmental surfaces; however, the CDC has also reported that swabbing is highly variable and that the interpretation of results should be conducted with caution.

Currently, hNoVs are most often detected by RT-qPCR due to its high sensitivity and low detection limits using measurements such as PCR amplifiable units (PCRU/ml). These PCRUs are determined by a standard curve produced from a 10-fold dilution series of the virus where one PCRU corresponds to the highest dilution with a quantifiable RT-qPCR value (or cycle threshold [C_T] value) (Knight et al., 2013; Tung et al., 2013). However, Knight et al. (2013) pointed out that the determination of PCRUs in correspondence to specific C_T values is dependent on the sample matrix and the standard used. Moreover, the cut-off C_T values (i.e. endpoint of detection) for hNoVs also vary across studies ranging from 32 to 40 (Knight et al., 2013). The presence of inhibitory components within some sample matrices could impact amplification efficiencies especially in contaminated food and environmental samples that typically have low viral loads (Knight et al., 2013; Sair et al., 2002). Regardless, RT-qPCR is primarily chosen for the analysis of viruses in environmental and food samples to allow for increased sensitivity to detect low viral concentrations that are typically present (Knight et al., 2013). However, as the authors of the review indicated, this method cannot determine infectivity since it may recognize intact or degraded viral nucleic acid, nonviable viruses, or defective viral particles (Knight et al., 2013). Consequently, the use of surrogates and other infectivity assays remain important in investigating enteric viral viability and infectivity in lab-based studies as further discussed in Section 2.3.2.

2.3. Factors impacting recovery of viruses from surfaces

Virus density, the rate of positive environmental samples of total samples collected, and exposure magnitude provide information about virus contamination on surfaces (Julian et al., 2011). However, these factors are impacted by the surface sampling method and detection assay selected. Subsection 2.3.1 to 2.3.5 will examine the variability among the many factors impacting recovery of viruses from surfaces, specifically surface type, virus type/density, drying time, elution buffers, and implement/recovery tool selection.

2.3.1. Surface type

Fomites are generally categorized as either nonporous or porous. Examples of nonporous surfaces are ceramic, glass, acrylic, and stainless steel, and examples of porous surfaces include carpets, lettuce, deli meats, wood, latex, and fruits. Surface type has been shown to have some effect on surface sampling recovery efficiencies (Table 1). Tung-Thompson et al. (2017) swabbed foods (cheese, apple, green pepper, tomato) and hard surfaces (stainless steel and ceramic) with wipes that were inoculated with 10 µl of varying PCR-units (PCRU)/ml of hNoV GII.4. The study obtained a mean range recovery efficiency of 74% to approximately 100% for all surfaces except for cheese, which was significantly different from the other surfaces with 29% to 69% recovery for high inoculum levels (10^4 to 10^6 PCRU) and no detection at low inoculum levels (10^2 to 10^3 PCRU) (Tung-Thompson et al., 2017). The authors were not able to determine if the lipid content of the cheese contributed to the possible absorption and recovery of the virus samples even though a previous study suggested this possibility for hNoVs (Fumian et al., 2009; Tung-Thompson et al., 2017).

Furthermore, surface properties can also impact recovery efficiencies in a variety of ways. For instance, stainless steel is a hydrophilic (contact angle of 58.2° in water, surface energy of 50.3 mJ/m^2) and negatively charged surface in which microorganisms have been shown to develop irreversible attachment within one minute potentially making surface recovery more difficult (Mafu et al., 1990; Mafu et al., 1991). The orientation of a surface could interfere with adequate surface sampling and collection as seen in a study involving vertical and horizontal stainless steel surfaces. Taku et al. (2002) determined that greater recovery efficiency could be obtained by allowing the elution buffer to sit on the surface for 15 min—something that cannot be performed on a vertical surface. The mean recovery for horizontal surfaces and sinks using the cell scraper-aspiration method ranged from 32% to 71% while vertical stainless steel surfaces only obtained a mean recovery of 11% since the buffer was not in contact with the surface long enough to facilitate virus recovery (Taku et al., 2002). Scherer et al. (2009) suggested physical properties of nonporous and porous could reduce virus recovery via trapping virus particles within the matrix/crevices or facilitate enhanced virus recovery by smooth/porous surfaces. Mattison et al. (2007) suggested the low mean recovery of feline calicivirus (FCV) from strawberries might be due to its surface texture and how the crevices may shield viruses against environmental conditions. Furthermore, the authors observed a pH change in the elution buffer from 7.2 to 5.5 when strawberries were immersed, which could impact virus recovery by either partial viral inactivation or interference with FCV recovery (Mattison et al., 2007). Overall, physical and chemical properties of nonporous and porous food and food contact surfaces could impact recovery efficiencies of enteric viruses. This review will focus on surface sampling techniques for enteric viruses from nonporous, inanimate surfaces.

Table 1
Surface sampling methods for human enteric viruses – nonporous and porous environmental surfaces (not food based).

Virus type	Method(s) evaluated	Surface type (s)	Conditions	Volume and concentration	Buffer Matrix(s)	Analysis	Recovery results	Reference
hNoV GII.4	Biowipes (cotton, polyester, polyamide fibers)	SS, ceramic	40 min drying time	10 µl of virus of varying PCRU (10^2 to 10^6)	Biowipes moistened in PBS (pH 8.0)	RT-qPCR	SS: 76.8% ± 19.7% (10^4) to 99.3% ± 1.1% (10^3) Ceramic: 42.4% ± 50.8% (10^3) to 96.6% ± 3.4% (10^5)	Tung-Thompson et al. (2017)
hNoV GII.7; mengovirus (MV)	- Swabs (3 types) - Cotton - Foamed cotton - Polyester	Laminated wooden fibreboard	1 h drying time	100 µl of either 10^5 PFU/ml MV or 10^3 GC/ml hNoV in PBS	1) Direct Nuclisens lysis buffer 2) Tris-HCl (100 mM) − glycine (50 mM)-beef extract buffer	RT-qPCR	Highest recovery = polyester swab with direct lysis Recovery rates of ≤ 13.7% dependent on concentration, virus type, and method used	Ibfelt et al. (2016)
TuV	Scraping – aspiration	SS, ABSS	Ambient conditions	50 µl of 5×10^4 PFU/ml	450 µl of PBS	PA	SS ~ 30% ABSS ~ 10%	Arthur and Gibson (2015)
Bacterio-phage PP7; MNV-1	Swab (rayon) – rinse protocol	RB, PF, NPF	3 h drying time at ambient conditions (RT; 50–80% RH)	50 µl of $10^3 - 10^6$	Culture medium	RT-qPCR	0.6% to 11.5% (PP7) and 12.2% to 77.0% (MNV-1) No significant difference between viruses	Ganime et al. (2015)
hNoV GII.4	Swab (4 types) – rinse protocol	SS	Ambient conditions (RT; 45–60% RH), 1–48 h drying time	50 µl virus suspension	2.5 ml PBST	RT-qPCR	SS (no drying): 16.6% ± 2.3% (polyester) to 43.5% ± 21.4% (macrofoam) decrease with drying time and surface area	Park et al. (2015)
FCV, MNV	Stomaching – sonication	Glass, cotton, polyester	Ambient conditions, 40 min drying time	200 µl 6.6–7 log PFU/ml FCV or 5.9-6.3 log PFU/ml MNV	10 ml PBST	PA	FCV: 0.15% (cotton) to 35.22% (glass) MNV: 0.85% (cotton) to 24.27% (glass)	Yeargin et al. (2015)
hNoV GI.4, GII.4	Swab (cotton) elution – extraction	HDPE	45 min drying in biosafety cabinet	100 µl of 10^4 GC of each virus	PBS (3 strategies differ when/how often swab moistened)	RT-qPCR	For both hNoVs, strategy 2 and 3 were significantly different from 1. Strategy 2 (Highest): 27.0% ± 26.5%	De Keuckelaere et al. (2014)
hNoV GII.4	Swab/cloth with semidirect lysis method	Low density PE, SS	Dry overnight at RT	100 µl of 10^{-4} to 10^{-6} GII.4 particles	2 ml of either PBS or 50 mM glycine buffer	RT-qPCR	PE: (highest) microfiber cloth 1 = 88.7% ± 2.7% (glycine) SS: (highest) microfiber cloth 1 = 79.0% ± 10.2% (glycine)	Rönnqvist et al. (2013)
MNV	Repeated pipetting (25x)	SS	20 min drying at RT	10 µl of 2×10^5 virus	1 ml EBSS	PA	37% recovery after 20 min drying	Fallahi and Mattison (2011)
Bacterio-phage MS2	- Swab – elution extraction - cotton swab - polyester swab - antistatic cloth	PVC, SS	45 min drying time (RT; 45–60% RH)	3.7 $\log_{10}$ in 5 µl	1) 0.85% saline 2) Ringer's solution 3) viral transport media 4) acid/base eluent	PA, RT-qPCR	Implement significantly influenced recovery lowest recovery = antistatic cloth highest recovery = swabs < 0.3 to 97% recovery for infective MS2	Julian et al. (2011)
hNoV GII.3; rotavirus	Swab protocol	HDPE, SS, ceramic, wood	15 min drying in laminar flow hood	100 µl of $10^5 - 10^7$ PCRU/ml of each virus	PBS	RT-qPCR	hNoV: 10.3–25.5% (wood) to 31–51.9% (ceramic) rotavirus: 5.4–10.2% (wood) to 45.9-57.7% (ceramic)	Scherer et al. (2009)
FCV	Vortex for 30 s in scintillation vials	SS	30 min drying in hood	10 µl of 3.0×10^7 PFU/ml	990 µl of EBSS	PA	33 and 11% after 0 and 30 min drying time, respectively	Mattison et al. (2007)
FCV	1) Swabbing 2) flooding − aspiration 3) scraping − aspiration	SS	15 min drying time	Not specified	1) 0.05 M glycine buffer, pH 6.5 2) 0.05 M glycine buffer, pH 9.5 3) Modified Eagle's medium, pH 7.2	RT-qPCR	Highest recoveries with 0.05 M glycine at pH 6.5 for FCV Scraping − aspiration method best Cell scraper mean recovery range on SS surfaces: 11% to 71%	Taku et al. (2002)

ABSS = acrylic-based solid surface; EBSS = Earle's buffered saline solution; FCV = feline calicivirus; GC = genomic copies; HDPE = high density polyethylene; hNoV = human norovirus; MNV = murine norovirus; NPF = non-porous formic;

PA = plaque assay; PBS = phosphate buffered saline; PBST = PBS + 0.02% Tween 80; PCRU = polymerase chain reaction units; PE = polyethylene; PF = porous formic; PFU = plaque forming units; RH = relative humidity; RB = rubberized surface; RT-qPCR = reverse transcription quantitative PCR; RT = room temperature; SS = stainless steel.

2.3.2. Virus type and density

Virus type and density may have varying effects on surface sampling techniques and recovery efficiencies. Traditionally, surrogates including murine norovirus strain type 1 (MNV-1), MS2 bacteriophage, Tulane virus (TuV), and FCV have been used for infectivity studies related to hNoVs (Rönnqvist and Maunula 2016). There has not been an *in vitro* cell culture system for hNoVs available until recently (Ettayebi et al., 2016), and until reproducible and readily available infectivity assays are developed, surrogates still provide much needed information on infectivity of hNoVs. Multiple surrogates are important for understanding infectivity due to variations in their genetic relatedness to hNoVs and the diversity among hNoV genotypes. Other cultivable viruses utilized in environmental persistence research include Aichi virus A (AiV) and HAV—both known human enteric pathogens (Cannon et al., 2006; Koopmans and Duizer, 2004, Yeargin et al., 2015Cannon et al., 2006Cannon et al., 2006; Koopmans and Duizer, 2004, Yeargin et al., 2015). Diversity among hNoV genotypes could impact the recovery efficiency from surfaces; however, studies focus mainly on hNoV GII.4 (Table 1). This focus is a result of GII.4 being the pandemic genotype of hNoV and accounting for over 80% of all hNoV outbreaks in the U.S. since 1996 (Glass et al., 2009). Surrogates provide essential information on hNoV infectivity in relation to viral persistence on food contact surfaces (FCS), and numerous studies have shown FCV, MNV, and TuV to remain infectious on multiple surfaces for at least 7 days or more (Arthur and Gibson 2015; Fallahi and Mattison 2011; Mattison et al., 2007).

Some studies have compared the recovery efficiency between different types of enteric viruses. Scherer et al. (2009) compared hNoV GII.3 and rotavirus recovery efficiencies using a cotton swab from various porous and nonporous FCS. Table 1 shows the recovery varied between virus types for a given surface. For instance, Scherer et al. (2009) reported the highest percentage of hNoV was recovered on ceramic (31–52%) while rotavirus was recovered at a slightly higher percentage (46–58%) on the same surface. The authors suggested the varying recovery rates observed between the two enteric viruses may be due to the abilities of the different viruses to adhere to the various surfaces as well as differences in virus properties affecting attachment (Scherer et al., 2009). A greater variety of surrogates and enteric viruses need to be evaluated for surface sampling to ensure accurate prevention and detection methods are being implemented.

Virus density could also impact the amount of virus recovered from a given surface. In general, higher starting densities of viruses equal greater recovery efficiencies—primarily due to the limit of detection of the downstream assay. Tung-Thompson et al. (2017) reported recovery efficiency variability by virus density when using wipes on food and nonporous food contact surfaces. The authors showed that recovery was consistent at high inoculum levels (10^4-10^6 PCRU/ml) of GII.4 while more variability was observed at lower inoculum levels ($10^2 - 10^3$ PCRU/ml). In contrast, Rönnqvist et al. (2013) also reported variability among lower concentrations of GII.4 with higher mean recoveries for hNoV GII.4 at 10^2 PCRU than 10^3 PCRU when evaluating four different swabs on environmental surfaces. For 10^3 PCRU of GII.4, there was no significance difference for recovery efficiency among the swabs evaluated except on latex surfaces with polyester swabs regardless of buffer type. Meanwhile, microfiber swabs combined with glycine buffer for elution was found to be a significantly better recovery method for 10^2 PCRU of GII.4 on all the surfaces (Rönnqvist et al., 2013). Scherer et al. (2009) reported that the mean recovery efficiencies for rotavirus and hNoV GII.3 were higher from various nonporous and porous surfaces using a cotton swab-rinse method at higher inoculum levels (2×10^5 PCRU for hNoV; 2×10^4 PCRU for rotavirus) than lower inoculum levels (2×10^4 PCRU for hNoV; 2×10^3 PCRU for rotavirus). The authors also mentioned how reverse transcription became less efficient at low inoculum levels resulting in an increase in statistical errors. Overall, the higher the inoculum level for all enteric viruses, the higher the mean recovery rate regardless of the variability among methods,

virus type, and high standard deviations of the mean recovery rates.

Additionally, organic matter such as coagulated food and other debris while on environmental surfaces may impact the effect of virus density on recovery efficiency. For instance, fatty foods such as cheese have been known to contribute to absorption and recovery of virus samples for hNoVs due to lipid content (Fumian et al., 2009). Furthermore, Abad et al. (1994) studied the effect of fecal matter on the persistence of enteric viruses and reported varying results between virus types and fomites. The authors found no effect on the persistence of HAV and human rotavirus with the exception of longer persistence of HAV on latex surfaces. Overall, Abad et al. (1994) observed longer persistence for adenovirus and poliovirus on nonporous fomites (china, glazed tile, aluminum, and latex), and a decrease in persistence of adenovirus and poliovirus on porous fomites (cotton cloth and paper).

For hNoVs, the preparation of stool samples (i.e. because hNoV does not have a routine culture method) is not always specifically stated in studies on virus persistence and recovery from surfaces. For example, Park et al. (2015) include a clarification step—a brief centrifugation to separate the large particulates from the viruses in 10% fecal suspensions—while others (De Keuckelaere et al., 2014; Ronnqvist et al., 2013) use hNoVs in the original 10% fecal suspension for their studies. The presence or absence of organic matter can certainly impact both virus persistence and recovery; however, it should also be noted that the presence of organic matter could also impact downstream analysis such as RT-qPCR via inhibition (Wilson 1997), also indicated in Section 2.2. Even though virus persistence and recovery from food matrices are not within the scope of this review, enteric virus recovery from nonporous environmental surfaces as a function of particle association (e.g., food and debris) is lacking and does need further study.

2.3.3. Drying time

Drying time for enteric virus surface sampling is highly variable and dependent on factors including volume of virus suspension and desiccation (Table 1). Drying times range from 15 min to overnight at ambient conditions with volumes ranging from 5 µl to 100 µl. Drying time impacts the recovery efficiencies of surface sampling methods, and generally, the longer a virus is on a surface, the harder it is to recover the virus from the surface. Mattison et al. (2007) tested recovery of FCV from stainless steel surfaces using vortexing at 30 min post inoculation versus immediate recovery after inoculation of 3.0×10^5 FCV in 10 µl. The difference in recovery between elution immediately following and after 30 min of drying was 33 and 11%, respectively—a three-fold difference. While this review is focused on FCS and not food, the authors did note that the difference between viral recovery from lettuce and stainless steel may be due to viruses being more influenced by the effects of air drying when on a flat nonporous surface. Park et al. (2015) observed a reduction in the recovery efficiency of hNoV GII.4 from stainless steel and toilet representative surfaces as a function of drying time. On stainless steel surfaces using macrofoam swabs, the recovery efficiency was 43.5% ± 21.4% without drying, 25.7% ± 10.6% at 1 h, 18.2% to 25.7% ≤ 24 h, and 10.0% ± 2.3% after 48 h (Park et al., 2015). Based on the evidence presented above, there is a need for uniformity among studies and standardization in drying time and inoculum amount in order to properly evaluate virus recovery and surface sampling methods.

2.3.4. Type of elution buffers

The recovery efficiencies for the numerous eluent-tool combinations are variable and often impacted by both intrinsic factors related to the actual tool and eluent types as well as the extrinsic factors already introduced (Sections 2.3.1–2.3.3). The differences in eluent formulations such as pH, salinity, and use of a surfactant can impact the recovery efficiency of viruses from surfaces. Ionic strength and pH of eluents have been known to impact the net charge of viral particles (Gerba, 1984). Rönnqvist et al. (2013) obtained slightly higher recovery efficiencies using an alkaline glycine buffer (pH 9.5) than eluting with PBS (pH 7.2). Conversely, Taku et al. (2002) recovered more FCV from stainless steel surfaces using a slightly acidic glycine buffer (pH 6.5) with a mean recovery of 42% compared to 28 and 10% recovery using glycine buffer (pH 9.5) or culture medium (pH 7.2), respectively.

Surfactants are another common component added to elution buffers. These are known to increase the water content of the surface, assist in solubilization of proteins and cells from the surface, and can disrupt hydrophobic interactions between charged viruses and surfaces thus enhancing virus recovery (Farrah 1982; Lukasik et al., 2000; Moore and Griffith 2007). Park et al. (2015) suggested that adding a surfactant (0.02% Tween 80) to the PBS elution buffer of a swab rinse protocol enhanced viral recovery efficiency of hNoV GII.4 even though no significance was observed. Meanwhile, another study found higher recovery of hNoV GII.7 and mengovirus from laminated wooden surfaces when using lysis buffer compared to 100 mM Tris-HCl − 50 mM glycine −1.5% beef extract (TGBE, pH 9.5); however, again no significance difference was observed (Ibfelt et al., 2016).

For MS2 recovery, two separate studies found the eluent type to not be significantly different (Casanova et al., 2009; Julian et al., 2011). Furthermore, eluent type for MS2 recovery was suggested to be selected based on experimental design such as considering eluents compatible with nucleic acid extraction for molecular detection-based sampling studies or with tissue culture for infectivity-based studies (Julian et al., 2011). Moreover, Rönnqvist et al. (2013) suggested an elution buffer be selected based on the specific situation with the consideration of factors such as the time elapsed between swabbing and sample analysis. Overall, eluent type can impact viral recovery, and thus eluent-tool combinations must be chosen with consideration of surface, virus, and eluent interactions for efficient surface sampling and recovery. Therefore, a matrix of elution buffers and when to apply given a certain situation or parameters would be a valuable resource.

2.3.5. Recovery tool options

The majority of tools used in laboratory-based studies for evaluation of surface sampling methods have focused on various types of swabs (Table 1). This finding comes as no surprise since swabbing is known as the gold standard for hNoV sampling and detection on FCS (ISO, 2017). Evaluation of swabs has shown varying recovery rates for enteric viruses; however, while the swab itself may be the primary driver in recovery, numerous other factors can play a role as indicated previously. More specifically, the material and properties of the recovery tool can impact recovery efficiencies. For example, the dying process of microfiber cloths can change its net surface charge, which could impact viral attachment and detachment from surfaces (Rönnqvist et al., 2013). Taku et al. (2002) suggested the selection of swabs are due to the ease of operation over small surface areas even though swabs yield consistently poor results in comparison to other methods evaluated, possibly due to surface area of the swab head and smearing virus over surfaces. Macrofoam, polyester-tipped, and/or cotton swabs have been shown to be more efficient among swabs tested in viral recovery from fomites depending on a given study's conditions and parameters (Ibfelt et al., 2016; Julian et al., 2011; Scherer et al., 2009). For instance, Julian et al. (2011) reported that polyester-tipped swabs recovered a greater amount of infectious MS2 than antistatic cloths. However, as indicated in Section 2.3.4, the elution buffer and tool combination complicates matters. For instance, Rönnqvist et al. (2013) reported that elution buffer type only impacted the recovery efficiency of microfiber cloths composed of polyester and polyamide materials where 50 mM glycine buffer (pH 9.5) performed better than PBS. Additionally, the authors reported better recovery of low inoculum hNoV GII.4 on latex surfaces when using polyester swabs, though it is unclear why. Unfortunately, it is difficult to compare swab types across studies due to differences among surface types, virus types, virus volume, and virus concentrations used for the evaluations of the swab sampling protocols.

3. Methods for recovery of enteric viruses from surfaces

As evidenced by Table 1, surface sampling methods used in the recovery of enteric viruses are highly variable and diverse. A majority of studies focus on swabbing for a variety of reasons. In fact, the International Organization of Standardization (2017) recommends hNoV sampling and detection on nonporous FCS to be collected with a cotton swab moistened with PBS followed by RNA extraction and reverse transcription – quantitative PCR (RT-qPCR) analysis. Other tools and methods such as repeated pipetting, cell scraping, and sonication/stomaching have been used for viral persistence and disinfection studies (Arthur and Gibson 2015; Mattison, 2011; Yeargin et al., 2015).

3.1. Outbreak sampling techniques – swabbing

Studies involving environmental surface sampling for applications in detecting viruses during outbreaks can be used as a baseline for standard surface sampling techniques for enteric viruses. Swabbing is the technique typically used for enteric virus studies involving applications in detection of viruses during outbreaks. Thus, studies have focused on evaluating swab protocols on surfaces associated with outbreaks such as on cruise ships and FCS (Table 1). Rönnqvist et al. (2013) evaluated four swab types (e.g. flocked nylon, cotton wool, microfiber, and polyester) in either PBS or glycine buffer at pH 9.5 for collecting hNoV GII.4 from stainless steel and plastic surfaces. Park et al. (2015) evaluated five swab types (e.g. cotton, rayon, polyester, antistatic cloth, and macrofoam) using hNoV GII.4 from stainless steel and toilet representative surfaces with macrofoam swabs producing the highest recovery efficiencies. During comparison of these two studies, microfiber performed better than macrofoam swabs with 79.0% ± 10.2% and 25.7% ± 10.6% recovery efficiency, respectively, when elution buffer (glycine buffer) and surface type (stainless steel) were the same. However, the amount and concentration of hNoV GII.4 varies between the two studies, and this could also impact recovery efficiencies as reviewed in Section 2.3.2. Rönnqvist et al. (2013) also provides information on using swabs on plastic surfaces. Overall, there is a need for more studies involving more viruses and nonporous surfaces to properly determine a standardized approach for surface sampling of enteric viruses during outbreaks.

3.2. Laboratory-based techniques for persistence and surface disinfection studies

Several different methods have been used to optimize recovery of enteric viruses from inanimate fomites in laboratory-based persistence studies. Furthermore, differences among the studies include virus types, volume and concentration of virus as well as tools, FCS, and type of analysis. In this subsection, we will further examine these differences and how they could contribute to the varying results of surface sampling method evaluation studies. Summaries of these studies are available in Table 1.

3.2.1. Swabbing

As stated in Section 3.1, swabbing has traditionally been the focus in studies on virus detection and persistence (Table 1). A few studies focused on evaluating one swab implement for use in recovering enteric viruses from a variety of surface types and virus inoculum levels. Scherer et al. (2009) evaluated a cotton swab with PBS (pH 7.2) elution buffer for collecting hNoV GII.3 and rotavirus from different FCS (i.e. stainless steel, ceramic, high-density polyethylene, and wooden chopping board) with recovery efficiencies ranging from 10.3 ± 13.0% (wood, 10^4 PCRU) to 51.9 ± 38.5% (ceramic, 10^5 PCRU) for GII.3 and 5.4 ± 1.5% (wood, 10^2 $TCID_{50}$) to 57.7 ± 25.9% (ceramic, 10^3 $TCID_{50}$) for rotavirus. The authors found recoveries for both hNoV and rotavirus to be higher from FCS than food surfaces at both inoculum concentrations (Scherer et al., 2009). Additionally, Ganime et al., (2015) evaluated the recovery rates of MNV-1 and bacteriophage PP7 from porous formic, non-porous formic, and rubberized surfaces using a rayon swab with culture media with recovery efficiencies ranging from 0.6 to 11.5% (PP7) and 12.2–77.0% (MNV-1). While these two studies evaluate how one particular swab performs, other studies expand their evaluations to provide a better comparison of different swabs and tools and their recovery of particular enteric viruses.

For example, Ibfelt et al. (2016) evaluated three different swabs (i.e. cotton, foamed cotton, and polyester) and two elution buffers (i.e. direct lysis or alkaline TGBE − pH 9.5) for recovery of hNoV GII.7 and mengovirus from 100 cm^2 laminated wooden surfaces. The authors found a significantly better virus recovery using polyester swabs with the direct lysis in comparison to other combinations tested; however, recovery efficiencies were ≤13% for all combinations. Ibfelt and others (2016) suggested their low recovery rates may be due to the size of the surface or differences in experimental design in comparison to other swab studies. Furthermore, Julian et al. (2011) also recommended the use of polyester swabs pre-moistened in either Ringer's or 0.85% saline solution for MS2 recovery from plastic and stainless steel surfaces following evaluation of three tools (cotton swab, polyester swab, and antistatic cloth) and four elution buffers (saline, Ringer's solution, viral transport media, and acid/base). Based on a meta-analysis of MS2 surface sampling, the authors noted that polyester swabs obtained significantly higher positive MS2 rates in comparison to rayon and cotton (Julian et al., 2011).

Conversely, De Keuckelaere et al. (2014) found cotton and polyester swabs to not be significantly different in their recovery efficiencies of hNoVs GI.4 and GII.4 from nitrile gloves, polyethylene, or neoprene rubber surfaces. Park et al. (2015) reported a similar result when evaluating the recovery efficiencies of four swab types (macrofoam, rayon, cotton, and polyester). The authors applied the different swabs for recovery of hNoV GII.4 from stainless steel and toilet representative surfaces and found that rayon, cotton, and polyester were not significantly different. However, macrofoam swabs obtained significantly higher recovery efficiencies of hNoV GII.4 in comparison to the other three swabs after 8 h of drying on a given surface (Park et al., 2015). Additionally, some studies found other tools and methods such as biowipes and cell scraper-aspiration methods to be potentially more efficient for enteric virus recovery from surfaces in comparison to cotton and/or polyester swabs. These studies are further examined in Sections 3.2.2 and 3.2.3 (De Keuckelaere et al., 2014; Taku et al., 2002).

3.2.2. Cloths and wipes

Cloths and wipes have also been introduced as possible alternatives to swabbing methods for obtaining higher recovery efficiencies of enteric viruses from surfaces. De Keuckelaere et al. (2014) evaluated two swabs (cotton and polyester) along with biowipes (Biomérieux, Lyon, France) composed of a mixture of fibers and microfibers (cotton, polyester, and polyamide fibers) moistened in PBS (pH 8.0) by recovering GI.4 and GII.4 hNoVs from FCS (high-density polyethylene, nitrile gloves, and neoprene rubber). There was no significant difference among any of the three tools evaluated based on recovery efficiency from polyethylene surfaces and nitrile gloves for hNoV GI.4. Meanwhile, the authors found significantly higher recovery efficiencies using biowipes (41.3 ± 12.4%) compared to cotton swabs (13.2 ± 5.2%) on the coarser rubber surface (De Keuckelaere et al., 2014). The authors also found that the mean recovery efficiency of biowipes for GI.4 from rubber surfaces was higher than using polyester swabs even though no significant difference was observed. For hNoV GII.4, there was no significant difference in recovery observed between all three tools tested on polyethylene surfaces and nitrile gloves even though the biowipes had significantly higher recovery efficiency (56.1 ± 12.5%) on rubber surfaces compared with both polyester (22.5 ± 8.7%) and cotton (16.9 ± 6.6%) swabs (De Keuckelaere et al., 2014). Another study further confirmed the effectiveness of these

biowipes in collecting hNoV GII.4 at various inoculum concentrations (10^2 to 10^6 PCRU) from stainless steel and ceramic FCS (Tung-Thompson et al., 2017). The authors reported a range of mean recovery efficiencies of GII.4 using biowipes (bioMerieux SA, Grenoble, France): 76.8–99.3% (stainless steel) and 42.4–96.6% (ceramic). It should be noted that recovery efficiencies reported by Tung-Thompson et al. (2017) were generally much higher than other studies included in Table 1.

However, a few studies showed certain swabs to be more efficient for recovery of enteric viruses than cloths. For example, macrofoam swabs had a higher recovery efficiency of hNoV GII.4 (7.08 ± 2.21%) from large (161.3 cm^2) stainless steel surfaces than antistatic cloths (0.33 ± 0.21%) (Park et al., 2015). Additionally, Julian et al. (2011) determined that polyester swabs obtained higher recoveries of infectious MS2 than antistatic cloths as well. Overall, cloths and wipes may be a valuable tool for collecting enteric viruses from FCS, and there is a need for further studies using cloths and wipes involving a greater variety of virus types, cloth types, surface types, and infectivity analyses.

3.2.3. Alternative methods for laboratory-based studies

Other surface sampling methods such as vortexing, repeated pipetting, stomaching/sonication, and cell scraping have been used for baseline information for viral persistence studies and disinfection studies (Table 1). The studies summarized in Table 1 use different surrogates, initial drying times, and elution buffers making it difficult to adequately compare the studies. Fallahi and Mattison (2011) recovered 37% of MNV-1 from stainless steel after a 20 min drying time using a repeated pipetting method with EBSS eluent. Mattison et al. (2007) recovered 11% of FCV from stainless steel after a 30 min drying time by vortexing for 30 s in EBSS eluent. Arthur and Gibson (2015) obtained recovery efficiencies of 10% and 30% for TuV from acrylic and stainless steel surfaces, respectively, after a drying time of 1 h using a cell scraping techniques. The cell scraping technique was confirmed as possible with TuV and has also been evaluated using FCV previously (Taku et al., 2002). Taku et al. (2002) found consistently better mean virus efficiencies for FCV using 50 mM glycine (pH 6.5) from stainless steel surfaces in comparison to 50 mM glycine (pH 9.5) and Modified Eagle's medium (pH 7.2) using the scraping-aspiration method. The mean FCV recovery efficiencies for the scraping-aspiration method from stainless steel were reported to be 42% (glycine pH 6.5), 28% (glycine pH 9.5), and 10% (Modified Eagle's medium). The authors suggested the modified Eagle's medium complex composition may have played a role in being less efficient than the glycine buffers (Taku et al., 2002). Taku et al. (2002) added cell scraping to the aspiration method for better recovery efficiencies speculating that cell scraping may facilitate release of virus from surface. In addition, Yeargin et al. (2015) recovered a range of 0.15% (cotton) to 35.22% (glass) for FCV and 0.85% (cotton) to 24.27% (glass) for MNV-1 from three surface types (i.e. polyester, cotton, and glass) using a stomaching/sonication method. The authors also found the recovery efficiencies to be highest for glass and lowest for polyester and cotton for both virus types. The recovery efficiencies were also reported to be significantly different among all surface types for the same virus type while only cotton swab recoveries showed a significant difference between MNV-1 and FCV (Yeargin et al., 2015). Similar to other techniques, more studies with inclusion of more virus types and standardized drying times are needed to provide information on using these alternative techniques for future persistence and environmental sampling studies.

4. Conclusions and recommendations

Surface sampling of enteric viruses varies across studies throughout the literature. This variability in results may exist due to varying human behavior, the tool used, and/or the elution buffer type used to recover the virus from the surface as well as numerous other factors outlined in the present review. Most surface sampling evaluations have focused on various swab types while there are limited studies focused on evaluation of other possible tools and techniques such as repeated pipetting and cell scraper application, historically used in a laboratory setting. As a result, food and environmental virology researchers may have difficulty in selecting the most appropriate surface sampling method for a particular study. Additionally, we found that no single standard approach to recover enteric viruses from FCS exists.

The following suggestions are based on our review to assist researchers in moving towards one standard methodology for optimizing the recovery of enteric viruses from fomite surfaces:

- Eluent buffer used to recover sample needs to be standardized.
- Concentrations and volumes of virus need to be more consistent and include standard low and high inoculum levels.
- The impact of organic materials on enteric virus recovery from surfaces needs further investigation.
- Infectivity assays such as plaque assays are highly recommended for the analysis of surface sampling optimization in order to distinguish infectious particles from non-infectious viral particles. However, this is currently only possible with cultivable viruses and hNoV surrogates.
- Results need to be reported in one standard form of measurement.
- More techniques and tools need to be evaluated along with the swab protocols and these evaluations should include of a variety of human enteric viruses and their surrogates.

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