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Evaluation of the swab sampling method to recover viruses from fomites

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The monitoring of virus contamination on fomites, especially at hospitals has been used for a more effective evaluation of the microbiological quality of surfaces. Swab sampling is the method used currently, although the use of an internal control process (ICP) has not yet been assessed. The aim of this study is to determine the recovery rate of murine norovirus 1 (MNV-1) and bacteriophage PP7 on different surfaces in order to assess their potential use as an ICP. For this purpose both viruses were spiked experimentally both on porous and non-porous formic as well as on rubberized surfaces. Quantitative PCR (qPCR) showed a variable efficiency with a percentage recovery ranging from 0.6 to 77% according to viruses and surfaces. A global analysis suggested that MNV-1 could be used as a potential ICP for the swab sampling method.

1. Introduction

The transmission of gastrointestinal and respiratory diseases through fomites especially for indoor environments such as schools, daycare centers, nursing homes and hospitals have been well reported in previous studies (Bean et al., 1982; Abad et al., 1994, 2001; Carducci et al., 2011; Ganime et al., 2012, 2014). The monitoring of surfaces is critical to highlight the spreading of viruses in hospital environment especially, thus contributing to elucidate nosocomial outbreaks by assessment of the microbiological quality of fomites, therefore helping to draw effective prevention measures and also appropriate patient management (Ganime et al., 2012).

The use of sensitive methods of viral detection, as well as obtaining suitable materials are crucial to recover from fomites, once they may be present in low concentration and distribute heterogeneously on the matrices (Rodríguez-Lázaro et al., 2012). The swab sampling assay associated to molecular methodologies are usually the first choice in this type of procedure, although the presence of inhibitors for molecular amplification procedures remains as a major challenge (Rodríguez-Lázaro et al., 2012). To overcome problems of inhibition for efficient monitoring which normally occur in environmental and food samples, studies to describe the use of bacteriophages and viruses as internal control processes (ICP) were carried out: MS2 (Dreier et al., 2005), P22 (Herzog et al., 2012) or PP7 (Rajal et al., 2007), Mengo virus (Uhrbrand et al., 2010) and murine norovirus 1 (MNV-1) (Wobus et al., 2006).

The aim of this study is to determine the recovery efficiency of bacteriophage PP7 and MNV-1 in samples obtained from rubberized (RB), porous formic (PF) and non-porous formic (NPF) surfaces using a swab sampling method associated to a quantitative amplification method (qPCR) to assess their potential use as an ICP.

2. Materials and methods

2.1. Viruses stock suspension

A bacteriophage PP7 (ATCC 15692-B2) provided by Dr Verónica Rajal (Salta University, Argentina) was replicated in *Pseudomonas aeruginosa* (ATCC 15692) using a protocol previously described (Rajal et al., 2007). The MNV-1 was propagated in RAW 264.7 cells as described previously (de Abreu Corrêa et al., 2012) and both, virus and cells, were kindly donated by Herbert W. Virgin, Department of Pathology and Immunology, Washington University School of Medicine (St. Louis, MO).

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Table 1
Primer and probe sets used to detect murine Norovirus 1 (MNV-1) and bacteriophage PP7 (PP7) by Quantitative amplification method (PCR).

Virus (Reference)	Primer or probe	Sequence (5′–3′)	Genome region
PP7 (Rajal et al., 2007)	247 F	GTTATGAACCAATGTGGCCGTTAT[a]	Replicase
	320 R	CGGGATGCCTCTGAAAAAAG	
	274 probe	FAM-TCGGTGGTCAACGAGGAACTGGAAC-TAMRA	
MNV-1 (Baert et al., 2008a, 2008b)	F – ORF1/ORF2	CAC GCC ACC GAT CTG TTC TG	ORF1-ORF2
	R – ORF1/ORF2	GCG CTG CGC CAT CAC TC	Junction region
	MGB – ORF1/ORF2	6FAM-CGC TTT GGA ACA ATG – MGBNFQ	

[a] International Union of Biochemistry code (W: A/T; R: A/G; B: C/G/T; Y: C/T; N: A/C/G/T); ORF: open reading frame; qPCR: quantitative PCR; RT: reverse transcription; F: forward. R: reverse.

2.2. Surfaces and swabs

RB, PF and NPF, with 63.6 cm^2 surfaces area were used for spiking experiments. Prior, the selected surface areas were washed and embedded overnight in Extran® 0.5% (Merck©, KGaA, Darmstadt, Germany), rinsed with sterile distilled water, dried at room temperature (22 ± 2 °C) and then left for 1 h under UV light.

Swabs used for recovering PP7 and MNV-1 were sterile, using the tip of rayon, and a plastic rod and packed individually (Alamar Técno Científica LTDA., Diadema, São Paulo, Brazil).

2.3. Nucleic acid extraction, cDNA synthesis and quantitative PCR (qPCR)

For nucleic acid extraction, the guanidinium thiocyanate-silica method (Boom et al., 1990) was adapted as described previously (Gallimore et al., 2004). The cDNA synthesis was conducted by RT using random primers (Prado et al., 2013). Quantitative PCR (qPCR) was performed to determine PP7 and MNV-1 concentrations (Rajal et al., 2007; Baert et al., 2008b). The sequence of primers and probes and the amplification region of the genome for both PP7 and MNV-1 quantification are described in Table 1.

2.4. Quantitative amplification method (qPCR) detection limit

Viral stocks and their 10-fold dilutions (10^2–10^5) were quantified in triplicate to evaluate the detection limit of each virus. To avoid cross-contamination, negative controls were included during all steps. To make sure that a specific test was carried out without cross contamination, PP7 samples were tested using the protocols for MNV-1 and vice versa.

2.5. Spiking experiments

For each experiment, MNV-1 and bacteriophage PP7 were inoculated separately in different dilutions of the three surfaces evaluated. Fifty μl of each 10-fold dilution of viral stocks (10^3–10^6) were spiked in triplicate at RB, PF and NPF using a biological safety cabinet class II (Bio IIA, Industrial TELSTAR, Terrassa, Spain). Three hours after incubation (necessary time to completely dry out viral stock dilutions inoculated on all surfaces), at room temperature (22 ± 2 °C) and relative ambient humidity of 50–80%, the swab dipped in the culture medium Dulbecco's Modified Eagle's Medium (DMEM–Gibco®, Grand Island, NY, USA) was rubbed over the surface for 10 s, stored in sterile tubes containing 1 ml of the DMEM and stored at −80 °C until molecular biology analysis.

2.6. Data analysis

The recovery of MNV-1 and bacteriophage PP7 was assessed both qualitative and quantitatively as described by Stals et al. (2011c). To evaluate the "recovery efficiency" (RE) and "recovery success rate" (RSR) of both viruses using a swab sampling method, identical sized of RB, NPF and PF surfaces were spiked artificially with different dilutions of the viral stocks.

Qualitative analysis was performed by comparing the number of samples with signal amplification (positive) using real-time PCR and the total number of PCR reactions performed in real time. Quantitative analysis was performed by comparing the average number of genome copies in samples recovered with the number of genome copies inoculated therein. The quantitative and qualitative analyses were expressed, respectively, as RE and RSR.

Statistical analysis was performed using the chi-square test as implemented by Epi info® software, version 3.5.1. Significance levels were set at 0.05.

3. Results

To evaluate the recovery of MNV-1 and PP7 bacteriophage using the swab sampling method, qPCR detection limits for both viruses were determined and RB, PF and NPF surfaces were spiked artificially with both viruses. qPCR detection limits for the PP7 were of 6.4E−01 to 4.2E+02 gc/mL with an average cycle threshold value (Ct) of 37; for the MNV-1, qPCR detection limits were of 4.3E+02 to 1.8E+03 gc/mL with an average value of 36.08 Ct (Table 2).

PP7 and MNV-1 were recovered in 100% of the contaminated surface samples (RB, PF and NPF), although the efficiency ranged from 0.6 to 11.5% and from 12.2 to 77.0%, respectively, according to the surface tested (Table 3). No significant difference was observed between the surfaces ($P > 0.05$) for both viruses.

4. Discussion

Fomites and surfaces can be important transmission means of viral diseases. The exposure occurs through touch and transfer of pathogens present on the fomite to the hands and then to the mouth, nasopharynx, and eyes (Abad et al., 1994, 2001; Carducci et al., 2011; Herzog et al., 2012). The detection of viruses on a large variety of surfaces and fomites, such as tables, door knobs, walls, toilets seats, thermometers, gloves, among others (Boone and Gerba, 2007; Ganime et al., 2012) has helped to demonstrate this route of transmission in sporadic cases and also in outbreaks (Ansari et al., 1988, 1991; Gallimore et al., 2004, 2005, 2006, 2008; Wu et al., 2005; Boxman et al., 2009). However, the method for recovering viruses from surfaces is not an easy task, mainly due to its heterogeneous distribution, as well as the low viral load and the eventual presence of inhibitors in these surfaces (Rodríguez-Lázaro et al., 2012). In this study RB, PF and NPF matrices were chosen to evaluate ICP in the swab sampling method once they represent almost all hospital surfaces which the hands come in contact with. The variability in the virus recovery efficiency from these surfaces was also demonstrated previously in different materials such as stainless steel, ceramic plates, polyethylene and wood surfaces (Carducci et al., 2002; Scherer et al., 2009). However, data from these experimental studies using different surfaces and viruses do not allow any comparison among recovery rates.

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