

# Application of a Swab Sampling Method for the Detection of Norovirus and Rotavirus on Artificially Contaminated Food and Environmental Surfaces

K. Scherer · D. Mäde · L. Ellerbroek · J. Schulenburg ·  
R. Johne · G. Klein

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**Abstract** Noroviruses and rotaviruses are the leading causes of non-bacterial gastroenteritis in humans worldwide. Virus-contaminated food and surfaces represent an important risk to public health. However, established detection methods for the viruses in food products are laborious and time-consuming. Here, we describe a detailed swabbing protocol combined with real-time RT-PCR for norovirus and rotavirus detection on artificially contaminated food and environmental surfaces. Recovery rates between 2 and 78% for norovirus and between 8 and 42% for rotavirus were determined for contaminated food surfaces of apple, pepper, cooked ham and salami. From contaminated environmental surfaces (stainless steel, ceramic plate, polyethylene, wood), recovery rates between 26 and 52% (norovirus) and between 10 and 58% (rotavirus) were determined. The results demonstrate the suitability of the swab sample method for virus detection on food and environmental surfaces. Compared to other methods, it is easy to perform

and significantly time-saving, predestining it for routine testing.

**Keywords** Norovirus · Rotavirus · Detection · Food · Environment · Real-time PCR

## Introduction

Noroviruses and rotaviruses are the leading cause of non-bacterial gastroenteritis worldwide. Norovirus gastroenteritis occurs in all age groups, whereas rotavirus mainly causes acute gastroenteritis in children. However, there is increasing evidence of clinically significant rotavirus infections in adults (Jansen et al. 2008). Outbreaks commonly occur in closed community situations with shared food and water sources, and the use of common facilities contributes to virus transmission (Hedlund et al. 2000; Keswick et al. 1983). Food-borne transmission accounts for a large proportion of norovirus outbreaks in many countries (Lopman et al. 2003). Mead et al. (1999) estimated that 67% of food-borne illness in the US is caused by viruses including hepatitis A virus, noroviruses, rotaviruses and that 40% of norovirus infections are food-borne. Noro- and rotaviruses are mainly transmitted through the faecal–oral route, either directly from person to person or indirectly via contaminated food, water, and surfaces (Ansari et al. 1991; Daniels et al. 2000; Gallimore et al. 2005; van Zyl et al. 2006). Both viruses are shed in large numbers in faeces of infected individuals ( $10^5$ – $10^{11}$  particles/g stool) (Bosch 1998) and are able to persist on environmental surfaces for a prolonged period (Cheesbrough et al. 1997; Sattar et al. 2001). The minimal infective dose of norovirus is believed to be as low as 10–100 virus particles (Caul 1996). For

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K. Scherer (✉) · G. Klein  
Institute of Food Quality and Food Safety,  
University of Veterinary Medicine Hannover, Bischofsholer  
Damm 15, 30173 Hannover, Germany  
e-mail: kathrin.scherer@bfr.bund.de

D. Mäde  
State Laboratory for Consumer Protection, Saxony-Anhalt,  
Freimfelder Str. 66/68, 061112 Halle, Germany

L. Ellerbroek · R. Johne  
Federal Institute for Risk Assessment, Berlin,  
Diedersdorfer Weg 1, 12277 Berlin, Germany

J. Schulenburg  
Central Institute of the Bundeswehr Medical Service,  
Kopperpähler Allee 120, 24119 Kiel, Germany

rotavirus, a minimal infectious dose of one cell culture-infective unit has been determined (Ward et al. 1986).

Viral contamination of food and water represents an important threat to public health. Many different types of food are implicated in food-borne outbreaks; however, shellfish, fruits, vegetables and cold foods are considered to be the most important. Fresh produce may be contaminated through the use of contaminated irrigation and wash water, but infected food handlers involved in the preparation and processing of food as well as contact with contaminated surfaces contribute to viral contamination (Anderson et al. 2001; Seymour and Appleton 2001). It could be assumed that the number of viral food-borne outbreaks far exceeds the number currently being reported. One reason for the failure to confirm the involvement of a viral agent is the lack of sensitive and reliable methods for the detection of viruses in food and contaminated surfaces (Svensson 2000; Koopmans and Duizer 2004). In addition, virus concentration in food and environmental samples may be too low for detection.

Low virus concentration, heterogeneous distribution of viruses in the samples and the presence of substances that may inhibit molecular detection make the identification of viruses in food and environment difficult. Currently used molecular methods for virus detection in food are often too expensive and too time-consuming for routine testing of foods (Lopman et al. 2002). Efforts to cultivate human norovirus have been reported (Straub et al. 2007). However, the method is difficult to reproduce. If virus contamination is the result of improper food handling and/or improper hygiene measures, a superficial contamination can be assumed in most food items. Rapid and sensitive methods for virus recovery from food and environmental surfaces are necessary to identify the source of infection helping to better understand viral transmission routes and outbreak dynamics (Bresee et al. 2002).

In our study, we tested the application of a swab sampling method combined with real-time RT-PCR for the detection of norovirus and rotavirus on artificially contaminated food and environmental surfaces. Comparison of the rates of virus recovery obtained by this method with those of other published methods will enable selection of an appropriate detection method dependent on food and surface type.

## Materials and Methods

### Virus Stocks

A stool sample containing norovirus genogroup (GG) II.3, isolated from a child suffering from enteric symptoms, was diluted to a 10% suspension in phosphate-buffered saline

(PBS, pH 7.2) and glycerol (10%). The amount of norovirus RNA in the virus stock was estimated by real-time PCR unit endpoint dilution as described by Richards et al. (2004). One real-time RT-PCR detectable virus unit (RT-PCRU) was defined as the highest dilution of the sample showing a  $C_T < 45$ . According to this, the 10% faecal stock contained approximately  $2 \times 10^7$  RT-PCRU per ml.

Rotavirus stock (bovine strain 4630, kindly provided by P. Otto, FLI, Jena, Germany) was propagated in MA 104 (African Green Monkey Kidney) cells using Eagle's minimum essential medium (MEM) (Gibco-Invitrogen, Carlsbad, CA) supplemented with 10% foetal calf serum, gentamicin (100 mg/ml), L-glutamine, non-essential aminoacids and trypsin (100 µg/ml) (Serva, Heidelberg, Germany) as described by Elschner et al. (2005). Cell debris was removed by centrifugation at  $1000 \times g$  for 10 min and the virus-containing supernatant was stored in 1 ml aliquots at  $-80^\circ\text{C}$  until use. Titration of the rotavirus was performed by an endpoint dilution method based on the occurrence of a cytopathogenic effect in MA-104 cells. By this, a titre of  $2 \times 10^4$  tissue culture infectious dose (TCID<sub>50</sub>)/ml was determined for the stock solutions used. The corresponding amount of rotavirus RNA in RT-PCR detectable virus unit (RT-PCRU) was determined as described above. According to this, the rotavirus stock contained approximately  $2 \times 10^5$  RT-PCRU per ml.

### Inoculation of Food Samples and Environmental Surfaces

Virus stock solutions and 1:10 dilutions in PBS were divided into 100 µl portions and inoculated on the surface of each 10 cm<sup>2</sup> food [outer surface of unsliced cucumber, apple, pepper, slices of cooked ham ("Gourmet Aufschnitt", Willms Fleisch GmbH, Germany) and pork salami ("1a Salami", Gut Bartenhof, Germany) and environmental surface commonly used during food preparation (stainless steel tray (SS 301), glazed ceramic plate (FC 30), high-density polyethylene (PE), wooden chopping board (maple)], and allowed to dry for 15 min in a laminar flow hood. Samples inoculated with 100 µl PBS were used as negative controls. Each experiment was repeated three times on different days for both norovirus and rotavirus.

### Virus Elution

A surface area of 10 cm<sup>2</sup> food and environmental sample was swabbed methodically with a sterile cotton swab (wood/cotton tipped, vwr, Darmstadt, Germany) moistened by dipping into PBS in a horizontal, vertical and diagonal direction five times each. The swab was turned to expose the whole swab during movement across the contaminated surface. Virus particles adhering to the swab were eluted by

three times pressing of the cotton into 500  $\mu$ l PBS. The same swab was used to repeat surface swabbing and virus elution two times.

### Viral RNA Extraction

The QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) was used to extract viral RNA from the norovirus stool sample and from the rotavirus stock used for inoculation as well as for RNA extraction from the eluted swab samples according to the manufacturer's instructions. In order to facilitate comparison between inoculation dose and virus recovery, 100  $\mu$ l of each swab sample eluate or virus suspension used for inoculation was mixed with 40  $\mu$ l PBS to obtain a sample volume of 140  $\mu$ l required for RNA extraction. Viral RNA was purified by adding extraction buffer AVL and ethanol following application to the QIAamp spin column. After two washes with buffers AW1 and AW2, RNA bound to the silica was eluted with 60  $\mu$ l elution buffer (AVE).

### Real-Time RT-PCR and Calculation of Virus Recovery Rate

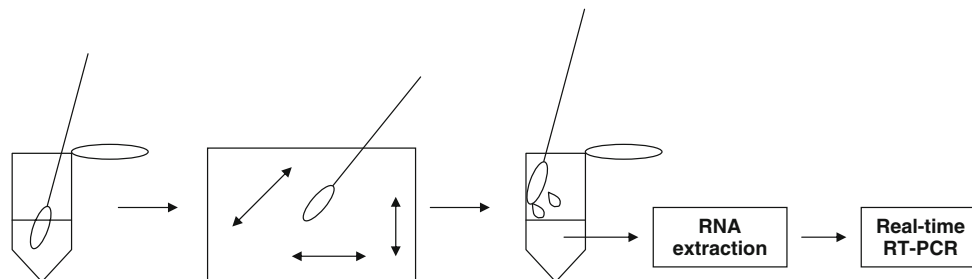
Real-time RT-PCR amplification reactions were performed using the QuantiTect Probe RT-PCR kit (Qiagen, Hilden, Germany) on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). For norovirus GGII detection, we used a TaqMan real-time RT-PCR assay described by Hühne and Schreier (2004). The real-time RT-PCR protocol used for the detection of rotavirus was done as previously described by Pang et al. (2004). Genome copy numbers were calculated on the basis of standard RNA curves for both norovirus and rotavirus. The suspensions that were used to inoculate food and environmental samples were analysed in parallel to the swab samples to determine the recovery rate of the swab method. Virus recovery rate (%) was calculated by the following formula: (genome copy number determined for

the eluate)  $\times 5$  / (genome copy number determined for the inoculum)  $\times 100$ . Presence of molecular detection inhibiting substances in the sample eluates was tested per matrix (food, surface) for both norovirus and rotavirus, by comparing  $C_T$  values of undiluted RNA to the respective 1:4 dilution. Samples showing a  $\Delta C_T$  between 2 and 2.5 were considered as inhibitor free.

### Results

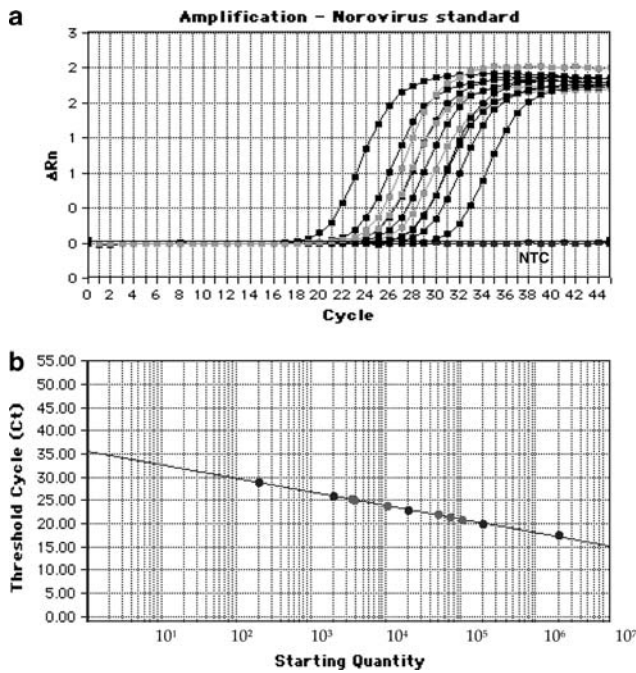
A total of five different types of food and four different environmental materials were inoculated onto 10 cm<sup>2</sup> of their surface with norovirus and rotavirus using two inoculation doses. The swab sample method used for virus detection is schematically shown in Fig. 1. Figure 2a shows an amplification plot used for the detection and quantitation of norovirus RNA isolated from selected swab samples as a typical example. The norovirus standard curve derived from this experiment is given in Fig. 2b. No inhibitors could be detected in the sample eluates by comparison of the  $C_T$  values derived from undiluted RNA and from a 1:4 dilution (data not shown). The mean recovery rates of three independent tests  $\pm$  the respective standard deviation (SD) of norovirus and rotavirus for the different types of food and environmental surfaces were calculated and are shown in Figs. 3 and 4, respectively.

Maximum recovery of norovirus from food surfaces at both inoculum levels could be detected with cucumber ( $77.9 \pm 6.7\%$  and  $31.6 \pm 9.8\%$  for  $2 \times 10^5$  and  $2 \times 10^4$  RT-PCRU, respectively), followed by salami ( $48.3 \pm 23.1\%$  and  $28.0 \pm 12.7\%$ ), apple ( $38.6 \pm 22.8\%$  and  $23.8 \pm 4.8\%$ ) and pepper ( $22.2 \pm 15.9\%$  and  $20.5 \pm 19.2\%$ ). Minimum recovery was observed for ham ( $1.5 \pm 1.3\%$  and  $2.4 \pm 2.1\%$ ). For environmental surfaces, highest percentage of norovirus was recovered at both inoculum levels with ceramic ( $51.9 \pm 38.5\%$  and  $31.0 \pm 18.7\%$  for  $2 \times 10^5$  and  $2 \times 10^4$  RT-PCRU, respectively), followed by PE ( $33.1 \pm 23.3\%$  and  $25.8 \pm 23.0\%$ ), and stainless steel ( $28.0 \pm$



**Fig. 1** Schematic presentation of the swab sampling method. The swab is moistened in PBS and used for swabbing a defined area methodically in a horizontal, vertical and diagonal direction. Virus is

eluted from the swab by repeated pressing of the cotton into PBS. Thereafter, RNA is isolated from the eluate and viruses are detected by real-time RT-PCR

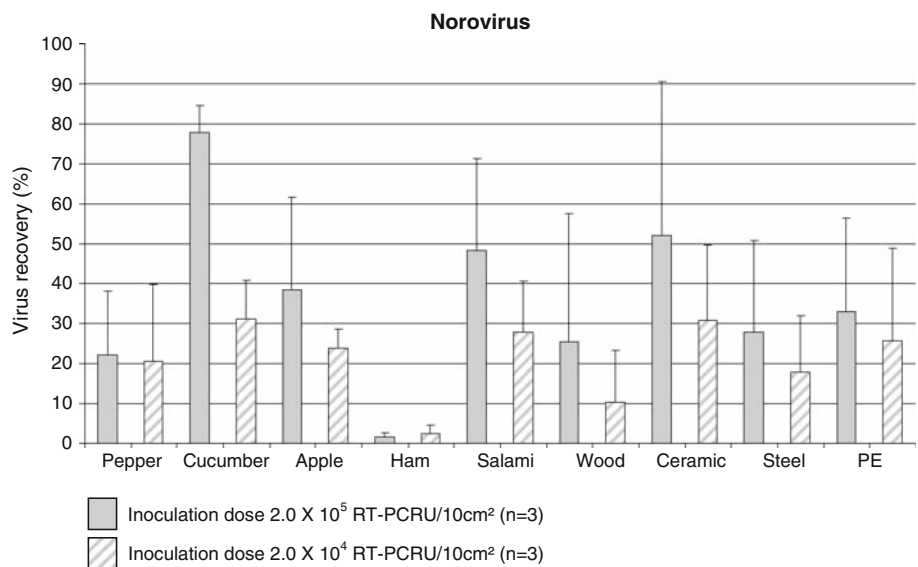


**Fig. 2** Detection and quantitation of norovirus RNA isolated from selected swab samples. **a** Amplification plot of ten-fold serial diluted norovirus stock solution and samples. **b** Norovirus standard curve generated using the data of (a). Slope:  $-2.9$ , Y-intercept:  $35.6$ , Correlation coefficient:  $0.999$ . Grey circle: Sample values; Black circle: Standards; NTC Negative control

$22.6\% \pm 18.0 \pm 14.0\%$ ). Lowest percentage of norovirus recovery was observed with wood ( $25.5 \pm 32.0\%$  and  $10.3 \pm 13.0\%$ ).

Rotavirus recovery rate for food surfaces was highest for salami ( $42.0 \pm 27.0\%$  and  $28.7 \pm 8.2\%$  for  $2 \times 10^3$  and  $2 \times 10^2$  TCID<sub>50</sub>, respectively), followed by apple ( $31.4 \pm 13.2\%$  and  $15.3 \pm 12.8\%$ ), cucumber ( $28.6 \pm 22.7\%$  and

**Fig. 3** Mean recovery rates  $\pm$  SD of norovirus from food and environmental surfaces after two different inoculation doses using the swab sampling technique



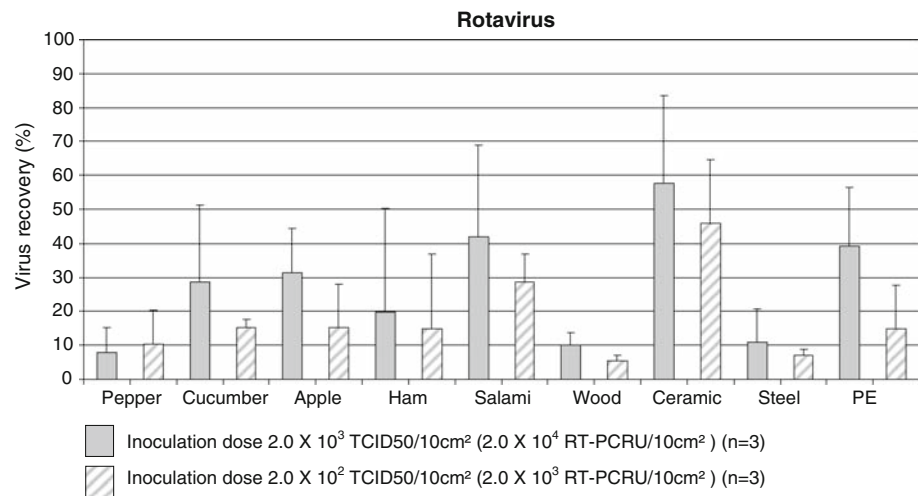
$15.1 \pm 2.6\%$ ) and ham ( $19.9 \pm 30.4\%$  and  $14.8 \pm 22.0\%$ ). The lowest recovery rate was attained for pepper ( $8.0 \pm 7.3\%$  and  $10.4 \pm 10.0\%$ ). Maximum recovery of rotavirus for environmental surfaces at both inoculum levels could be detected with ceramic ( $57.7 \pm 25.9\%$  and  $45.9 \pm 18.6\%$  for  $2 \times 10^3$  and  $2 \times 10^2$  TCID<sub>50</sub>, respectively), followed by PE ( $39.3 \pm 17.0\%$  and  $14.8 \pm 12.8\%$ ), and stainless steel ( $11.1 \pm 9.6\%$  and  $7.0 \pm 1.7\%$ ). The lowest recovery rate was observed with wood ( $10.2 \pm 3.6\%$  and  $5.4 \pm 1.5\%$ ).

**Discussion**

Many reports of disease outbreaks indicate that in addition to vehicular transmission of viruses through contaminated environmental surfaces, contamination of food and water significantly contribute to virus spread (Koopmans 2008; Said et al. 2008). Previous studies have suggested an environmental persistence of norovirus, as viral RNA has been detected on environmental surfaces such as sinks, commodes and carpets for several days after initial contamination (Liu et al. 2003). For rotavirus, similar environmental stability was shown (Abad et al. 1994) and infectious particles could be recovered from several environmental surfaces (Butz et al. 1993).

Conventionally used methods for virus detection in food are mainly based on elution concentration, ultrafiltration or ultracentrifugation procedures and combinations of these systems (Baert et al. 2008; Butot et al. 2007b; Dubois et al. 2007; Rzezutka et al. 2006). Various protocols of virus extraction, concentration and detection have been published so far. Most of them are laborious and time consuming since multiple steps and various reagents are included and results of virus recovery diverge depending on the food matrices

**Fig. 4** Mean recovery rates  $\pm$  SD of rotavirus from food and environmental surfaces after two different inoculation doses using swab sampling technique



and the methods used (Boxman et al. 2007; Le Guyader et al. 2004; Rutjes et al. 2006; Sair et al. 2002). Here, we show that the swab sampling method is rapid and simple to perform and can be applied to various food surfaces. Using this method, recovery rates of food surfaces ranged from 2% (ham) to 78% (cucumber) and from 8% (pepper) to 42% (salami) for norovirus and rotavirus, respectively. As direct comparison of virus recovery rates obtained by the presented swabbing method with those reported by the use of other protocols may be difficult due to different sampling procedures, different calculations of recovery rates as well as different virus strains used. Park et al. (2008) recovered 14% of a norovirus GGII strain from artificially contaminated strawberries using an immunomagnetic separation technique. Dubois et al. (2002) reported recovery rates of 13% for a norovirus strain from frozen raspberry surfaces using an elution-concentration method. Since food is being contaminated indirectly through virus-shedding persons, through contaminated surfaces or by contaminated water, one can assume that viral particles are mainly present on the food surface. Thus, wiping of contaminated surfaces by using swabs is a sensible technique to monitor viral contamination. Moreover, inhibitors in the sample eluate may be reduced as swabbing is more gentle compared to homogenising or rinsing methods, especially since food matrix remains intact.

For virus detection on environmental surfaces, the recommended procedure and the technique most commonly applied is based on the swab-rinse technique (Favero et al. 1968). Studies have shown the swab-rinse technique to be suitable for hepatitis B virus detection on environmental surfaces. Using a swab protocol for norovirus detection on environmental surface, Mäde et al. (2005) were able to detect as little as 3 RT-PCR/10 cm<sup>2</sup> glass or paper. Moreover, they detected norovirus in environmental samples during an outbreak investigation using this method. In

our study, recovery rates of environmental surfaces ranged from 26% (wood) to 52% (ceramic) and from 10% (wood) to 58% (ceramic) for norovirus and rotavirus, respectively, confirming the applicability of this method for environmental samples.

The diverging recovery rates of norovirus and rotavirus observed with different surfaces may be due to different abilities of viruses to adhere to the respective surface. Virus recovery of both norovirus and rotavirus was higher from environmental surfaces than from food surfaces at both inoculum levels and varied depending on the respective matrix. Factors such as virus type, pH, ionic concentration, surface charge, and organic matter are thought to be responsible for virus attachment to surfaces (Dowd et al. 1998; Grant et al. 1993; Redman et al. 1997). Electrostatic interactions, van der Waals forces and hydrophobic effects are assumed to be involved in the interactions between virus particles and solid substrates (Vega et al. 2005, 2008). Vega et al. (2008) stated that the primary force of virus adsorption to lettuce is based on electrostatic interaction; moreover, they showed that virus attachment to lettuce surface varies amongst virus types (Vega et al. 2005). Butot et al. (2007a) reported that adsorption of enteric viruses in bottled water depends on several factors such as virus type, chemical composition of water and presence of autochthonous bacteria. The low recovery rate of norovirus from ham compared to that of rotavirus determined in our study may therefore be explained by different virus properties which affect virus attachment to surfaces.

It is obvious that physical properties of the surface could further reduce recovery as virus particles are trapped within the matrix, especially if the surface is porous, and that smooth surfaces facilitate virus recovery. This may serve as an explanation for the high virus recovery from ceramic having a hard and smooth surface compared to wood where



crevices and pores may entrap virus particles resulting in possibly incomplete removal of viruses from the surface. In a previous study, Buttner et al. (2007) observed similar results as greater recovery rates from smooth nonporous material, such as glass and metal via surface swabbing, were obtained although the test organism and analysis methods were different.

Bidawid et al. (2004) suggested that at least 50–80 infectious viral units could be transferred to food via contaminated hands which may be sufficient to initiate infection in susceptible individuals. Therefore, it is important that the method used is sensitive enough to detect low levels of virus. Regarding results of different virus inoculation doses, recovery for both norovirus and rotavirus was more efficient at higher inoculum levels except for ham (norovirus) and pepper (rotavirus), indicating a decreased sensitivity of the swab method if contamination level is low. It should be kept in mind that at low inoculation levels the statistical error rises, and, in addition, the reverse transcription becomes less effective. Results of our study show that the swab sampling method revealed a remarkable variability of recovery rates as evidenced by the relatively high standard deviation (SD) values. This is consistent with previous studies where the swab sampling method revealed high SD values relative to the mean recovery rate (Brown et al. 2007; Rose et al. 2004). This fact may be due to errors inherent to the sampling mechanism itself, e.g. swab material, surface composition and mechanical removal action, as well as collecting and processing errors. In addition, inhomogeneous surface deposition and variable attachment of virus to the surface may introduce errors. Also the relative low number of repetitive experiments must be taken into consideration. It is difficult to standardise the sampling technique as the speed of sampling and the pressure applied to the swab during sampling varies individually and may lead to great variability in results (Moore and Griffith 2007). Further research is needed, especially whether different swab material and different methods for the elution of viruses from the swabs can increase virus recovery.

In conclusion, the swab sample method is suitable for the detection of norovirus and rotavirus from hard-surfaced food and environmental surfaces. It is easy to perform and significantly time-saving when compared to other methods. However, chemical and physical surface properties as well as virus type may affect recovery efficiency. Regarding the interpretation of swab sample data, one should consider that positive swab samples indicate surface contamination implying a potential risk of exposure whereas negative swab samples do not completely assure absence of infectious particles and hence absence of the potential risk of exposure.

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