Semi-direct lysis of swabs and evaluation of their efficiencies to recover human noroviruses GI and GII from surfaces

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# Abstract

Enteric viruses such as noroviruses (NoV) continue to be the cause of widespread viral outbreaks due to person-to-person transmission, contaminated food and contaminated surfaces. In order to optimize swabbing methodology for the detection of viruses on (food) contact surfaces, three swab elution/extraction strategies were compared in part one of this study of which one strategy was based on the recently launched ISO protocol (ISO/TS 15216-1) for the determination of hepatitis A virus and NoV in food using real-time RT-PCR (RT-qPCR). These three swab elution/extraction strategies were tested for the detection of GI.4 and GII.4 NoV on high density polyethylene (HD PE) surfaces with the use of cotton swabs. For detection of GI.4 and GII.4, the sample recovery efficiency (SRE) obtained with the direct lysis strategy (based on ISO/TS 15216-1) was significantly lower than the SRE obtained with both other strategies. The semi-direct lysis strategy was chosen to assess the SRE of two common swabs (cotton swab and polyester swab) compared to the biowipe (Biomérieux, Lyon, France) on three surfaces (HD PE, neoprene rubber and nitrile gloves). For both surfaces HD PE and nitrile gloves, no significant differences in SRE of GI.4 and GII.4 NoV were detected between the three different swabs. For the coarser neoprene rubber, biowipes turned out to be the best option for detecting both GI.4 and GII.4 NoV.

**Keywords**: norovirus, swab, environment, real-time RT-PCR, neoprene rubber, high density polyethylene, gloves

# 1. Introduction

Monitoring surface hygiene is a well known quality control measurement within the food industry. Surfaces are not only swabbed for the traditional hygiene assessment (based on total aerobic count), but also for the detection/quantification of bacterial pathogens, allergens, and ATP bioluminescence as an alternative measure for surface hygiene, and viruses (Moore and Griffith 2002; Wang et al. 2010; Daelman et al. 2013; Boxman et al. 2011). Environmental surfaces are in fact a well known transmission route for (foodborne) viral outbreaks (Boone and Gerba 2007; Cheesbrough et al. 2000; Isakbaeva et al. 2005; Patterson et al. 1997; Stals et al. 2013).

Recent studies have been positive about the use of environmental swabs for the detection of enteric viruses in food producing areas and healthcare centers (Boxman et al. 2011; Carducci et al. 2011; Wu et al. 2005). While detection methods for viruses have only recently become available in the area of food safety, in medical healthcare the usage of swabs for the detection of viruses is a well established tool to take clinical samples (Green et al. 1998, Nakanishi et al. 2009) and surface samples in hospital settings (Carducci et al. 2002, 2011; Wu et al. 2005). The use of swabs on food contact surfaces for the detection of (pathogenic) bacteria is well known and has resulted in the International Standard ISO 18593, describing surface sampling methods for the detection or enumeration of bacteria in food processing area and equipment (Anonymous 2004) and continues to be a topic of further research as different swabs and (food) surfaces remain to be tested (Moore and Griffith 2007; Hedin et al. 2010; Lutz et al. 2013). However, only a limited amount of studies regarding the recovery of enteric viruses (or surrogates) on food (contact) surfaces has been published (Scherer et al. 2009; Taku et al. 2002; Julian et al. 2011; Jones et al. 2012; Rönnqvist et al. 2013) and a new ISO protocol (ISO/TS 15216-1) (Anonymous 2012) has only recently been adopted as a technical specification with a sampling procedure for the detection of hepatitis A virus and norovirus on food surfaces.

As such, the presented study (1) provides new data on the comparison of different swab elution/extraction strategies, of which one closely resembles the protocol suggested in the ISO/TS, and (2) provides data on the efficiency of three different swabs for environmental sampling of enteric viruses on different surfaces frequently found at the farm level during harvesting (gloves, transport rubber bands, plastic crates) using the semi-direct lysis method (strategy 3) which is similar as the one recently described by Rönnqvist et al. (2013). In contrast to other studies, no NoV surrogate viruses were used for determining the sample recovery efficiency (SRE) (Julian et al. 2011; Taku et al. 2002; Herzog et al. 2012) on different surfaces. As actual quantitative data on norovirus detection on surfaces by swabbing is scarce and comparing data from one swab study to another is challenging due to the large number of parameters influencing the SRE, this study aimed to compare three swabs (cotton swab, polyester swab and biowipes) in their efficiency for the detection of GI.4 and GII.4 noroviruses (NoV) on three different test surfaces (neoprene rubber, high density polyethylene and nitrile gloves).

# 2. Material and methods

### 2.1. Surfaces and swabs

The three surfaces used in this study were high density polyethylene (HD PE), neoprene rubber (NR) and powder free blue nitrile (PF 240) gloves (GL) (Shield Scientific, B.V., Malaysia). These surfaces are likely to come in contact with fresh produce during harvesting or further processing. For the HD PE and neoprene surfaces, areas of 100 cm² were denoted and prior to each experiment these surfaces were decontaminated by the use of antiviral RBS Viro spray (Sigma Aldrich, Steinheim, Germany) and soaking (10 min) and rinsing with boiling water. In case of the nitrile gloves, areas of 25 cm² were marked after the insertion of a piece of carton in the glove and administration of clamps to keep the surface in a stretched position. No decontamination prior to the inoculation experiment was performed as each glove was only used once.

The three sterile swabs under study were the cotton swab (150C) (Copan, Italy), the polyester swab (159C) (Copan, Italy) and the recent biowipe (Biomérieux, Lyon, France). Biowipes (2.5 by 3.5 cm) (Biomérieux) are composed of a mixture of fibers and microfibers (cotton, polyester and polyamide fibers) wetted in PBS buffer (pH 8.0).

### 2.2. Virus stocks

Both NoV GI.4 and GII.4 stool samples were kindly provided by the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). 10% suspensions in phosphate-buffered saline (PBS, pH 7.2, Lonza, Verviers, Belgium) of both stocks were diluted (final dilution was approx. 1% of the initial stool sample for each pathogen) and eventually mixed until a stock concentration of approx. 3 x 104 genomic copies of GI.4 NoV/100 µl and approx. 9 x 104 genomic copies of GII.4 NoV/100 µl was obtained for experiment part 1 in which different swab elution/extraction strategies were compared in their SRE of NoV on HD PE. In part 2, in which the SRE of different swabs was obtained for the detection of NoV on different surfaces, the stock concentration contained approx. 2 x 105 genomic copies GI.4 NoV/100 µl and approx. 1 x 105 genomic copies GII.4 NoV/100 µl. Viral stocks were quantified by molecular methods using a standard curve as described in paragraph 2.4. Aliquots of this stock concentration were stored at -80°C until use.

### 2.3. Study design

Demarcated areas on each surface were spiked with 100 µl NoV suspension in case of HD PE and NR and 25 µl in case of the nitrile gloves (GL). The viral stock solution was administered in little droplets on the surface and smeared all over the surface with a sterile inoculation pin, after which the surface was allowed to dry for 45 min in a biosafety cabinet.

During one experiment 12 surfaces of the same material (either HD PE, NR or GL) were spiked on three subsequent inoculation times (3 batches). For each batch one random surface was spiked with NoV free PBS, which acted as negative control surface, and three surfaces were spiked with the described viral stock solution. In one batch, each of the three types of swab elution/extraction strategies or types of swabs was used to randomly analyze one of the three spiked surface. Each surface was swabbed in three directions, horizontal, vertical and diagonal, in such a way that the whole surface came each time in contact with the whole swab. During one experiment each swab elution/extraction strategy or type of swab was used to analyze one of the three surfaces used as negative control surface and 100 µl of the spike was extracted for calculation of the SRE.

#### 2.3.1. Set-up Part 1: Comparison of different swab elution/extraction strategies

For the comparison of different swab elution/extraction strategies, the cotton swabs were used on 100 cm² HD PE surfaces. During each of the three batches one surface was swabbed according to each of the following strategies. *Strategy 1*: the swab was moisturized only once in the beginning in PBS and the demarked area was swabbed according to the above stated protocol. Afterwards the used swab was stored in an empty 15 ml centrifuge tube on ice until the lysis step and the RNA extraction process. *Strategy 2*: the swab was moisturized each time before swabbing the surface in a different direction (horizontally, vertically, diagonally) by dipping in 1.0 ml PBS in a 15 ml centrifuge tube and afterwards removing excess liquid by pressing the swab against the wall. This dipping and pressing cycle was repeated each occasion three times. At the end of the swabbing the swab was eluted in the PBS by 60 sec vortexing and pressing the swab against the wall to remove excess liquid. The swab was removed and the swab eluate (in the 15 ml tube) was stored on ice until the lysis step. *Strategy 3*: likewise as in strategy 2, the swab was moisturized before swabbing the surface in each of the three directions. This time 0.5 ml of PBS was used in a 15 ml centrifuge tube. After swabbing the swab was stored in the liquid, on ice until the lysis step.

The maximum storage time of the samples on ice was approx. 2.5 h, before the start of the lysis step. The lysis step for all three strategies comprised of the adding of the NucliSENS easyMAG lysis buffer (BioMérieux, Boxtel, The Netherlands), which was respectively 3 ml, 2 ml and 2.5 ml in case of strategy 1, 2 and 3, and subsequent incubation of 10 min at room temperature after a short mixing by vortexing. The lysis step took place immediately after the swabbing of the 12 surfaces during one experiment. For strategy 1, this lysis step resulted in a direct lysis method of the swab itself which is in accordance with the protocol suggested in the recently released ISO method for the detection of NoV in food using real-time RT-PCR (ISO/TS 15216-1:2012(E)). The exact protocol as stated in the current ISO/TS method was not included as this experiment predates the arrival of the ISO/TS method. Although, this protocol only diverged a small part compared to the new standard protocol as here the swab was lysed during 10 min instead of a simple immersion and pressing cycle which should be repeated three to four times according to the ISO/TS. For strategy 2 the lysis buffer was added to the eluate and for strategy 3 this protocol resulted in a semi-direct lysis method as both the eluate and swab (present in one tube) were lysed by the addition of 2.5 ml of lysis buffer.

After incubation (10 min, RT), the lysis buffer was removed and RNA extraction was performed using the automated NucliSens® EasyMAGTM system 2.0 (Biomérieux, Boxtel, the Netherlands), following generic 2.0.1 protocol for off-board lysis incubation according to manufacturer’s guidelines. During each run of the automated NucliSens EasyMAG one well was reserved as negative control (addition of 500 µl PBS) to control for cross-contamination and contaminated reagents. The final elution volume was 25 µl which was adequately stored at -80°C.

#### 2.3.2. Set-up Part 2: Comparison of SRE of different swabs tested on different surfaces

The SRE obtained with cotton swabs, polyester swabs and biowipes for the detection of GI and GII NoV on three different surfaces (HD PE, nitrile gloves and neoprene rubber), was explored in part 2. Swab elution/extraction strategy 3, the semi-direct lysis method, was used for the cotton swabs and the polyester swabs because of the positive results in part 1 and as this strategy closely resembles the elution/extraction strategy for the biowipes (which was done according to manufacturers guidelines). As such, in case of the biowipe, moistening in PBS was not necessary in the beginning and in between swabbing of different directions as these biowipes were stored moisturized in their individual wraps. Before the usage of a new biowipe, fresh gloves were administered as these biowipes came in direct contact with the gloves during swabbing. After swabbing the used biowipe was stored in a 15 ml centrifuge tube on ice until lysis step. In case of the biowipe 3 ml of lysis buffer (biomérieux) was added. After 10 min incubation at room temperature the lysis buffer was added to the sample strip of the NucliSENS EasyMAG after which the off-board protocol was followed as previously stated.

For each of the three surfaces the described experiment was repeated two times separately in time, each with triplicate samples per swab type. In total six repeats were performed for every surface/swab combination, which were combined in one data unit. To reduce operator bias (due to e.g. a difference in applied pressure), one person carried out all experiments.

### 2.4. Real-time RT-PCR

The two-step RT-qPCR was performed as described in Stals et al. (2009). The RT-step was performed in double for each sample: once for the undiluted RNA extract and once for the 1/4 diluted RNA extract. Each time 3 µl RNA was included in a total volume of 20 µl reaction mix. All cDNA preparations were stored at -20°C.

The qPCR assay was used as a duplex qPCR for the detection of GI and GII NoV. For real-time quantification, 5 µl of template cDNA was included in 25 µl of reaction mix and was performed on the SDS 7300 Real-time PCR System (Applied Biosystems). Ten-fold serial dilutions ranging from 107 to 101 copies of the control plasmids for GI and GII NoV described by Stals et al. (2009) were used to prepare the standard curves. Standard curves were subjected in duplicate and amplification data was collected and analyzed using the SDS 7300 instrument’s software. Aliquots of the spike were analyzed in quadruplicate (Part 1) or duplicate (Part 2). Mean values were used for calculation of the SRE. The used fluorophore/quencher combinations for GI and GII NoV probes were respectively 6-FAM/BHQ-1 (Integrated DNA Technologies, Leuven, Belgium) and HEX/BHQ-2 (Integrated DNA Technologies).

### 2.5. Data analysis

Inhibition assessment was performed by the dilution approach. In essence, this approach is focused on the Ct difference between undiluted RNA and in this case ¼ diluted RNA extracts. Samples showing a ∆Ct between 2.0 – 0.2 and 2.0 + 0.2 were considered as inhibitor free and in this case the recovery efficiency was calculated using the data obtained with the undiluted RNA extract. If ∆Ct < 1.8 the recovery efficiency was calculated using the data obtained with the ¼ diluted RNA extract. The sample recovery efficiency (SRE) of the spiked viruses was calculated using the following equation: percentage of recovery = the number of recovered viruses/the number of seeded viruses x 100.

In order to perform the correct statistical test, the normality was checked each time using the Shapiro-Wilk test and the equality of variances was tested with the Levene’s test. When both assumptions were met, ANOVA was used to define significant differences. In case of a significant difference, the Bonferroni test was used as post hoc multiple comparison test. When the assumptions for ANOVA were not met, the Kruskal-Wallis (KW) test was used and when significant differences were found, the applied post hoc tests were Mann-Withney (MW) tests with the use of a Bonferroni correction.

Statistical analysis was performed using SPSS software, version 20 (SPSS Inc. Chicago, IL, USA). p-Values ≤0.05 were deemed statistically significant.

# 3. Results

## 3.1. Comparison of different swab elution/extraction strategies

The mean SRE and standard deviation for the detection of GI and GII NoV on HD PE according to the three different swab elution/extraction strategies using a cotton swab are depicted in Fig. 1. For both detection of GI and GII NoV there was a significant difference in recovery efficiencies obtained with the three swab elution/extraction strategies (ANOVA, p=0.002 for GI, p=0.001 for GII). For both viruses strategy 1 was significantly less efficient (Bonferroni, p < 0.020) than strategy 2 and 3, resulting in a mean recovery efficiency of 27.0% ± 26.5% and 18.9% ± 14.3%, respectively for GI and GII NoV. For both strains, there was no significant difference in SRE obtained with strategy 2 or 3 (Bonferroni, p=0.836 for GI, p=0.073 for GII). Both strategies obtained high recovery efficiencies (SRE > 70%). Mean recovery efficiencies >100% can be attributed to measurement uncertainty on the determination of the number of genomic copies, and has been observed in literature (Julian et al. 2011; Stals et al. 2011a). Due to measurement uncertainty the genomic copies detected in the spike used in part 1 (spike was analyzed in quadruplicate) for example, consisted out of a mean value with a standard deviation of which the mean value was used for calculation of the SRE.

## 3.2. Comparison of recovery efficiencies of different swabs tested on different surfaces

**Detection of GI NoV**

**For the detection of GI NoV (Fig. 2), the SRE was significantly dependant on the type of surface tested (KW-test, p=0.000). As such the SRE for GI NoV detection on both HD PE and gloves was significantly higher than for GI NoV detection on neoprene rubber (MW-test, p<0.008). No significant difference in SRE was detected between HD PE and gloves (MW-test, p=0.066).**

When the performance of the three different swabs under study (cotton swab, polyester swab and biowipe) were compared for each test surface, no significant difference in SRE was observed on the surfaces HDPE (ANOVA, p=0.125) and nitrile gloves (ANOVA, p=0.094). On neoprene rubber, the coarser surface, the use of biowipes (SRE = 41.3% ± 12.4%) resulted in a significant higher recovery efficiency than when cotton swabs (SRE = 13.2% ± 5.2%) were used for the detection of GI NoV (KW-test, p=0.007; MW-test, p<0.008). But no significant difference in the performance of biowipes and polyester swabs (SRE = 19.8% ± 12.4%) could be detected (MW-test, p=0.010), although the mean SRE obtained with biowipes was considerably higher than the SRE obtained when using polyester swabs.

**Detection of GII NoV**

For the detection of GII NoV (Fig. 2), the SRE was likewise significant depending on the type of surface tested (KW-test, p=0.000). Similar as for the detection of GI NoV, for GII NoV no significant difference in SRE was detected between the surfaces HD PE and gloves (MW-test, p=0.000). But both previous surfaces obtained a significant higher SRE of GII NoV than neoprene rubber (MW-test, p=0.963).

For GII NoV no significant difference was observed for the performance of the three tested swabs on HD PE and on gloves (ANOVA, respectively p=0.144 and p=0.881). On neoprene rubber there was a significant difference between the SRE obtained with the different swabs (ANOVA, p=0.000). In this case the biowipe (SRE = 56.1% ± 12.5%) performed significantly better than both cotton swab (SRE = 16.9% ± 6.6%) (Bonferroni, p=0.000) and polyester swab (SRE = 22.5% ± 8.7%) (Bonferroni, p=0.000). Cotton swab and polyester swab performed equally (Bonferroni, p=0.979).

Significant differences in SRE between both viral strains (GI and GII NoV) on the different surfaces were only observed for the nitrile gloves (t-test, p=0.027). For both other surfaces, HD PE (MW-test, p=0.696) and neoprene rubber (MW-test, p=0.335), no significant difference in SRE between both viral strains could be detected.

**Inhibition control**

In this study the dilution approach was used to assess inhibition of the RT-qPCR detection step, instead of the use of an RT-PCR control as suggested by ISO/TS. The choice to use the dilution approach was based on the results obtained when MNV-1 RNA was added as an RT-PCR control to a subset of the samples of this experiment Part 2. An MNV-1 RT-PCR control was added to the undiluted and 1:4 diluted RNA extracts of half of the repeats of each swab/surface combination as described earlier (Stals et al. 2011b ). When the inhibition assessment according to the ISO/TS proposal (RT-PCR control recovery efficiency > 25% = no inhibition) was performed and compared to the inhibition assessment obtained with the dilution approach, the latter was judged more sensitive as according to the RT-PCR control approach all samples (1:1 and 1:4 diluted RNA) were not inhibited while according to the dilution approach in approximately halve of the undiluted samples some inhibition (∆Ct < 1.8) played a role. As such, due to the detected inhibition when using the dilution approach, in approx. halve of the samples the SRE obtained with the 1:4 diluted RNA were used, which lead to higher SRE compared to when inhibition would be assessed according to the ISO/TS protocol which used >25% as threshold for recovery efficiency of the RT-PCR control (data not shown). Diluting until 1:4 was judged sufficient, as inhibition of the undiluted RNA extract was minor according to the dilution approach and non-existing in the undiluted and 1:4 diluted RNA according to the threshold level of >25% recovery efficiency of the RT-PCR control proposed by the ISO/TS protocol.

# 4. Discussion

Next to traditional bacteriology analysis of surfaces, swabbing for the detection of enteric viruses is increasingly used during outbreak investigations (Wadl et al. 2010; Cheesbrough et al. 2000; Repp et al. 2013; Boxman et al. 2009) and in environmental research studies (Boxman et al. 2011; Akhter et al. 1995; Russell et al. 2006). Contaminated (food) surfaces have led/contributed in the past to widespread/prolonged NoV outbreaks (Isakbaeva et al. 2005; Repp et al. 2013; Patterson et al. 1997; Evans et al. 2002; Kuusi et al. 2002). As such, swabbing for enteric viruses such as noroviruses, has proven to be useful during outbreak investigations, but could also play a role in prevention strategies by the analysis of critical control points during food preparation. However, people should be careful regarding the interpretation of swab sample data. As one should consider a positive swab sample as an indicator of surface contamination, which implies 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 (Scherer et al. 2009). Compliance with Good Hygienic Practices (GHP) and the following of precautionary principles in case of an infected food handler is still advised.

A first ISO/TS protocol for the determination of hepatitis A virus and NoV in food using RT-qPCR has been launched recently (ISO/TS 15216-1:2012), including a section for the swabbing of food surfaces and the use of appropriate controls to prevent false positive results due to cross-contamination and false negative results due to inhibition of the molecular detection assay. It should be noted that the latter ISO protocol is until today still a technical specification, meaning that improvements to the proposed protocols can still easily be inserted and as such further research into these detection protocols can contribute to a better proposal and in time a final international standard. As research on different swabbing techniques/swab extraction techniques for the detection of enteric viruses is rare, this study aimed to contribute to this research topic.

In the first part of this study three different swab elution/extraction strategies were compared. Both strategy two and three provided high SRE and performed significantly better than strategy one for the detection of GI and GII NoV on HD PE. While the differences between the design of strategy one and three were rather limited. Their difference in recovery efficiency may be due to the difference in storage conditions after swabbing which was in liquid in case of strategy three, although the applied storage time was rather limited in this study (max. 2.5 h on ice). A second hypothesis is that the repeated wetting of the swab itself between swabbing directions in strategy three improved the recovery/removal of the dried NoV from the surface as when strategy one was applied the swab seemed desiccated at the end of the swabbing of the larger 100 cm² surfaces. The application of more moisture before swabbing has been proven to be beneficial for the recovery of bacteriophage P22 on different surfaces (Herzog et al. 2012). In this study by Herzog et al. (2012) the addition of a wetting step, before swabbing with a premoistened antistatic wipe, has resulted in a doubling of the SRE results in the majority of the cases. In case of viral transfer efficiency, a wet acceptor surface (e.g. lettuce) has been proven to be more effective for viral transfer than a dry acceptor surface in case of the transfer of Feline calicivirus from stainless steel (donor surface) towards lettuce (acceptor surface) (D'Souza et al. 2006). Both previous studies show (indirectly) that the difference in the degree of moisture on the swab could lead to a different SRE for the detection of NoV on HD PE.

However, due to the significant lower SRE obtained with strategy 1, which is in close agreement with the new ISO/TS protocol, one can question the efficiency of the method proposed in this new ISO/TS for the detection of NoV on larger (100 cm²) surfaces.

Strategy 3 was chosen over strategy 2 as it was our goal (in part two) to compare common swabs such cotton swab and polyester swab against the biowipe (Biomérieux) for which the protocol also included direct lysis of the biowipe in lysis buffer. The elution/lysis technique applied in strategy 3 is almost equal to the one recently applied by Rönnqvist et al. (2013), although both studies were performed independently. As such, this semi-direct lysis strategy applied for the cotton and polyester swab in part 2 differs significantly from other studies as here the swab itself, together with the eluate, is subjected to the lysis step.

When viewing the mean recovery efficiencies for the different swabs on different surfaces (Fig. 2), a relatively high standard deviation (SD) can be noted, even though the surface/swab combination stays constant and all experiments were carried out by one person. This variability in recovery efficiencies for swabbing was also noted in previous studies (Scherer et al. 2009; Rönnqvist et al. 2013; Moore and Griffith 2002) and can be due to a variety of factors, both inherent or extrinsic to the used sampling mechanism, as described by Scherer et al. (2009). In spite of this huge variability in recovery efficiencies, biowipes had the intensity to perform better on coarser surfaces such as the neoprene rubber, than cotton swabs and polyester swabs. On other surfaces (HD PE and gloves) no significant differences could be detected in the performance of the different swabs although the recovery efficiencies obtained with the biowipes were consistently higher than those obtained with cotton swabs and polyester swabs. The predomination of microfiber cloth over polyester and cotton swabs for the detection of viral RNA was previously observed by Julian et al. (2011).

In comparable studies, such as detection of GII.3 NoV on HD PE with the use of a cotton swab performed by Scherer et al. (2009) or the detection of MS2 RNA on PVC plastic using a cotton swab performed by Julian *et al.* 2011, the obtained mean recovery was respectively 33.1 ± 23.3% and 7 – 13% (depending on the eluent type) which is considerable lower to the 77.5 ± 17.5% recovery obtained in this study. Despite this difference, direct comparison is difficult because of other differences besides the swab elution/extraction strategy such as incubation time and type of virus. When the results obtained in this study for detection of GII.4 on HD PE with cotton (77.5% ± 17.5%) and polyester tipped swabs (61.6% ± 24.4%) are compared with does obtained by Rönnqvist et al. (2013) (in both cases SRE = ca. 30%) on low density polyethylene, again a higher recovery was noted in the present study. Although it has to be noted that the incubation time applied in the study of Rönnqvist et al. (2013) was significantly longer (incubation overnight) than the 45 min incubation time applied in this study.

In conclusion, efficient swabbing continues to be a challenge due to the large variations in SRE, inherent to the swabbing process, and the possibility of substantial differences in SRE depending on the type of surface swabbed. The used biowipes (Biomérieux) in this study had the tendency to perform better than the commonly used cotton and polyester swabs, especially on coarser surfaces. In case of the use of normal cotton/polyester swabs, the semi-direct lysis method turned out to be more effective for the detection of NoV on larger surfaces than direct lysis (strategy 1) which is proposed by ISO/TS. As such, one can question the efficiency of the direct lysis method proposed in the recently launched ISO/TS 15216-1:2012 for the detection of NoV on larger (100 cm²) surfaces. The semi-direct lysis method has proven its effectiveness as well in this study as in the study of Rönnqvist, M. et al.(2013), for the detection of NoV on different types of surfaces.

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# Figures

Fig 1. The mean sample recovery efficiencies for the detection of GI and GII NoV on high density polyethylene according to three different swab elution/extraction strategies. Error bars represent the standard deviation. For each spike, means (n=3) marked with the same letter are not significantly different.

Fig 2. The mean sample recovery efficiency (SRE) (%) for detection of GI NoV (left) and GII NoV (right) when three different swabs were used on three different surfaces: HD PE: high density polyethylene; GL: gloves; NR: neoprene rubber. Error bars represent the standard deviation. For each type of surface, means (n=6) marked with the same letter are not significantly different.

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# Figures



A

B

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b

a

Fig. 1. The mean sample recovery efficiencies for the detection of GI and GII NoV on high density polyethylene according to three different swab elution/extraction strategies. Error bars represent the standard deviation. For each spike, means (n=3) marked with the same letter are not significantly different.



**Fig. 2.** The mean sample recovery efficiency (SRE) (%) for detection of GI NoV (left) and GII Nov (right) when three different swabs were used on three different surfaces: HD PE: high density polyethylene; GL: gloves; NR: neoprene rubber. Error bars represent the standard deviation. For each type of surface, means (n=6) marked with the same letter are not significantly different.

