AEM Accepts, published online ahead of print on 31 August 2012
Appl. Environ. Microbiol. doi:10.1128/AEM.02144-12

Resubmission of AEM02144-12 to AEM, Public Health Microbiology

Article type: Original research long-form article

Title: **Residual viral and bacterial contamination of surfaces after cleaning and disinfection.**

Running Title: **Viral contamination after cleaning and disinfection.**

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Abstract (250 words)

Environmental surfaces contaminated with pathogens can be sources for indirect transmission, and cleaning and disinfection are common interventions focused on reducing contamination levels. We determined efficacy of cleaning and disinfection procedures for reducing contamination by noroviruses, rotavirus, poliovirus, parechovirus, adenovirus, influenza virus, *Staphylococcus aureus* and *Salmonella enterica* from artificially contaminated stainless steel surfaces. After a single wipe with water, liquid soap or 250 ppm free chlorine solution, the numbers of infective viruses and bacteria were reduced by 1$\log_{10}$ for poliovirus to close to 4 $\log_{10}$ for influenza virus. There was no significant difference in residual contamination after wiping with water, liquid soap or 250 ppm chlorine solution.

When a single wipe with liquid soap was followed by a second wipe using 250 or 1000 ppm chlorine, an extra 1 to 3 $\log_{10}$ reduction was achieved and, except for rotavirus and norovirus genogroup I, no significant additional effect of 1000 ppm compared to 250 ppm was found. A reduced correlation between reduction in PCRU and reduction in infectious particles suggests that at least part of the reduction achieved in the second step is due to inactivation instead of removal alone. We used data on infectious doses and transfer efficiencies to estimate a target level to which the residual contamination should be reduced and found that a single wipe with liquid soap followed by a wipe with 250 ppm free chlorine solution was sufficient to reduce the residual contamination to below the target level for most of the pathogens tested.

Key words: hygiene; disinfection; norovirus; parechovirus; influenza virus; rotavirus

52 **Introduction**

53 Viruses are the most common cause of infectious disease acquired in the
54 indoor environment in hospitals, schools and households (4) causing considerable impact
55 on human health. Transmission of enteric and respiratory viruses is assumed to occur
56 predominantly direct from person to person followed by indirect transmission through
57 contaminated surfaces (7, 40, 48, 54). The risk of infection resulting from transmission
58 through contaminated surfaces depends on a number of factors, including the level of
59 shedding of infective particles, their stability on surfaces and resistance to
60 decontamination procedures and low dose required for infection. Among the enteric
61 viruses, human noroviruses (NoVs) and rotaviruses are most notorious for causing
62 outbreaks of gastroenteritis within hospitals, nursing homes and cruise ships and are
63 significant cause of hospitalization (13, 36, 44). Human NoV outbreaks are often
64 prolonged and re-occurring (5) due to the high levels of shedding of over 10^7 NoV
65 particles/g in stool (46) or vomitus (43), and the low number of particles required for
66 infection (53). Noroviruses are found at different types of surfaces (floor, table, door
67 knobs, handles, bed rails, carpets and curtains) in health care facilities, in food production
68 facilities, schools and in the community (7, 25, 60). Moreover, the NoV, and many other
69 enteric viruses, stay infectious for up to several weeks (15, 38, 57), which is considered
70 another important factor in the environmental transmission.
71 Besides human NoV, other enteric viruses like poliovirus and rotavirus, and respiratory
72 viruses like influenza and adenovirus may also be transmitted through contaminated
73 surfaces (7). Influenza A virus was frequently associated with epidemics and occasional
74 pandemics. Adenovirus type 5 is a recommended test organism for testing disinfectants
75 (45) as well as an interesting virus since it can be detected in respiratory excretions and in
76 feces (49). Parechovirus infections have commonly been associated with mild
77 gastrointestinal symptoms in young children and are excreted in feces as well (6). The

transmission of parechovirus through contaminated surfaces has not been reported yet but an indirect transmission route is likely to play a role in its spreading, given its similarities with enteroviruses.

Cleaning and disinfection of contaminated surfaces is one of the frequently implemented measures to control transmission of pathogens in indoor environments (17, 24, 31). The effectiveness of cleaning and disinfection practices is often monitored by determining reductions for bacteria such as Gram positive *Staphylococcus aureus* in hospital setting and *Staphylococcus aureus* and Gram negative *Salmonella* Enteritidis in food preparation facilities (16, 18). Additionally, the importance of environmental cleaning to control NoV outbreaks in health care settings is widely accepted (5, 28, 33) and decontamination of food production facilities may reduce the number and size of food borne outbreaks (8, 19). However, the reduction levels achieved for bacterial contaminations do not necessarily correlate to reduction levels for viral contaminations and as recently reported by Greig and Lee (30) the scientific proof supporting effectiveness of implemented intervention measures is limited. Therefore, effective science-based control measures to reduce environmental contamination are urgently needed to reduce the burden of disease of these viruses.

To be able to implement the most effective viral decontamination method, it is necessary to have quantitative data on residual contamination levels after commonly applied cleaning and disinfection practices for some of the most relevant viruses, and preferably, these data should be comparable to data for some bacteria. Thus in the present study, we assessed the effects of different cleaning and disinfection procedures on stainless steel carriers that were artificially contaminated with poliovirus Sabin 1, parechovirus 1, NoV GI.4, GII.4 and its cultivable surrogate MNV 1 (10), simian rotavirus SA 11, influenza A (H1N1) virus, adenovirus type 5 and the bacteria *St. aureus* and *S.* Enteritidis. The experiments were designed to reflect the order of magnitude of the

104 levels of contamination that may result from common events such as toilet flushing (3),
105 poor hygiene, or environmental dispersal of viral particles through droplets generated
106 during a vomiting accidents (12) or remain after removal of visible contamination.
107 The residual contamination was quantified by (cell) culture and PCR assays. As human
108 NoV cannot be cultured (22), the residual contamination of these viruses was determined
109 by quantitative PCR only.
110
111 **Materials and Methods**
112
113 **Test organisms and stocks**
114 Viruses used for the test were poliovirus Sabin 1 (vaccine strain), simian
115 rotavirus SA 11 (ATCC nr.VR-1565), adenovirus type 5 (Hu/adenovirus/type
116 5/6270/1988/Ethiopia), influenza A (H1N1) virus (Hu/influenza A
117 /266/2008/Netherlands (H1N1) virus), parechovirus 1 (Hu/parechovirus/type
118 1/147/2008/Netherlands), MNV 1 (Mu/NoV/GV/MNV1/2002/USA), human NoV GI.4
119 (Hu/NoV/GI.4/946/2009/ Netherlands) and human NoV GII.4
120 (Hu/NoV/GII.4/1803/2008/Netherlands). The bacterial test organisms were
121 *Staphylococcus aureus* (196E, toxin producer, human isolate) and *Salmonella enterica*
122 serovar Enteritidis (phage type 4).
123 Virus stocks were prepared as described before (58) and stored at -80°C. The
124 stocks used contained: poliovirus Sabin 1: 7.2×10^8 50 % Tissue Culture Infective Dose /
125 ml ($TCID_{50}$ / ml) and 5.3×10^{11} PCR units (PCRU) / ml), adenovirus type 5: 2.8×10^7
126 ($TCID_{50}$ / ml) and 6.7×10^9 PCRU / ml), parechovirus 1: 3.9×10^8 (6.7×10^{10} PCRU /
127 ml) , rotavirus 5: 1.4×10^8 (6.7×10^9 PCRU / ml), influenza A (H1N1) virus: 2.3×10^7
128 (2.0×10^9 PCRU / ml) and MNV 1: 4.9×10^6 50 % Tissue Culture Infective Dose

129 ($TCID_{50}$) / ml (1.2×10^9 PCRU / ml). The human NoVs GI.4 and GII.4 stocks were $6.6 \times$
130 10^8 and 1.1×10^8 PCRU / ml, respectively.

131 *St. aureus* and *S.* Enteritidis were cultured in Brain Heart Infusion broth
132 (Difco, USA) and enumerated on Tryptone Soy Agar (Oxoid, England) as described
133 before (37). Bacterial stocks contained *St. aureus*: 8.8×10^9 and *S.* Enteritidis; 4.2×10^8
134 colony forming units (CFU) / ml and the detection limit of both bacteria was 10 colony
135 forming units per contaminated spot.

136

137 **Preparation of sterile stool suspension**

138 The sterile stool suspension from a healthy volunteer was prepared (58) and the
139 suspension was free of rotaviruses, enteric adenoviruses, astroviruses and sapoviruses as
140 determined by PCR (51).

141

142 **Cleaning and disinfection experiments**

143 The cleaning and disinfection experiments were performed on 2.2 cm × 2.2 cm stainless
144 steel carriers (AISI type 304 standard, Netherlands). The carriers were degreased by
145 dipping into acetone for 10 min, followed by five times rinsing under running tap water.
146 Thereafter, the carriers were soaked in 70% alcohol and dried. The carriers were then
147 sterilized by autoclaving (121°C for 15 min). The viscose wiping cloth was cut into
148 pieces (approximately 4 cm × 3.5 cm) and sterilized by autoclaving.
149 One chlorine tablet (Suma tab D4, Germany) was dissolved in 1000 ml sterile water.
150 From this solution, 250 and 1000 ppm chlorine solutions were freshly prepared and free
151 chlorine concentrations were measured using a HATCH colorimeter kit (HANNA HI
152 96771, Romania).

153 BSA (3% w/v in water) or sterile stool suspension (20% w/v) were added to
154 the virus stock to perform the experiments in clean and dirty conditions. Final

concentrations were 0.03% BSA and 1% stool respectively. Since human stool is not the natural matrix for influenza A virus, this experiment was performed in clean conditions only. The human NoVs were used as 10% (w/v) stool suspensions and no extra feces was added. Stainless steel carriers were contaminated by spreading 30 µl of each virus suspension in 0.03% w/v BSA or 1% w/v stool separately (contaminated spot) and thereafter dried inside a biosafety cabinet for 1 h at room temperature (22-25°C, 40-45% RH). Then the following cleaning and disinfection procedures were applied:

Single wiping: 1000 ml of each cleaning and disinfection solution was prepared. The cloth pieces were soaked into water, water with liquid soap or 250 ppm or 1000 ppm free chlorine solutions separately and excess liquid was squeezed out by hand. With this wet cloth the contaminated carriers were wiped once by hand and sampled 20 min after wiping.

Double wiping**:** The carriers contaminated with viruses and bacteria were wiped once with the cloth soaked in water with liquid soap as described in procedure single wiping and followed by wiping once again with clothes that were soaked in 250 or 1000 ppm free chlorine solution and wrenched. The carriers were sampled after 20 min. Gloves were worn throughout the cleaning process and changed after each wiping.

For sampling, the carrier was kept in a sterile flat bottom tube (Sarstedt 60.597.001, Germany) with the wiped surface facing upwards and 2 ml cold DMEM (4-8°C) with 10% fetal bovine serum (DMEM-FBS) was added for neutralization. For the carriers that were wiped with chlorine solutions, 500 µl 7% w/v sodium thiosulphate solution in water was added for neutralization first and then 1500 µl DMEM-FBS was added. Thereafter the virus was extracted by vortexing at maximum speed for 30 s and flushing the carrier with the medium several times. The suspensions were then collected and infective viruses were enumerated by cell culture assays. Additionally, quantitative PCR assays were performed on samples obtained from wiping with water with liquid soap, 1000 ppm free

chlorine solution (single wiping) and on samples obtained from wiping with water with liquid soap followed by wiping with 1000 ppm free chlorine solution (double wiping) to quantify the genomic copies left.

Spot disinfection: If infective virus could still be detected after wiping with water with liquid soap followed by wiping with 1000 ppm free chlorine solution, virus inactivation was further tested by spot disinfection in dirty conditions to determine if extra contact time with the chlorine solution would result in lower residual contamination levels. After wiping the contaminated carrier with water with liquid soap, 800 µl 1000 ppm free chlorine solution was added onto the carrier so that the carrier was completely covered with the chlorine solution for 5, 10 and 20 min. After the exposure time, the chlorine solution was neutralized with an equal volume of 7% w/v sodium thiosulphate solution in water and 400 µl DMEM-FBS was added to make a total volume of 2 ml.

Untreated carriers were kept as control. For neutralization control, compounds (liquid soap or chlorine solutions) were diluted with DMEM-FBS or neutralized with 7% sodium thiosulphate solution before addition to the virus. The experiments were also performed with *St. aureus* and *S.* Enteritidis. Neutralized bacteriological peptone water (Oxoid, England) was used instead of DMEM-FBS. As stool is not the natural matrix for *St. aureus*, the experiment was done only in clean conditions.

$TCID_{50}$ determination

The viruses were enumerated by titration in 96 well plates on sensitive cells as described before (58).

Real time PCR

To allow comparison of virus reduction between the cultivable viruses and the non-cultivable human NoVs (22), quantitative PCR assays were performed. Viral nucleic acid

extraction was performed using Magna Pure total nucleic acid extraction kit as described before (51). Real time PCR assays were performed as described before for poliovirus Sabin 1 (20), adenovirus type 5 (34), rotavirus SA 11 (51), parechovirus 1 (56). MNV1 (2), human NoV GI.4 (51) and NoV GII.4 (58). Amplifiable PCRU were determined by slopes of standard curves made for each virus. The standard curve was made by plotting cyclic threshold (Ct) values verses log PCRU of 10 fold dilutions of the virus stocks. The highest dilution giving a positive result was assigned a value of 1 PCRU.

Residual contamination

In order to provide data that will allow for risk assessments we present data on basis of residual contamination instead of pathogen reduction. The number of pathogens present on the carrier after cleaning or after cleaning and disinfection was considered the residual contamination. The reduction of the pathogens was calculated as: ($\log_{10}$ pathogens on the control carrier) - ($\log_{10}$ pathogens on wiped carrier). The control carriers were contaminated and dried but not subjected to the treatments. All the experiments were performed in triplicate and repeated for confirmation (n = 6).

Data analysis

Statistical analysis was performed by using the student's t-Test. The $\log_{10}$ values of infectivity (x) and PCRU (y) reduction for cleaning with liquid soap, 1000 ppm chlorine solution and wiping with liquid soap followed by wiping with 1000 ppm chlorine solution were plotted to compare with the line of equality y = x.

231 **Results**

232 **Calculation of the residual contamination target level**

233 The residual contamination on the carrier after cleaning and disinfection possess a risk
234 when enough infectious microorganisms can be transferred to individuals to cause either
235 infection or to continue transmission indirectly through handling. The data for transfer of
236 microorganisms from contaminated surfaces to human hand (fingerpad) have been
237 determined for rotavirus and hepatitis A virus (1, 42) and shown to be approximately 20
238 % after 20 min drying (1). The number of viruses required for peroral infection is
239 estimated as 10-100 infectious particles for rotavirus, norovirus, poliovirus, parechovirus,
240 and influenza A virus change (23, 32, 52, 61), and approximately 150 infectious particles
241 for adenovirus virus (29). An estimated 10-100 cells are required for peroral *S.* Enteritidis
242 infection (50) and *St. aureus*. If we assume 20% transfer from fomite to fingers for all
243 microorganisms tested, then the risk of infection will be small if the residual
244 contamination is less than 5 times the particles required for infection; this level may
245 result in an infection only in the unlikely event that a contaminated finger is directly put
246 in the mouth. We therefore assumed that at residual contamination levels of infective
247 particles of less than 50 (1.7 $\log_{10}$) for rotavirus, MNV1, poliovirus, parechovirus and
248 influenza A (H1N1) virus, *S.* Enteritidis and for *St. aureus* and less than 750 (2.9 $\log_{10}$)
249 for adenovirus type 5, per contact spot, the probability of continued transmission or
250 getting infected is low (but not zero). On the basis of this assumption, lines indicating the
251 residual contamination target levels were drawn in Figure 1.

252

253 **Residual contamination after cleaning – single wiping**

254 The recovery of the viruses and bacteria from the stainless steel carriers after drying for
255 one hour ranged from 24 to 76 %. After wiping the surfaces were visibly dry within 3
256 min. The residual contaminations of infective viruses and bacteria in clean and dirty

conditions after single and double wiping are shown in Figure 1. There was no significant difference in residual contamination after wiping with water or water with liquid soap. Only for poliovirus and rotavirus there was a minor but significantly higher residual contamination when feces were present compared to clean conditions. We found little or no effect of the use of 250 ppm chlorine solutions instead of liquid soap in the cleaning step; only for rotavirus under dirty conditions and influenza A virus (i.e. in only 2 out of 14 pathogen-matrix combinations tested), a lower residual contamination was seen when 250 ppm chlorine was used.

The residual contamination after wiping with 1000 ppm chlorine solution was significantly lower ($p < 0.05$) than wiping with water or liquid soap in 10 out of 14 pathogen-matrix combinations. Additionally, in 7 out 14 pathogen-matrix combinations the wipe with 1000 ppm chlorine solutions resulted in a significantly lower residual contamination when compared to wiping with 250 ppm chlorine solution.

Residual contamination after cleaning and disinfection – double wiping

The residual contamination after wiping with liquid soap followed by wiping with 250 ppm chlorine solution (double wiping) was significantly lower ($p < 0.05$) than after wiping with liquid soap alone (single wiping) in most of the viruses (except MNV1 and rotavirus) and bacteria tested (Figure 1). After the double wiping procedure there was no significant difference ($p > 0.05$) in residual contamination between 250 or 1000 ppm chlorine solution in 12 out of 14 pathogen-matrix combinations. Only for rotavirus the reduction achieved with 1000 ppm was better than the reduction achieved with 250 ppm chorine solution, resulting in a residual contamination of less than 2 infectious particles per spot (detection limit; > 6 $\log_{10}$ reduction).

Reduction of genomic copies of norovirus after cleaning and disinfection

283 As human NoVs could not be cultured, the reductions in genomic copies were quantified
284 by PCR assays. The reductions in genomic copies of NoV GI.4, GII.4 and MNV1 are
285 shown in Figure 2. For MNV1, all the treatments resulted in a comparable reduction
286 while for NoV GI.4 and GII.4 we observed a significant higher reduction in PCRU with
287 the double wiping protocol. In 5 out of 6 treatments the reduction in PCRU for NoV GI.4
288 and MNV1 differed, in 3 out of 6 they differed between NoV GI.4 and NoV GII.4 and in
289 2 out of 6 between NoV GII.4 and MNV1.
290
291 **The reduction of infective load and genomic copies**
292 The PCRU reductions of poliovirus Sabin1, adenovirus type 5, parechovirus 1, MNV1,
293 rotavirus SA 11 and influenza A (H1N1) virus by wiping with water with liquid soap,
294 with 1000 ppm chlorine solution and wiping with water with liquid soap followed by
295 wiping with 1000 ppm chlorine solution were also determined. The equality between
296 reduction of genomic copies and reduction of infectivity of the tested viruses in clean
297 condition is shown in Figure 3. After wiping with water with liquid soap there was a
298 correlation between the infectivity and PCRU reduction except for rotavirus SA11 and
299 influenza A (H1N1) virus. The infectivity reduction was higher than the PCRU reduction
300 (i.e. deviating from the equality line) on wiping with 1000 ppm chlorine solution and
301 with liquid soap followed by wiping with 1000 ppm chlorine solution in case of
302 parechovirus 1, rotavirus SA 11, MNV1, adenovirus type 5 and influenza A (H1N1) virus.
303
304 **Residual contamination after spot disinfection**
305 Since there was residual contamination of MNV1, poliovirus Sabin 1, adenovirus type 5,
306 parechovirus 1 and *S.* Enteritidis after the double wiping procedure using 1000 ppm
307 chlorine, spot disinfection of the bacteria and viruses in dirty conditions by 1000 ppm
308 chlorine solution after cleaning with water with liquid soap was tested to determine if this

309 treatment would result in a residual contamination that is below the detection limit. The
310 residual contamination was reduced to below the detection limit of 10 particles of MNV1
311 in 5 min (a reduction of 5 $\log_{10}$), poliovirus Sabin 1 (6.9 $\log_{10}$) and adenovirus type 5 (5.3
312 $\log_{10}$) in 10 min. The infective loads of parechovirus 1 and *S.* Enteritidis were reduced
313 with 3.2 ± 0.1 and 4.9 ± 0.4 $\log_{10}$ respectively within 20 min of disinfection. Genomic
314 copies of NoVs GI.4 (6.7 $\log_{10}$ PCRU) and GII.4 (5.2 $\log_{10}$ PCRU) were reduced to below
315 the detection limit of 60 PCRU/spot after 10 and 5 min respectively. MNV1 was reduced
316 with 6.9 ± 0.7 $\log_{10}$ PCRU within 20 min of disinfection with 1000 ppm free chlorine
317 solution.
318
319
320

Discussion

Our data indicate that in case of an outbreak of gastroenteritis, by either NoV, rotavirus or *Salmonella*, a cleaning step with liquid soap followed by a wipe using a 1000 ppm chlorine solution most consistently results in the lowest residual contamination level of all treatments tested. However, if we assume that an equivalent of 1 in 2 NoV PCRUs is infectious (data for NoV GI.1(53)), the residual infectivity of NoV GI.4 and GII.4 will be approximately 5×10^2 or 5×10^3 infectious particles (approximately 1×10^3 or 1×10^4 PCRU), respectively, per contaminated spot, which is well above the level we defined as target level. Increasing the contact time between pathogen and the 1000 ppm chlorine solution to at least 5 min (as studied by spot disinfection) did result in residual contamination below the target levels of NoV and rotavirus and may be considered to be an effective intervention strategy in controlling gastro-enteric pathogens transmission via hard surfaces, although it may be impractical. Our data suggest that *S.* Enteritidis may still be present at loads above our target levels, however, the low prevalence of *S.* Enteritidis in non-food and health-care related outbreaks (59) suggests that transmission via hard surfaces is not a main route of transmission for this pathogen. We did not find clear differences in the reduction in infective enteric viruses or viable bacteria in our experiments, indicating that the apparent greater outbreak potential of NoV and rotavirus is not due to a higher resistance to cleaning and disinfection, but more likely due to the extremely high infectivity of NoV and the high levels of shedding for rotavirus.

Due to the inability to cultivate the human NoVs in vitro, several cultivable viruses such as feline calicivirus (FCV), canine calicivirus (CaCV), MS2 bacteriophage and MNV1 have been used as surrogates to study NoV inactivation (11, 21, 47). However, NoV GI and GII viruses differ in binding properties to for example shellfish tissues and lettuce surfaces (41, 55), but also in resistance to freeze-drying and heat treatment (9, 35), making it unlikely that one model virus will be a valuable surrogate for

NoV GI and NoV GII. This was confirmed in our studies that showed inconsistencies in the level of correlation of MNV results with those for NoV GII.4 and GI.4 in complex situations such as this study where removal and disinfection were combined. In the absence of cultivation method for the human NoV we postulate that especially for quantitative risk assessment purposes, the use of any model virus should be accompanied by a PCR based method to allow comparison.

The two picornaviruses tested (poliovirus and parechovirus) showed remarkable differences in residual contamination and thus risk of infection remaining after cleaning, however this was mainly caused by a 2 $\log_{10}$ difference in starting contamination level. Since differences in levels of shedding do occur (14, 39), these data may reflect real variation in levels of contamination after cleaning and disinfection. Spot disinfection showed a remarkable resistant parechovirus fraction as some could still be cultured after 20 min exposure to 1000 ppm chlorine solution. Such a very resistant virus fraction, representing 0.01% of the stock suspensions used, was also shown to exist during thermal inactivation at 73°C (56). Due to the low infectious dose, these resistant fractions may represent a risk when present in foods or on surfaces when very high levels are shed.

In this study we confirmed the higher sensitivity of the enveloped respiratory influenza A virus to chlorine disinfection, compared to sensitivity of the non-enveloped enteric viruses (57) and the complete removal of infectious influenza virus after a single wipe, with a 1000 ppm confirms a recent study that showed complete inactivation of human influenza A viruses by wipes containing 1% bleach (sodium hypochlorite and sodium hydroxide) (27). The two step procedure consisting of a single wipe with liquid soap followed by a disinfection step using 250 ppm chlorine solution is likely to be a good intervention strategy in case of viral respiratory disease outbreaks since it reduced the infectivity of both respiratory viruses tested to well below the target level.

373 Efficacy of cleaning and disinfection is not only determined by the intrinsic
374 effectiveness of the method applied but also by the appropriateness of the surfaces treated.
375 Cleaning and disinfecting should be focused on the critical spots, i.e. the surfaces really
376 involved in transmission. Reducing the infective load on critical spots such as door knobs,
377 handles, light switches and other frequently touched surfaces is more likely to have a
378 profound impact on transmission than treating rarely touched surfaces. Interestingly, a
379 recent study on the removal of viruses from hard surfaces found a comparable reduction
380 of infective MNV1 after wiping the surfaces 6 times (26) as we found after a single wipe,
381 indicating that surface cleaning and disinfection can be performed quite efficiently.
382 Nonetheless, manual cleaning and disinfection procedures will always be more labor
383 intensive than for example room disinfection using hydrogen peroxide vapor (58) and for
384 the control of outbreaks a combination of both methods is most likely needed.
385 In this study we performed cleaning and disinfection by wiping as it may be
386 carried out in health care settings. Since these procedures will be carried out by different
387 individuals, variability in residual contamination levels is likely. Additional variation will
388 occur due to differences in level of shedding, differences in temperature and humidity
389 and types of contaminated surfaces. However, tests like these, even if just describing one
390 scenario, provide the scientific background for evidence based cleaning and disinfection
391 guidelines or protocols.
392 In health care facilities cleaning may be performed according to different
393 protocols: general cleaning performed on a day to day basis and more stringent cleaning,
394 often in combination with disinfection procedures, during outbreaks. Our findings show
395 that in all cases a single wipe with a wet cloth with either water or liquid soap resulted in
396 a significant reduction ($> 1 \log_{10}$) of the infective load of all pathogens tested, but the
397 residual contaminations indicate that further transmission may still occur. Adding a
398 wiping step with 250 or 1000 ppm chlorine solution resulted in an additional reduction of

399 the infective load, most likely through inactivation of the pathogens rather than by
400 particle removal, as indicated by the discrepancy between infectivity and PCRU
401 reduction. Pre-cleaning before disinfection of the contaminated surfaces is recommended
402 and the removal and disinfection together will often result in residual contamination
403 levels below the target levels of residual contamination.
404
405

Acknowledgement

The authors would like to thank Rina Puspitasari for excellent contribution to the laboratory work.

Conflict of interest

None to declare

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602 Figure 1

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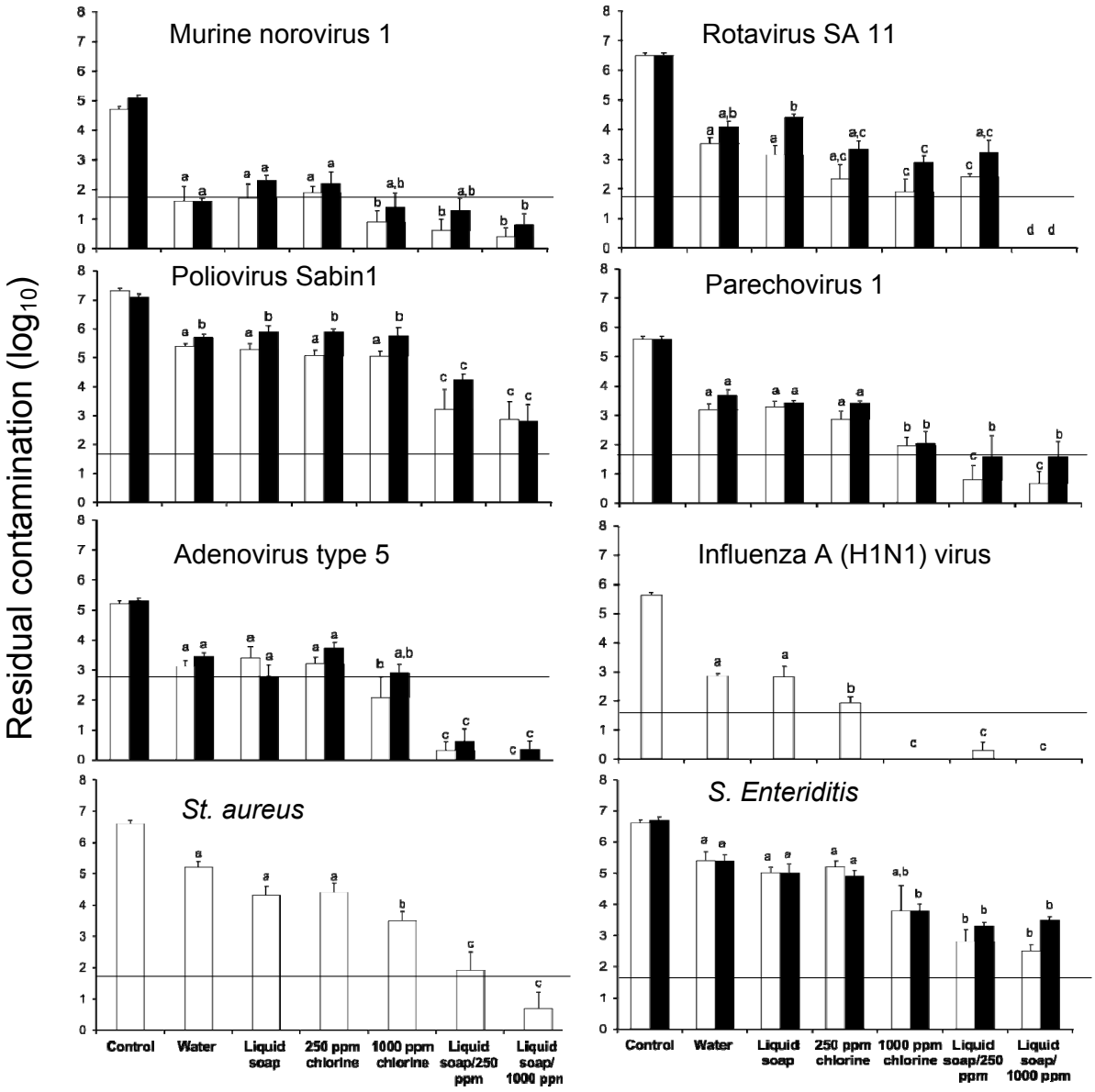

Figure 1: Residual contamination of different pathogens on stainless steel carrier in clean (white) and dirty (black) conditions after different cleaning and disinfection methods. Control is the recovery after 1 h of drying. Water, Liquid soap, 250 ppm chlorine and 1000 ppm chlorine indicate the suspensions used to wet a wipe for the one-wipe (cleaning) procedure. Liquid soap/250 ppm and Liquid soap/1000 ppm indicate the consecutive suspensions used to wet wipes for the two-step (cleaning and disinfection) procedure. Error bars indicate standard deviation of the mean and the means with a different letter differ significantly ($p < 0.05$) ($n = 6$). The horizontal lines in the figures indicate the residual contamination target levels.

643 **Figure 2**
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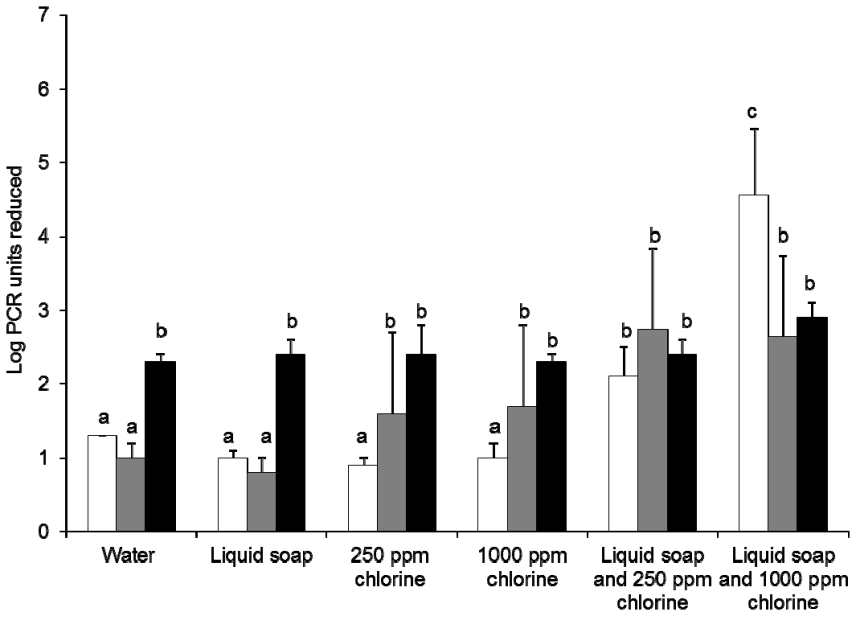

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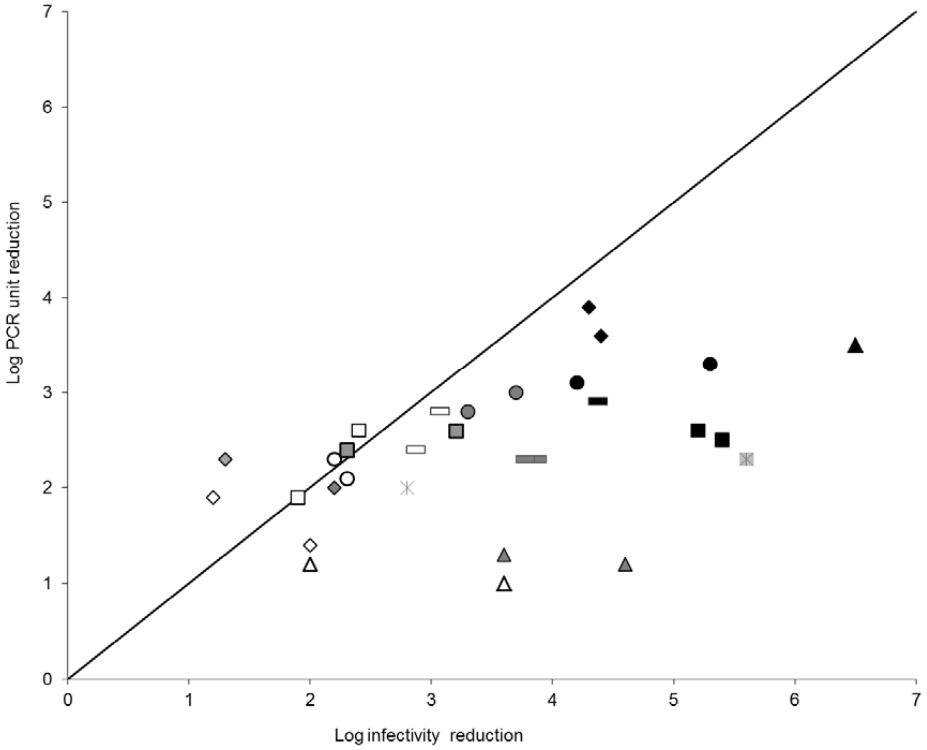
649 **Figure 2:** Reduction of genomic copies of human NoVs GI.4 (white), GII.4 (grey) and
650 MNV1 (black) in dirty condition after different cleaning methods. Water, Liquid soap,
651 250 ppm chlorine and 1000 ppm chlorine indicate the suspensions used to wet a wipe for
652 the one-wipe (cleaning) procedure. Liquid soap/250 ppm and Liquid soap/1000 ppm
653 indicate the consecutive suspensions used to wet wipes for the two-step (cleaning and
654 disinfection) procedure. Error bars indicate standard deviation of the mean and the means
655 with a different letter differ significantly ($p < 0.05$) ($n = 6$).
656

657 **Figure 3**



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661 **Figure 3**: Correlation between PCR units and infectivity reduction by different cleaning
662 methods in clean and dirty conditions of poliovirus Sabin 1 (◊), adenovirus type 5 (□),
663 parechovirus 1 (O), rotavirus SA 11(Δ), MNV1 (▭) and influenza A (H1N1) virus (×)
664 (n = 6). White, grey and black symbols represent the reduction by wiping with liquid
665 soap, 1000 ppm chlorine solution and wiping with liquid soap followed by wiping with
666 1000 ppm chlorine solution, respectively. In some cases only one data point is visible due
667 to overlap of data points.
668