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1 **Pathogen Transfer and High Variability in Pathogen Removal by Detergent**

2 **Wipes**

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18 Running title: Efficacy of detergent wipes

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23 ABSTRACT

24 The rise in healthcare associated infections has placed a greater emphasis on
25 cleaning and disinfection practices. The majority of policies advocate using
26 detergent based products for routine cleaning, with detergent wipes increasingly
27 being utilized; there is no information about their ability to remove and
28 subsequently transfer pathogens in practice.

29 Seven detergent wipes were tested for their ability to remove and transfer *S.*
30 *aureus*, *A. baumannii* and *C. difficile* spores using the 3-stage wipe protocol.
31 The ability of the detergent wipes to remove *S. aureus*, *A. baumannii* and *C.*
32 *difficile* spores from a stainless steel surface ranged from 1.50 log₁₀ (range,
33 0.24-3.25), 3.51 log₁₀ (range, 3.01-3.81) and 0.96 log₁₀ (range, 0.26-1.44)
34 respectively following a 10 s wiping time. All wipes repeatedly transferred
35 significant amount of bacteria/spores over three consecutive surfaces, even
36 though the percentage of total microorganisms transferred from the wipes after
37 wiping was low for a number of products. Detergent based wipe products have
38 two major drawbacks: their variability in removing microbial bioburden from
39 inanimate surfaces and their propensity to transfer pathogens between surfaces.
40 The use of additional complimentary measures such as combined detergent-
41 disinfectant based product and/or antimicrobial surfaces need to be considered
42 for appropriate infection control and prevention.

43

44 **Keywords:** surface cleaning, disinfection, detergent wipes, *Clostridium difficile*,

45 *Acinetobacter baumannii*, *Staphylococcus aureus*

46

47

48 **INTRODUCTION**

49

50 A detergent is a group of chemical compounds (synthetic or organic) which are
51 liquid or water soluble. Unlike soaps, detergents are not prepared from animal
52 and vegetable fats and oils and are not inactivated by hard water. The major
53 components in cleaning products are surfactants (surface-active agents);
54 detergent surfactants are now commonly made from petrochemicals and/or
55 oligochemicals. Surfactants can be classified into four groups depending on the
56 polar head group; anionics, cationics, non-ionics and zwitterionics.¹ The majority
57 of cleaning products will be formulated to contain one or more surfactants in
58 combination with additional compounds, such as preservatives, enzymes and
59 perfume.

60

61 The majority of current UK infection control policies advocate the use of
62 detergent and water or microfiber and water for cleaning of soiled/contaminated
63 surfaces.² Detergent wipes are increasingly being utilised, serving as a
64 convenient, ready-to-use disposable product for environmental cleaning. The
65 ability of microorganisms such as methicillin-resistant *Staphylococcus aureus*,
66 vancomycin resistant *Enterococci* and *Clostridium difficile* to persist on inanimate
67 surfaces for prolonged periods is well recognized,^{3,4} with common healthcare
68 associated pathogens frequently isolated from surfaces in close proximity to the
69 patient (high touch points). There is a growing body of evidence demonstrating

70 the importance of environmental contamination in the transmission of clinically
71 relevant pathogens.^{5,6} Although multiple studies have investigated the efficacy of
72 microfiber cloths^{7,8 and 9} and antimicrobial wipes,^{10,11,12,13,14,15 and 16} to the best of
73 our knowledge no study has yet investigated the efficacy of detergent wipes.
74 Although it has been suggested that a 'one wipe, one surface, one direction'
75 approach be implemented, in practice a wipe (detergent or disinfectant based) is
76 likely to be used on multiple surfaces. The purpose of any cleaning wipe is to
77 firstly ensure the efficient removal of microorganisms from a surface and
78 secondly to ensure the microorganisms are retained on the wipe, thus preventing
79 the transfer of pathogenic microorganisms. The aim of this study was to test
80 using a modified 3-stage protocol¹³ the efficacy of a number of commercially
81 available detergent wipes to remove *S. aureus*, *A. baumannii* and *C. difficile*
82 spores from surfaces and prevent their transfer between surfaces.

83

84 **MATERIALS AND METHODS**

85 **Detergent Wipes**

86 Seven detergent wipes currently used in healthcare facilities in the UK were
87 obtained from different manufacturers. Details of wipe ingredients and
88 manufacturers are summarized in Table 1.

89

90 **Bacterial strains**

91 The following organisms were used in this study: *S. aureus* NCIMB 9518 (PHE,
92 UK), *A. baumannii* NCTC 10788 (NCIMB Ltd, UK) and *C. difficile* NCTC 11209
93 (PHE, UK). *S. aureus* and *A. baumannii* were grown overnight in Tryptone Soya
94 Broth (Oxoid, UK), centrifuged at 5,000 *g* for 20 min at 4°C and the pellet
95 resuspended in phosphate buffered saline (PBS)+0.1% Tween-80 (PBST)
96 (Fisher Scientific) before use. For the preparation of the *C. difficile* spores, the
97 method by Perez *et al.*,¹⁷ was followed with the following modifications; multiple
98 colonies of *C. difficile* 11209 were inoculated into 20 mL of reduced Brain Heart
99 Infusion (BHI) broth (Oxoid, UK) and cultured overnight at 37°C under anaerobic
100 conditions (5% H₂: 10% CO₂: 85% N₂) in a Whitley MG500 workstation (DW
101 Scientific, UK). The overnight culture was gently vortexed and 1% was added to
102 500 mL of reduced Clospore and incubated for 7 days. The spore preparation
103 was centrifuged at 10,000 *g* for 20 min at 4°C. Spores were purified as
104 described by Perez *et al.*,¹⁷ assessed by phase contrast microscopy and heat
105 shock at 60°C for 20 min. Spores were enumerated by diluting in PBST and
106 plated onto Brain Heart Infusion (BHI) agar supplemented with 0.1% (w/v)
107 sodium taurocholate (BHIS) (Fisher Scientific). Purified spores were stored at
108 4°C until use.

109

110 **Bactericidal and Sporicidal Activity**

111 Bactericidal and sporicidal activity was determined using a protocol based on the
112 European standard method for chemical disinfectants EN 13727.¹⁸ All testing

113 was conducted on fluid expressed from wipes; a single wipe was placed in a
114 sterile 20 mL syringe; solution from the wipe was collected by applying pressure
115 for 30-60 seconds. The process was repeated until sufficient fluid had been
116 collected and used within 5 minutes. For bactericidal activity, the test organism
117 was cultured in 10 mL of TSB, after 24 h of incubation at 37°C the cell
118 suspension was centrifuged and re-suspended in PBST and combined with
119 bovine serum albumin so that the organic load in the test was 3 g/L ('dirty
120 conditions'). The average number of cells/spores in the test was 7.91 ± 0.12
121 Log_{10} , $8.14 \pm 0.20 \text{ Log}_{10}$ and $5.43 \pm 0.54 \text{ Log}_{10}$ CFU/mL, for *S. aureus*, *A.*
122 *baumannii* and *C. difficile*, respectively. The test suspension was held at 20°C
123 for 1 min and enumerated. To conduct the test 0.1 mL of bacterial or spore
124 inoculum was added to 0.9 mL wipe solution. After a contact time of 1 min 0.1 mL
125 of the test solution was transferred to 0.9 mL of a neutralizing solution consisting
126 of saponin (Sigma) 30 g/L, polysorbate 80 (Sigma) 30 g/L, azolectin from
127 soybean (Sigma) 3 g/L, L-Histidine (Sigma) 1 g/L and sodium dodecyl sulphate
128 (Sigma) 5 g/L, 5 g/L sodium thiosulphate prepared in de-ionised water.
129 Neutraliser toxicity and neutraliser efficacy were determined in suspension using
130 the protocol described by Knapp *et al.*¹⁹

131

132

133 **Efficacy test protocol – 3-stages protocol**

134 The 3-stage protocol described in Williams *et al.*¹³ was adapted, utilizing the
135 'Wiperator®' system (<http://www.filtaflex.ca/wiperator.htm>; accessed 9 January
136 2014). Wipes were cut aseptically in squares of 2 x 2 cm for testing.

137 *Measurement-1 - efficacy of wipes to remove microorganisms from surfaces:*
138 microorganisms (10 µL) were inoculated onto clean magnetized, brush stainless
139 sterile steel discs (AISI Type 430 (European equivalent X6Cr17 and number
140 1.4016); Group 2; No. 4 finish (EN 10088-2 1J/2J)) and dried for 30 min at 37°C.
141 A detergent wipe was attached to a plastic boss to allow an elliptical mechanical
142 rotation for 10 s exerting a weight of 150 g. Steel discs were transferred into
143 bottles containing neutralizer (1 mL) and glass beads (1 g; 3 mm diameter;
144 Sigma). After horizontal shaking (150 rpm for 1 min) and neutralization for 5 min,
145 the suspension was serially diluted and used to inoculate appropriate agar. *S.*
146 *aureus* and *A. baumannii* were counted after 24 h incubation at 37°C and *C.*
147 *difficile* after 48 h anaerobic incubation. The log₁₀ cell removal from the disk
148 surfaces was calculated by subtracting the mean log₁₀ number of cells recovered
149 from the disc after using the wipes from the number of cells recovered from the
150 dry control.

151 *Measurement-2 - bacterial transfer from wipes:* Following the application of wipes
152 to the contaminated surfaces as described above, the subsequent transfer of
153 contamination onto three consecutive stainless steel discs was measured
154 together with the effect of the mechanical action (10 s wipe, 150 g pressure).
155 Steel discs were placed in neutraliser and bacterial colonies enumerated.

156 *Dry control:* Prior to the use of wipes, cell deposited and dried on the surface of
157 the disk were recovered into bottles containing neutralizer and glass beads as
158 described above. After horizontal shaking (150 rpm for 1 min) for 5 min, the
159 suspension was serially diluted and used to inoculate appropriate agar.

160

161 **Biological Replicates and Statistical Analysis**

162 All data presented in this manuscript represent the results of three independent
163 experiments. Data were checked visually for normality and homogeneity of
164 variance using a histogram, Q-Q plots and fitted values. A one-way ANOVA at
165 the 95% confidence interval with a post hoc Tukey's test was performed or a
166 paired-sample t-test. All analyses were completed in SPSS Statistics 20.

167

168 **RESULTS**

169 In this study, *S. aureus*, *A. baumannii* and *C. difficile* spores were used to firstly
170 assess the microbicidal activity of seven detergent wipes and secondly the ability
171 of the wipes to remove and transfer microorganisms onto three consecutive
172 surfaces. Prior to use a modified EN13727 suspension test, the neutralizer
173 toxicity and neutralizer efficacy to quench the active contained in the wipe were
174 assessed. The neutralizer did not display any toxicity and was found to be
175 efficacious in quenching the activity of the wipe with $<1 \log_{10}$ reduction reported
176 for all organisms tested (data not shown). Unsurprisingly expressed solution

177 from the seven wipes tested displayed no bactericidal or sporicidal activity (data
178 not shown).

179 In order to test the impact of drying on the organisms tested, a paired-samples t-
180 test was conducted. No statistically significant difference was found between the
181 viable counts pre and post drying for *S. aureus* ($p = 0.418$, two-tailed) and *C.*
182 *difficile* ($p = 0.419$, two-tailed). A statistically significant decrease was found for
183 *A. baumannii* pre ($7.13 \pm 0.40 \log_{10}$) and post ($6.00 \pm 0.33 \log_{10}$) drying, with the
184 eta squared statistic (0.91) indicating a large effect size. For this reason all
185 calculations for removal utilized the dry control values. Initial analysis by means
186 of a two-way ANOVA between groups assessed the impact of wipes and bacteria
187 on removal. The interaction effect between wipes and bacteria was found to be
188 significant ($F(12, 42) = 10.34$, $p < 0.001$), thus all subsequent analysis was
189 undertaken with a one-way analysis of variance. The detergent wipes tested in
190 this study showed marked differences in their ability to remove microbial
191 bioburden from surfaces following a 10 second wipe, as shown in Figure 1. The
192 average removal of *S. aureus* from a stainless steel surface by the wipes tested
193 was $1.45 \log_{10}$ (range: 0.24-3.25). Wipe D removed significantly more (ANOVA,
194 *post hoc* Tukey's test, $p < 0.05$) *S. aureus* from the stainless steel disk than the
195 other wipes. All the wipes repeatedly transferred large number of *S. aureus* onto
196 three consecutive surfaces except wipe G for which transfer of bacteria was
197 below the limit of detection for this test (<17 CFU; recorded as 0.00% transfer;
198 Table 2). The average removal of *A. baumannii* by the wipes tested was 3.51

199 \log_{10} (range: 3.01-3.81). No statistically significant difference was observed in
200 the efficacy of the wipes to remove *A. baumannii* from a stainless steel surface
201 (Fig. 1). The wipes tested were particularly poor at preventing the transfer of *S.*
202 *aureus* but much better in preventing the transfer of *A. baumannii* with the
203 exception of wipe C, which performed poorly with both bacteria. Of the three
204 microorganisms tested, the wipes removed the least number of spores from the
205 surface (0.96 \log_{10} , range: 0.26-1.44). Wipes A, D, E, and G removed
206 significantly more spores than Wipes B and C (ANOVA, *post hoc* Tukey's test, p
207 < 0.05). As with the vegetative bacteria, all wipes tested failed to retain the
208 spores. Between 117 and 34377 spores were transferred onto surfaces
209 (corresponding to 1.29% transfer, wipe G and 114.95% transfer, wipe C; Table
210 2). Wipes A and C performed particularly poorly and wipe G performed better
211 than the others. The percentage of bacteria (CFU) transferred was estimated
212 based on the assumption that the difference in the number of CFU on the
213 stainless steel disk before and after wiping ended up into the wipe (Table 2). On
214 three occasions the percentage exceeded 100%, which would indicate that the
215 number of CFU picked up by the wipes were underestimated. The percentages of
216 bacteria/spores transferred onto 3 surfaces were at times very low, particularly
217 with *A. baumannii*, indicating that this microorganism is retained better regardless
218 of the wipe material and formulation (Table 2). It can also be noted that the
219 percentage of *C. difficile* spores transferred is high despite the calculated low
220 spore number on the wipes.

221

222 **DISCUSSION**

223 The lack of microbicidal activity demonstrated by the wipes was unsurprising
224 given the wipes composition (Table 1). The lack of activity needed to be
225 evaluated to ensure that the propensity of the wipes to remove and/or transfer
226 microbial bioburden from surfaces was not affected by any intrinsic wipe
227 microbicidal activity. The Gram-positive *S. aureus* and spores of *C. difficile* were
228 not affected by drying, however the Gram-negative *A. baumannii* was. These
229 results support findings of other studies, which have demonstrated Gram-positive
230 organisms are more tolerant of desiccation than Gram-negative organisms.^{20,21,22}
231 and²³ It is important to take into consideration the impact a dry inoculum can have
232 when assessing the efficacy of a product, it would be misleading to associate a
233 mean difference of 1.4 log₁₀ between pre and post drying of *A. baumannii* to the
234 product being tested. In order to overcome such issues a higher starting inoculum
235 can be used, the inoculum can be combined with proteins in order to stabilize the
236 organism^{20,21} and²² or the impact of drying can be stated and taken into
237 consideration during analysis.

238 The efficacy of the detergent wipes to remove microbes from a surface varied
239 considerably; for example Wipe A removed the greatest amount of *A. baumannii*
240 3.81 log₁₀, 1.23 log₁₀ *C. difficile* but only 0.25 log₁₀ *S. aureus*, demonstrating the
241 ability of the wipe to remove bioburden from a stainless steel surface is
242 dependent on the microorganism tested. This interaction effect has also been

243 observed when assessing the efficacy of microfiber cloths.²⁴ In the
244 aforementioned study methicillin-resistant *Staphylococcus aureus* (MRSA) was
245 consistently more difficult to remove than *C. difficile* spores and *E. coli*; these
246 findings are somewhat akin to our findings in that the Gram-negative organism
247 (*A. baumannii*) was consistently removed by all detergent wipes tested, whereas
248 *C. difficile* spores and *S. aureus* (with the exception of Wipe D) proved to be
249 more difficult to remove. Although it should be noted that in the study by Smith *et*
250 *al.*,²⁴ a wet inoculum was utilized and although an automated cleaning rig was
251 utilized the pressure employed in the study was not specified. In a study
252 performed by Tuladhar *et al.*,²⁵ the log₁₀ reduction of *S. aureus* was ~2.30 log₁₀
253 with liquid soap applied to a viscose cleaning cloth, this is 1 log₁₀ higher than the
254 median value obtain in this study (1.45 log₁₀). This difference may be due to the
255 material tested, the strain used or the method of wiping the surface (hand vs.
256 automated system). In a previous study comparing the efficacy of a detergent
257 wipe to a disinfectant wipe using the 3-stage protocol, both wipes were found to
258 remove on average ~1.72 (± 0.32) and 1.74 (± 0.96) *S. aureus* respectively, in
259 dirty conditions.¹⁴ Here, among the seven wipes tested an average of 1.45 (±
260 1.15) was observed. This suggests that disinfectant wipes may outperform
261 detergent wipes in removing *S. aureus*, although the protocol used in most of
262 these studies were different, which makes comparison difficult. The variability in
263 results reflects the differences in the ability of the detergent wipes tested to
264 remove this bacterium.

265 The wipes tested in this study are generally composed of non-ionic surfactants,
266 preservatives and perfume, therefore they would be expected to perform on par
267 with each other (Table 1). However, from the data presented above this is not
268 the case, the performance of the detergent wipes may be influenced by the type
269 of nonwoven, quality of the raw materials and non-woven, the liquid to wipe ratio
270 and the packaging of the product.²⁶ Indeed the difference in performance
271 between wipe B and wipe G might be explained by the use of viscose in wipe G.
272 The other factors were not investigated in this study but the differences in
273 efficacy of the wipes tested suggests there is scope for further development of
274 these products, which are increasingly being utilized in the healthcare setting.
275 Furthermore the formulation of the detergent and its compatibility with the non-
276 woven may impact the efficacy of the wipe as seen with cotton towels and
277 disinfectant based cleaners.²⁷

278 Although all detergent wipes tested removed microbial bioburden from a stainless
279 steel surface, they repeatedly transferred a large amount of bacteria/spores on
280 three consecutive transfers. Only wipe G performed better than the others with
281 the vegetative bacteria, where no transfer was detected. On the other hand wipe
282 C caused the highest release of bacteria and spores. On three occasions the
283 number of bacteria/spores transferred were higher than the calculated number of
284 bacteria/spores on the wipe. It is possible that bacteria/spores are in the form of
285 dense aggregates given the high concentration of the starting inoculum used in
286 this study (~8 and 5 log₁₀ for bacteria and spores, respectively) combined with

287 the desiccation process when the inoculum is deposited on the surfaces. Despite
288 using saponin, polysorbate 80 and sodium dodecyl sulfate in the neutralizer and
289 glass beads, the presence of aggregates cannot be ruled out. The presence of *C.*
290 *difficile* spores aggregates during wipe efficacy testing has been reported
291 previously.¹⁰ It is conceivable that the surfactant-based formulation of the wipe
292 tested breaks up releases aggregates,¹⁰ although it is interesting to note that the
293 Gram-negative *A. baumannii* was not concerned with these observations. These
294 results highlight the need to assess the efficacy of wipes to both remove and
295 transfer microbes. This is particularly pertinent with the release of *C. difficile*
296 spores, since the infectious dose was estimated to be as low as < 5
297 spores/cm².²⁸ Although the calculated spores number in the wipes was relatively
298 low (when compared to the vegetative bacteria) from 5,000 and 90,000 spores,
299 the lowest number of spores transferred was 117 (corresponding to 1.29%
300 transfer; wipe G, table 3). While this is not the first study to demonstrate the
301 transfer of microbes to clean surfaces by wipes,^{10,11,13,14 and 16} it is the first
302 instance where the transfer of microorganisms onto multiple surfaces has been
303 quantified in this way and the percentage transfer estimated. The potential
304 repeated seeding of the healthcare environment by wipes is of concern and
305 raises questions as to how best to use wipes in practice; should a 'one wipe, one
306 surface, one direction' approach be universally and strictly implemented as
307 already recommended? Although infection control teams provide some guidance
308 on product use, surely a standard policy document is required. Currently the

309 closest guidance document available on wipes was issued by the Royal College
310 of Nursing.²⁹ Manufacturers are also providing comprehensive guidance
311 documents and training packages for their products, but could do more to
312 educate the end users on the appropriate use of their products.⁴ In view of the
313 findings from our study, additional complimentary ways to decrease surface
314 microburden should be explored including the use of combined detergent-
315 disinfectant wipes and antimicrobial surfaces.^{10,11,13,14 and 16} The later is showing
316 promising results in significantly reducing microorganisms from environmental
317 surfaces in healthcare settings.³⁰

318

319 **CONCLUSION**

320 In conclusion the efficacy of commercially available detergent wipes to remove
321 microbial bioburden from surfaces was found to be variable between products.
322 The efficacy of the wipes to remove *A. baumannii* from surfaces was appropriate,
323 but far to be satisfactory with *S. aureus* and spores of *C. difficile*. Worryingly all of
324 the wipes repeatedly transferred bacteria and spores onto multiple surfaces.
325 Given that detergent cleaning is advocated in many national guidance documents
326 it is imperative that such recommendations and guidance take into account the
327 wipe limitations found in this study. The issue of potential transfer onto multiple
328 surfaces needs to be addressed to avoid the potential spread of microbial
329 pathogens.

330

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338

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436 **Table 1.** Detergent wipes' ingredients

Wipe	Composition ^a	Product	Manufacturer
Wipe A	Amongst other ingredients; <5% non-ionic surfactants, parfum, DMDM hydantoin, iodopropynyl butylcarbamate.	Azodet™ Detergent Wipe	Synergyhealth, Derby, UK
Wipe B	<5% non-ionic surfactants and preservatives (old formulation). ^b	Clinell® Detergent Wipe	GAMA Healthcare, London, UK
Wipe C	Dimethyl oxazolidine, parfum.	Sani Cloth Detergent Wipe	PDI Europe, Flint, UK
Wipe D	<5% non-ionic surfactant, DMDM hydantoin, iodopropynyl butylcarbamate.	Aquamed MA Detergent Wipe	Marshal Curtis, Didcot, UK
Wipe E	<5% non-ionic surfactant, DMDM hydantoin, iodopropynyl butylcarbamate.	Clinitex® Detergent Wipe	Techtex®, Manchester, UK
Wipe F	Amongst other ingredients; Parfum, DMDM hydantoin, iodopropynyl butylcarbamate.	Tuffie Detergent Wipe	Vernacare, Bolton, UK
Wipe G	<5% non-ionic surfactants and preservatives (new formulation). ^b	Clinell® Detergent Wipe	GAMA Healthcare, London, UK

437 ^a Composition noted from packaging

438 ^b Difference between wipe B and G is the material used (viscose) wipe G

Table 2: CFU and % transfer in *S. aureus*, *A. baumannii* and *C. difficile* onto three consecutive surfaces. Mean values from 3 biological repeats.

Wipes	CFU/spores on wipes*	Transfer 1 st surface	Transfer 2 nd surface	Transfer 3 rd surface	Total % transferred
		% microbial/spore transfer			
<i>S. aureus</i>					
A	66890	66.43	82.28	64.74	213.45
B	3633282	11.01	9.75	13.14	33.90
C	5078282	8.58	66.05	44.83	119.46
D	4941786	0.04	0.03	0.04	0.11
E	14537759	0.43	0.39	0.37	1.20
F	13388894	0.09	0.07	0.21	0.37
G	16705056	0.00	0.00	0.00	0.00
<i>A. baumannii</i>					
A	13388894	0.02	0.01	0.01	0.04
B	1505426	0.02	0.01	0.02	0.05
C	3442779	8.00	0.03	0.02	8.05
D	1505426	0.01	0.01	0.01	0.03
E	507976	0.03	0.02	0.03	0.08
F	507804	0.02	0.02	0.02	0.06
G	777048	0.00	0.00	0.00	0.00
<i>C. difficile</i>					
A	92684	2.88	13.10	11.68	27.66
B	24111	2.89	7.18	2.69	12.76
C	29907	114.95	71.78	36.52	223.25
D	25275	8.16	20.88	1.76	30.80
E	5928	5.34	3.09	2.53	10.96
F	5360	16.61	20.42	31.10	68.13
G	9070	5.33	6.43	1.29	13.05

* Average number of bacteria/spore on the wipe following wiping – calculated

from the difference between bacteria left on surface before and after wiping.

Figure 1: Mean log₁₀ bacterial removal from disks using the 3-step method examining the efficacy of detergent wipes against *S. aureus* (■), *A. baumannii* (■) and *C. difficile* (spores) (■). Data is a mean of 3 biological repeats, bars represent SD of replicates.

