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Impact of test protocols and material binding on the efficacy of antimicrobial wipes

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SUMMARY

Background: The use of effective cleaning/disinfectant products is important to control pathogens on healthcare surfaces. With the increasing number of wipe products available, there is a concern that combination of a formulation with the wrong material will decrease the efficacy of the product. This study aimed to use a range of efficacy test protocols to determine the efficacy of four formulations before and after binding to three commonly used wiping materials.

Methods: Two quaternary ammonium (QAC)-based products, one hydrogen-peroxide-based product and one neutral cleaner were combined with microfibre, cotton or non-woven materials and tested for efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with two surface tests (ASTM E2197-17 and EN13697-15) and two 'product' tests (ASTM E2967-15 and EN16615-15).

Findings: Overall, the impact of using different materials on formulation efficacy was limited, except for an alkyl(C_{12-16})dimethylbenzylammonium chloride-based product used at 0.5% v/v. The hydrogen peroxide product was the most efficacious regardless of the material used. The results from wipe test ASTM E2967-15 were consistent with those from the surface tests, but not with EN16615-15 which was far less stringent.

Conclusions: The use of different wiping cloth materials may not impact severely on the efficacy of potent disinfectants, despite the absorption of different volumes of formulation by the materials. QAC-based formulations may be at higher risk when a low concentration is used. There were large differences in efficacy depending on the standard test performed, highlighting the need for more stringency in choosing the test to make a product claim on label.

Introduction

The control of microbial bioburden on surfaces is recognized as an important part of infection control [1—4]. It is now well established that pathogens can survive for a long time on surfaces despite regular cleaning and disinfection [1,5—7]. A limit for the number of viable aerobic bacteria and pathogens on surfaces post-cleaning and disinfection has been proposed as 2.5 colony-forming units (cfu)/cm^2 [8—10]. Recent studies have highlighted that bacterial pathogens may survive on environmental dry surfaces in healthcare settings, embedded in complex biofilms with a majority of non-pathogenic species [11—13]. Healthcare environmental surfaces including high-touch surfaces need to be cleaned regularly, or cleaned and disinfected [4,14]. Cleaning and disinfection is imparted on surfaces with

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formulations delivered with materials [15]. The use of purposely designed antimicrobial or cleaning wipes has increased dramatically over the years. Recent evidence suggests that wipe products are better for controlling bacterial pathogens on surfaces than the mere use of some materials combined with a disinfectant [16]. Indeed, a double crossover study highlighted that purposely designed antimicrobial wipes were better at controlling total bacterial bioburden, including multi-drug-resistant organisms, than the combination of sodium hypochlorite in a bucket and some cloth [16]. With the number of biocidal formulations and materials available today, the impact of different materials on formulations has received little attention to date, although the percentage of a biocidal formulation adsorbed on to different materials can be significant [17].

One of the most important changes in recent years was the introduction of efficacy test protocols that reflected the use of a product rather than a formulation [15]. The introduction of the purposely designed antimicrobial wipe test ATSM 2197-15 [19] and to some extent the EN16615-15 [18] provides manufacturers with an appropriate test platform that palliates some of the deficiencies associated with US-driven wipe tests [15]. Despite these tests, some consumers and regulators are still demanding for formulations to be tested for efficacy on their own. One concern is that some formulation ingredients could remain in the material, decreasing the microbicidal efficacy of the formulation on surfaces. This study aimed to evaluate the performance of approved disinfectants using standardized ASTM, EPA and EN test methods after material binding.

Materials and methods

Bacterial strains

Staphylococcus aureus ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 were used in this study. Bacterial stocks were stored at -80°C and revived in tryptone soya broth (Oxoid Ltd, Basingstoke, UK) following incubation at 37°C for 24 h. Culture purity was checked on tryptone soya agar (Oxoid Ltd) following incubation at 37°C for 24 h. *S. aureus* test inoculum was prepared in accordance with EN 13697-15 [20]. *P. aeruginosa* test inoculum was prepared in a glycerol diluent (1 g/L tryptone, 8.5 g/L NaCl, 2 g/L glycerol) according to EN16615-15 [18]. For EN 16615-15, the start-up inocula for *S. aureus* and *P. aeruginosa* were 6.31 $\pm$ 0.34 $\log_{10}$ cfu/mL and 6.69 $\pm$ 0.74 $\log_{10}$ cfu/mL, respectively.

Study design

This study aimed to understand the impact of formulation retention in materials on bactericidal efficacy on surfaces. A number of commercially available formulation and material combinations were tested using the protocol described in Figure 1. This protocol enabled efficacy testing using different standardized efficacy tests (ASTM2197-11 and EN13697-15) at different points of formulation/material interactions, ultimately using a standardized product (i.e. wipe) test protocol (ASTM2967-15 and EN16615-15).

Briefly, the study was divided into three parts: testing the efficacy of commercially available formulations before and after the use of materials, and testing the bactericidal activity of combined formulations/materials with standardized efficacy tests. Two litres of the commercially available formulation were added to a 4-L container. The solution was used within 1 h of dispensing into the container. In parallel, 10 mL of formulation was tested directly with ASTM2197-11 [21] and EN13697-15 [20]. Dry materials were weighed before being submerged for 5 min in the formulation. The material was then wrung out lightly until it was no longer dripping, and weighed to determine how much formulation was adsorbed into the cloth. The soaked and wrung material (formulation/material

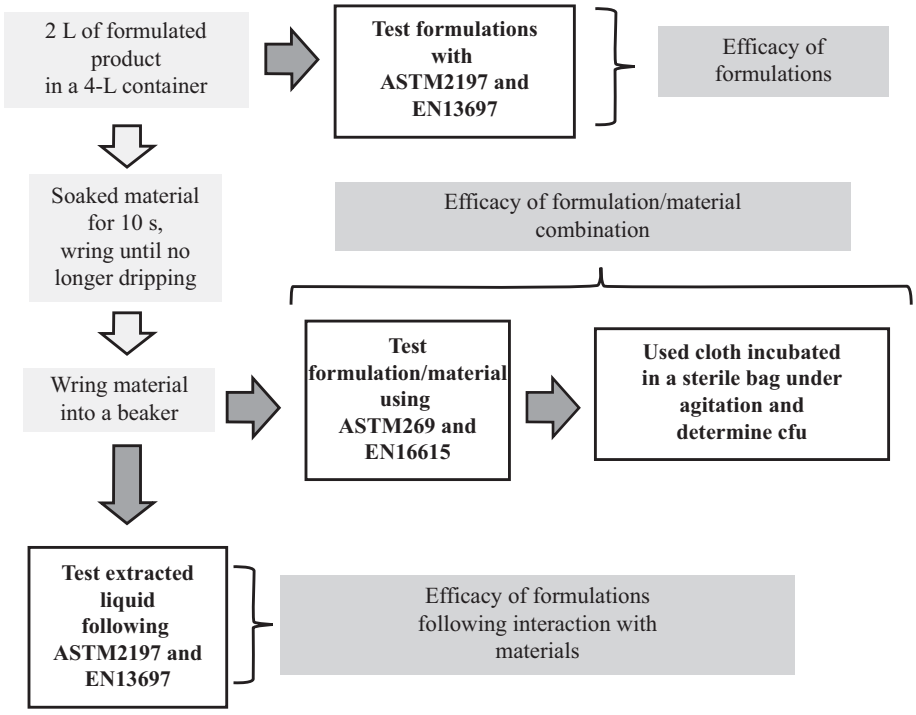

Figure 1. Study design to understand the impact of materials on product efficacy.

combination) was tested using ASTM2697-15 [19] and EN16615-15 [18], or the formulation from the material was eluted following tighter wringing but ensuring not to wring the material dry. The material was weighed to measure how much formulation was left in the material. The formulation was then tested using ASTM2197-11 or EN13697-15.

The formulations tested consisted of: (i) an alkyl(C_{12-16}) dimethylbenzylammonium-chloride-based product (Formulation A; active concentration: 0.5% w/v); (ii) a didecyldimethylammonium-chloride-based product (Formulation B; active concentration 0.3% w/v); (iii) an hydrogen-peroxide-based product (Formulation C; active concentration 7.2% v/v); and (iv) a neutral cleaner (Formulation D; 8% v/v). Materials used were a microfibre cloth (Material A), a non-woven cloth (Material B) and a cotton cloth (Material C).

Standardized test protocols

The following four protocols were investigated: ASTM2197-11 [21], ASTM2967-15 [19], EN13697-15 [20] and EN16615-15 [18]. For all protocols, a test temperature of 20°C was used. A 'universal' neutralizer containing saponin (30 g/L), L-histidine (1 g/L), polysorbate-80 (30 g/L), azolectin from soybean (3 g/L) and sodium thiosulphate (5 g/L) was used with all products. The neutralizer efficacy to quench the efficacy of each product was validated using EN13697-15. A 5-min contact time was used with ASTM29896-12 and EN13697-15. A 10 sec wiping followed by a 5 min contact time was used with the ASTM E2967-15 and EN16615-15. This wiping time and contact time does not follow EN16615-15, but was deemed appropriate for this study. Brushed stainless steel discs (2-cm diameter), AISI type 304, were used with EN13697-15, and brushed stainless steel disks (1-cm diameter), AISI type 430, were used with ASTM2197-11 and ASTM2967-15. Soiling consisted of bovine serum albumin 3 g/L or 0.3 g/L for ASTM2697-15 and the EN tests, or bovine serum albumin + mucin (5% equivalent serum) for ASTM2197-11.

Each testing standard required a different demonstration in $\log_{10}$ reduction to pass the test. EN16615 required $\geq$5 $\log_{10}$ reduction and EN13679 required $\geq$4 $\log_{10}$ reduction. ASTM 2197-11 and ASTM 2967-15 do not state a pass or fail requirement limit. For the purpose of thise study, the pass criterion was set as a $\geq$4 $\log_{10}$ reduction. For the transfer experiment, EN16615 states <50 cfu/25 cm^2 for a pass. This is equivalent to 1.7 $\log_{10}$.

Wiping materials

Three commercially available testing cloths were used. Material A was an ultra microfibre cloth with a thickness of 1.1 mm. Material B was a non-woven cloth with a thickness of 0.2 mm. Material C was a standard cotton bar mop cloth with a thickness of 2.1 mm. Where appropriate, wiping materials were cut into dimensions stated by each standard.

Statistical analysis

Each test was performed in triplicate unless otherwise stated. Data were analysed using one-way analysis of variance (StatPlus 6.0) at the 95% confidence level to compare the efficacy of product combinations to inactivate, remove and transfer bacteria. The $\log_{10}$ reduction was used for the statistical analyses, enabling comparison between material/formulation combinations, and differences in results between the different standards used.

Results

Amount of formulations adsorbed and released from materials

There was a clear difference in the amount of formulation adsorbed and released from different materials after the light wringing (Table I). The cotton material (Material C) adsorbed the greatest amount of formulation regardless of the product, while the non-woven material (Material B) adsorbed the least. The microfibre and cotton materials adsorbed a larger quantity of Formulation A. After light wringing, Material C contained the largest amount of formulation, followed by Material A. Material C released the lowest amount of formulation, while the non-woven material (Material B) released the highest quantity for Formulations B, C and D (Table I).

Table I
Weight of formulation adsorbed by different materials before and after wringing

Material/ formulation	Formulation A (weight; g)			Formulation B (weight; g)			Formulation C (weight; g)			Formulation D (weight; g)		
	Dry	Wet	Lightly wrung	Dry	Wet	Lightly wrung	Dry	Wet	Lightly wrung	Dry	Wet	Lightly wrung
Material A	40.52	199.00	171.52	39.73	176.67	148.63	38.93	164.10	143.23	39.62	194.47	166.22
Material B	2.90	21.63	16.16	2.93	19.26	14.13	2.66	20.70	15.23	2.85	29.41	22.20
Material C	65.23	324.66	291.53	64.96	322.36	285.30	64.56	300.76	263.00	64.70	313.60	282.96
	% formulation adsorbed on the material following light wringing[a]											
	Formulation A			Formulation B			Formulation C			Formulation D		
Material A	86			84			87			85		
Material B	75			73			74			75		
Material C	90			89			87			90		

Formulation A, alkyl(C_{12-16})dimethylbenzylammonium-chloride-based product; Formulation B, didecyldimethylammonium-chloride-based product; Formulation C, hydrogen-peroxide-based product; Formulation D, neutral cleaner; Material A, microfibre cloth; Material B, non-woven cloth; Material C, cotton cloth.

[a] % formulation extracted from material $= \left(\frac{Weight\ of\ the\ material\ after\ light\ wringing}{Weight\ of\ wet\ material}\right) \times 100$

Efficacy tests

The neutralizer was shown to have no toxicity and was efficacious to neutralize all the formulations tested before combination with materials (data not shown).

The start-up inoculum concentration for *S. aureus* was consistent for both EN13697-15 and ASTM21967-11 at 7.51 ± 0.42 cfu/mL, but was lower for *P. aeruginosa* at 6.57 ± 0.74 cfu/mL. The concentration used for ASTM2967-15 was lower than 7 $\log_{10}$ cfu/mL for both bacteria: 6.11 ± 0.26 for *S. aureus* and 5.25 ± 0.40 for *P. aeruginosa*. These different inocula concentrations between the two bacteria resulted from the propagation step. Since the study aimed to compare the methods rather than the activity of the products, the difference in start-up inocula had no impact on the results.

When the bactericidal efficacy of the quaternary ammonium (QAC)-based formulation (Formulation A) was evaluated, its combination with the different materials showed a significant reduction ($P = 0.0082$) in efficacy (Table II). When the reproducibility of bacterial inactivation was evaluated before material binding, there were a few discrepancies in the results. Inactivation results with *S. aureus* and *P. aeruginosa* were consistent with EN13697-15 but not with ASTM2197-11: from 0.53 ± 0.45 to 1.83 ± 0.20 $\log_{10}$ reduction in viability for *S. aureus* and from 0.17 ± 0.01 to 2.30 ± 0.21 $\log_{10}$ reduction for *P. aeruginosa* (Table II). When the formulation was combined with different materials, there was a significant difference in bacterial removal from surfaces ($P = 0.001$) between ASTM2697-15 and EN16615-15 (Table II). The non-woven material (Material B) seemed to be better at preventing transfer of bacteria from the material to other surfaces (Table II). Overall, Formulation A before material binding did not pass EN13697-15 as < 4 $\log_{10}$ reduction in bacterial viability was observed following a 5-min contact time. However, Formulation A combined with any of the materials satisfied the pass criteria of EN16615-15, demonstrating a >4 $\log_{10}$ removal of *P. aeruginosa* from surfaces and the absence of significant transfer, with the exception of its combination with the microfibre and cotton materials to reduce *S. aureus* from surfaces.

When the didecyldimethylammonium-chloride-based product (Formulation B) was tested, there was no evidence of a material binding effect ($P = 0.4471$) for all the materials tested, with the exception of Formulation B activity against *P. aeruginosa* when combined with the non-woven material (Material B) evaluated with EN13697-15 (Table III). There was some variability in inactivation results before material binding with Formulation B with both bacteria: from 1.67 ± 0.23 to 4.19 ± 0.17 $\log_{10}$ reduction with EN13697-15, and from 1.18 ± 0.18 to 3.25 ± 0.05 $\log_{10}$ reduction with ASTM21297-11 for *P. aeruginosa* (Table III). All formulation/material combinations meet the pass criteria of the EN16615-15, except for the transfer of *S. aureus* when the product was combined with the cotton material. The formulation/material combinations to remove bacteria from surfaces were not as efficient when tested with ASTM2967-15, although none of the combinations transferred bacteria post-wiping to other surfaces.

The use of hydrogen-peroxide-based formulation (Formulation C) produced the best activity against both bacteria. Pre-binding inactivation results were mostly consistent for *S. aureus* (all >4 $\log_{10}$ reduction). Formulation C combined with any materials generally performed well (>3 $\log_{10}$ removal)

Table II
Efficacy of the alkyl($C_{12\text{-}16}$)dimethylbenzylammonium-chloride-based product (Formulation A) before and after combination with materials

	EN13697-15		ASTM 2197-11		ASTM 2967-15		EN 16615-15	
Bacterial strain	Before binding	After binding	Before binding	After binding	Removal	Transfer	Removal	Transfer
Combination with microfibre (Material A)								
P. aeruginosa	1.12 (0.34)	0.24 (0.12)	2.30 (0.21)	0.39 (0.07)	1.66 (0.63)	1.69 (0.63)	6.32 (0.71)	1.06 (1.20)
S. aureus	1.86 (0.15)	0.03 (0.19)	1.83 (0.20)	0.70 (0.08)	0.74 (0.10)	4.67 (0.15)	4.07 (0.07)	3.36 (0.04)
Combination with non-woven material (Material B)								
P. aeruginosa	1.11 (0.79)	0.57 (0.73)	0.17 (0.01)	0.32 (0.06)	1.76 (0.26)	0.00 (0.00)	7.09 (0.10)	0.00 (0.00)
S. aureus	1.34 (0.19)	0.78 (0.20)	1.13 (0.04)	0.94 (0.13)	0.72 (0.53)	0.00 (0.00)	7.55 (0.05)	0.00 (0.00)
Combination with cotton (Material C)								
P. aeruginosa	0.85 (0.14)	0.66 (0.11)	1.96 (0.17)	0.72 (0.24)	2.40 (1.05)	0.00 (0.00)	5.95 (0.43)	0.31 (0.36)
S. aureus	1.36 (0.28)	0.64 (0.14)	0.53 (0.45)	0.18 (0.04)	1.15 (0.11)	2.26 (0.50)	3.96 (0.79)	3.95 (0.45)

P. aeruginosa, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.
Red indicates a fail and green indicates a pass (see text). For ASTM2197-11 and ASTM2967-15 for which there are no pass/fail criteria, a result <4 $\log_{10}$ reduction was considered as a fail for consistency with the other standards. For the transfer data, a transfer >1.5 $\log_{10}$ was considered to be a fail.

Table III
Efficacy of the didecyldimethylammonium-chloride-based product (Formulation B) before and after combination with materials

	EN13697-15		ASTM 2197-11		ASTM 2967-15		EN 16615-15	
Bacterial strain	Before binding	After binding	Before binding	After binding	Removal	Transfer	Removal	Transfer
Combination with microfibre (material A)								
P. aeruginosa	2.19 (0.56)	2.55 (0.48)	1.18 (0.18)	1.31 (0.23)	1.87 (0.18)	0.00 (0.00)	6.64 (0.16)	0.67 (0.76)
S. aureus	2.22 (0.18)	2.49 (0.25)	1.58 (0.05)	1.17 (0.16)	1.69 (0.19)	0.00 (0.00)	6.74 (0.04)	1.15 (0.13)
Combination with non-woven material (Material B)								
P. aeruginosa	4.19 (0.17)	2.95 (0.23)	3.02 (0.12)	2.88 (0.09)	3.40 (0.02)	0.00 (0.00)	6.71 (0.49)	0.38 (0.65)
S. aureus	3.09 (0.76)	3.51 (0.11)	3.18 (0.06)	2.79 (0.48)	1.96 (0.19)	0.00 (0.00)	6.91 (0.42)	0.07 (0.13)
Combination with cotton (Material C)								
P. aeruginosa	1.67 (0.23)	1.88 (0.07)	3.25 (0.05)	3.25 (0.07)	2.40 (0.02)	0.00 (0.00)	6.71 (0.41)	0.07 (0.13)
S. aureus	3.10 (0.15)	3.47 (0.17)	3.09 (0.08)	3.01 (0.06)	2.12 (0.08)	0.00 (0.00)	5.90 (0.15)	2.14 (0.04)

P. aeruginosa, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.
Red indicates a fail and green indicates a pass (see text). For ASTM2197-11 and ASTM2967-15 for which there are no pass/fail criteria, a result <4 $\log_{10}$ reduction was considered as a fail for consistency with the other standards. For the transfer data, a transfer >1.5 $\log_{10}$ was considered to be a fail.

with ASTM2697-15, with the exception of activity against *S. aureus* when combined with the cotton material. EN16615-15 also showed a high performance (passing the test criteria) of all combinations against both bacteria (Table IV).

The neutral cleaner (Formulation D) failed to inactivate both bacteria within a 5-min contact time before and after binding to materials (Table V). The use of ASTM2967-15 showed >1 $\log_{10}$ removal with *P. aeruginosa* regardless of the material used. All materials transferred a high number of bacteria post-wiping. In contrast, the use of EN16615-15 showed a 3.80–5.81 $\log_{10}$ removal of bacteria from surfaces. Although the neutral cleaner combined with any material failed to pass the test, which requires >5 $\log_{10}$ removal, the $\log_{10}$ removal achieved was significantly greater ($P = 0.001$) than that obtained with ASTM2697-15.

Discussion

This study aimed to understand the impact of using different materials on the efficacy of formulations. Microfibre, non-woven and cotton cloths are used most commonly in health-care settings. This study also provided information on using different standard tests to evaluate the efficacy of formulations or formulation/material combinations.

This study found that there was little impact on activity when different formulations were combined with a range of materials, with the exception of the QAC-based formulation used at 0.5% with any of the materials tested. Overall, there is little information in the literature about the impact of material on formulations. The efficiency of water-wetted microfibre materials to remove *S. aureus* from stainless steel surfaces has been shown to vary between microfibre materials, and not to be better than a non-woven material [22]. In addition, Moore and Griffiths [22] reported that all materials had a risk of recontaminating surfaces with organic soil and micro-organisms. In a food setting, water-wetted cellulose/cotton material was shown to be better at removing *Listeria monocytogenes* from stainless steel (5.40–5.69 $\log_{10}$ cfu/cm^2) and formica (2.78–3.62 $\log_{10}$ cfu/cm^2) surfaces than microfibre, scouring cloth, non-woven fabric and terry towel [23]. A recent in-situ study showed that a pre-formulated antimicrobial wipe performed better at reducing bacterial pathogens from surfaces than a cotton cloth soaked in a bucket of sodium hypochlorite 1000 ppm [16]. Although material binding did not seem to affect the efficacy of the formulations at the concentrations tested, with the exception of the QAC-based formulation, the material itself had an effect on activity. The appropriate combination of an antimicrobial formulation and wipe material has been deemed essential to achieve the best product activity, measured as microbial removal from surfaces and prevention of microbial transfer from the wipe material [15,16,24]. ASTM2697-15 showed that the hydrogen-peroxide-based product was more effective ($P = 0.00052$) when combined with the microfibre material or the non-woven material than the cotton material. This was not necessarily the case with the other QAC-based formulations.

The different materials used in this study adsorbed different quantities of formulations, with the non-woven material adsorbing the least. Despite that, the formulations combined with the non-woven materials did not perform worse than when combined with other materials. Likewise, the three materials released different quantities of formulation after wringing. There was no apparent correlation between the amount released and formulation activity.

Table IV
Efficacy of hydrogen-peroxide-based product (Formulation C) before and after combination with materials

	EN13697-15		ASTM 2197-11		ASTM 2967-15		EN 16615-15	
Bacterial strain	Before binding	After binding	Before binding	After binding	Removal	Transfer	Removal	Transfer
Combination with microfibre (Material A)								
P. aeruginosa	6.03 (0.10)	6.03 (0.10)	6.34 (0.12)	6.34 (0.12)	4.38 (0.07)	0.00 (0.00)	6.63 (0.31)	0.27 (0.47)
S. aureus	5.20 (0.06)	4.60 (0.31)	4.77 (0.51)	3.74 (0.81)	4.59 (0.04)	0.00 (0.00)	5.85 (0.43)	0.00 (0.00)
Combination with non-woven material (Material B)								
P. aeruginosa	6.06 (0.43)	6.06 (0.43)	5.97 (0.18)	5.77 (0.26)	3.30 (0.50)	0.00 (0.00)	6.89 (0.11)	0.21 (0.36)
S. aureus	4.20 (0.09)	2.51 (0.14)	6.76 (0.02)	6.05 (0.61)	3.48 (0.90)	2.13 (0.75)	6.05 (0.24)	0.00 (0.00)
Combination with cotton (Material C)								
P. aeruginosa	3.64 (0.30)	2.63 (1.27)	2.56 (0.13)	2.47 (0.17)	3.17 (0.75)	0.00 (0.00)	6.44 (0.07)	0.17 (0.30)
S. aureus	4.00 (0.11)	3.44 (1.31)	4.09 (0.09)	3.30 (0.62)	2.43 (0.68)	0.00 (0.00)	6.06 (0.17)	0.00 (0.00)

P. aeruginosa, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.
Red indicates a fail and green indicates a pass (see text). For ASTM2197-11 and ASTM2967-15 for which there are no pass/fail criteria, a result <4 $\log_{10}$ reduction was considered as a fail for consistency with the other standards. For the transfer data, a transfer >1.5 $\log_{10}$ was considered to be a fail.

Table V
Efficacy of neutral cleaner (Formulation D) before and after combination with materials

	EN13697-15		ASTM 2197-11		ASTM 2967-15		EN 16615-15	
Bacterial strain	Before binding	After binding	Before binding	After binding	Removal	Transfer	Removal	Transfer
Combination with microfibre (Material A)								
P. aeruginosa	0.21 (0.24)	0.14 (0.08)	0.08 (0.05)	0.15 (0.19)	1.43 (0.1)	3.45 (0.15)	5.81 (1.13)	1.01 (1.42)
S. aureus	0.24 (0.03)	0.28 (0.13)	0.12 (0.04)	0.15 (0.01)	0.31 (0.1)	3.44 (0.08)	4.18 (0.36)	0.76 (0.34)
Combination with non-woven material (Material B)								
P. aeruginosa	0.12 (0.14)	0.11 (0.23)	0.15 (0.13)	0.02 (0.12)	1.88 (0.23)	3.32 (0.41)	4.55 (0.41)	1.20 (0.83)
S. aureus	0.11 (0.12)	0.30 (0.21)	0.18 (0.08)	0.24 (0.22)	0.92 (0.28)	5.75 (0.66)	3.69 (1.01)	1.83 (0.99)
Combination with cotton (Material C)								
P. aeruginosa	-0.12[a] (0.14)	-0.06[a] (0.32)	0.28 (0.09)	0.22 (0.01)	1.15 (0.04)	3.36 (0.10)	4.50 (0.27)	1.51 (0.64)
S. aureus	0.40 (1.13)	0.36 (0.12)	0.30 (0.11)	0.34 (0.10)	0.62 (0.18)	5.64 (0.03)	3.81 (0.31)	2.33 (0.49)

P. aeruginosa, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.
Red indicates a fail and green indicates a pass (see text). For ASTM2197-11 and ASTM2967-15 for which there are no pass/fail criteria, a result <4 $\log_{10}$ reduction was considered as a fail for consistency with the other standards. For the transfer data, a transfer >1.5 $\log_{10}$ was considered to be a fail.
[a]Denotes no reduction in viability.

These results differ from the results of Engelbrecht *et al.* [25] who measured a decrease in efficacy of three QAC-based formulations combined with cotton towels. In their study, they observed an 85.3% decrease in QAC concentration after exposure to the material. Such a reduction in concentration likely impinged on the efficacy of the formulation measured with a germicidal spray test. In the present study, it is conceivable that the active ingredient(s) in the biocidal

formulations (A–C) were still in excess following wringing to deliver some bactericidal activity, which was measurable with the standards used in this study.

In addition, using the 'wipe' test standards ASTM2697-15 and EN16615-15, the viability of *P. aeruginosa* was less than that of *S. aureus* when tested. The use of *P. aeruginosa* for testing on surfaces is problematic as it does not survive well desiccation, increasing result variability. A higher start-up inoculum or the use of glycerol is needed [18].

Efficacy tests were performed on different days over a 12-month period, and some differences in inactivation using the same bacterial inoculum and standard tests were observed. These differences were not imparted to the inoculum concentration, despite the fact that lower start-up bacterial inocula were used for ASTM2967-15. There was no identifiable pattern for these differences in inactivation (Tables II–IV). Results obtained for the QAC-based and hydrogen-peroxide-based products and the neutral cleaner, regardless of the material used, were generally consistent between the two surface tests. Discrepancies between the two tests were highlighted with the didecyldimethylammonium-chloride-based product, for which ATSM2197-11 showed better inactivation when the product was combined with the non-woven or the cotton materials, although the product failed ATSM2197-11 with an artificial pass criterion set as >4 $\log_{10}$ reduction. Likewise, for the products that showed limited (<4 $\log_{10}$ reduction in cfu/mL) or no activity with the surface test, there was a clear difference when data from ASTM2697-15 and EN16615-15 were compared. Differences in inactivation results depending on the standard test used have been reported recently [26]. The 'four field test' uses a 2-kg weight on the surface [17], whereas ASTM2697-15 [19] uses a 300-g weight. It could be argued that this difference in pressure exerted on the material will increase friction and the ability of the material to remove more bacteria from the surface [15], in essence making EN16615-15 a less stringent protocol. It is particularly interesting that the ASTM2697-15 results correlated better with the results from both surface tests EN13697-15 and ASTM2197-11, although the protocol differs markedly in terms of the mechanical action in ASTM2697-15 as well as the formulation.

It has been recommended that with a combination of material and formulation, not only the removal/killing of bacteria on surfaces needs to be evaluated, but also the risk of transfer of bacteria from the material to other surfaces [15,27,28]. Hence, ASTM2697-15 and EN16615-15 have a transfer component as part of the protocol. The type of formulation will affect the transfer of micro-organisms, particularly surfactant-/detergent-based formulations [24,27,29]. Here, the QAC-based formulation in combination with the microfibre material showed a high transfer rate of *S. aureus* and *P. aeruginosa*. The combination of microfibre and the didecyldimethylammonium-chloride-based product did not result in the transfer of bacteria. The neutral cleaner, perhaps not surprisingly, showed the highest transfer of micro-organisms with either ASTM2697-15 or EN16615-15. Other cleaner-/detergent-based products have been shown to have a high transfer rate post-wiping [24,27].

In conclusion, this study highlighted that materials can impact on formulation activity, but failed to provide evidence that certain types of materials contribute to a decrease in bactericidal efficacy. This study aimed to mimic product usage, so in-use dilutions of products were used. The concentration of active ingredient(s) likely remained high enough to demonstrate changes in bactericidal efficacy. Unfortunately, the concentration of active ingredients was not measured post-wringing. However, this study highlighted discrepancies in results between standard tests, with EN16615-15 constantly showing better efficacy of product/material combinations. Conversely, ASTM2697-15 provided results which were more in line with the results for the surface tests.

Conflict of interest statement
None declared.

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