Impact of test protocols and material binding on antimicrobial wipes efficacy

Wesgate R, Robertson A, Barrell M and Maillard J-Y*
Cardiff school of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, UK

*address for correspondence
Cardiff school of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII avenue, Cardiff CF10 3NB, Wales, United Kingdom
Email: maillardj@cardiff.ac.uk

Keywords: wipes, test protocols, efficacy, material

Short title: Microbicidal formulations efficacy depend on materials

Journal of hospital infection

Summary

Background: The use of effective cleaning/disinfectant product 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 product efficacy. This study aims 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.

Method: Two quaternary ammonium and one hydrogen peroxide-based products, and one neutral cleaner, were combined with microfiber, cotton or non-woven materials and tested for efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with two surface tests (ASTM E2197-17; 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 the wipe-test ASTM E2967-15 were consistent with those from the surface tests but not with the EN16615-15 which was far less stringent.

Conclusions: The use of different wiping cloth materials might 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 more at 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 recognised as an important part of infection control.[1-4] It is now well established that pathogens can survive a long time on surface despite the regular use of cleaning and disinfection.[1,5-7] A limit as to the number of viable aerobic bacteria and pathogens on surfaces post-cleaning and disinfection has been proposed as 2.5 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 regularly cleaned or cleaned and disinfected.[4,14] Cleaning and disinfection is imparted on surfaces with formulations delivered with materials.[15] The use of purposely designed formulation/material, the antimicrobial or cleaning wipes has increased dramatically over the year. Recent evidence suggests, that wipe products are better in controlling bacterial pathogens on surfaces than the mere use of some materials combined with a disinfectant.[16] Indeed, a double cross-over study highlighted that purposely designed antimicrobial wipes were better at controlling total bacterial bioburden including multi-drug resistant organisms (MDRO) 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 material on formulation has yet received little attention, although the percentage of a biocidal formulations adsorbed on different material can be significant.[17]

One of the most important change 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 the EN16615-15[18] “four field test” and to some extend the ATSM 2197-15[19] has been impactful for manufacturers despite the existence of other US-driven tests, that nevertheless presented a number of negative issues for testing formulated wipes.[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 aims to evaluate the performance of approved disinfectants using standardized ASTM, EPA and EN test methods after material binding.

Materials and Methods

Bacterial strains

The following bacteria were used: *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 15442. Bacterial stocks were stored at -80°C and revived in tryptone soya broth (TSB, Oxoid Ltd., Basingstoke, UK) following incubation at 37°C for 24h. Culture purity was checked on tryptone soya agar (TSA; Oxoid Ltd., Basingstoke, UK) following incubation at 37°C for 24h. *S. aureus* test inoculum was prepared in accordance to the 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 the EN16615-15.[18] For the EN 16615-15, the start-up inoculum for *S. aureus* and *P. aeruginosa* were 6.31 ± 0.34 $\log_{10}$ CFU/mL and 6.69 ± 0.74 $\log_{10}$ CFU/mL respectively.

Study design

The study aims to understand the impact of formulation retention in materials on bactericidal efficacy in surfaces. To do so, a number of commercially available formulation and material combinations were tested including using the protocol described in figure 1. This protocol enabled efficacy test using different standardised efficacy tests (ASTM2197-11; EN13697-15) at different points of formulation/material interactions, ultimately using standardised 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 standardised 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 directly tested with the ASTM2197-11[21] and EN13697-15.[20] Dry materials were weighted before being submerged for 5 min in the formulation. The material was then wrung lightly until no longer dripping and its weight measured to determine how much formulation was adsorbed into the cloth. The soaked and wrought material (formulation/material combination) was tested using the 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 then weighed to measure how much formulation was left in the material. The formulation was then tested using the ASTM2197-11 or the EN13697-15.

The formulations tested consisted of i) an alkyl($C_{12\text{-}16}$)dimethylbenzylammonium chloride-based product (formulation A; concentration of active: 0.5% w/v), ii) a didecyldimethylammonium chloride-based product (formulation B; concentration of active 0.3% w/v) ii) an hydrogen peroxide-based product (formulation C; concentration of active: 7.2% v/v), and iv) a neutral cleaner (formulation D used at 8% v/v). Materials used were a microfiber cloth (material A), a nonwoven material (material B) and a cotton cloth (material C).

Standardised test protocols

The following four protocols/bacteria will be investigated: ASTM2197-11[21] and ASTM2967-15,[19] EN13697-15[20] and EN16615-15.[18] For all protocols the test temperature of 20°C was used. A ‘universal’ neutraliser containing saponin (30 g/L), L-histidine (1 g/L), Polysorbate-80

(30 g/L), azolectin from soybean (3 g/L) and sodium thiosulfate (5 g/L) was used with all products. The neutraliser efficacy to quench the efficacy of each products was validated using the EN13697-15. A 5 min contact time was used with the ASTM29896-12 and EN13697-15, and a 10 sec wiping followed. By a 5 min contact time was used with the ASTM2697-15 and EN16615-15. This wiping time and contact time does not follow the EN16615-15 standard but was deemed appropriate for this study. Stainless steel discs (2 cm diameter) brushed, AISI type 304 were used with the EN13697-15 protocol and stainless steel disks (1 cm diameter) brushed, AISI type 430 were used with the ASTM2197-11 and ASTM2967-15. Soiling consisted of bovine serum albumin 3 g/L or 0.3 g/L for the ASTM2697-15 and EN tests, or BS + mucin (5% equivalent serum) for the ASTM2197-11.

Each testing standard required a different demonstration in $\log_{10}$ reduction to pass the test. EN16615 required ≥5 $\log_{10}$ reduction and EN13679 requires a ≥4 $\log_{10}$ reduction. ASTM 2197-11 and ASTM 2967-15 do not state a pass or fail requirement limit. For the purpose of the study, the pass criterion was set as a ≥4 $\log_{10}$ reduction. For the transfer experiment the EN16615 states a < 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 is an Ultra Microfiber cloth with a thickness of 1.1mm. Material B is a non-woven cloth with a thickness of 0.2mm. Material C is a standard cotton bar mop cloth with a thickness of 2.1mm. 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 a one-way ANOVA (StatPlus 6.0) at the 95% confidence level) to compare the efficacy of product combination to inactivate, remove and transfer bacteria. The use of the $\log_{10}$ reduction was used for the statistical analyses, enabling comparison between material/formulation combination, 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 1). The cotton material (Material C) adsorbed the largest amount of formulation regardless of the product, while the non-woven material (Material B) adsorbed the least. The microfiber 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 released the highest quantity for formulations B, C and D (Table 1).

Efficacy tests

The neutraliser was shown to have no toxicity and it was efficacious to neutralise 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 tests with 7.51 ± 0.42 CFU/mL but lower for *P. aeruginosa* with 6.57 ± 0.74 CFU/mL. The concentration used for the 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 inoculum concentrations between the two bacteria resulted from the propagation step. Since the study aimed to compare the methods and not the activity of the products, the difference in start-up inocula had no impact on the results.

When the bactericidal efficacy of the quaternary-based formulation (A) was evaluated its combination with the different materials showed a significant reduction (P=0.0082) in efficacy (Table 2). When the reproducibility of bacterial inactivation was evaluated before material binding, there were a few discrepancies in results. Inactivation results with *S. aureus* and *P. aeruginosa* were consistent with the EN13697-15, but not with the ASTM2197-11; from 0.53 ± 0.45 to 1.83 ± 0.20 $\log_{10}$ reduction in viability for *S. aureus* and 0.17 ± 0.01 to 2.30 ± 0.21 $\log_{10}$ reduction for *P. aeruginosa* (Table 2). When the formulation was combined with different materials, there was a significant difference in bacterial removal from surfaces (P=0.001) between the ASTM2697-15 and the EN16615-15 (Table 2). The non-woven material seems better at preventing transfer of bacteria from the material to other surfaces (Table 2). Overall, formulation A before material binding did not pass the EN13697-15 as <4 $\log_{10}$ reduction in bacterial viability was observed following a 5 min contact time. Formulation A combined with any of the materials however satisfied the pass criteria of the EN16615-15 demonstrating a >4 $\log_{10}$ removal of *P. aeruginosa* from surface and the absence of significant transfer, with the exception of its combination with the microfiber and cotton materials to reduce *S. aureus* from surfaces.

When the didecyldimethylammonium chloride-based product (B) was tested, there was no evidence of 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 evaluated with the EN13697-15 standard (Table 3). 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 the EN13697-15 test, and 1.18 ± 0.18 to 3.25 ± 0.05 $\log_{10}$ reduction

with the ASTM21297-11 for *P. aeruginosa* (Table 3). All formulation/material combinations performed well with EN16615-15, meeting the test pass criteria, but for the transfer of *S. aureus* when product was combined with the cotton and evaluated with the EN16615-15. The formulation/material combinations to remove bacteria from surfaces was not as efficient when tested with the ASTM2967-15 test, although none of the combinations transferred bacteria post-wiping to other surfaces.

The use of hydrogen peroxide-based formulation (C) produced the best activity against both bacteria. Pre-binding inactivation results were mostly consistent with *S. aureus* (all >4 $\log_{10}$ reduction). Formulation C combined to any materials generally performed well (> 3 $\log_{10}$ removal) with the wipe test ASTM2697-15 with the exception of activity against *S. aureus* when combined with the cotton material. The EN16615-15 again showed a high performance (passing test criteria) of all combinations against both bacteria (Table 4).

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

Discussion

This study aimed to understand the impact of using different materials on the efficacy of formulations. Microfiber, non-woven and cotton represented the most used materials in healthcare settings. The study also provided information on using different standard tests on evaluating the efficacy of formulations or formulation/material combinations.

Here, we showed that there was little impact on activity when different formulations were combined with a range of materials, with the exception of the quaternary-ammonium based formulation used at 0.5% with any of the material tested. There is overall 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] observed that all materials were shown to carry the risk to re-contaminate surfaces with organic soil and micro-organisms.[22] In a food setting, showed that water hydrated cellulose/cotton material was 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 a microfiber, 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 the use of 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 concentration tested, with the exception of the quaternary-based one, 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] The ASTM2697-15 showed that the hydrogen peroxide-based product was more effective (P=0.00052) when combined with the microfibre or the non-woven material than the cotton one. This was not necessarily the case with the other quaternary ammonium -based formulations.

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

Our results differ from the study from Engelbrecht and colleagues[25] who measured a decrease in efficacy of three QAC-based formulations combined with cotton towels. In their study they observed an 85.3% decreased 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. Here, 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 standard used in our study.

In addition, using the “wipe” test standards ASTM2697-15 and EN16615-15, the viability of *P. aeruginosa* was less than *S. aureus* when tested. *P. aeruginosa* does not survive well dehydration and *using P. aeruginosa* for surface testing is problematic as the start-up inoculum needs to be higher to encompass for a loss a viability due to desiccation or glycerol needs to be added to the inoculum on surfaces[18] it can be more prone to results variability.

Efficacy tests were performed on different days over a 12 months 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 lower start-up bacterial inocula were used for the ASTM2967-15 test. There was no identifiable pattern for these differences in inactivation (Tables 2-4). Results obtained for the quaternary-based and hydrogen-peroxide-based products, and the cleaner regardless of the material combination, were consistent between the two surface tests. Discrepancies between the two tests were

highlighted with the didecyldimethylammonium chloride-based product for which the ATSM2197-11 showed better inactivation when the product was combined with the non-woven or the cotton materials, although the product failed the ATSM2197-11 with an artificial pass criterion set as >4 $\log_{10}$ reduction. Likewise, for the product that show limited (< 4 $\log_{10}$ reduction in CFU/ML) or no activity with the surface test, there was a clear difference when data from the ASTM2697-15 and the EN16615-15 were compared. Differences in inactivation results depending on the standard test used have recently been reported.[26] The 'four field test" uses a 2 kg weight on surface[17] whereas the ASTM2697-15[19] test uses 300 g. 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 the EN16615-15 standard 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 the ASTM2197-11, although the protocol differs markedly in that the mechanical action in the ASTM2697-15 as well as the formulation.

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

This study highlighted that materials can impact on formulation activity but failed to produce evidence that certain type of materials contributed to a decrease in bactericidal efficacy. Here we wanted to mimic product usage and as such in use dilution of products were used. The concentration of active ingredient(s) likely remained high enough to demonstrate changes in bactericidal efficacy. Unfortunately, we did not measure the concentration of active ingredients post-wringing. Our study however highlighted discrepancies in results between standard tests with the use EN16615-15 constantly showing a better efficacy of the product/material combination. Conversely, the ASTM2697-15 test provided results which were more in line with the results for the surface tests.

References

[1] Gebel J, Exner M, French G, Chartier Y, Christiansen, Gemein S et al. The role of surface disinfection in infection prevention. GMS Hyg Infect Control 2013;8:1-12.

[2] Donskey CJ. Does improving surface cleaning and disinfection reduce health care-associated infections? Am J Infect Control 2013;41:S12-9.

[3] Loveday HP, Wilson JA, Pratt RJ, Golsorkhi M, Bak A, Browne J et al. epic3: national evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. J Hosp Infect 2014;86:S1-S70.

[4] Siani H, Maillard J-Y. Best practice in healthcare environment decontamination. *Eur J Clin Microbiol Infect Dis* 2015;34:1-11.

[5] Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol 2011;32:687-99.

[6] Weber DJ, Anderson DJ, Sexton DJ, Rutala WA. Role of the environment in the transmission of Clostridium difficile in health care facilities. Am J Infect Control 2013;41:S105-10.

[7] Kundrapu S, Sunkesula V, Jury LA, Kundrapu S, Sunkesula V, Jury LA. Daily disinfection of high-touch surfaces in isolation rooms to reduce contamination of healthcare workers' hands. Infect Control Hosp Epidemiol 2012;33:1039-42.

[8] Lewis T, Griffith C, Gallo G, Weinbren M. A modified ATP benchmark for evaluating the cleaning of some hospital environmental surfaces. J Hosp Infect 2008;69:156-63.

[9] White LF, Dancer SJ, Robertson C, McDonald J. Are hygiene standards useful in, assessing infection risk? Am J Infect Control 2008;36:381-4.

[10] Mulvey D, Redding P, Robertson C, Woodall C, Kingsmore P, Bedwell D et al. Finding a benchmark for monitoring hospital cleanliness. J Hosp Infect 2011;77:25-30.

[11] Vickery K, Deva A, Jacombs A, Allan J, Valente P, Gosbell I. Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J Hosp Infect 2012;80:52-5.

[12] Hu H, Johani K, Gosbell I, Jacombs A, Almatroudi A, Whiteley G et al. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy. J Hosp Infect 2015;91:35-44.

[13] Ledwoch K, Dancer, Otter JA, Kerr K, Roposte D, Rushton L et al. Beware Biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multicentre study. *J Hops Infect* 2018, in press.

[14] Dancer SJ. Hospital cleaning in the 21st century. Eur J Clin Microbiol Infect Dis 2011;30:1473–81.

[15] Sattar SA, Maillard J-Y. The crucial role of wiping in decontamination of high-touch environmental surfaces: review of current status and directions for the future. Am J Infect Control 2013;4:S97-104.

[16] Siani H, Wesgate R, Maillard J-Y. Impact of antimicrobial wipe compared with hypochlorite solution on environmental surface contamination in a healthcare setting: a double crossover study. Am J Infect Control 2018; DOI: https://doi.org/10.1016/j.ajic.2018.03.020.

[17] Bloß R, Meyer S, Kampf G. Adsorption of active ingredients of surface disinfectants depends on the type of fabric used for surface treatment. J Hosp Infect 2010;75:56-61.

[18] EN16615-15. Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2). British Standard Institute 2015; London.

[19] ASTM2197-15 Standard Test Method for Assessing the Ability of Pre-wetted Towelettes to Remove and Transfer Bacterial Contamination on Hard, Non-Porous Environmental Surfaces Using the Wiperator. ASTM International 2015.

[20] EN13697-15. Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2, step 2). British Standard Institute 2015; London.

[21] ASTM2197-11. Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. ASTM International 2011.

[22] Moore G, Griffith C. A laboratory evaluation of the decontamination properties of microfibre cloths. J Hosp Infect 2006;64:379-85.

[23] Koo, O-K, Martin, Martin EM, Story R, Lindsay D, Ricke SC, Crandall PG. Comparison of cleaning fabrics for bacterial removal from food-contact surfaces. Food Control 2013;30:292-7.

[24] Siani H, Cooper C, Maillard J-Y. Efficacy of “sporicidal” wipes against *Clostridium difficile.* Am J Infect Control 2011;39:212-8.

[25] Engelbrecht K, Ambrose D, Sifuentes L, Gerba C, Weart I, Koenig D. Decreased activity of commercially available disinfectants containing quaternary ammonium compounds when exposed to cotton towels. Am J Infect Control 20143;41:908-11.

[26] Wesgate R, Rauwell G, Criquelion J, Maillard J-Y. Impact of standard test protocols on sporicidal efficacy. J Hosp Infect 2016;93:256-62.

[27] Ramm L, Siani H, Wesgate R, Maillard J-Y. Pathogen transfer and high variability in pathogen removal by detergent wipes. Am J Infect Control 2015;43:724-8.

[28] Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard J-Y. The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with *Staphylococcus aureus*. J Hosp Infect 2007;67:329-35.

[29] Cadnum JL, Hurless KN, Kundrapu , Donskey CJ. Transfer of *Clostridium difficile* spores by nonsporicidal wipes and improperly used hypochlorite wipes: Practice + Product = Perfection. Infect Control Hosp Epidemiol 2013;34:441-2.

Funding

This project was funded by Diversey.

Conflict of interest

None

Table 1 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*											
	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 extracted from material $= \left(\frac{Weight\ of\ the\ material\ after\ light\ wringing}{Weight\ of\ wet\ material}\right) \times 100$

Table 2 Efficacy of the alkyl(C_{12-16})dimethylbenzylammonium chloride-based product (A) before and after combination to materials. Colours (red): fail; (green): pass (see text). For the ASTM2197-11 and ASTM2967-15 for which there is 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.

	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 microfiber (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 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)

Table 3 Efficacy of the didecyldimethylammonium chloride-based product (B) before and after combination to materials. Colours (red): fail; (green): pass (see text). For the ASTM2197-11 and ASTM2967-15 for which there is 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.

	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 microfiber (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 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)

Table 4 Efficacy of hydrogen peroxide-based product (C) before and after combination to materials. Colours (red): fail; (green): pass (see text). For the ASTM2197-11 and ASTM2967-15 for which there is 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.

	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 microfiber (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 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)

Table 5 Efficacy of neutral cleaner (D) before and after combination to materials. Colours (red): fail; (green): pass (see text). For the ASTM2197-11 and ASTM2967-15 for which there is 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.

	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 microfiber (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 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*** (0.14)	**-0.06*** (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)

* denote no reduction in viability

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