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Efficacy of five 'sporicidal' surface disinfectants against *Clostridioides difficile* spores in suspension tests and 4-field tests

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SUMMARY

Background: A sporicidal surface disinfection is recommended both for the outbreak and the endemic setting but a comparative evaluation on the efficacy of 'sporicidal' surface disinfectants using suspension tests and 4-field tests has not been performed.
Aim: To determine the efficacy of five 'sporicidal' surface disinfectants (three ready-to-use wipes (A, B, E), two concentrates (C, D) based on peroxides or aldehydes against *C. difficile* spores.
Methods: The efficacy was determined under clean conditions using a suspension test and the 4-field test. Each test was performed in duplicate in two separate laboratories. Wipes were wrung to collect the solution for the suspension tests.
Results: Product A (peracetic acid; 5 min), product C (peracetic acid; 2% solution in 15 min or 1% solution in 30 min) and product D (peracetic acid; only 2% solution in 15 min) were effective with at least a 4 $\log_{10}$-reduction of *C. difficile* spores in suspension and on surfaces. Product B (hydrogen peroxide) was not effective in suspension (0.9 $\log_{10}$ after 15 min; 3.2 $\log_{10}$ after 1 h) and on surfaces (2.8 $\log_{10}$ after 15 and 60 min). Product E based on glutaraldehyde, (ethylendioxy)dimethanol and DDAC demonstrated 0.9 $\log_{10}$ after 4 h in suspension and 4.5 $\log_{10}$ after 4 h on surfaces.
Conclusions: Not all surface disinfectants with a sporicidal claim were effective against *C. difficile* spores in standardized suspension tests and in the 4-field test. In clinical

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practice preference should be given to products that reliably pass the efficacy criteria of both types of tests.

Introduction

A sporicidal surface disinfection is recommended both for the outbreak and the endemic setting [1] as one of many elements usually applied in bundles with the aim of controlling nosocomial transmission [2,3]. Only few biocidal agents such as peroxides, aldehydes or chlorine dioxide, sometimes in formulated products, have been described to be effective against spores of *Clostridioides difficile* in suspension or surface tests [4—8]. An effective treatment against spores on surfaces has been demanded to reduce the risk of *C. difficile* spore transmission in healthcare [9]. Some manufacturers of surface disinfectants claim a sporicidal activity of their product based on results obtained with spores of *Bacillus subtilis* in suspension tests. In the meantime, however, test protocols have been developed and approved to measure the sporicidal activity of surface disinfectants with spores of *C. difficile* in suspension tests and under practical conditions (4-field test) [6]. Both experimental designs have been described to yield reproducible efficacy data [6]. A comparative evaluation on the efficacy of 'sporicidal' surface disinfectants using both types of tests has, to our knowledge, never been performed. The aim of this study was therefore to determine the efficacy of five 'sporicidal' surface disinfectants against *C. difficile* spores in both suspension tests and 4-field tests.

Material and methods

Laboratories

Six laboratories participated in the study. All products were tested in laboratory 1 which has the largest experimental experience in sporicidal efficacy testing. A second test of each product was pereformed in one of the other five laboratories such that finally two data sets from different laboratories were available for each product.

Test organism and culture conditions

C. difficile NCTC 13366 was used in all experiments. It was chosen because of its clinical relevance (ribotype 027) and mostly lower susceptibility to disinfectants compared with other strains of the species [10]. Three spore suspensions were prepared centrally at Bonn University according to EN 17126 [11] and supplied to the participating laboratories. Briefly, a subculture was prepared from the stock culture by streaking on to BHIYT-L agar plates. After anaerobic incubation of the plates for 48 h at 36 °C an isolated colony was suspended in 5 mL pre-reduced Columbia broth and incubated in an anaerobic jar for 24 h at 36 °C. A 50 µL aliquot of the culture was inoculated into 20 mL of pre-reduced Columbia broth and incubated anaerobically for 20 h at 36 °C. The entire inoculum was then transferred into a 500-mL culture flask with the liquid sporulation medium. The flasks were incubated anaerobically for 10 d at 36 °C. Finally, the cells were washed, vegetative cells and debris digested enzymatically with trypsin and lysozyme. The spores were stored at 2 °C to 8 °C and were only used after a storage time of at least 8 weeks. The spore suspension adjusted to a cell count of 1.5—5.0 × 10^8/mL. The spore suspension was microscopically visually checked. Slight single debris was observed, the purity was approximately 97%. Laboratories were advised to keep the spore suspension at 2—8 °C.

Test products and sample size

Five products from German manufacturers with sporicidal claims were used and are described in Table I. Two of the products were from Ecolab Deutschland GmbH, one from Schülke & Mayr GmbH, one from Dr. Schumacher GmbH and one from Bode Chemie GmbH. Each product was tested twice on two different days and in two different laboratories. In addition, Bioban™ GA 50 Antimicrobial (Dow Chemical Company Ltd., Staines, UK, 50% glutaraldehyde) was used as glutaraldehyde standard. Lerasept® spezial (Stockmeier Chemie GmbH & Co. KG, Bielefeld, Germany, 4.9% peracetic acid and 25.5% hydrogen peroxide) was used as peracetic acid standard. The two standards were used to measure the susceptibility of the *C. difficile* spore preparation as described previously [6].

Determination of susceptibility of the prepared C. difficile spores for internal control

For internal quality-control purposes, a test using the validation solutions in its lower specified concentration 1% glutaraldehyde and 0.01% peracetic acid was performed at least once in each laboratory using a suspension test according to EN 17126 [11]. Briefly, 8 mL of the test product was thoroughly mixed with 1 mL of water of standard hardness and 1 mL of the test suspension (1.5—5.0 × 10^6 cfu/mL) controlled at 20 °C. Towards the end of the exposure time the tube contents were mixed again. After the exposure time of 15 min, an aliquot of 1 mL of the mixture was removed and transferred to a tube containing 9 mL of an appropriate neutralizer solution. Immediately afterwards, 10^{-1} and 10^{-2} dilutions were prepared in neutralizer solution. The following neutralizers were used: polysorbate 80 (10 g/L) with glycine (20 g/L) in 0.25 M phosphate buffer for glutaraldehyde, and polysorbate 80 (10 g/L) with sodium thiosulphate (3 g/L) in 0.25 M phosphate buffer for peracetic acid. The suitability of the neutralizers for the test products was validated with *C. difficile* spores according to VAH method 18 [18]. After a neutralization time of 5 min the solution was mixed again and 1 mL taken out in duplicate. The 1 mL samples were poured into separate Petri dishes. Fifteen to 20 mL of melted BHIYT-L agar was added and cooled to 45 °C. Plates were then incubated in anaerobic jars for five days at 36 °C followed by counting the colonies per plate, followed by calculating the number of cfu per mL on a $\log_{10}$ scale. The difference from the number of cells in the test mixture at the

Table I
Test products with a sporicidal claim from the manufacturer and validated neutralizing agents

Test product	Active biocidal ingredient(s)	Manufacturers sporicidal claim		Validated neutralizing agents
		2017	2021	
A*	Peracetic acid (0.06%, w/w)	Sporicidal in 15 min according to EN 13704**** Effective in 5 min against *C. difficile* spores****	Effective in 5 min against *C. difficile* spores according to EN 17126 under clean conditions	1% Tween 80, 0.3% sodium thiosulphate, 0.025% catalase
B*	Hydrogen peroxide (1.5%, w/w)***	Sporicidal in 60 min according to EN 13704 under clean conditions Effective in 15 min against *C. difficile* spores according to EN 13704under clean conditions Sporicidal in 15 min according to modified EN 16615 under clean conditions	Sporicidal in 60 min according to EN 17126 under clean conditions Effective in 60 min against *C. difficile* spores according to EN 17126 under clean and dirty conditions	1% Tween 80, 0.3% lecithin, 0.3% histidine, 0.3% sodium thiosulphate, 0.025% catalase
C**	Peracetic acid, made from a powder containing disodium carbonate, compound with hydrogen peroxide and citric acid; a 2% solution (w/v) contains > 0.1% peracetic acid	Sporicidal in 15 min at 2% according to EN 13704**** Effective in 10 min at 1% in a practical procedure*****	Sporicidal in 15 min at 2% and effective in 15 min at 1% against *C. difficile* spores according to EN 17126 under clean and dirty conditions	1% Tween 80, 0.3% lecithin, 0.3% histidine, 0.3% sodium thiosulphate
D**	Peracetic acid, made from a powder containing sodium percarbonate, citric acid and sodium carbonate; a 1% solution (w/v) contains >0.075% peracetic acid	Effective in 15 min at 2%, 30 min at 1% and 60 min at 0.5% against *C. difficile* spores according to EN 13704 under clean conditions	Effective in 5 min at 1.5% and 15 min at 1% against *C. difficile* spores according to EN 17126 under clean and dirty conditions Effective in 60 min at 0.5% against *C. difficle* spores according to a modified EN 16615 under clean and dirty conditions	1% Tween 80, 0.3% lecithin, 0.3% histidine, 0.3% sodium thiosulphate
E*	(Ethylendioxy)dimethanol (0.282%, w/w), didecyldimethylammoniumchloride (0.16%, w/w), glutaraldehyde (0.1%, w/w)	Effective in 4 h against *C. difficile* spores****	No updated information found	1% Tween 80, 0.3% lecithin, 0.3% histidine, 2% glycine

* Ready-to-use wipe.
** Powder concentrate.
*** Contains hydrogen peroxide (<8%) and acetic acid (1–5%) as additional ingredients.
**** No information on organic load.
***** No information on test method and requirements.

beginning of the contact time is reported as the $\log_{10}$ reduction. The susceptibility of the *C. difficile* test spores is considered to be validated if the mean $\log_{10}$ reduction is <1.5 with 1% glutaraldehyde and 0.01% peracetic acid.

Efficacy of test products in suspension tests

The efficacy of the surface disinfectants against *C. difficile* spores was determined with organic load (0.03% albumin w/v; 'clean conditions') in each laboratory in duplicate using a suspension test according to EN 17126 [11]. Clean conditions were chosen because most manufacturers provided sporicidal efficacy data only under clean conditions. Briefly, test products A, B and E were wrung inside the package in order to ensure that the ready-to-use surface disinfectant solution had no additional contact with other materials. The volume per package was sufficient to perform the suspension test and (typically 30 mL or more) was used within 1 h. Products C and D were diluted with water of standard hardness to the required use concentration. An 8 mL aliquot of the test product was then thoroughly mixed with 1 mL of 0.3% (w/v) sterile filtered albumin solution and 1 mL of the test suspension ($1.5–5.0 \times 10^7$ cfu/mL) controlled at 20 °C. Then 9.7 mL of the wrung ready-to-use solutions of A, B and E were mixed with 0.2 mL of 1.5% (w/v) albumin solution and 0.1 mL of the test suspension ($1.5–5.0 \times 10^8$ cfu/mL). Towards the end of the exposure time the tube content was mixed again. After the product-specific exposure time an aliquot of 1 mL of the mixture was removed and transferred to a tube containing 9 mL of an appropriate neutralizer solution. Immediately afterwards, 10^{-1} and 10^{-2} dilutions were prepared in neutralizer solution. The selected neutralizers are described in Table I. The suitability of the neutralizers for the test products was validated with *C. difficile* spores according to EN 17126 [11]. After a neutralization time of 5 min the solution was mixed again and a 1-mL solution was taken out in duplicate. The 1-mL samples were poured into separate Petri dishes, then 15–20 mL of melted BHIYT-L agar were added and cooled to 45 °C. Plates were then incubated in anaerobic jars for five days at 36 °C followed by counting the colonies per plate and calculating the number of cfu per mL on a $\log_{10}$ scale. The difference in the number of cells in the water control without product exposure and the number of cells after product exposure is described as the $\log_{10}$ reduction.

Efficacy of test products in the 4-field test

The efficacy of the surface disinfectants against *C. difficile* spores was determined using a practical test according to VAH method 19 [12] which is based on EN 16615 [11] because a European norm for sporicidal efficacy on surfaces with wiping is currently not available, only a work item (WI 000216139) which corresponds to VAH method 19. PVC pieces (20 × 50 cm; Forex classic, thyssenkrupp Plastics GmbH, Essen, Germany) were prepared simulating a surface to be treated with a surface disinfectant [13]. Four areas of 5 × 5 cm were marked. The first field was contaminated with 50 µL of a mixture containing 0.9 mL of the test suspension ($1.5–5.0 \times 10^7$ cfu/mL) and 0.1 mL of the organic load (0.03% albumin w/v; 'clean conditions'). Clean conditions were chosen because most manufacturers provided sporicidal efficacy data only under clean conditions (Table I). The inoculum was spread with a glass spatula and allowed to dry at room temperature for up to 60 min. Test products A, B and E were used directly. Test products C and D were diluted with water of standardized hardness to the appropriate use dilutions (Table I). A standard wipe (16.5 × 30 cm, TORK Low-Lint Cleaning Cloth, Essity Professional Hygiene Germany GmbH, Mannheim, Germany) based on 55% cellulose and 45% polyethylene terephthalate (PET) was used for products C and D. Each wipe was soaked for 30 min in 16 mL of the use dilutions of the disinfectant prior to the surface treatment following EN 16615. The volume of 16 mL for impregnating the standard wipe is a specification from EN 16615. The unitary weight (granite block, 2.5 kg) was covered with parafilm on the bottom. The soaked wipe, folded once, was placed on the protected area with parafilm and fixed with a rubber band. The hand pushed the weight over the test surface without applying additional pressure. The wiping procedure started in front of test field 1 and went on to fields 2, 3 and 4 within 1 s. Immediately afterwards it returned to field 1, crossing fields 4, 3 and 2 within another second (Figures 1 and 2). After the product-specific contact time, each test field was carefully swabbed using a cotton swab soaked with neutralizer in accordance with EN 16615 [14]. The suitability of the neutralizers for each test product was validated with *C. difficile* spores according to EN 17126, as described in Table I [11]. The entire test field 1 was wiped with a cotton swab moistened with neutralizer in a horizontal, vertical and diagonal direction. This recovery process was repeated using the same swab after it had been washed out in neutralizer. Subsequently, the lower half of the swab was transferred to the neutralizer test tube containing 5 mL of neutralizer by cutting it off at the edge of the neutralizer test tube. The recovery process was repeated once on the same test field with a second, dry cotton swab until the test field was visibly dry. The lower half of the swab was likewise transferred to the same neutralizer test tube and mixed. The recovery process took roughly 1 min per test field. The two cotton swabs used were combined in 5 mL of neutralizer per test field and vortexed thoroughly for approx. 1 min. Recovery from test fields 2 to 4 takes place in the same way. The swab was then put into a vial containing 5 mL of neutralizer. With a second dry swab the entire test field was carefully swabbed once more until the test field was visibly dry. This swab was put into the same neutralizer vial which was then vortexed for 1 min. After a 5-min neutralization time, aliquots of 1 mL were taken out in duplicate and poured into separate Petri dishes. For the sample obtained from the contaminated test field a

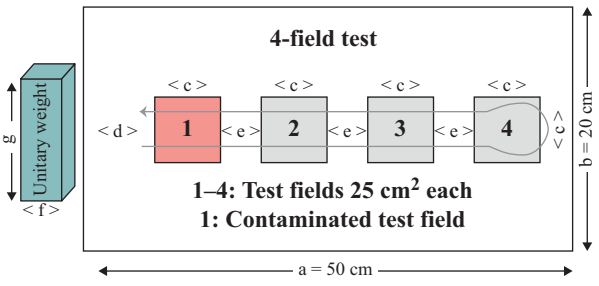

Figure 1. The 4-field test. Test surface (20 × 50 cm) with four test fields (5 × 5 cm) and stipulated wiping route of the wiping cloth. a = 50 cm, b = 20 cm, c = 5 cm, d = 10 cm, e = 5 cm, dimensions of the unitary weight f × g at least 8.6 cm × 12.1 cm.

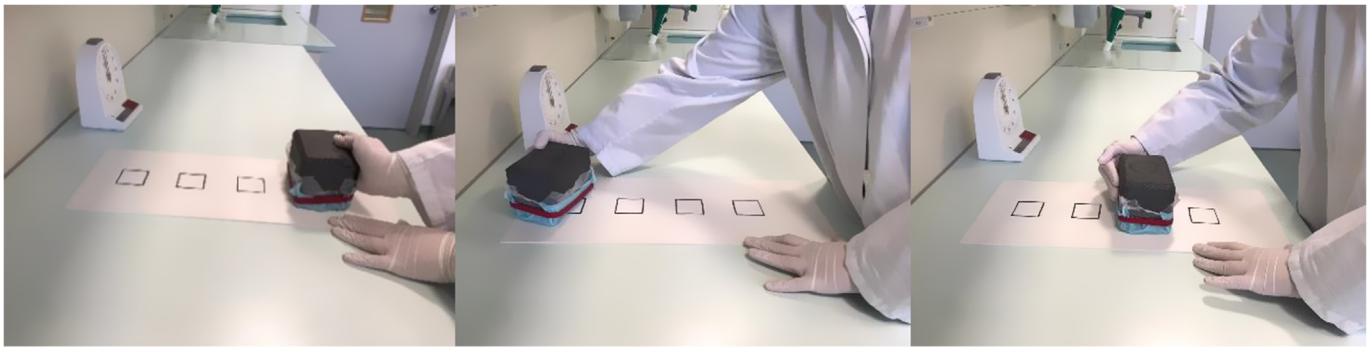

Figure 2. Wiping process in the 4-field test. The hand pushed the weight over the test surfaces without applying additional pressure.

1:10 dilution in neutralizer was prepared in addition. The samples were processed as described above for the suspension test. A $\log_{10}$ reduction of $\geq$ 4.0 on test field 1 is regarded as sufficient sporicidal activity. The numbers of cfu from the three other primarily uncontaminated test fields were also evaluated. A mean number of $\leq$ 50 cfu per 25 cm^2 was regarded as a sufficiently low residual contamination. An additional experiment using water of standardized hardness instead of the surface disinfectant revealed a mean number on test fields 2 to 4 of $\geq$ 10 cfu per 25 cm^2, demonstrating the lack of sporicidal activity. The applied volume of surface disinfectant was calculated by measuring the difference in weight of each wipe immediately before and after the wiping procedure.

Statistical evaluation

Colony counts between 1 and 330 were used for calculation. The mean and standard deviations were calculated for the $\log_{10}$-reductions per product, type of application and laboratory. The mean and median were calculated for the number of colonies on test fields 2 to 4 (4-field test results).

Results

The number of *C. difficile* spores in the test suspension used for the suspension tests was within the set limit for concentrates between 1.5 and 5.0 $\times$ 10^7 cfu/mL and ready-to-use products between 1.5 and 5.0 $\times$ 10^8 cfu/mL, as described in EN 17126 (laboratory 1: 3.0 $\times$ 10^7 resp. 3.3 $\times$ 10^8 cfu/mL; laboratory 2: 2.4 $\times$ 10^8; laboratory 3: 2.5 $\times$ 10^8; laboratory 4: 4.1 $\times$ 10^7; laboratory 5: 1.8 $\times$ 10^7; laboratory 6: 1.8 $\times$ 10^8). In the 4-field tests the mean number of cfu after the contact time (drying control 't') was 4.1 $\times$ 10^5 per 25 cm^2 (laboratory 1), 5.9 $\times$ 10^5 per 25 cm^2 (laboratory 2), 7.2 $\times$ 10^5 per 25 cm^2 (laboratory 3), 5.5 $\times$ 10^5 per 25 cm^2 (laboratory 4), 4.8 $\times$ 10^5 per 25 cm^2 (laboratory 5) and 1.1 $\times$ 10^5 per 25 cm^2 (laboratory 6).

Susceptibility of the C. difficile spore preparation

The internal quality control of the *C. difficile* test spores is considered to be valid if the mean $\log_{10}$ reduction is $<$1.5 with 1% glutaraldehyde and 0.01% peracetic acid [11]. This requirement was fulfilled in all laboratories with a mean $\log_{10}$ reduction of 0.59 with 1% glutaraldehyde (mean pH: 6.11 $\pm$ 0.77), a mean $\log_{10}$ reduction of 0.69 with 0.01% peracetic acid (mean pH: 4.92 $\pm$ 0.28) and a test suspension within the set limit between 1.5 and 5.0 $\times$ 10^6 cfu/mL.

Effect of water control

The water control led to a reduction in *C. difficile* spores between 1.68 and 3.14 $\log_{10}$ (mean: 2.14 $\log_{10}$; median: 2.10 $\log_{10}$). The residual contamination on the three non-contaminated test fields was $\geq$10 cfu/25 cm^2 in all laboratories (range of means: 28–518 cfu/25 cm^2).

Efficacy of test products

The solution of product A (ready-to-use wipes) reduced the *C. difficile* spore count in both laboratories by at least 4.0 $\log_{10}$ in the suspension tests in 5 min (Table II). In the 4-field test the wipes reduced the spore counts on field 1 in 5 min by at least 4.0 $\log_{10}$, on test fields 2 to 4 the spore count was consistently less than 50 cfu per 25 cm^2. The product solution and the wipe therefore fulfilled the efficacy criteria of both test methods and can be regarded as effective against *C. difficile* spores in 5 min under clean conditions. The applied amount was on average 0.37 mL.

The solution of product B (ready-to-use wipe) was not effective in the suspension tests and showed after 15 min a mean $\log_{10}$-reduction of 0.90 and after 60 min of 3.22 (Table II). In the 4-field tests the spore reduction on field 1 was similar after 15 min (2.82 $\log_{10}$) and 60 min (2.83 $\log_{10}$), suggesting that the additional 45-min exposure time did not add any sporicidal effect to the contaminated surface. On test fields 2 to 4 the spore counts were mostly above 50 cfu per 25 cm^2 indicating a substantial carry-over effect of the spores without a sufficient sporicidal effect. The product solution and the wipe therefore did not fulfil the efficacy criteria of both test methods and cannot be regarded to have sufficient efficacy against *C. difficile* spores in 15 or 60 min, respectively. The applied amount was on average 0.55 mL (15 min application time) and 0.41 mL (60 min application time).

Product C is a concentrate and reduced *C. difficile* spores in suspension by 5.40 $\log_{10}$ (2% solution, 15 min) and 5.03 $\log_{10}$ (1% solution, 30 min; Table II). In the 4-field test the product reduced the spore counts on field 1 by 5.16 $\log_{10}$ (2% solution, 15 min) and 4.36 $\log_{10}$ (1% solution, 30 min). On test fields 2 to 4 the spore count was consistently less than 50 cfu per 25 cm^2. The product at 2% and 1% therefore fulfilled the efficacy criteria of both test methods and can be regarded as effective under clean conditions against *C. difficile* spores in 15 or 30 min, respectively. The applied amount was on average 1.01 mL (15 min application time) and 0.94 mL (30 min application time).

Table II
Mean $\log_{10}$-reductions obtained with five products against *Clostridioides difficile* spores in suspension tests and the 4-field test under clean conditions

Test product	Exposure time (min)	Laboratory	Suspension test	4-field-test	cfu per 25 cm^2 on fields 2 to 4 (mean/median)	Mean released liquid onto test surfaces (g; mean and SD)
			Log_{10}-reduction (mean and SD)	Log_{10}-reduction on field 1 (mean and SD)		
A*	5	1	5.60 ± 0.15	5.24 ± 0.42	10/8	0.39 ± 0.06
		2	5.36 ± 0.13	4.71 ± 0.47	1/0	0.35 ± 0.02
		Both	**5.48 ± 0.18**	**4.98 ± 0.50**	**5/3**	**0.37 ± 0.05**
B*	15	1	0.72 ± 0.47**	3.29 ± 0.31**	13**/10**	0.78 ± 0.43
		3	1.08 ± 0.15	2.35 ± 0.49	822/896	0.32 ± 0.06
		Both	**0.90 ± 0.37**	**2.82 ± 0.64**	**418/68**	**0.55 ± 0.36**
B*	60	1	3.43 ± 0.62	3.03 ± 0.18	83/81	0.49 ± 0.11
		3	3.01 ± 0.11	2.64 ± 0.09	401/419	0.32 ± 0.04
		Both	**3.22 ± 0.47**	**2.83 ± 0.25**	**242/101**	**0.41 ± 0.12**
C (2%)	15	1	5.29 ± 0.37	5.51 ± 0.07	3/3	1.08 ± 0.12
		4	5.52 ± 0.27	4.82 ± 0.73	8/7	0.93 ± 0.18
		Both	**5.40 ± 0.33**	**5.16 ± 0.61**	**6/3**	**1.01 ± 0.16**
C (1%)	30	1	5.25 ± 0.20	4.43 ± 0.40	7/3	0.92 ± 0.06
		4	4.80 ± 0.38	4.30 ± 0.18	5/5	0.96 ± 0.23
		Both	**5.03 ± 0.37**	**4.36 ± 0.30**	**6/4**	**0.94 ± 0.15**
D (2%)	15	1	5.42 ± 0.60	4.29 ± 0.28	0/0	0.78 ± 0.08
		5	5.27 ± 0.11	3.95 ± 0.08	20/21	0.89 ± 0.41
		Both	**5.35 ± 0.41**	**4.12 ± 0.26**	**10/8**	**0.83 ± 0.28**
D (0.5%)	60	1	5.09 ± 1.00	2.83 ± 0.16	19/18	0.86 ± 0.04
		5	5.33 ± 0.12	3.35 ± 0.15	73/75	1.08 ± 0.29
		Both	**5.21 ± 0.67**	**3.09 ± 0.31**	**46/43**	**0.97 ± 0.23**
E*	240	1	1.12 ± 0.00	5.05 ± 0.49	22/5	0.48 ± 0.06
		6	0.68 ± 0.14	3.89 ± 0.29	8/8	0.28 ± 0.10
		Both	**0.90 ± 0.25**	**4.47 ± 0.73**	**15/6**	**0.38 ± 0.13**

SD, standard deviation.
Bold: log10-reduction (mean and SD)obtained for both laboratories.
* Ready to use wipe.
** Based on $n = 3$.

Product D is also a concentrate and reduced *C. difficile* spores in suspension by 5.35 $\log_{10}$ (2% solution, 15 min) and 5.21 $\log_{10}$ (0.5% solution, 60 min; Table II). In the 4-field test the 2% product solution reduced the spore counts in 15 min on field 1 by 4.12 $\log_{10}$ although the mean $\log_{10}$-reduction was just below 4.0 in one of the two laboratories. The 0.5% product was less effective in 60 min and reduced the spore counts by 3.09 $\log_{10}$. On test fields 2 to 4 the overall spore count was consistently less than 50 cfu per 25 cm^2 for the 2% and 0.5% product solution although the counts were above 50 per 25 cm^2 for the 0.5% product solution in one laboratory. The 2% product solution (15 min) but not the 0.5% product solution therefore fulfilled the efficacy criteria of both test methods and can be regarded as having sufficient efficacy under clean conditions against *C. difficile* spores. The applied amount was on average 0.83 mL (15 min application time) and 0.97 mL (30 min application time).

Product E (ready-to-use wipe) revealed a mean $\log_{10}$-reduction of 0.9 after 4 h in the suspension test (Table II). In the 4-field test the product reduced the spore counts in 4 h on field 1 by 4.47 $\log_{10}$ although the mean $\log_{10}$-reduction was just below 4.0 in one of the two laboratories. On test fields 2 to 4 the spore count was consistently less than 50 cfu per 25 cm^2. The product solution therefore did not fulfil the efficacy criteria of both test methods and cannot be regarded as having sufficient efficacy against *C. difficile* spores in 4 h. The applied amount was on average 0.38 mL.

Discussion

Although the manufacturers of all five products claimed that their surface disinfectant has sporicidal activity, we found that only two of them (products A and C) had sufficient efficacy under clean conditions against *C. difficile* spores in suspension and under practical conditions. Product D at 2% (15 min) was also sufficiently effective but not at 0.5% (1 h). Product E fulfilled only the efficacy criteria after 4 h under practical conditions but not in the suspension tests. Product B did not fulfil the efficacy criteria of both test methods. Comparable results were found in two different laboratories and thus the study data are considered to be reliable. In addition, the spore suspensions were visually checked by microscopy, internal quality controls ensured the required chemical tolerance of the spores and valid neutralization. The results also show that a manufacturer's sporicidal claim often only based on suspension tests does not necessarily mean that the surface disinfectant exhibits sufficient sporicidal efficacy against *C. difficile* spores under practical conditions. That is why testing provides more reliable data [15]. A similar overall result with 10 different 'sporicidal' wipes has been described previously [16].

A major limitation of the study was that all experiments were performed under clean conditions. Clean conditions were chosen because most manufacturers provided sporicidal efficacy data under clean conditions. In clinical practice it is likely

that the surroundings of *C. difficile* patients are contaminated with some organic load with the spores. That is why it is uncertain whether the same efficacy can be assumed in the presence of organic load such as faeces. It has been described previously that the bactericidal efficacy is lower for 1.4 mM peroxynitric acid and 1.1 mM hypochlorous acid in the presence of protein [17]. The effect of ethanol, n-propanol or isopropanol against the murine norovirus, however, was not impaired in a carrier test under dirty conditions [18]. For clinical practice, however, efficacy data against *C. difficile* spores obtained under dirty test conditions are from our perspective more reliable and should preferably be used.

In clinical practice it will be relevant to avoid that the wiping itself contributes to the spread of the *C. difficile* spores on the treated surfaces [19]. That is why the results from test fields 2 to 4 have relevant practical implications. The wiping itself will carry over some of the local contamination to the neighbouring parts of the surface. That is why the disinfectant solution remaining on the treated surface should keep some sporicidal activity, which is considered and evaluated with this 4-field test [12].

Another limitation of our results may be the long exposure time of some products. EN 14885 specifies that the minimum efficacy should be proven for use on surfaces around the patient within 15 min, on other surfaces it may take up to 60 min [20]. A disinfectant requiring 240 min is therefore outside the maximum exposure time for this type of application. In particular, the use of higher concentrations with shorter contact times, however, requires the careful balancing of the advantage of a fast sporicidal efficacy with possible harms to the health of the cleaning staff and patients. A stronger sporicidal efficacy typically requires a higher concentration of the product with all possible side effects for occupational health. This aspect should be taken into account when evaluating suitable formulations for a sporicidal surface disinfection.

C. difficile-infected patients usually harbour between 1.5 and 5.5 $\times$ 10^6 *C. difficile* spores per g in their faeces [21]. It is therefore plausible to assume that the requirement for a 4 $\log_{10}$-reduction of spores is reasonable. The surface contamination in the direct surroundings of *C. difficile*-infected patients, however, has been described to be rather low with a mean of 5.1 cfu per swab [22]. Weber *et al.* reported in 2013 that surfaces were mostly contaminated with <1 to 2 $\log_{10}$ *C. difficile*. Two studies reported >2 $\log_{10}$ *C. difficile* on surfaces, of which one study that sampled different surfaces with a sponge found more than 1300 colonies [23]. No additional data were found. Even if the contamination level is low in some cases, Lawley *et al.* [24] found in an experiment with mice that 5—10 *C. difficile* spores per cm^2 (125—250 cfu/25 cm^2) are sufficient to infect 50% of the mice within 1 h of exposure. With this background, a practical consideration of the possible distribution of *C. difficile* spores in the environment is urgently expected and can be illustrated with this 4-field test [12]. For the human medical area, a 4 log reduction was set [11,12,25] and seems to be reasonable and sufficient in this context.

All three ready-to-use wipes used in our study released a rather small volume of the surface disinfectant solution (0.37—0.55 mL). When a standard wipe was soaked with 16 mL of a surface disinfectant solution, the release per wipe was higher at 0.83—1.10 mL. It has been shown previously that a larger volume of a disinfectant results in a higher $\log_{10}$-reduction on test field 1 [26]. It seems possible therefore that a larger volume of product per wipe may yield more favourable results for those products that failed to meet the efficacy requirements of the 4-field test.

'The results obtained with product E are somewhat confusing. The wrung product solution itself revealed consistently only a poor activity against *C. difficile* spores in suspension within 4 h. But when the wipe was applied in the 4-field test it demonstrated an overall sufficient sporicidal activity on the surface including the low carry-over effect shown in test fields 2 to 4. This discrepancy cannot be explained currently although it may be possible that a larger proportion of spores adhered to the tissues soaked with the slightly sticky product solution.

Another interesting observation was made with product B. The product solution showed a higher $\log_{10}$-reduction after 60 min in suspension compared with 15 min in suspension. This finding is expectable. At the same time, however, the wipe revealed no difference in efficacy on the surface whether spores were exposed for 15 min (2.82 $\log_{10}$) or for 60 min (2.83 $\log_{10}$). The data indicate that no additional sporicidal effect was achieved after 15 min. A possible explanation is that hydrogen peroxide requires the presence of water to act as a sporicidal substance.

Overall, product A (ready-to-use wipes, 5 min), product C (2% in 15 min or 1% in 30 min) and product D (2% in 15 min) were found to be effective against *C. difficile* spores in suspension and on surfaces. For surface disinfection of existing *C. difficile* infections, disinfectants with proven efficacy against *C. difficile* spores should be used [27]. This efficacy test should be carried out in a standardized manner according to VAH method 18 [25] or EN 17126 [11] and under practical conditions according to VAH method 19 [12].

In conclusion, not all surface disinfectants with a sporicidal claim from the manufacturer are effective against *C. difficile* spores in standardized suspension tests and in the 4-field test. In clinical practice, preference should be given to products that reliably pass the efficacy criteria of both types of tests.

Conflict of interest statement

The authors declare to have no conflict of interest related to the content of the manuscript.

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