American Journal of Infection Control 47 (2019) 27–32

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

There is no additional bactericidal efficacy of Environmental Protection Agency–registered disinfectant towelettes after surface drying or beyond label contact time

Alyssa M. West MS [a], Peter J. Teska BS, MBA [b], Haley F. Oliver PhD [a,*]

[a] Department of Food Science, Purdue University, West Lafayette, IN
[b] Diversey Inc., Charlotte, NC

Key Words:
Disinfectant
Towelette
Contact time

Background: Disinfectant towelettes are commonly used for surface disinfection to prevent health care–associated infections; however, there is limited consensus as to whether a surface needs to remain wet for the full label contact time after the disinfectant towelette has been used in order for complete efficacy to be achieved. The purpose of this study was to quantify the effect of contact time, including times before and after a product dries, on bactericidal efficacy of 6 towelette products registered by the Environmental Protection Agency .

Methods: Six disinfectant towelette products were tested at varying contact times, including defined label contact times. Quantitative Environmental Protection Agency MB-33-00 was used to measure disinfectant efficacy against *Staphylococcus aureus* on Formica. Complete dry time for each disinfectant was measured gravimetrically.

Results: There were significant differences in dry times among the towelette products; contact time did not have a significant effect on bactericidal efficacy. There was no longitudinal effect when a disinfectant's contact time was greater than defined label contact time, irrespective of whether the product was wet or dry on the surface.

Discussion: Overall, bactericidal efficacy varied by towelette product tested and surface area wiped. Wiping larger surface areas may lead to decreased bactericidal efficacy but is product dependent.

Conclusions: There was no additional bactericidal effect after a product dried, indicating that extended contact times beyond when the product dries will not enhance disinfection.

BACKGROUND

Health care–associated infections (HAIs) are estimated to affect hundreds of millions of patients around the world every year.[1] In 2011 it was estimated that there were 721,800 HAIs in U.S. acute care hospitals.[2] A study that analyzed 1,022 HAI outbreaks determined that 2 of the most frequent sources of the infections were medical equipment or devices.[3] Many studies have shown that the environment contributes to these infections and that pathogenic microorganisms can be transferred from gloved hands without direct patient contact.[4-6] Proper cleaning and disinfection of environmental surfaces and patient care equipment is an important element in preventing the transmission of HAI-related microorganisms. This can be accomplished through the use of disinfectant towelettes, which provide an easy, ready-to-use version of traditional sprays. This is especially important for efficiency in fast-paced health care environments. However, there is increasing evidence that there are significant lapses in procedures and quality of health care cleaning and disinfection, despite the presence of institutional policies consistent with national guidance.[7] These can include not leaving the disinfectant on the surface for the full contact time listed on the label.[8,9] There are conflicting viewpoints on the impact of wet contact time on disinfectant efficacy. Some published works indicate that surfaces must remain wet for the full disinfection contact time to achieve the sufficient bactericidal efficacy.[8-11] Alternatively, a recently published commentary by Rutala and Weber[12] indicated that wet contact time is not necessar`y for disinfectant

* Address correspondence to Haley F. Oliver, PhD, Department of Food Science, 745 Agriculture Mall Dr, Purdue University, West Lafayette, IN 47907.
E-mail address: hfoliver@purdue.edu (H.F. Oliver).

https://doi.org/10.1016/j.ajic.2018.07.005

towelette products to meet Environmental Protection Agency (EPA) efficacy requirements. A white paper by Clayton[13] also agreed with this claim. Overall there is no consensus among disinfectant manufacturers regarding the impact of wet contact time on bactericidal efficacy.[11-14] Furthermore, there have been no peer-reviewed studies conducted to our knowledge that specifically investigate the impact of wet versus dry contact time on bactericidal efficacy using EPA methodology**.** The objective of this study was to better understand the relationship between contact time, dry time, and efficacy of towelettes using an EPA-approved method. We hypothesized that disinfectants that dried before the label contact time would have reduced bactericidal efficacy. We further hypothesized that there would be no additional kill occurring after the time point at which the disinfectant had dried on the surface.

METHODS

Disinfectants and bacteria used in the study

This study tested 6 disinfectant towelette products described in Table 1. Diversey EasyWipes (dry wipes) wetted with phosphate-buffered saline solution (PBS; 15.1 mL per towelette) were used as a control. EPA standard strain *Staphylococcus aureus* strain ATCC CRM-6538 was used in this study to measure towelette efficacy.

Drying time method

Formica squares 6″ × 6″ were used to perform the dry testing. Briefly, the Formica square was preweighed on a Mettler-Toledo AG204 analytical balance (Mettler-Toledo, Columbus, OH) (accurate to 0.0001 g). For disinfectant application, the first towelette from the disinfectant container was thrown away, and the second one was used to ensure the tested towelette was fully wet. The surface was wiped with consistent pressure, from left to right, in 4 passes back and forth. The weight of the Formica square was recorded starting at t = 0 second and every 15 seconds thereafter until the weight did not change for at least 1 minute, indicating the square was dry. Dry time testing was completed independently 5 times for each disinfectant product and the PBS control.

Towelette efficacy testing method

A modified version of EPA SOP MB-33-00 (Quantitative Petri Plate Method for Determining the Effectiveness of Antimicrobial Towelettes Against Vegetative Bacteria on Inanimate, Hard, Non-porous Surfaces) was used to evaluate towelette efficacy on inoculated 97-mm–diameter Formica discs.[15] The Formica discs were disinfected in 100% ethanol for 1 minute before inoculation with a soil load composed of 67.3% bacterial culture, 19.2% mucin, 8.7% yeast, and 4.8% bovine serum albumin. Inoculated discs were dried in a 37°C incubator for approximately 25 minutes to adhere the bacteria to the surface. After EPA SOP MB-33-00, the Formica was wiped with a disinfectant towelette and the discs were left to sit for a predetermined contact time.[15] Ten contact times were tested: 30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 20 minutes, 30 minutes, and 60 minutes. After the contact time was reached, the discs were swabbed with a PUR-Blue Swab Sampler (World Bioproducts, Libertyville, IL) containing 10 mL of neutralizing buffer. We did not independently validate the neutralizing buffer on the sterile swabs, because this had been done previously by the manufacturer. The swab samplers were vortexed to release the bacteria, and the solution was vacuum-filtered onto a filter membrane following the EPA protocol.[15] Membrane filters were plated on tryptone soya agar, incubated for 24-48 hours at 37°C, then enumerated. Five biologic replicates were conducted for quat-based products and 3 biologic replicates for all others. Three technical replicates were performed within each biologic replicate.

Statistical analyses

Statistical Analysis System (version 9.4; SAS Institute Inc, Cary, NC) was used for all analyses. A generalized linear model was generated for total dry time values. Least squares means with Tukey-Kramer adjustment for multiple comparisons were used to determine whether there was a significant difference in disinfectant total drying time among the products tested ($\alpha = 0.05$). Disinfectant efficacy data were transformed into log10 reduction values for all analyses. A generalized linear mixed model (GLMM) was used to determine if the disinfectant product, contact time, or variable interaction had a significant impact on disinfectant efficacy ($\alpha = 0.05$). Least squares means with Tukey-Kramer adjustment for multiple comparisons were used to determine whether there were significant differences in efficacy among disinfectant products. One-way analysis of variance was used to determine whether there were significant differences in bactericidal efficacy between defined label contact time and calculated dry time ($\alpha = 0.05$). The means analysis with Tukey's honest significant difference (HSD) test was used to determine whether there was a significant difference in log10 reduction values among the defined label contact time, the calculated dry time, and each extended contact time point tested.

RESULTS

Disinfectant dry time significantly varied among products tested

Five of the 6 products tested remained wet until the label contact time was reached. However, the 0.28% quat product

Table 1
Active ingredients and contact time for towelettes tested in study

Disinfectant product*	Disinfectant active ingredient(s)	Label contact time†
0.233% quat + 14.3% alcohol	0.233% diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride 14.3% isopropanol	3 min
0.76% quat + 22.5% alcohol	0.76% didecyldimethylammonium chloride 7.5% ethanol 15% isopropanol	1 min
0.25% quat	0.125% n-alkyl dimethyl ethylbenzyl ammonium chlorides 0.125% n-alkyl dimethyl benzyl ammonium chlorides	3 min
0.28% quat	0.14% n-alkyl dimethyl ethylbenzyl ammonium chlorides 0.14% n-alkyl dimethyl benzyl ammonium chlorides	3 min
0.55% sodium hypochlorite	0.55% sodium hypochlorite	30 s
1.4% hydrogen peroxide	1.4% hydrogen peroxide	1 min

*This naming scheme was used to identify the disinfectant on towelettes throughout the study.
†Defined label contact time for *Staphylococcus aureus*.

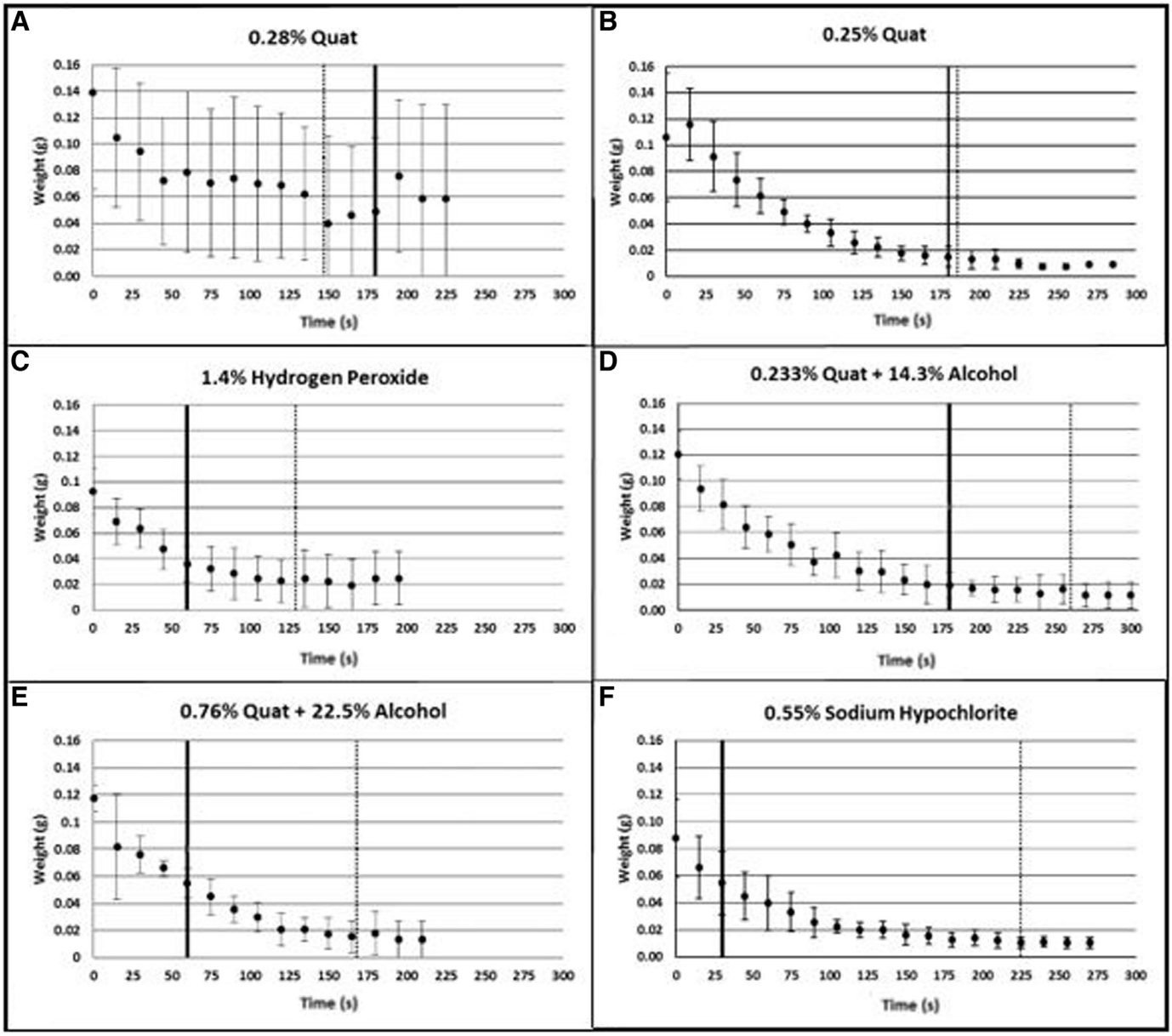

Fig 1. Drying time curve based on surface wetness, by weight measurements, after application of disinfectant for 6 disinfectant products. Bold vertical line indicates label contact time point for each product. Dashed vertical line indicates the time point at which the disinfectant was determined to be fully dry. (A) Drying time curve for 0.28% quat product. (B) Drying time curve for 0.25% quat product. (C) Drying time curve for 1.4% hydrogen peroxide product. (D) Drying time curve for 0.233% quat + 14.3% alcohol. (E) Drying time curve for 0.76% quat + 22.5% alcohol product. (F) Drying time curve for 0.55% sodium hypochlorite product.

(Fig 1A) dried at 2 minutes and 45 seconds under these test conditions; it has a label contact time of 3 minutes. Furthermore, the 0.25% quat product (Fig 1B) and 1.4% hydrogen peroxide product (Fig 1C) remained wet 10 seconds less than the label contact time.

There were significant differences among the times it took for the disinfectant to dry fully. The 0.233% quat + 14.3% alcohol product (Fig 1D) had a significantly longer drying time compared with the 0.76% quat + 22.5% alcohol (GLMM with Tukey-Kramer correction; $P_{adj} = .0005$) (Fig 1E), 0.25% quat ($P_{adj} = .0057$), and 0.28% quat products ($P_{adj} < .0001$), *and* 1.4% hydrogen peroxide ($P_{adj} < .0001$). The 0.55% sodium hypochlorite product (Fig 1F) had a significantly longer drying time compared with the 0.76% quat + 22.5% alcohol ($P_{adj} = .0398$), 1.4% hydrogen peroxide ($P_{adj} = .0002$), and 0.28% quat products ($P_{adj} = .0025$). The 1.4% hydrogen peroxide product had a significantly longer drying time compared with the 0.25% quat product ($P_{adj} = .0398$).

Disinfectant mode of action had a significant effect on bactericidal efficacy

Overall, there were significant differences in bactericidal efficacy among products, which all had different modes of action (GLMM; $P < .0001$). Contact time and the interaction between contact time and product were not significant. Overall, the 0.28% quat product (Fig 2A) achieved a significantly higher log10 reduction compared with the 0.76% quat + 22.5% alcohol product ($P_{adj} < .0001$). The 0.233% quat + 14.3% alcohol achieved a significantly higher

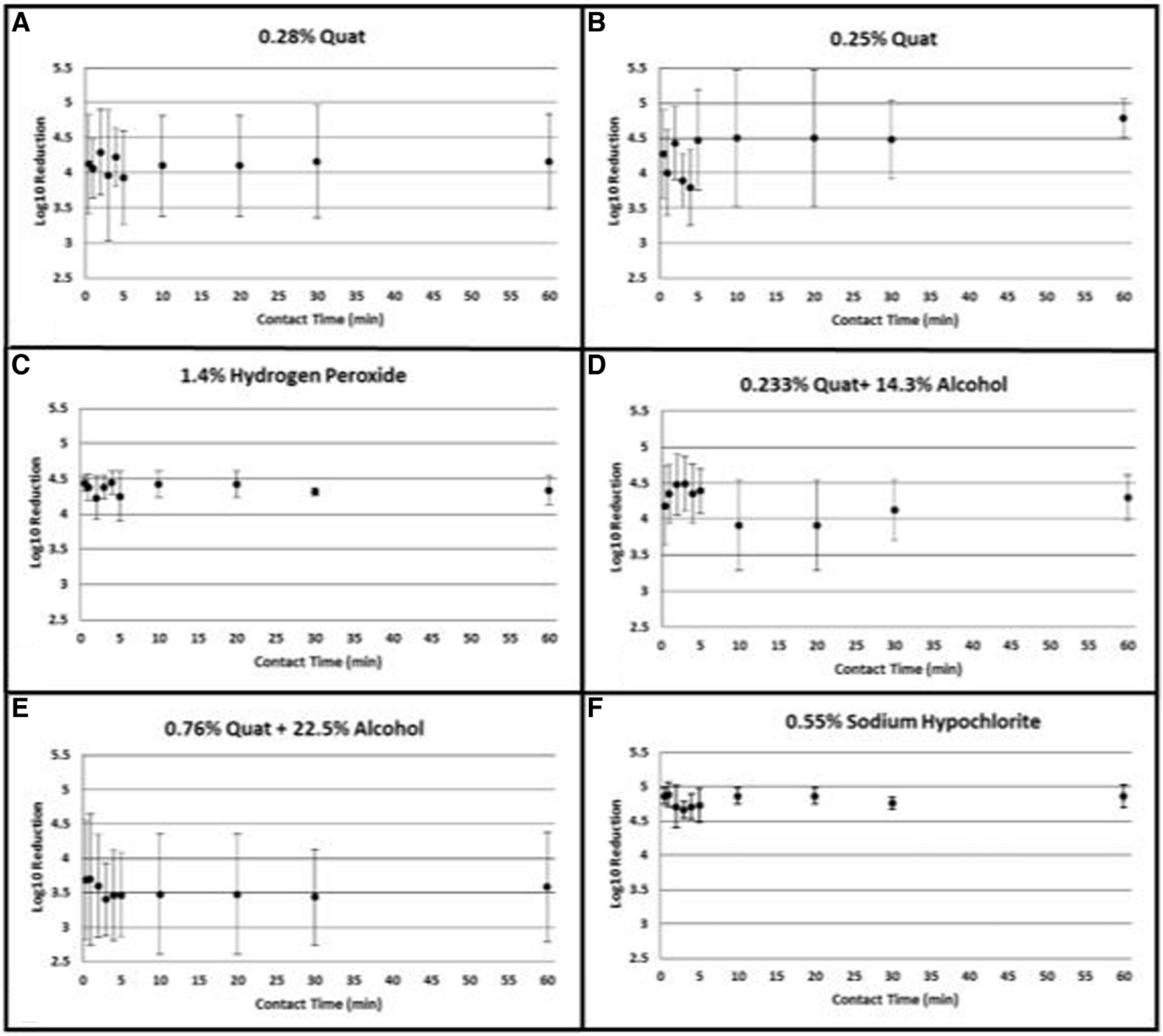

Fig 2. Bactericidal efficacy, based on Log10 reduction values, of 6 different disinfectant products with varying contact times. Ten contact time points were tested for each product. (A) Bactericidal efficacy of a 0.28% quat product over time. (B) Bactericidal efficacy of a 0.25% quat product over time. (C) Bactericidal efficacy of a 1.4% hydrogen peroxide product over time. (D) Bactericidal efficacy of a 0.233% quat + 14.3% alcohol product over time. (E) Bactericidal efficacy of a 0.76% quat + 22.5% alcohol product over time. (F) Bactericidal efficacy of 0.55% sodium hypochlorite disinfectant product over time.

bactericidal efficacy compared with the 0.25% quat (P_{adj} = .0148) (Fig 2B). The 1.4% hydrogen peroxide product (Fig 2C) also achieved a significantly higher log10 reduction than the 0.25% quat (P_{adj} = .0145) and the 0.76% quat + 22.5% alcohol products (P_{adj} < .0001). The 0.233% quat + 14.3% alcohol (Fig 2D) achieved a significantly higher $\log_{10}$ reduction as compared with 0.76% quat + 22.5% alcohol (Fig 2E) (GLMM with Tukey-Kramer Correction; P_{adj} < .0001). The 0.55% sodium hypochlorite product (Fig 2F) achieved a significantly higher log10 reduction as compared with the 0.25% quat product (P_{adj} < .0001) and the 0.76% quat + 22.5% alcohol product (P_{adj}< .0001). The PBS-wetted control towelettes achieved an approximate reduction value of 3 log10 in bacteria overall. Only 4 of the 6 products tested had significantly higher bactericidal efficacy compared with the control towelette wetted with PBS (all P_{adj} < .05). The 0.25% quat product and the 0.76% quat + 22.5% alcohol product did not achieve significantly different log10 reduction values compared with wiping with a PBS-wetted towelette.

Bactericidal efficacy did not significantly change after product was dry on surface or after label contact time was reached

There was no significant difference in bactericidal efficacy at defined label contact time and calculated dry time for all products tested. This included the 0.28% quat product, which dried before its label contact time was reached. Specifically, there were no significant differences between the log10 reduction at the time point when the disinfectant dried and each time point tested thereafter (means comparison with Tukey HSD; P_{adj} > .05). It was also determined that there

were no significant differences in log10 reduction at the disinfectant label contact time and each time point tested after defined label contact time (Means comparison with Tukey HSD; $P_{adj} > .05$).

Discussion

In this study we tested 6 disinfectant towelette products to determine the impact of dry time and contact time on bactericidal efficacy, using EPA method MB-33-00.[15] We found significant differences in the time it took each product to fully dry on Formica surfaces. We also found that bactericidal efficacy varied among the products tested, although label contact time did not impact efficacy. There are limited peer reviewed studies on disinfectant towelette performance, and, to our knowledge, this work is among the first quantitative investigations to evaluate the impact of dry time on efficacy.

Disinfectant mode of action, not contact time, significantly affected bactericidal efficacy

It was determined that disinfectant mode of action, not contact time, was a significant variable. During dry testing the 0.28% quat product dried before its label contact time was reached. This could be owing to the presence of other inactive ingredients, because other quat-based products tested did not dry before the label contact time was reached. Although the product dried before the defined label contact time, contact time did not impact the bactericidal efficacy. These findings contradict the conclusion that wet contact time is crucial for complete disinfection of a surface.[9] Our study was limited to testing one *S aureus* strain; therefore we cannot definitively say that wet contact time doesn't have a significant impact.

Overall, the 0.55% sodium hypochlorite product achieved the highest bactericidal efficacy and the 0.76% quat + 22.5% alcohol product was the least effective against *S aureus* at defined label concentration and contact time. Four of the 6 products tested had significantly higher bactericidal efficacy compared with the control towelette wetted with PBS. The 0.25% quat and the 0.76% quat + 22.5% alcohol products did not achieve a significantly higher log10 reduction than the control towelette wetted with PBS. The disinfectant towelettes tested in this study were composed of multiple substrates; thus we cannot rule out that the towelettes themselves impacted bactericidal efficacy. There are more than 50 wiping substrates available to disinfectant manufacturers to select from when developing a disinfectant towelette product. Substrate selection has been shown to be extremely important for quat-based products; certain substrate types will bind with the quat compounds, preventing full efficacy from occurring.[16,17] We believe the physical wiping action of the material is disrupting the bacteria and that the wipe substrate is physically removing the bacteria from the surface. Therefore further research is needed to determine the extent of the effect towelette composition has on a disinfectant's bactericidal efficacy.

No additive bactericidal effect beyond the dry time and the label contact time

We found that the disinfectant towelettes did not achieve any statistically significant additional kill after the disinfectant dry time or label contact was reached using a quantitative method. The 0.55% sodium hypochlorite product's bactericidal efficacy reached the detection limit (approximately 5log reduction) in this study; thus we cannot definitively assess the longitudinal effect of 0.55% sodium hypochlorite under these conditions. A recently published commentary article by Rutala and Weber[12] concluded that a surface does not need to remain wet for the full label contact time of a disinfectant towelette product for bactericidal efficacy to be achieved. A white paper recently published by a disinfectant towelette manufacturer concluded the same as the aforementioned article.[13] Clayton[13] stated that "the EPA does not require test surfaces to remain wet during the test method [for registration]" and "regardless of whether the surface is wet, dry, or somewhere in between, the efficacy is assured to be in line with the EPA registration." Furthermore, the current EPA registration testing method referenced in both articles is qualitative; the testing method used in this study quantified bactericidal efficacy.[12,13] Sans an exception noted in Clayton, [13] the EPA[18] does not explicitly state in the testing protocol that the surface must remain visibly wet. The exception is the testing procedure for *Clostridium difficile* and *Candida auris* sporicide towelette products. This guidance document states, "A determination for the amount of time the carrier remains wet should be made. This wetness determination will be used to generate the contact time."[19] The EPA is advising all pesticide registrants that the contact time should be shorter than the time the surface remains wet irrespective of target organism (EPA: Efficacy Evaluation Team, 2018, personal communication). Furthermore, the EPA is recommending that disinfectant manufacturers conduct visual and gravimetric wetness tests (although they are not required to do so) (EPA: Efficacy Evaluation Team, 2018, personal communication).

CONCLUSIONS

Overall, in this study, we found differences in disinfectant efficacy among products with different modes of action, which was not influenced by label contact time. Furthermore, we found that there was no additional bactericidal efficacy of EPA-registered disinfectant towelettes achieved after surface drying or beyond label contact time. Our data highlight the effect of dry time on disinfectant efficacy and underscore the need for consistent language and guidance on wet contact time.

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