STUDY TITLE

Assessment of the Combined Activity of Spray and Wiping for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Healthcare-Associated Pathogens

TEST ORGANISM

Clostridium difficile spores (ATCC 43598), *Staphylococcus aureus* (ATCC 6538) *and Serratia marcescens* (ATCC 13880)

TEST SAMPLE IDENTITY

- PCS 200

TEST Method

Quantitative carrier test – Tier 3 or QCT-3

AUTHOR

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Study Director

STUDY COMPLETION DATE

Nov. /06/18

PERFORMING LABORATORY

CREM Co. Labs. Units 1-2, 3403 American Dr., Mississauga, Ontario, Canada L4V 1T4

SPONSOR

Process Cleaning Solutions

STUDY NUMBER

PCS181024-01

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was not conducted in compliance with U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.

Submitter: _____ Date: _____

Sponsor: _____ Date: _____

Study Director: _____ Date: _____

STUDY PERSONNEL

STUDY DIRECTOR: Syed A. Sattar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Bahram Zargar, PhD
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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of the Combined Activity of Spray and Wiping for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Healthcare-Associated Pathogens
Study Number:	PCS181024-01
Sponsor	Process Cleaning Solutions (PCS), Inc.
Testing Facility	CREM Co Labs Unit 1-2, 3403 American Drive, Mississauga, ON, Canada L4V 1T4

TEST SUBSTANCE IDENTITY

Test Substance Name: PCS 200
Lot/Batch(s): Lot #

STUDY DATES

Date Sample Received:	Oct/24/18
Study initiation date:	Oct/25/18
Experimental Start Date:	Oct/25/18
Experimental End Date:	Nov/04/18
Study Completion Date:	Nov/15/18

I. RATIONALE

Routine manual cleaning of hard, non-porous environmental surfaces in healthcare and other settings often does not achieve the desired level of their microbial decontamination (Carling 2016; Sattar and Maillard 2013). Also effectiveness of a function of the way that the products are applied (eg, spraying vs wiping) and the work practices and conditions with which they are used is different. Some institutions have implemented a double-clean process in an effort to achieve higher levels of microbial decontamination. Others now use sporicidal formulations for the terminal disinfection of isolation rooms.

Current testing of environmental surface disinfectants does not incorporate the often used wiping component (Sattar 2010), which is crucial as a physical step to enhance the process of surface decontamination by adding pressure as well as by contributing to the removal of soiling. There is, therefore, a need to generate test data on such formulations by combining the physical action of wiping with the disinfection process. Such information would better inform infection preventionists of the field-relevant potential of environmental surface decontamination processes.
In spraying, less solution is used, and often the same disinfectant solution can be used for general disinfection of operatory surfaces and may provide an acceptable alternative to other cleaning/disinfecting method. The combined use of spraying and wiping with disinfection could also

lead to reductions in the amounts of chemicals used, thereby adding further to environmental and workplace safety.

In most field situations, the target pathogens on environmental surfaces in healthcare settings in particular are unknown and may often be present as mixtures as released from those infected. This reality is also not considered when assessing environmental surface disinfectants for registration purposes. We have previously approached this matter by developing mixtures of relevant pathogens for simultaneous exposure to the test formulation (Best et al., 1994; Sabbah et al., 2010).

II. OBJECTIVES

The objective of this study was to:

a. Conduct laboratory-based testing on the combined use of a spray and microfiber fabric using chlorine-based disinfectant (PCS 200) diluted in a neutral pH buffer for the microbial decontamination of hard, non-porous environmental surfaces representing those found in healthcare settings. The aim here was to evaluate the efficacy of a cleaning/sanitizing process using spray and wipe with PCS 200 cleaner.

SUMMARY OF RESULTS

Test Substance:	PCS 200 neutral PH oxidizing disinfectant
Test Carriers	1 cm diameter disks of brushed stainless steel.
Dilution:	PCS 200 was tested as Ready-to-Use (RTU), No dilution was required.
Test Organism	Mixture of *Clostridium difficile spores* (ATCC 43598), *Staphylococcus aureus* (ATCC 6538) and *Serratia marcescens* (ATCC 13880)
Exposure Time:	No exposure time was considered. In the “Spray-Wipe” technique, the disks of each platform were transferred to neutralization solution immediately at the end of wiping.
Exposure Temperature:	Ambient temperature (22±2ºC)
Soil Load:	In accordance with the ASTM standard E2197, a mixture of bovine mucin, bovine serum albumin, and yeast extract was used to give a total protein concentration equal to that in 5% bovine serum in test microbial suspension.

TEST SYSTEM

1. Test Microorganisms

Three strains were used in a mixture:

- The spores of *Clostridium difficile* (ATCC # 43598), a Gram-positive, obligate anaerobe and a major nosocomial pathogen of world-wide concern. Due to its strict anaerobic requirements, the infectious and transmissible morphotype is the dormant spore. In susceptible patients, *C. difficile* spores germinate in the colon to form vegetative cells that initiate *C. difficile* infections (CDI). During CDI, *C. difficile* induces a sporulation pathway that produces more spores; these spores are responsible for the persistence of *C. difficile* in patients and horizontal transmission between hospitalized patients. While important to the *C. difficile* lifecycle, the *C. difficile* spore proteome is poorly conserved when compared to members of the *Bacillus* genus.

- *Staphylococcus aureus* (ATCC 6538), a Gram-positive coccus, is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning.

- *Serratia marcescens* (ATCC 13880), is a species of rod-shaped gram-negative bacteria in the family *Enterobacteriaceae*. A human pathogen, *S. marcescens* is involved in hospital-acquired infections (HAIs), particularly catheter-associated bacteremia, urinary tract infections and wound infections. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. Due to its abundant presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found growing in bathrooms (especially on tile grout, shower corners, toilet water line, and basin), where it manifests itself as a pink, pink-orange, or orange discoloration and slimy film feeding off phosphorus-containing materials or fatty substances such as soap and shampoo residue.

2. Test Medium

The recovery test medium used in this study was Brain Heart Infusion (BHI) agar with yeast extract (5 g/L), and sodium taurocholate (1 g/L) to grow the test organisms *S. aureus, S. marcescens* and recover *C. difficile.* Trypticase soy broth (TSB) was used to culture both test organism *S. aureus,* and *S. marcescens.*

3. Preparation of Test Organisms

To prepare a broth culture of *S. aureus* and *S. marcescens*, a 100 µL volume of the stock culture of the test organism was added to 10.0 mL of TSB in a tube and incubated aerobically for 24±2 h at 36±1°C. The culture of *S. marcescens* was incubated in a shaker incubator. Stock of *C. difficile* was used directly.

4. Preparation of Test Inocula

To prepare the mixture of test organisms for inoculation, equal volumes of each individual culture were mixed directly with soil load (mixture of bovine mucin, yeast extract and BSA).

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TEST METHOD

1. **Preparation of Test Substances**

The efficacy tests were performed on Ready-To-Use PCS 200 as specified by the Sponsor.

2. Test Procedure

A quantitative test system to closely simulate the field-application of the environmental surface decontamination process (quantitative carrier test – Tier 3 or QCT-3) was applied. Such a system aims to standardize the wiping of the target surface in terms of simulating the style of wiping in the field as well as the pressure applied during the wiping. Disks (1 cm diameter) of brushed stainless was used as archetypical environmental surfaces. Sterile disks were placed on the platform (dimensions 1 ft. x 2 ft. (~30.0 x 6 0.5 cm). The platforms are constructed in a way to allow the retrieval of the disks simultaneously in an eluent/neutralizer immediately at the end of the exposure time. The disks were then eluted and the eluates assayed for viable organisms.

Each metal disk on the platform was contaminated with 10 µL of the test inoculum with a soil load (ASTM protocol E2197) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC) for 120±10 minutes. A separate platform with sterile disks was used as a clean surface (transfer platform).

"Spray-Wipe" method,

Starting with the contaminated platform, both platforms were sprayed in an 'S' shaped pattern once as instructed by the Sponsor and then wiped in two steps in a pre-determined manner (as instructed by manufacturer). Both steps were performed with one piece of dry microfiber cloth (14" x 14"). Wiping was started right after spray, starting from the contaminated platform back and forth to the end of transfer platform. In the second step, wiping was continued from transfer platform using the dry side of the same microfiber and ended up to the beginning of contaminated platform. Constant pressure of 2-3 lbs was applied during wiping process.

The separate platform (transfer platform) was used to determine if, and how much, microbial contamination could be transferred to uncontaminated surfaces in the immediate vicinity.

To recover the inocula from the disks simultaneously, using the retrieval mechanism, each disk on the platform was placed into a separate vial containing 10 mL of a neutralizer/eluent/diluent (PBST+0.1% Sodium thiosulfate) and vortex mixed for 30±5 seconds to recover the inocula from the carriers (10^0 dilution). Ten-fold dilutions were prepared for each carrier. Depending on the initial inoculum level and the level of microbicidal activity expected, the number of dilutions was different for test and control eluates. The selected dilutions of treated carriers were membrane-filtered using a vacuum, then the vial was rinsed with 10 mL of PBS. The membranes were washed with 10 mL PBS first and washed with 40 mL of PBS after pouring the contents of each vial. Finally, each membrane was plated aseptically on the surface of a BHI agar plate.

The plates were incubated anaerobically at 36±1°C for 48±4 hours and the colonies of the test organism on each plate were counted. The plates were reincubated for three hours aerobically to distinct between the colonies of the three different strains.

"Spray-Wipe" test was repeated once and the average of the two tests is reported here as the final result

Experimental Design

a) Efficacy Test

1. Two platforms were used in testing of each method, one as a contaminated platform by inoculating all 9 disks with 10 µL of test organisms suspension and the second one as transfer plate with clean disks.
2. Platforms were left inside an operating BSC for 2 hrs to dry.
3. **To clean/disinfect surfaces:**

   "Spray-Wipe": One bottle of PCS 200 was poured to the pump device. Both platforms were sprayed in an 'S' shaped form with equal speed (~ 4 seconds). The surfaces of both platforms were wiped with one micro fiber cloth (2-folded), starting from contaminated platform to the end of transfer one with a constant pressure between 2-3 lbs ; and rewiped with the dry side of the same microfiber cloth from transfer platform back to the contaminated platform.

4. The contamination was retrieved from each disk by filtration and incubation of the membrane filters on the brain heart infusion agar plates at 36±1 for 48±2 hrs.
5. Three control disks were included in each test to estimate the initial contamination on the platform. The test was initiated with processing one control before the processing test carriers, one in the middle of the test (after processing contaminated platform disks) and ended up with the third control (after processing transfer platform disks). This was done to take into the account the changes in the input level of the test organisms during the experiment.

DATA ANALYSIS

Calculation of Percent Reduction

$$Percent\ Reduction = \left(1 - \frac{\frac{\mathrm{CFU}_{contaminated}}{\mathrm{A}_{disk}}}{\frac{\mathrm{CFU}_{initial}}{\mathrm{A}_{platform}}}\right) x100$$

$$Percent\ Transfer = \left(\frac{\frac{\mathrm{CFU}_{transfer}}{\mathrm{A}_{disk}}}{\frac{\mathrm{CFU}_{initial}}{\mathrm{A}_{platform}}}\right) x100$$

Where
$\mathrm{CFU}_{initial}$= average of CFU on the two control disks
$\mathrm{CFU}_{contaminated}$= average of CFU on the five disks retrieved from contaminated platform
$\mathrm{CFU}_{transfer}$= average of CFU on the five disks retrieved from transfer platform
$\mathrm{A}_{platform}$= Area of the platform (cm^2)
A_{disk}= Area of the disk (cm^2)

STUDY ACCEPTANCE CRITERIA
No product acceptance criterion was specified for this range-finding study.

TEST RESULTS

The results of testing are reported here in three different sections: Section a) vegetative bacteria only, Section b) *C. difficile*, and Section c); the total of three types of microorganisms.

A) Vegetative Bacteria (*S. aureus* and *S. marcescens*)

Table 1 shows the results of total vegetative cells for each sample tested and the average of percent reduction and percent transfer of the two tests.

	(CFU/cm^2)			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	27,000	0	0	100	0	100	0
Test #2	35,000	0	0	100	0		

B) *C. difficile* spores

Table 2: The result of C. difficile for each sample test and the average of percent reduction and percent transfer of two tests

	(CFU/cm^2)			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	27,000	3.57	0	99.99	0	99.95	0
Test #2	9,240	8.15	0	99.91	0		

C) The total of three types of microorganisms

Table 3: The result of total colony forming units for each test and the average of percent reduction and percent transfer of two tests

	(CFU/cm^2)			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	45,900	3.57	0	99.99	0	99.985	0
Test #2	44,200	8.15	0	99.98	0		

APPENDIX

Result of QCT-3 efficacy test using spraying PCS 200 and wiping, exposure to mixture of *C. difficile* (spores), *S. aureus* and *S. marcescens* *on* an inanimate surface.

Table 4: Spraying PCS 200 and wiping Test 1, Vegetative bacteria, *S. aureus* and *S. marcescens*, first test

Dilution	C1	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C2	C3
10^{0}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	58	-	-	-	-	-	-	-	-			54	36
10^{-6}	6	-	-	-	-	-	-	-	-			5	6

Table 5: Spraying PCS 200 and wiping Test 1, *C. difficile,* first test

Dilution	C1	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C2	C3
10^{0}	-	1	0	8	1	4	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	45	-	-	-	-	-	-	-	-	-	-	43	42
10^{-6}	3	-	-	-	-	-	-	-	-	-	-	4	2

Table 5: Spraying PCS 200 and wiping Test 2, Vegetative bacteria, *S. aureus* and *S. marcescens*, second test

Dilution	C1	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C2	C3
10^{0}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	56	-	-	-	-	-	-	-	-			48	43
10^{-6}	8	-	-	-	-	-	-	-	-			11	8

Table 6: Spraying PCS 200 and wiping Test 2, *C. difficile,* second test

Dilution	C1	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C2	C3
10^{0}	-	1	0	29	2	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	15	-	-	-	-	-	-	-	-	-	-	13	12
10^{-6}	3	-	-	-	-	-	-	-	-	-	-	3	1

References

1. Carling P.C. (2016). Optimizing Health Care Environmental Hygiene, Infect Dis Clin North Am. Sep;30(3):639-660.
2. Sattar, S. A. and Maillard J.-Y.(2013). 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. May;41(5 Suppl):S97-104.
3. Sattar, S.A. (2010). Promises & pitfalls of recent advances in chemical means of preventing the spread of nosocomial infections by environmental surfaces. Am J Infect Control 38: S34-40.