STUDY TITLE

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

TEST ORGANISM

Clostridium difficile spores (ATCC 43598), *Staphylococcus aureus* (ATCC 6538) *and Salmonella choleraesuis* (ATCC 10708)

TEST SAMPLE IDENTITY

- PCS 250
- CX
- CI

TEST Method

Quantitative carrier test – tier 3 or QCT-3

AUTHOR

Dr. Syed A. Sattar
Study Director

STUDY COMPLETION DATE

Oct. 16/17

PERFORMING LABORATORY

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

SPONSOR

PCS

STUDY NUMBER

PCS170417-02

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

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STUDY REPORT

GENERAL STUDY INFORMATION

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

Study Number: PCS170417-02
Protocol Number: PCS170417
Sponsor Process Cleaning Solutions (PCS) Inc.

Testing Facility CREM Co Labs
Unit 1-2, 3403 American Drive, Mississauga, ON, Canada

TEST SUBSTANCE IDENTITY

Test Substance Name: PCS 250, CX, and CI
Lot/Batch(s): Lot #

STUDY DATES
Date Sample Received: Aug/19/17
Study initiation date: Aug/25/17
Experimental Start Date: Aug/30/17
Experimental End Date: Oct/10/17
Study Completion Date: Oct/16/17

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). 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. The combined use of wiping and 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 basic objectives of this study were to:

a. Conduct laboratory-based testing on the combined use of a microfiber fabric with a chlorine-based disinfectant (PCS 250) 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 in comparison with two other types of pre-moistened commercial wipes; one based on 1.4% hydrogen peroxide (CX) and the other containing a mixture of ethanol and quaternary ammonium disinfectant (CI). It also investigated the possibility of reducing environmental contamination to safe levels with lower concentrations of chemicals, thereby reducing occupational exposure to high concentration of chemicals, damage to equipment and environmental surfaces and occupational exposure to high levels of chemicals.

b. Perform the testing on disinfecting wipes as instructed by the manufacturers, and evaluate the efficacy of a cleaning/decontaminating process of each product in comparison to using a microfiber cloth with PCS 250.

SUMMARY OF RESULTS

Test Substance:	PCS 250 neutral PH oxidizing disinfectant , hydrogen peroxide 1.4% pre-moistened wipe (CX) and an ethanol and quaternary ammonium disinfectant wipe (CI)
Test Carriers	1 cm diameter disks of brushed stainless steel.
Dilution:	The samples were tested as Ready-to-Use (RTU)
Test Organism	Mixture of *Clostridium difficile spores* (ATCC 43598)*,* *Staphylococcus aureus* (ATCC 6538) *and Salmonella choleraesuis* (ATCC 10708)
Exposure Time:	As described in the respective wipe manufacturer's instructions
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 Microorganism

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.

- *Salmonella choleraesuis* (ATCC 10708), non-typhoid species is an important cause of reportable food-borne infections. Among more than 2,000 serotypes, *Salmonella enterica* serotype Choleraesuis shows the highest predilection to cause systemic infections in humans. The most feared complication of serotype Choleraesuis bacteremia in adults is the development of myotic aneurysm, which previously was almost uniformly fatal.

2. Test Medium

The 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, Salmonella choleraesuis* and recover *C. difficile.*

3. Preparation of Test Organisms

To prepare a broth culture of *S. aureus* and *Salmonella choleraesuis*, 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 *Salmonella choleraesuis* 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 volume 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 the 3 different samples PCS 250, CX and CI 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 (~0.305 m x 0.61 m). 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). Starting with the contaminated platform, both platforms were then wiped in two steps in a pre-determined manner (as instructed by manufacturer) with a piece of microfiber cloth dampened with PCS250 or disinfecting towelettes (CX and CI). In testing PCS 250, 30 mL of the test disinfectant was added to dampen the microfiber cloth (7" x 14") to keep the wiped surface damp for at least 30 seconds. 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 (UN-1) 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.

The test on PCS 250 was repeated once and the average of the two tests is reported here as the final result

Experimental Design

a) Input

Please refer to the report of study # PCS170417-01

b) Efficacy Test

1. Two platforms were used in testing of each test sample, 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 surfaces:**

   PCS250: 30 mL of the test sample was added to a PCS microfiber cloth. The cloth was rolled up to distribute the liquid, and then was folded once to have 2 sides. Starting with the first platform (contaminated platform), the surface was wiped twice (back and forth) with the folded microfiber cloth applying a pressure of between 2 -3 lbs, continuing until all surfaces of both platforms were wiped, then the microfiber cloth was flipped over to clean side and the surfaces was rewiped staring with the transfer platform back to the contaminated platform.

   CX: Starting with the first platform (contaminated platform), the surface was wiped twice (back and forth) with one pre-saturated CX towelette applying a pressure of between 2 -3 lbs, continuing until all surfaces of both platforms were wiped.

   CI: Starting with the first platform (contaminated platform), the surface was wiped twice (back and forth) with one pre-saturated CI towelette was used directly to preclean the surface

4. **To disinfect cleaned surface:**

   PCS250: A new microfiber cloth was moistened with 30 mL of test sample. The surface was wiped as described in section 4 only once starting from the contaminated platform and ending up with the transfer platform. At the end of wiping, the surfaces were left to air dry under BSC for not more than 5 minutes before transferring each disk to the neutralizer.

   CX: To disinfectant the pre-cleaned surface, a second CX towelette was pulled out and used directly to wipe both platforms and allow to remain wet for 1 minute.

   CI: To disinfectant the pre-cleaned surface, a second CI towelette was pulled out and used directly to wipe both platforms and allow to remain wet for 3 minutes.

5. 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.

6. Two 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 and ended up with the second control. 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 result of testing is reported here in three different sections: Section a) vegetative bacteria only, Section b) *C. difficile*, and Section c) The total microorganisms.

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

Table 1 shows the result of total vegetative cells for each sample test (Average of two tests on PCS 250, CX, and CI)

	Control CFU/cm^2	Contaminated CFU/cm^2	Transfer CFU/cm^2	Percentage Transfer	Percent Reduction
250 ppm	10,810	0	0	0	100
CX	14,000	1.27	0	0	99.991
CI	34,400	2.54	0	0.00096	99.993

B) *C. difficile* spores

Table 2: The result of *C. difficile* for each sample test (Average of two tests on PCS 250, CX, and CI)

	Control CFU/cm^2	Contaminated CFU/cm^2	Transfer CFU/cm^2	Percentage Transfer	Percent Reduction
250 ppm	741	3.44	2.33	0.18	99.5
CX	1,150	14.33	15.3	1.33	98.75
CI	664	263	161	24.25	60.39

Table 3: The result of total colony for each sample test (Average of two tests on PCS 250, CX, and CI)

	Control CFU/cm^2	Contaminated CFU/cm^2	Transfer CFU/cm^2	Percentage Transfer	Percent Reduction
250 ppm	10,810	3.44	2.325	0.0215	99.97
CX	15,500	14.3	15.3	0.099	99.91
CI	34,400	263	115	0.33	99.24

APPENDIX

Result of QCT3 efficacy test on 3 samples (PCS 250, CX, and CI) exposure to mixture of *Clostridium difficile* (spores), *Staphylococcus aureus* and *Salmonella choleraesuis* on an inanimate surface.

Table 4: PCS 250, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, first test

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-2}	-	0	0	0	0	-	-	-	-	-	-	-	-
10^{--3}	-	0	0	0	0	-	-	-	-	-	-	-	-
10^{--4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{--5}	27	-	-	-	-	-	-	-	-			26	31
10^{-6}	3	-	-	-	-	-	-	-	-			1	4

Table 5: PCS 250, *C. difficile,* first test

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	1	0	0	0	1	TNTC	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	3*	0	0	0	0	-	-
10^{-2}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{--3}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{--4}	4	-	-	-	-	-	-	-	-	-	-	11	TNTC
10^{--5}	1	-	-	-	-	-	-	-	-	-	-	1	15
10^{-6}	0	-	-	-	-	-	-	-	-	-	-	0	0

*The value is out of acceptable tolerance and is considered as an outlier which happened due to experimental error

Table 5: PCS 250, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, second test

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	0	0	0	0	0	0	0	-	-
10^{-2}	-	0	0	0	0	-	-	-	-	-	-	-	-
10^{--3}	-	0	0	0	0	-	-	-	-	-	-	-	-
10^{--4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{--5}	28	-	-	-	-	-	-	-	-			27	46
10^{-6}	3	-	-	-	-	-	-	-	-			1	4

Table 6: PCS 250, *C. difficile,* second test

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

*The value is out of acceptable tolerance and is considered as an outlier which happened due to experimental error

Table 7: CX, Vegetative bacteria, *S. aureus* and *S. choleraesuis*,

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	0	0	1	TNTC	0	0	0	0	0	0	-	-
10^{-1}	-	0	0	0	18*	0	0	0	0	0	0	-	-
10^{-2}	-	0	0	0	3*	0	0	0	0	0	0	-	-
10^{-3}	-	0	0	0	1*	0	-	-	-	-	-	-	-
10^{-4}	TNTC	0	0	0	0	0	-	-	-	-	-	TNTC	TNTC
10^{-5}	27	-	-	-	-	-	-	-	-			26	31
10^{-6}	3	-	-	-	-	-	-	-	-			1	4

*The value is out of acceptable tolerance and is considered as an outlier which happened due to experimental error

Table 8: CX, *C. difficile*,

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	6	9	0	TNTC	9	6	10	11	1	5	-	-
10^{-1}	-	10	2	1	TNTC	5	4	3	11	1	1	-	-
10^{-2}	-	2	0	0	25*	1	0	2	4	0	1	-	-
10^{-3}	-	0	0	0	5*	0	-	-	-	-	-	-	-
10^{-4}	4	0	0	0	2*	0	-	-	-	-	-	11	TNTC
10^{-5}	1	-	-	-	-	-	-	-	-	-	-	1	15
10^{-6}	0	-	-	-	-	-	-	-	-	-	-	0	0

*The value is out of acceptable tolerance and is considered as an outlier which happened due to experimental error

Table 9: CI, Vegetative bacteria, *S. aureus* and *S. choleraesuis*,

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2
10^{0}	-	1	9	0	0	0	0	0	0	0	0	-
10^{-1}	-	0	0	0	0	0	0	0	0	0	0	-
10^{-2}	-	0	0	0	0	0	0	0	0	0	0	-
10^{-3}	-	0	0	0	0	0	-	-	-	-	-	-
10^{-4}	TNTC	0	0	0	0	0	-	-	-	-	-	TNTC
10^{-5}	60	-	-	-	-	-	-	-	-			59
10^{-6}	6	-	-	-	-	-	-	-	-			9

Table 10: CI, *C. difficile*,

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2
10^{0}	-	TNTC	TNTC	TNTC	TNTC	TNTC	0	0	0	0	0	-
10^{-1}	-	32	11	9	TNTC	24	0	0	0	0	0	-
10^{-2}	-	4	2	0	8	3	0	0	0	0	0	-
10^{-3}	-	0	0	0	2	0	0	0	0	0	0	-
10^{-4}	TNTC	0	0	0	0	0	-	-	-	-	-	27
10^{-5}	1	0	0	0	0	0	-	-	-	-	-	1
10^{-6}	0	-	-	-	-	-	-	-	-	-	-	0

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