STUDY TITLE

Assessment of Activity of PCS Toraysee™ Cleaning Cloths for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with *Clostrioides difficile* spores (ATCC 43598) as representative Healthcare-Associated Pathogens

TEST ORGANISM

Clostrioides difficile spores (ATCC 43598)

TEST SAMPLE IDENTITY

PCS 1000 and Toraysee™ wipe

TEST Method

Quantitative carrier test – Tier 3 or QCT-3

AUTHOR

Bahram Zargar, PhD
Study Director

STUDY COMPLETION DATE

Sep/22/21

PERFORMING LABORATORY

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

SPONSOR

Process Cleaning Solutions, Ltd.
2060 Fisher Drive, Peterborough, ON, Canada, K9J 8N4

STUDY NUMBER

PCS210901-01

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: Bahram Zargar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Khyati Sodvadiya, MSc
Jimikumar Patel, MSc
Payal Virani, BSc

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of Activity of PCS Toraysee™ Cleaning Cloths for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with *Clostrioides difficile* spores (ATCC 43598) as representative Healthcare-Associated Pathogens
Study Number:	PCS210901-01
Sponsor	Process Cleaning Solutions, Ltd. 2060 Fisher Drive, Peterborough, ON, Canada, K9J 8N4
Testing Facility	CREM Co Labs Unit 1-2, 3403 American Drive, Mississauga, ON, Canada L4V 1T4

TEST SUBSTANCE IDENTITY

Test Substance Name: PCS 1000 and Toraysee™ wipe
Lot/Batch(s): Lot #

STUDY DATES

Date Sample Received:	Aug/26/21
Study initiation date:	Aug/25/21
Experimental Start Date:	Sept/01/21
Experimental End Date:	Sept/07/21
Study Completion Date:	Sept/22/21

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 is a function of the way that the products are applied (e.g., spraying vs wiping) and the work practices and conditions with which they are used imay be 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.

While reuse of a regular single-use wipe for decontamination/cleaning of multiple surfaces can

result in transfer of microbial contamination, a reusable wipe, which does not transfer the contamination and at the same time can be decontaminated for multiple times using a strong disinfectant, can be a better alternative for decontaminating surfaces in healthcare settings.

II. OBJECTIVES

The objectives of this study were to:

a. Conduct laboratory-based testing on PCS Toraysee™ Cleaning Cloths 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 PCS Toraysee™ Cleaning Cloths and PCS 1000 as the wetting agent.

b. Compare the efficacy of single time wiping of PCS Toraysee™ Cleaning Cloths and PCS 1000 with that of double time wiping

SUMMARY OF RESULTS

Test Substance:	PCS Toraysee™ Cleaning Cloths and PCS 1000
Test Carriers	1 cm diameter disks (AISI 430) of brushed stainless steel.
Dilution:	PCS 1000, the wetting agent, was tested as Ready-to-Use (RTU); no dilution was required.
Test Organism	*Clostrioides difficile* spores (ATCC 43598)
Exposure Time:	No exposure time was considered. In the cleaning technique, the disks on each platform were transferred to a neutralization solution immediately at the end of wiping.
Exposure Temperature:	Ambient temperature (22±2ºC)
Soil Load:	3 part soil was used as specified in ASTM International's standard E2197-17.

TEST SYSTEM

1. **Test Microorganism**

Spores of *Clostridioides difficile* (ATCC # 43598): The spores of *Clostrioides 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.

2. Test Medium

The test media used in this study for *C. difficile* was Brain Heart Infusion (BHI) agar with yeast extract (5 g/L), and sodium taurocholate (1 g/L).

3. Preparation of Test Inocula

To prepare the spores for inoculation, the spore stock was mixed directly with the soil load (the mixture of bovine mucin, yeast extract and bovine serum albumin). Dilutions of spore mixture were prepared using PBST (PBS plus 0.5% Tween-80).

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TEST METHOD

1. **Preparation of Test Substance**

One single piece of cloth was used for the test. The efficacy test was performed using a pre-wetted PCS Toraysee™ Cleaning Cloth by PCS 1000. The PCS Toraysee™ Cleaning Cloth was dipped in PCS 1000 before the test and the excess liquid was squeezed out.

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 (AISI 430) were used as archetypical environmental surfaces. Sterile disks were placed on the platform (dimensions 1 ft. x 2 ft. (~30.0 x 60.5 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 the soil load (3-part soil load based on ASTM protocol E2197) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC). A separate platform with sterile disks was used as a clean surface (transfer platform).

Wipe method,

Before starting the efficacy test, the PCS Toraysee™ cloth was dipped in a container with 250 mL of PCS 1000. The cloth was squeezed out and used for testing. In the first test, starting with the contaminated platform, both platforms were wiped in one step in a pre-determined manner (as instructed by the manufacturer). Wiping was started from the

contaminated platform in one direction twice to the end of transfer platform (each section of platform was wiped twice in one direction before moving to the adjacent section). Constant pressure of 2-3 lbs was applied during wiping process. In the second test, after finishing the first series of wiping, the cloth was folded again and the two platformed were wiped using the clean side in the same manner as explained above.

A 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 neutralizer/eluent/diluent (Letheen broth with 0.2% sodium thiosulfate) and vortex mixed for 30±5 seconds to recover the inocula from the carriers (10^0 dilution). A ten-fold dilution series were prepared for each carrier and control eluate using PBS-T. Depending on the initial inoculum level and the level of sporicidal 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 placed 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.

Experimental Design

a) Input

The viability of the stock spores utilized in the testing was titrated by 10-fold serial dilution and assayed to determine the starting titer of the spore. The results of this control were for informational purposes only.

b) Neutralization Test (LB with 0.5% sodium thiosulfate)

Confirmation of neutralization of the test formulation was also carried out using Letheen broth as neutralizer containing 0.5% sodium thiosulfate with the PCS 1000 test sample and 100 µL of 10^{-5} dilution of countable colonies of the spores. In addition, PBS-T as control and the neutralizer were included individually to rule out any microbicidal or microbistatic action of the neutralizer itself.

c) 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 organism's suspension and the second one as the transfer platform with clean disks.
2. Platforms were left inside an operating biological safety cabinet (BSC) to dry for 2 hours.
3. **To clean/disinfect surfaces:**

Wipe: Before starting each efficacy test, the PCS Toraysee™ cloth was dipped in a container with 250 mL of PCS 1000. The cloth was squeezed out and used for testing. In the first test, wiping was started from the contaminated platform in one direction twice to the end of transfer platform (each section of platform was wiped twice in one direction before moving to the adjacent section). Constant pressure of 2-3 lbs was applied during wiping process. In the second test, after reaching to the end of transfer platform, the wipe was folded again and the wiping was repeated with the clean side as explained above. A separate platform (transfer platform) was used to determine if, and how much, microbial contamination could be transferred to uncontaminated surfaces in the immediate vicinity.

4. The contamination was retrieved from the eluate of 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
$\mathrm{CFU}_{\ or\ PFU\ transfer}$= 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

Table 1summarize the result of efficacy tests *C. difficile* spores.

Table 1: *C. difficile* spores inactivating/removing activity using PCS Toraysee™ cloth.

	CFU on Platform			Percent	
	Control	Contaminated	Transfer	Reduction	Transfer
Test #1 (wiping once)	1.70 x10^7	16,568	473	99.902	0.0028
Test #2 (wiping twice)	4.23 x10^7	0	0	100*	0*

*=No CFU were detected in the eluents tested.

Conclusions

The results of this study showed that, under the test conditions specified, PCS Toraysee™ cloth with PCS 1000 could efficiently decontaminate the contaminated platform and also prevent the transfer to the clean platform of *C. difficile* spores when the wipe was folded again and the wiping process was repeated with the clean side.

APPENDIX

Result of QCT3 efficacy test on PCS Toraysee™ cloth exposure to *, C. difficile spores* on an inanimate surface.

Table 2: PCS Toraysee™ cloth, *C. difficile spores,* test #1

Dilution	C1	CUL	CBL	CM	CUR	CBR	TUL	TBL	TM	TUR	TBR	C2	C3
10^{0}	-	0	2	0	4	29	0	0	0	0	1	-	-
10^{-1}	-	0	0	0	0	4	0	0	0	0	0	-	-
10^{-2}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-3}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	15	-	-	-	-	-	-	-	-	-	-	18	15
10^{-6}	5	-	-	-	-	-	-	-	-	-	-	1	0

Table 2: PCS Toraysee™ cloth, *C. difficile spores,* test #2

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	0	-	-	-	-	-	-	-
10^{-3}	-	0	0	0	0	0	-	-	-	-	-	-	-
10^{-4}	TNTC	-	-	-	-	-	-	-	-	-	-	TNTC	TNTC
10^{-5}	38	-	-	-	-	-	-	-	-	-	-	35	46
10^{-6}	5	-	-	-	-	-	-	-	-	-	-	4	5

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.