STUDY TITLE

Assessment of the Durability and Activity of PCS Toraysee™ Cleaning Cloths for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Coronavirus 229E (ATCC VR-740), Murine Norovirus (Strain S99), and *Clostrioides difficile* spores (ATCC 43598) as representative Healthcare-Associated Pathogens

TEST ORGANISM

Coronavirus 229E (ATCC VR-740*), Murine Norovirus (Strain S99), and Clostrioides difficile* spores (ATCC 43598)

TEST SAMPLE IDENTITY

PCS 5000 and Toraysee™ wipe

TEST Method

Quantitative carrier test – Tier 3 or QCT-3

AUTHOR

Bahram Zargar, PhD
Study Director

STUDY COMPLETION DATE

July/15/21

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

PCS210426-01

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
Mussarat Mussarat, BSc

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title:	Assessment of the Durability and Activity of PCS Toraysee™ Cleaning Cloths for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Coronavirus 229E (ATCC VR-740), Murine Norovirus (Strain S99), and *Clostrioides difficile* spores (ATCC 43598) as representative Healthcare-Associated Pathogens
Study Number:	PCS210426-01
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 5000 and Toraysee™ wipe
Lot/Batch(s): Lot #

STUDY DATES

Date Sample Received:	April/19/21
Study initiation date:	April/20/21
Experimental Start Date:	April/20/21
Experimental End Date:	June/27/21
Study Completion Date:	July/16/21

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 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.

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 strong disinfectant, can be a good alternative for decontaminating surfaces in healthcare settings.

II. OBJECTIVES

The objective of this study was 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 durability and efficacy of a cleaning/sanitizing process using PCS Toraysee™ Cleaning Cloths.

b. Test a single PCS Toraysee™ Cleaning Cloth in multiple studies to evaluate the efficacy of the cloth when it is used over and over and is decontaminated using PCS 5000 after each use.

SUMMARY OF RESULTS

Test Substance:	PCS Toraysee™ Cleaning Cloths and PCS 5000
Test Carriers	1 cm diameter disks of brushed stainless steel.
Dilution:	PCS 5000 was tested as Ready-to-Use (RTU), No dilution was required.
Test Organism	Coronavirus 229E (ATCC VR-740), Murine Norovirus (Strain S99), and *Clostrioides difficile* spores (ATCC 43598)
Exposure Time:	No exposure time was considered. In the cleaning 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:	5% fetal bovine serum (FBS) in test microbial suspension. For *C. difficile* 3 part soil was used as explained in ASTM E2197.

TEST SYSTEM

1. Test Microorganism

Coronavirus 229E (ATCC VR-740): Coronavirus 229E (ATCC VR-740) is an enveloped virus in the genus Coronavirus. Members of this genus can cause acute respiratory infections such as SARS-1 and SARS-2 (19-nCOV). Unlike Coronavirus 229E, SARS-1, SARS-2 and Middle-East Respiratory Syndrome (MERS) virus require Biosafety Level 3 labs. Therefore, Coronavirus 229E is frequently used as surrogate for them to assess the activity of different technologies for infection prevention and control (IPAC).

Murine Norovirus (MNV): MNV is a non-enveloped RNA virus in the family Caliciviridae. It is the most prevalent viral infection in mice. There are 4 described strains designated MNV-1, MNV-2, MNV-3, and MNV-4, as well as multiple field strains. The virus causes enteric infections and can also exit the gut to replicate in macrophages and dendritic cells in multiple organs, including mesenteric lymph nodes and liver. Since human noroviruses are difficult to culture in the lab, MNV is frequently used as its surrogate.

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. Host Cell Line

MRC5 or RAW 264. cells were used as hosts to support the replication and quantitation of 229E and MNV, respectively.

The cells were seeded into 12-well multi-well cell culture plates containing modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 36±1°C in a humidified atmosphere of 5% CO_2. Efficacy test was performed when the cell monolayer reached >90% confluency.
The cells were seeded into 12-well multi-well cell culture plates contained modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 36±1°C in a humidified atmosphere of 5% CO_2. Efficacy test was performed when the cell monolayer reached >90% confluency.

3. 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). Th test media used for Raw cells and

MRC-5 cells was 1X and 2 X Minimum Essential Medium (MEM) supplemented with L-glutamine, 10% (v/v) FBS, antibiotics and 7.5% sodium bicarbonate to grow the host cell and the virus after infection.

4. **Preparation of Test Inocula**

To prepare the virus for inoculation, the virus stock was mixed directly with the soil load (5% FBS). For *C. difficile*. To prepare the mixture of bovine mucin, yeast extract and BSA was used as soil load. Dilution of the virus mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4) and for C. difficile in 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 all tests. The efficacy tests were performed using a pre-wetted PCS Toraysee™ Cleaning Cloth by PCS 5000. The PCS Toraysee™ Cleaning Cloth was dipped in PCS 5000 before the test and the excess liquid was squeezed out. After each efficacy test the cloth was decontaminated by dipping in PCS 5000 for 5 minutes and then was squeezing out to remove the excess liquid. The piece of cloth was then rinsed with DI water and left in a BSC to air dry. The cloth was kept in the BSC between the tests and was reused in the next efficacy test.

2. **Test Procedure**

Before starting the efficacy tests a single PCS Toraysee™ cloth was placed in a container with 250 mL of PCS 5000 for ten days and the ppm of PCS 5000 was monitored using Extra High Level Chlorine strip sensor (Indigo Instruments, SKU: 33815-10K).

For Efficacy tests, 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 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 a soil load (5% FBS for virus and 3 part soil load based on ASTM protocol E2197 for *C. difficile*) 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,

One single piece of wipe was used for all efficacy tests and was decontaminated in PCS 5000 after each test.

Before starting each efficacy test, the PCS Toraysee™ cloth was dipped in a container with 250 mL of PCS 5000. The cloth was squeezed out and used for testing. 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.

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 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) (1 mL of neutralizer was used for virus and 10 mL for C. difficile). A ten-fold dilution series were prepared for each carrier and control eluate using EBSS or PBS-T. Depending on the initial inoculum level and the level of virucidal activity expected, the number of dilutions was different for test and control eluates.

For viruses, the appropriate dilutions were inoculated onto monolayers of host cells and incubate at 36±1°C for 60 minutes for virus adsorption. after virus adsorption, overlay medium was added to each well and the plates left in a BSC for the overlay to solidify. They were then incubated at 36±1°C in an incubator with 5% CO_2. The monolayers were fixed and stained after 40-44 hrs of incubation and the plaques on them counted and recorded to determine percentage reduction in the viability and transfer of the virus to clean platform.

For *C. difficile*, 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.

Experimental Design

a) Input

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

b) Cytotoxicity Control

Prior to test, cytotoxicity control and control for interference with virus infectivity were performed to determine if the test substance causes any apparent degeneration

(cytotoxicity) of the host cell line and to assess if the neutralizer in any way reduces or enhances such cytotoxicity. Control monolayers received an equivalent volume of EBSS (without any neutralizer) only.

c) 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 5000 test sample and 100 µL of 10^{-5} dilution of the test virus or countable colonies of spores. In addition, EBSS or PBS-T as control and the neutralizer were included individually to rule out any microbicidal or microbistatic action of the neutralizer itself.

d) 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 transfer plate with clean disks.
2. Platforms were left inside an operating BSC to dry (one hour for virus and 2 hours for *C. difficile* spores).

3. **To clean/disinfect surfaces:**

   Wipe: One single wipe was used for all efficacy tests and was decontaminated in PCS 5000 after each test. Before starting each efficacy test, the PCS Toraysee™ cloth was dipped in a container with 250 mL of PCS 5000. The cloth was squeezed out and used for testing. 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. 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.

4. For virus, the host cells in multi-well culture plates were inoculated with 100 µL of the dilutions prepared from test and control samples. Uninfected indicator cell cultures (cell controls) were inoculated with 100 µL EBSS alone. The cultures were incubated at 36±1°C in a humidified atmosphere of 5% CO_2 for 40-44 hrs before fixing and staining them for counting plaques.

5. For *C. difficile* spores, 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. 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

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

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

Where

$\text{CFU or PFU}_{\ initial}$= average of CFU or PFU on the two control disks

$\text{CFU or PFU}_{\ contaminated}$= average of CFU or PFU on the five disks retrieved from contaminated platform

$\text{CFU}_{\ or\ PFU\ transfer}$= average of CFU or PFU on the five disks retrieved from transfer platform

$\text{A}_{platform}$= 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 1,2,and 3 summarize the result of efficacy tests on *229E*, *MNV* and *C. difficile* spores, respectively.

Table 1: 229E virus inactivating/removing activity using PCS Toraysee™ cloth.

	PFU on Platform			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	3,458	0	0	100*	0*	100	0
Test #2	8,292	0	0	100*	0*		

*=No PFU were detected in the eluents tested.

Table 2: MNV virus inactivating/removing activity using PCS Toraysee™ cloth.

	PFU on Platform			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	71,111	0	0	100*	0*	100	0
Test #2	142,500	0	0	100*	0*		

*=No PFU were detected in the eluents tested.

Table 3: *C. difficile* spores inactivating/removing activity using PCS Toraysee™ cloth.

	CFU on Platform			Percent		Average Percent	
	Control	Contaminated	Transfer	Reduction	Transfer	Reduction	Transfer
Test #1	4.76 x10^6	0	0	100*	0*	100	0
Test #2	2.87 x10^6	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 5000 efficiently decontaminated the contaminated platform and also prevented the transfer of infectious virus and C. difficile spores to the clean platform.

The PCS Toraysee™ cloth's integrity and efficacy also was not affected in 6 separate efficacy tests on the three microorganisms in two months.

The stability test also shows the potency of PCS 5000 did not dropped bellow the acceptable range (<5000 ppm) when the PCS Toraysee™ cloth was kept in PCS 5000 in a closed container for 10 days.

APPENDIX

Result of QCT3 efficacy test on PCS Toraysee™ cloth exposure to *Coronavirus 229E* (ATCC VR-740) on an inanimate surface.

Table 4: PCS Toraysee™ cloth, Test 1, *Coronavirus 229E* (ATCC VR-740)

Dilution	C1	C2	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C3	C4
10^{0}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	-	-
10^{-1}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	-	-	-	-	-	-	-
10^{-2}	2,4,5	4,4,7	-	-	-	-	-	-	-	-	-	-	7,6,0	3,3,4
10^{-3}	0,0,0	0,0,0	-	-	-	-	-	-	-	-	-	-	0,0,0	0,0,0

Table 5: PCS Toraysee™ cloth, Test 2, *Coronavirus 229E* (ATCC VR-740)

Dilution	C1	C2	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C3	C4
10^{0}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	-	-
10^{-1}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	-	-	-	-	-	-	-
10^{-2}	6,7,2	5,6,3	-	-	-	-	-	-	-	-	-	-	5,6,5	7,7,9
10^{-3}	1,1,1	2,3,2	-	-	-	-	-	-	-	-	-	-	0,0,2	1,0,0

Table 6: PCS Toraysee™ cloth, Test 1, *Murine Norovirus* (Strain S99)

Dilution	C1	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C3	C4
10^{0}	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC
10^{-1}	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC
10^{-2}	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC
10^{-3}	7,12,4	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	7,5,10	6,3,10

Table 7: PCS Toraysee™ cloth, Test 2, *Murine Norovirus* (Strain S99)

Dilution	C1	C2	CBL	CBR	CM	CUL	CUR	TBL	TBR	TM	TUL	TUR	C3	C4
10^{0}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC
10^{-1}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC
10^{-2}	TNTC	TNTC	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	14,18,15	7,7,9
10^{-3}	13,18,18	16,16,12	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	14,18,15	13,18

Table 7: 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	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}	29	-	-	-	-	-	-	-	-	-	-	38	28
10^{-6}	7	-	-	-	-	-	-	-	-	-	-	4	7

Table 8: 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}	21	-	-	-	-	-	-	-	-	-	-	31	40
10^{-6}	0	-	-	-	-	-	-	-	-	-	-	3	4

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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.