**STUDY TITLE**

Assessment of the Combined Activity of Spray and Wiping for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Mouse Norovirus (MNV) as a representative Healthcare-Associated Pathogen

**TEST ORGANISM**

*Murine Norovirus (MNV-RVB 651)*

**TEST SAMPLE IDENTITY**

PCS 200

**TEST Method**

Quantitative carrier test – Tier 3 or QCT-3

**AUTHOR**

Dr. Syed A. Sattar
Study Director

**STUDY COMPLETION DATE**

Dec. /17/18

**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
Saeideh Naderi, PhD

**STUDY REPORT**

**GENERAL STUDY INFORMATION**

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|---|---|
| **Study Title:** | Assessment of the Combined Activity of Spray and Wiping for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing with Mouse Norovirus (MNV) as a representative Healthcare-Associated Pathogen |
| **Study Number:** | PCS181024-01 |
| **Sponsor** | Process Cleaning Solutions (PCS), Inc. |
| **Testing Facility** | CREM Co Labs<br>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:** | **Nov**/20/18 |
| **Study initiation date:** | **Nov**/20/18 |
| **Experimental Start Date:** | Nov/24/18 |
| **Experimental End Date:** | Dec/16/18 |
| **Study Completion Date:** | Dec/17/18 |

## 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.

## 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 (virus) 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** | Murine Norovirus (MNV) |
| **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 Microorganism**

   Murine Norovirus (MNV): MNV is a non-enveloped RNA virus in the family Caliciviridae. Members of this family can cause acute diarrhea and vomiting. Unlike MNV, the human norovirus cannot be readily cultured in the lab. Therefore, MNV is often used as surrogate for it to assess the activity of microbicidal chemicals.

2. **Host Cell Line**

RAW 264.7 cells were used as host cell to support the replication of murine noroviruses. This cell line is a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice. This cell line is a commonly used model of mouse macrophages for the study of efficacy test of disinfectants.

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 were RPMI-1640 for passaging the cell line, 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 (mixture of bovine mucin, yeast extract and BSA). Dilution of the mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4).

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## TEST METHOD

1. **Preparation of Test Substance**

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 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 (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 the manufacturer). Both steps were performed with one piece of dry

microfiber cloth (14" x 14"). Wiping was started right after spraying, 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 950 µL of a neutralizer/eluent/diluent (Letheen broth with 0.5% 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 EBSS. Depending on the initial inoculum level and the level of virucidal activity expected, the number of dilutions was different for test and control eluates. The appropriate dilutions were inoculated onto monolayers of RAW 264.7 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.

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

**Experimental Design**

**a) Input**

The stock virus utilized in the testing was titrated by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. 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 PCS200 test sample and 100 µL of $10^{-5}$ dilution of the test virus. In addition, EBSS 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 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 into 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 RAW 264.7 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. 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**

Table 1 shows the result of virus inactivating/removing activity of each sample test.

**Table 1**: virus inactivating/removing activity using Spray PCS200

| | (PFU/$cm^2$) | | | Percent | | Average Percent | |
|---|---|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | Transfer | Reduction | Transfer |
| Test #1 | 4,333 | 0 | 0 | 100* | 0 | 100 | 0 |
| Test #2 | 18,386 | 0 | 0 | 100* | 0 | | |

*=No PFU were detected in the eluents tested.

**Conclusions**

The results of this study showed that, under the test conditions specified, spray and wiping with PCS200 efficiently decontaminated the contaminated disks and also prevented the transfer of infectious virus to the clean disks.

## APPENDIX

Result of QCT3 efficacy test on test sample (PCS 200) exposure to MNV on an inanimate surface.

**Table 2:** Spraying PCS 200 and wiping Test 1, MNV

| 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 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | - | - |
| $10^{-1}$ | - | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | 11,5 | - | - | - | - | - | - | - | - | - | - | 11,10 | 4,13 |
| $10^{-5}$ | 3,2 | - | - | - | - | - | - | - | - | | | 3,1 | 0,0 |
| $10^{-6}$ | 0,0 | - | - | - | - | - | - | - | - | | | 0,0 | 6 |

**Table 3:** Spraying PCS 200 and wiping Test 2, MNV

| 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 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | - | - |
| $10^{-1}$ | - | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | 19,12,17 | - | - | - | - | - | - | - | - | - | - | 20,15,18 | - |
| $10^{-5}$ | 5,5,7 | - | - | - | - | - | - | - | - | - | - | 1,1,2 | - |
| $10^{-6}$ | 2,2,1 | - | - | - | - | - | - | - | - | - | - | 1,0,0 | - |

## 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.

## 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 7000
PCS 1000
Saline $T_{80}$

## TEST Method

Quantitative carrier test – tier 3 or QCT-3

**AUTHOR**
Dr. Syed A. Sattar
Study Director

## STUDY COMPLETION DATE

Aug/06/17

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

## SPONSOR

PCS

## STUDY NUMBER

PCS170417-01

## 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
Saeideh Naderi, PhD

**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:** | PCS170417-01 |
| **Protocol Number:** | PCS170417 |
| **Sponsor** | Process Cleaning Solutions (PCS) Inc. |
| **Testing Facility** | CREM Co Labs<br>Unit 1-2, 3403 American Drive, Mississauga, ON, Canada |

**TEST SUBSTANCE IDENTITY**

**Test Substance Name:** PCS 7000, PCS 1000, and Saline $T_{80}$
**Lot/Batch(s):** Lot #

**STUDY DATES**

| | |
|---|---|
| **Date Sample Received:** | Apr/19/17 |
| **Study initiation date:** | Apr/15/17 |
| **Experimental Start Date:** | June/11/17 |
| **Experimental End Date:** | Aug/06/17 |
| **Study Completion Date:** | Aug/21/17 |

## 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 setting 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 7000) diluted in a neutral pH buffer for the microbial decontamination of hard, non-porous environmental surfaces representing those found in healthcare settings.
b. Perform the testing to determine if the concentration of the bleach (as hypochlorous acid) could be reduced for economy as well as environmental and workplace safety.

The aim here was to evaluate the efficacy of a cleaning/sanitizing process in comparison to cleaning/disinfecting with PCS 7000. 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.

### SUMMARY OF RESULTS

| | |
|---|---|
| **Test Substance:** | PCS 7000, pH-neutral solutions of PCS 7000 (1000 ppm) and Saline $T_{80}$ |
| **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:** | N/A |
| **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 Agar with yeast extract (5 g/L), horse blood (70 mL/L) and sodium taurocholate (1 g/L) (BHIY-HT) , pre-reduced, to grow the test organisms *S. aureus* and *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 amount 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 7000 as a positive control, PCS 1000, and Saline $T_{80}$ as negative control) as specified by the Sponsor using pieces of the PCS microfiber cloths

**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 with a piece of microfiber cloth dampened with test substance. In this process, 60 mL of the test disinfectant or control substance was added to dampen the microfiber cloth 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. As control, two separate tests were performed, one using Saline $T_{80}$ as a negative control and the other with undiluted PCS 7000 as a positive control.

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 Saline$T_{80}$. The membranes were washed with 10 mL Saline$T_{80}$ first and washed with 40 mL of Saline$T_{80}$ after pouring the contents of each vial. Finally, each membrane was plated aseptically on the surface of a BHIY-HT 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.

**Experimental Design**

**a) Input**

The titer of each microorganism was measured separately. The initial titer of the three microorganism appeared to be around $10^9$

**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 under BSC for 2 hrs to dry.
3. 60 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 twice to have 8 sides.
4. **To clean surfaces:** 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.
5. **To disinfect cleaned surface:** A new microfiber cloth was moistened with 60 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.
6. 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 24 hrs.
7. Two control disks were included for each test sample 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

$$Percent\ Reduction = \left(1 - \frac{\frac{CFU_{contaminated}}{A_{disk}}}{\frac{CFU_{initial}}{A_{platform}}}\right) x100$$

$$Percent\ Reduction = \left(1 - \frac{\frac{CFU_{transfer}}{A_{disk}}}{\frac{CFU_{initial}}{A_{platform}}}\right) x100$$

Where
$CFU_{initial}$= average of CFU on the two control disks
$CFU_{contaminated}$= average of CFU on the five disks retrieved from contaminated platform
$CFU_{transfer}$= average of CFU on the five disks retrieved from transfer platform
$A_{platform}$= 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 section. In section a) just vegetative bacteria is reported, in Section b) just *C. difficile* is reported and in Section c) The result of the three microorganisms is reported.

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

Table 1 shows the result of total vegetative cells for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$). Table 2 shows the average of the two tests and percent reduction and percent transfer of each sample.

**Table 1:** The result of total vegetative bacteria (*Staph aureus* and *S. choleraesuis*) for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | Test #1 (CFU/cm$^2$) | | | Test #2 (CFU/cm$^2$) | | |
|---|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Control | Contaminated | Transfer |
| **Saline $T_{80}$** | 7000 | 33.9 | 0 | 22300 | 28.3 | 0 |
| **1000 ppm** | 7000 | 0.764 | 0 | 12900 | 0 | 0 |
| **7000 ppm** | 7140 | 0 | 0 | 4290 | 0 | 0 |

**Table 2:** The average of the two tests and reduction and transfer percent for total viable vegetative bacteria for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | (CFU/cm$^2$) | | | Percent | |
|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | Transfer |
| **Saline $T_{80}$** | 14,650 | 31.1 | 0 | 99.79 | 0 |
| **1000 ppm** | 9,950 | 0.382 | 0 | 99.996 | 0 |
| **7000 ppm** | 5,715 | 0 | 0 | 100 | 0 |

## B) *C. difficile* spores

**Table 3:** The result of *C. difficile* for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | Test #1 (CFU/cm$^2$) | | | Test #2 (CFU/cm$^2$) | | |
|---|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Control | Contaminated | Transfer |
| **Saline $T_{80}$** | 13,600 | 2,840 | 228 | 16,700 | 4,290 | 364 |
| **1000 ppm** | 9,960 | 24.46 | 0.51 | 14,900 | 105.22 | 13.25 |
| **7000 ppm** | 13600 | 4.08 | 0.61 | 5890 | 0.51 | 0 |

**Table 4:** The average of the two tests and reduction and transfer percent for *C. difficile* for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | (CFU/cm$^2$) | | | Percent | |
|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | transfer |
| **Saline $T_{80}$** | 15,150 | 3,565 | 296 | 76.47 | 1.95 |
| **1000 ppm** | 12,430 | 64.84 | 6.88 | 99.75 | 0.055 |
| **7000 ppm** | 9745 | 2.30 | 0.31 | 99.976 | 0.0032 |

**Table 5:** The result of total colony for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | Test #1 (CFU/cm$^2$) | | | Test #2 (CFU/cm$^2$) | | |
|---|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Control | Contaminated | Transfer |
| **Saline $T_{80}$** | 39,000 | 4,320 | 364 | 20,600 | 4,010 | 349 |
| **1000 ppm** | 18,800 | 10.4 | 0.51 | 27,900 | 105 | 13.2 |
| **7000 ppm** | 21,100 | 3.74 | 0.51 | 13,400 | 0.51 | 0 |

**Table 6:** The average of the two tests and reduction and transfer percent for Total Colony for each sample test (PCS 1000 and PCS 7000) and control (Saline$T_{80}$)

| | (CFU/cm$^2$) | | | Percent | |
|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | Transfer |
| **Saline $T_{80}$** | 29,800 | 4,170 | 365.5 | 0.8600 | 1.22651 |
| **1000 ppm** | 23,600 | 57.7 | 6.86 | 0.9976 | 0.0291 |
| **7000 ppm** | 17,300 | 2.13 | 0.25 | 0.9998 | 0.00145 |

## APPENDIX

Result of QCT3 efficacy test on 3 samples (PCS 1000, Saline $T_{80}$ and PCS 7000) exposure to mixture of *Clostridium difficile* (spores), *Staphylococcus aureus* and *Salmonella choleraesuis* on an inanimate surface.

Table 7: PCS 1000, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, Test #1

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

Table 8: PCS 1000, *C. difficile*, Test #1

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 0 | 0 | 0 | 2 | 5 | 0 | 0 | 1 | 0 | 0 | - |
| $10^{-1}$ | - | 1 | 5 | 0 | 15 | 3 | 1 | 0 | 0 | 0 | 0 | - |
| $10^{-2}$ | - | 0 | 1 | 0 | 8 | 6 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-3}$ | - | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 17 | - | - | - | - | - | - | - | - | - | - | 12 |
| $10^{-6}$ | 2 | - | - | - | - | - | - | - | - | - | - | 3 |

Table 9: PCS 1000, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-1}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 35 | - | - | - | - | - | - | - | - | - | - | 35 |
| $10^{-6}$ | 3 | - | - | - | - | - | - | - | - | - | - | 1 |

Table 10: PCS 1000, *C. difficile*, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 64 | 1 | 184 | 120 | 44 | 40 | 2 | 10 | 0 | 0 | - |
| $10^{-1}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 31 | - | - | - | - | - | - | - | - | - | - | 35 |
| $10^{-6}$ | 3 | - | - | - | - | - | - | - | - | - | - | 2 |

Table 11: PCS 7000, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, Test #1

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

Table 12: PCS 7000, *C. difficile*, Test #1

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 2 | 4 | 2 | 8 | 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 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 17 | - | - | - | - | - | - | - | - | - | - | 12 |
| $10^{-6}$ | 2 | - | - | - | - | - | - | - | - | - | - | 3 |

Table 13: PCS 7000, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, 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 | - | - |
| $10^{-1}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | 37 | - | - | - | - | - | - | - | - | - | - | 35 | TNTC |
| $10^{-5}$ | 3 | - | - | - | - | - | - | - | - | - | - | 8 | 14 |
| $10^{-6}$ | 2 | - | - | - | - | - | - | - | - | - | - | 0 | 2 |

Table 14: PCS 7000, *C. difficile*, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - |
| $10^{-1}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC | TNTC |
| $10^{-5}$ | 12 | - | - | - | - | - | - | - | - | - | - | 7 | 24 |
| $10^{-6}$ | 4 | - | - | - | - | - | - | - | - | - | - | 0 | 1 |

Table 15: Saline $T_{80}$, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, Test #1

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 3 | 87 | 0 | 12 | 1 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-1}$ | - | 0 | 21 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-2}$ | - | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-3}$ | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 17 | - | - | - | - | - | - | - | - | - | - | 17 |
| $10^{-6}$ | 0 | - | - | - | - | - | - | - | - | - | - | 2 |

Table 16: Saline $T_{80}$, *C. difficile*, Test #1

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | TNTC | TNTC | 36 | TNTC | 63 | TNTC | TNT | TNT | 0 | TNT | - |
| $10^{-1}$ | - | TNTC | TNTC | 6 | TNTC | 7 | 33 | 28 | 31 | 0 | 33 | - |
| $10^{-2}$ | - | 18 | 56 | 1 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-3}$ | - | 4 | 7 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 20 | - | - | - | - | - | - | - | - | - | - | 18 |
| $10^{-6}$ | 3 | - | - | - | - | - | - | - | - | - | - | 4 |

Table 17: Saline $T_{80}$, Vegetative bacteria, *S. aureus* and *S. choleraesuis*, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | TNTC | 0 | 0 | TNTC | TNTC | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-1}$ | - | TNTC | 0 | 0 | 2 | TNTC | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-2}$ | - | 2 | 0 | 0 | 0 | TNTC | - | - | - | - | - | - |
| $10^{-3}$ | - | 0 | 0 | 0 | 0 | 103* | - | - | - | - | - | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 28 | - | - | - | - | - | - | - | - | - | - | 30 |
| $10^{-6}$ | 6 | - | - | - | - | - | - | - | - | - | - | 6 |

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

Table 18: Saline $T_{80}$ *C. difficile*, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC | TNT | TNT | TNT | TNT | - |
| $10^{-1}$ | - | TNTC | 20 | TNTC | 73 | TNTC | 55 | 20 | 10 | 56 | 2 | - |
| $10^{-2}$ | - | 41 | 0 | 9 | 7 | TNTC | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-3}$ | - | 1 | 1 | 1 | 0 | 37* | 0 | 0 | 0 | 0 | 0 | - |
| $10^{-4}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-5}$ | 17 | - | - | - | - | - | - | - | - | - | - | 26 |
| $10^{-6}$ | 4 | - | - | - | - | - | - | - | - | - | - | 5 |

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

## STUDY TITLE

Assessment of the Combined Activity of Wiping and Disinfection for Decontaminating Hard, Non-Porous Environmental Surfaces using the Murine Norovirus (MNV)

## TEST ORGANISM

*Murine Norovirus* (Strain S99)

## TEST SAMPLE IDENTITY

PCS 250 with Microfiber Cloth
Hydrogen Peroxide-based Wipes
Saline $T_{80}$ with Microfiber cloth

## TEST Method

Quantitative Carrier Test – Tier 3 or QCT-3

**AUTHOR**
Dr. Syed A. Sattar
Study Director

**STUDY COMPLETION DATE**
Dec/08/17

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

**SPONSOR**
Process Cleaning Solutions (PCS) lnc.

**STUDY NUMBER**
PCS171025-01

## 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
Saeideh Naderi, PhD

# STUDY REPORT

## GENERAL STUDY INFORMATION

| | |
|---|---|
| **Study Title:** | Assessment of the Combined Activity of Wiping and Disinfection for Decontaminating Hard, Non-Porous Environmental Surfaces using the Murine Norovirus (MNV) |
| **Study Number:** | PCS171025-01 |
| **Protocol Number:** | PCS171025 |
| **Sponsor** | Process Cleaning Solutions (PCS) Inc. |
| **Testing Facility** | CREM Co Labs<br>Unit 1-2, 3403 American Drive, Mississauga, ON., Canada |

## TEST SUBSTANCE IDENTITY

**Test Substance Name:** PCS 250, Hydrogen peroxide-based disinfectant, and Saline $T_{80}$
**Lot/Batch(s):** Lot #

## STUDY DATES

| | |
|---|---|
| **Date Sample Received:** | Apr/19/17 |
| **Study initiation date:** | Oct/25/17 |
| **Experimental Start Date:** | Oct/25/17 |
| **Experimental End Date:** | Nov/26/17 |
| **Study Completion Date:** | Dec/09/17 |

## 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.

## OBJECTIVES

The basic objectives of this study were to:

a. Access the virus inactivating/removing activity by conducting laboratory-based test on the combined use of a microfiber fabric with a diluted chlorine-based disinfectant (PCS 250) in a neutral pH buffer for the microbial decontamination of hard, non-porous environmental surfaces representing those found in healthcare settings.
b. Perform the testing to determine if the low concentration of the bleach (as hypochlorous acid) could be used for economy as well as environmental and workplace safety.

The aim here was to evaluate the virus inactivating/removing efficacy of a cleaning/sanitizing process of PCS 250 in comparison to cleaning/disinfecting with commercial hydrogen peroxide-based wipe and with Saline-$T_{80}$ using a microfiber cloth as a control.

## SUMMARY OF RESULTS

| | |
|---|---|
| **Test Substance:** | PCS 250, pH-neutral solutions of diluted PCS7000, hydrogen peroxide-based wipe (HPW) and Saline $T_{80}$ |
| **Test Carriers** | 1 cm diameter disks of brushed stainless steel. |
| **Dilution:** | The samples were tested as Ready-to-Use (RTU) |
| **Test Organism** | Murine Norovirus (MNV; Strain S99) |
| **Exposure Time:** | N/A |
| **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

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.

1. **Host Cell Line**
   RAW 264.7 cells were used as host cell to support replication of MNV. This cell line is a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice. This cell line is a commonly used model of mouse macrophages for the study of efficacy test of disinfectants.

   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.

2. **Test Medium**
   The test medium used in this study 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.

3. **Preparation of Test Organism**
4. To prepare the virus for inoculation, the virus stock was mixed directly with soil load (mixture of bovine mucin, yeast extract and BSA). Dilution of the mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4).

**TEST METHOD**

1. **Preparation of Test Substances**
   The efficacy tests were performed on the 3 different samples - PCS 250, Saline $T_{80}$ as negative control as specified by the Sponsor using pieces of the PCS microfiber cloths, and a hydrogen peroxide-based wipe.

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 aim 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 inocula with a soil load (ASTM protocol E2197) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC) for 120±10 minutes. The separate platform with sterile disks was used as a clean surface (transfer platform). Starting with contaminated platform, both platforms were then wiped in two steps in a pre-determined manner with a piece of PCS microfiber cloth dampened with test substance. In this process, 30 mL of the test disinfectant or control substance was added to dampen the PCS microfiber cloth to keep the wiped surface damp for at least 30 seconds. The ready-to-use hydrogen peroxide-based wipe was used in two steps according to the manufacturer's instruction. 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.

In each test, as a control, individual disks were inoculated and dried similarly. These disks were considered as the initial level of contamination of the platform before wiping.

To recover the inocula from the disks simultaneously, using the retrieval mechanism each disk on the platform was placed into a separate vial containing 950 µL of a neutralizer/eluent/diluent (Letheen broth with 0.1% 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 EBSS. Depending on the initial inoculum level and the level of virucidal activity expected, the number of dilutions was different for test and control eluates. The appropriate dilutions were inoculated onto monolayers of RAW 264.7 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 plaque on them counted and recorded to determine percentage reduction in the viability and transfer of the virus to clean platform.

**Experimental Design**

**a) Input**

The stock virus utilized in the testing was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. 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 0.1% Sodium Thiosulfate)**

Confirmation of neutralization of the test formulation was also carried out using Letheen broth as neutralizer containing 1% Sodium thiosulfate with the PCS250 test sample and 100 µL of $10^{-5}$ dilution of the test virus. In addition, EBSS as control and the neutralizer were included individually to rule out any microbicidal or microbistatic action of the neutralizer itself.

**d) Range-Finding Test**

A preliminary range-finding test was conducted using test samples PCS250 and HPW to determine the number of dilutions needed for each carrier of contaminated, transfer (clean) platforms and control as well.

Number of Carriers per platform: 9
Number of Carriers retrieved per platform: 5
Number of negative Controls: 3
Contact Time: N/A

**e) Efficacy Test**

1. Two platforms were used in testing of each test sample, one as a contaminated platform by inoculating all 9 disks with 10µL of test organisms suspension and the second one as transfer plate with clean disks.
2. Platforms were left under BSC for 2 hrs to dry.
3. 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 4 sides.
4. **To clean surfaces:** Starting with the first platform (contaminated platform), surface was wiped twice (back and forth) with folded microfiber cloth applying pressure between 2 -3 lb, continuing until all surface 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.
5. **To disinfect cleaned surface:** A new microfiber cloth was moistened with 30 mL of test sample. The surface was wiped as described in section 4 only once started from the contaminated platform and ended up with the transfer platform. At the end of wiping, surfaces were left allowed to air dry under BSC for not more than 5 minutes before transferring each disk to the neutralizer.
6. The RAW 264.7 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 30 hrs before fixing and staining them for counting plaques.
7. Three control disks were included for each test to estimate the initial contamination on the platform. The test was initiated with processing one control before the processing test carriers, one before processing transfer platform's carrier and ended up with the third 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**

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

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

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

**STUDY ACCEPTANCE CRITERIA]'**

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

**TEST RESULTS**

Table 1 shows the result of virus inactivating/removing activity of each sample test (PCS 250 and HPW) and control (Saline$T_{80}$).

Table 1: the result of virus inactivating/removing activity of each sample test (PCS 250 and HPW) and control (Saline$T_{80}$).

| | (PFU/$cm^2$) | | | Percent | |
|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | Transfer |
| **Saline $T_{80}$** | 4,480.48 | 3.40 | 7.67 | 99.92 | 0.17 |
| **Saline $T_{80}$** | 4,480.48 | 3.40 | 8.49 | 99.92 | 0.19 |
| **250 ppm test 1** | 3,894.07 | 3.82 | 9.34 | 99.90 | 0.24 |
| **250 ppm test 2** | 5,529.96 | 0.42 | 7.64 | 99.99 | 0.14 |
| **HPW** | 5,529.96 | 0.85 | 8.49 | 99.98 | 0.15 |

**Table 2:** The average of the two tests and reduction and transfer percent for **virus inactivating/removing** activity of each sample test (PCS 250 and HPW) and control (Saline$T_{80}$)

| | (PFU/$cm^2$) | | | Percent | |
|---|---|---|---|---|---|
| | Control | Contaminated | Transfer | Reduction | Transfer |
| **Saline $T_{80}$** | 4480.48 | 3.40 | 8.08 | 99.92 | 0.18 |
| **250 ppm test 1** | 4,712.02 | 2.12 | 8.49 | 99.96 | 0.18 |
| **HPW** | 5,529.96 | 0.85 | 8.49 | 99.98 | 0.15 |

## APPENDIX

Result of QCT3 efficacy test on 3 samples (PCS 250, Saline $T_{80}$ and HPW) exposure to MNV on an inanimate surface.

Table 3: PCS 250 Test #1

| Dilution | C1 | C2 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | - | 1,1,0 | 0,0,1 | 1,0,1 | 1,1,1 | 0,0,1 | 1,0,0 | 1,1,1 | 1,0,1 | 1,1,1 | 0,1,1 | - |
| $10^{-1}$ | - | - | 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 | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | 43,72 | 45,46 | - | - | - | - | - | - | - | - | - | - | 35,33 |
| $10^{-4}$ | 13,9,9 | 9,8,9 | - | - | - | - | - | - | - | - | - | - | 10,8,11 |
| $10^{-5}$ | 0,2,0 | 1,1,0 | - | - | - | - | - | - | - | - | | | 3,1,1 |
| $10^{-6}$ | 0,0,0 | 0,0,0 | - | - | - | - | - | - | - | - | | | 0,0,0 |

Table 4: PCS 250 Test #2

| Dilution | C1 | C2 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | - | 0,0,0 | 0,0,0 | 1,0,0 | 0,0,0 | 0,0,0 | 0,0,1 | 0,1,1 | 1,1,0 | 1,1,0 | 0,1,1 | - |
| $10^{-1}$ | - | - | 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 | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | TNTC | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-4}$ | 19,11,15 | 12,9,14 | - | - | - | - | - | - | - | - | - | - | 11,16 |
| $10^{-5}$ | 0,1,0 | 3,0,1 | - | - | - | - | - | - | - | - | | | 1,1 |
| $10^{-6}$ | 0,0,0 | 0,0,0 | - | - | - | - | - | - | - | - | | | 0,0 |

Table 5: HPW

| Dilution | C1 | C2 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | - | 1,1,0 | 0,0,0 | 0,0,0 | 0,0,0 | 0,0,0 | 1,1,1 | 1,1,1 | 0,0,1 | 0,0,0 | 1,0,2 | - |
| $10^{-1}$ | - | - | 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 | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | TNTC | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-4}$ | 19,11,15 | 12,9,14 | - | - | - | - | - | - | - | - | - | - | 11,16 |
| $10^{-5}$ | 0,1,0 | 3,0,1 | - | - | - | - | - | - | - | - | | | 1,1 |
| $10^{-6}$ | 0,0,0 | 0,0,0 | - | - | - | - | - | - | - | - | | | 0,0 |

Table 6: Saline $T_{80}$ , Test #1

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 3,2,1 | 0,0,0 | 1,0,0 | 1,0,0 | 0,0,0 | 0,0,0 | 1,1,0 | 0,1,1 | 1,0,2 | 1,1,0 | - |
| $10^{-1}$ | - | 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 | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-4}$ | 6,10,7 | - | - | - | - | - | - | - | - | - | - | 8,8,7 |
| $10^{-5}$ | 1,0,1 | - | - | - | - | - | - | - | - | - | - | 3,1,1 |
| $10^{-6}$ | 0,0,0 | - | - | - | - | - | - | - | - | - | - | 0,0,0 |

Table 7: Saline $T_{80}$, Test #2

| Dilution | C1 | CUL | CBL | CM | CUR | CBR | TUL | TBL | TM | TUR | TBR | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $10^{0}$ | - | 1,1,1 | 1,0,0 | 1,0,0 | 1,0,0 | 1,1,0 | 0,1,0 | 2,2,1 | 0,0,1 | 2,1,0 | 0,0,0 | - |
| $10^{-1}$ | - | 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 | - |
| $10^{-2}$ | - | - | - | - | - | - | - | - | - | - | - | - |
| $10^{-3}$ | TNTC | - | - | - | - | - | - | - | - | - | - | TNTC |
| $10^{-4}$ | 6,10,7 | - | - | - | - | - | - | - | - | - | - | 8,8,7 |
| $10^{-5}$ | 1,0,1 | - | - | - | - | - | - | - | - | - | - | 3,1,1 |
| $10^{-6}$ | 0,0,0 | - | - | - | - | - | - | - | - | - | - | 0,0,0 |

## 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

PROFESSIONAL PERSONNEL INVOLVED: Bahram Zargar, PhD
Saeideh Naderi, PhD

**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<br>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 ($cm^2$)
$\mathrm{A}_{disk}$= 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 |

## 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

Dr. Syed A. Sattar
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
Saeideh Naderi, PhD

**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<br>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).

.

## 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 ($cm^2$)
$\mathrm{A}_{disk}$= 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.

**Date: March 17, 2017**

# SAFETY DATA SHEET

## SECTION 1 - IDENTIFICATION

**Product identifier used on the label:** Diluted neutral PH solution containing 32 parts water and 1 part PCS 7000 Oxidizing Disinfectant/disinfectant Cleaner

**Other means of Identification:** none

**Recommended use of the chemical and restrictions on use:** For professional use only.

**Manufacturer/Supplier:**
Process Cleaning Solutions Ltd.
**Address:**
2060 Fisher Dr.
Peterborough, On K9J 8N4

**Telephone:** 705-740-2880
**Fax:** 705-745-1239

**24 Hr. Emergency Tel. #:** Infotrac 1-800-535-5053 (North America), 011-1-352-323-3500 (International)

## SECTION 2 - HAZARDS IDENTIFICATION

**Classification of the chemical:**

**This chemical does not meet the hazardous criteria set forth by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200). However, this Safety Date Sheet (SDS) contains valuable information critical to the safe handling and proper use of this product. This SDS should be retained and available for employees and other users of this product.**

## SECTION 3 - COMPOSITION/INFORMATION ON INGREDIENTS

**The product contains no substances which, at their given concentration, are considered to be hazardous to health.**

## SECTION 4 - FIRST-AID MEASURES

**Description of first aid measures:**

**If swallowed:** Rinse mouth. Do NOT induce vomiting. Immediately call a poison center or doctor/physician.

**If on skin (or hair):** If skin irritation occurs get medical advice/attention. Wash contaminated clothing before reuse.

**If inhaled:** Remove person to fresh air and keep comfortable for breathing. If respiratory issues develop call doctor/physician.

**If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If irritation persists get medical advice/attention.

**Most Important symptoms and effects, both acute and delayed:** None

**Indication of any immediate medical attention and special treatment needed:** Treat symptomatically

## SECTION 5 - FIRE-FIGHTING MEASURES

**Extinguishing media:**

***Suitable extinguishing media:*** Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

***Unsuitable extinguishing media*:** Not determined

**Special hazards arising from the substance or mixture:** None known

**Flammability classification:** Not flammable

**Hazardous combustion products:** Carbon oxides, unidentified organic compounds.

**Special protective equipment and precautions for firefighters:**

***Protective equipment for fire-fighters:*** Firefighters should wear proper protective equipment and self-contained breathing apparatus with full face piece operated in positive pressure mode.

***Special fire-fighting procedures:*** Move containers from fire area if safe to do so. Cool closed containers exposed to fire with water spray. Do not allow run-off from firefighting to enter drains or water courses. Dike for water control.

## SECTION 6 - ACCIDENTAL RELEASE MEASURES

**Personal precautions, protective equipment and emergency procedures:** All persons dealing with the clean-up should wear the appropriate chemically protective equipment. Keep people away from and upwind of spilt/leak. Restrict access to area until completion of clean-up. Refer to protective measures listed in sections 7 and 8.

**Methods and material for containment and cleaning up:** Do not allow material to contaminate ground water system. If necessary, dike well ahead of the spill to prevent runoff into drains, sewers, or any natural waterway or drinking supply. Ventilate the area. Prevent further leakage or spillage if safe to do so. Soak up with inert absorbent material. Do not use combustible absorbents, such as sawdust. Pick up and transfer to properly labeled containers. Contaminated absorbent material may pose the same hazards as the spilled product. Contact the proper local authorities.

**Special spill response procedures:** In case of a transportation accident, contact Infotrac 1-800-535-5053 (North America), 011-1-352-323-3500 (International). If a spill/release in the US in excess of the EPA reportable quantity is made into the environment, immediately notify the national response center in the United States (phone: 1-800-424-8802).

## SECTION 7 - HANDLING AND STORAGE

**Precautions for safe handling:** Handle in accordance with good industrial hygiene and safety practice. Use protective equipment recommended in section 8. Avoid contact with eyes. Do not breathe dust/fume/gas/mist/vapors/spray.

**Conditions for safe storage:** Keep container tightly closed and store in a cool, dry and well-ventilated place. Store locked up. Keep out of reach of children.

**Incompatible materials:** Oxidizing agents. Do not mix with other chemicals or cleaners

## SECTION 8 – EXPOSURE CONTROLS AND PERSONAL PROTECTION

**Exposure controls:**

**Ventilation and engineering measures:** Not required under normal use conditions. For production facilities use only in well-ventilated areas. Apply technical measures to comply with the occupational exposure limits. Where reasonably practicable this should be achieved by the use of local exhaust ventilation and good general extraction.

**Personal Protective Equipment:**

**Respiratory protection:** Not required with normal use. If airborne concentrations are above the permissible exposure limit or irritation occurs, use NIOSH-approved respirators. Respirators should be selected based on the form and concentration of contaminants in air, and in accordance with OSHA (29 CFR 1910.134). Advice should be sought from respiratory protection specialists.

**Protective Measures:** For consumer use, no unusual precautions are necessary. In a work environment, if a splash is likely, chemical goggles may be needed.

**Other protective equipment:** Other equipment may be required depending on workplace standards.

**General hygiene considerations:** In an industrial work environment, avoid eye and prolonged skin contact. Do not eat, drink or smoke when using this product. Handle in accordance with good industrial hygiene and safety practice.

## SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

**Appearance:** Clear Colorless liquid

**Odor:** Slight Bleach

**Odor threshold:** No applicable information available

**pH:** 5.8-7.0

**Melting/Freezing point:** No applicable information available

**Initial boiling point and boiling range:** No applicable information available

**Flash point:** No applicable information available

**Flashpoint (Method):** No applicable information available

**Evaporation rate (BuAe = 1):** Similar to water

**Flammability (solid, gas): Not flammable**

**Lower flammable limit (% by vol.):** Not Flammable

**Upper flammable limit (% by vol.):** Not Flammable

**Vapor pressure:** No applicable information available

**Vapor density:** No applicable information available

**Relative density:** 1.00

**Solubility in water:** Soluble

**Other solubility(ies):** No applicable information available

**Partition coefficient:** No applicable information available

**Auto ignition temperature:** No applicable information available

**Decomposition temperature:** No applicable information available

**Viscosity:** Water thin.

**Volatile organic Compounds (%VOC's):** No applicable information available

**Other physical/chemical comments:** No applicable information available

## SECTION 10 - STABILITY AND REACTIVITY

**Reactivity:** Not normally reactive

**Chemical stability:** Stable

**Possibility of hazardous reactions:** No hazardous polymerization

**Conditions to avoid:** Keep out of reach of children. Do not use in areas without adequate ventilation. Avoid contact with incompatible materials.

**Incompatible materials:** Fluorine, strong oxidizing or reducing agents, bases, metals, sulfur trioxide, phosphorus pentoxide

**Hazardous decomposition products:** None known. Refer to 'Hazardous Combustion Products' in Section 5

## SECTION 11 - TOXICOLOGICAL INFORMATION

**Information on likely routes of exposure:**

**Routes of entry - inhalation:** Avoid breathing vapors or mists

**Routes of entry - skin & eye:** Avoid contact with eyes

**Routes of entry - Ingestion:** Do not taste or swallow

**Potential Health Effects:**

**Signs and symptoms of short term (acute) exposure:**

***Symptoms:*** Please see section 4 of this SDS sheet for symptoms*.*

**Potential Chronic Health Effects:**

**Mutagenicity:** Not expected to be mutagenic in humans.

**Carcinogenicity:** No components are listed as carcinogens by ACGIH, IARC, OSHA or NTP.

**Reproductive effects:** No applicable information available

**Sensitization to material:** No applicable information available

**Specific target organ effects:** No data available to indicate product or components will have specific target organ effects.

**Medical conditions aggravated by overexposure:** No applicable information available

**Toxicological data:**

See the following table for individual ingredient acute toxicity data.

| Chemical name | CAS # | $LD_{50}$ (Oral, rat) | $LD_{50}$ (Dermal. Rabbit) | $LC_{50}$ (4hr, Inhal., rat) |
|---|---|---|---|---|
| No applicable information | | | | |

*All empty cells no applicable information available

Other important toxicological hazards: None reported.

## SECTION 12 - ECOLOGICAL INFORMATION

**Ecotoxicity:** No applicable information available.

**Persistence and degradability:** No applicable information available

**Bioaccumulation potential:** No applicable information available.

**Mobility in soil:** No applicable information available.

**Other Adverse Environmental effects:** No applicable information available.

## SECTION 13 - DISPOSAL CONSIDERATIONS

**Handling for disposal:** Handle in accordance with good industrial hygiene and safety practice. Refer to protective measures listed in sections 7 and 8. Empty containers should be rinsed before disposal.

**Methods of disposal:** Dispose in accordance with all applicable federal, state, provincial and local regulations. Contact your local, state, provincial or federal environmental agency for specific rules.

**RCRA:** This product, as supplied, is not considered hazardous waste defined under RCRA, Title 40 CFR 261. It is the responsibility of the waste generator to determine the proper waste identification and disposal method. For disposal of unused or waste material, check with local, state and federal environmental agencies.

## SECTION 14 - TRANSPORTATION INFORMATION

**US 49 CFR/DOT information:**

***UN No.:*** Not Regulated

***UN Proper Shipping Name:*** Not Regulated

***Transport Hazard Class(es):*** Not Regulated

***Packing Group:*** Not Regulated

***Special Transportation Notes:*** None

## SECTION 15 - REGULATORY INFORMATION

**TSCA information:** All listed ingredients appear on the Toxic Substances Control Act (TSCA) inventory.

**Canadian Environmental Protection Act (CEPA) information:** All ingredients listed appear on the Domestic Substances List (DSL).

## SECTION 16 - OTHER INFORMATION

**Legend:**
**ACGIH:** American Conference of Governmental Industrial Hygienists
**CAS:** Chemical Abstract Services
**CERCLA:** Comprehensive Environmental Response, Compensation, and Liability Act of 1980
**CFR:** Code of Federal Regulations
**CSA:** Canadian Standards Association
**DOT:** Department of Transportation
**ECOTOX:** U.S. EPA Ecotoxicology Database
**EINECS:** European Inventory of Existing Commercial chemical Substances
**EPA:** Environmental Protection Agency
**HSDB:** Hazardous Substances Data Bank
**IARC:** International Agency for Research on Cancer
**IUCLID:** International Uniform Chemical Information Database
**LC:** Lethal Concentration
**LD:** Lethal Dose
**NIOSH:** National Institute of Occupational Safety and Health
**NTP:** National Toxicology Program
**OECD:** Organization for Economic Co operation and Development
**OSHA:** Occupational Safety and Health Administration
**PEL:** Permissible exposure limit
**RCRA:** Resource Conservation and Recovery Act
**RTECS:** Registry of Toxic Effects of Chemical Substances
**SARA:** Superfund Amendments and Reauthorization Act
**SDS:** Safety Data Sheet Material Safety Data Sheet
**STEL:** Short Term Exposure Limit
**TOG:** Canadian Transportation of Dangerous Goods Act & Regulations
**TLV:** Threshold Limit Values
**TWA:** Time Weighted Average
**WHMIS:** Workplace Hazardous Materials Identification System

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### DISCLAIMER



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