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 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)
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 (SalineT_{80}).

Table 1: the result of virus inactivating/removing activity of each sample test (PCS 250 and HPW) and control (SalineT_{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 (SalineT_{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