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

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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
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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 Mouse Norovirus (MNV) as a representative Healthcare-Associated Pathogen
Study Number:	PCS181024-01
Sponsor	Process Cleaning Solutions (PCS), Inc.
Testing Facility	CREM Co Labs 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.