ATS LABS

STUDY REPORT

STUDY TITLE

Evaluation of Antimicrobial Activity of a Cold Plasma Generator

Virus: Feline Calicivirus

PRODUCT IDENTITY

GPS-2400-1 Cold Plasma Generator

AUTHOR

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STUDY COMPLETION DATE

May 28, 2013

PERFORMING LABORATORY

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SPONSOR

Global Plasma Solutions
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PROJECT NUMBER

A14991

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Evaluation of Antimicrobial Activity of a Cold Plasma Generator

Project Number: A14991

TRF Number: GPS01042913.FCAL

TEST SUBSTANCE IDENTITY

Test Substance Name: GPS-2400-1 Cold Plasma Generator

STUDY DATES

Date Sample Received: May 9, 2013
Study Initiation Date: May 9, 2013
Experimental Start Date: May 10, 2013
Experimental End Date: May 17, 2013
Study Completion Date: May 28, 2013

TEST PARAMETERS

Product Preparation: The middle support bracket was attached to the bar containing one GPS-2400-1 Cold Plasma Generator at each end of the bar. The generators were placed, with the carbon fiber brushes pointing down, in the back of a hood with the hood sash closed.

Virus: Feline Calicivirus, ATCC VR-782, Strain F-9

Exposure Time: 30 minutes

Exposure Temperature: Room temperature (22.0°C)

Organic Soil Load: 1% fetal bovine serum

Test Medium: Minimum Essential Medium (MEM) supplemented with 5% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 10 µg/mL gentamicin, and 2.5 µg/mL amphotericin B

Indicator Cell Cultures: Feline kidney (CRFK) cells

EXPERIMENTAL DESIGN

Preparation of Virus Films
Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of four 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were air-dried at 20.0°C in a relative humidity of 50% until visibly dry (20 minutes).

Input Virus Control (TABLE 1)
On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Treatment of Virus Films with the Test Substance (TABLE 2)
For each of the two replicates, the bar containing one GPS-2400-1 Cold Plasma Generator at each end of the bar was placed in the back of the hood. For each replicate, one GPS-2400-1 Cold Plasma Generator was placed directly over the top of one of the two carriers (petri dishes) containing the dried virus films and the generator was plugged in. (Only one carrier was placed under each GPS-2400-1 Cold Plasma Generator.) The carbon fiber brushes on the GPS-2400-1 Cold Plasma Generators were pointing downward toward the carriers at a distance of approximately 1 inch from the carriers. The carriers were held open under the GPS-2400-1 Cold Plasma Generator at room temperature (22.0°C) for the 30 minute exposure time. The sash on the hood was closed for the exposure and the green light, between the brushes of each GPS-2400-1 Cold Plasma Generator, was illuminated. At the end of the exposure time, a 2.00 mL aliquot of test medium was added to each of the two carriers and the carriers were individually scraped with a cell scraper to resuspend the contents of the carrier. The contents of each carrier were immediately passed through an individual Sephadex column utilizing the syringe plunger in order to detoxify the mixture. Each filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

Dried Virus Control (TABLE 1)
Two virus control films were run in parallel to the test virus but a 2.00 mL aliquot of test medium was added in lieu of exposure to the GPS-2400-1 Cold Plasma Generator. The virus control films were held covered for the 30 minute exposure time at room temperature (22.0°C). Just prior to the end of each exposure time, the virus films were individually scraped to resuspend the contents and at the end of the exposure time the mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity.

Cytotoxicity Control (TABLE 3)
A cytotoxicity control was performed for the GPS-2400-1 Cold Plasma Generator in parallel to the test, however test medium containing the Sponsor requested organic soil load was dried on a 100 X 15 mm sterile glass petri dish in lieu of virus. The petri dish with the dried test medium film was held open under the GPS-2400-1 Cold Plasma Generator at room temperature (22.0°C) for 60 minutes. At the end of the exposure time, a 2.00 mL aliquot of test medium was added to the petri dish and the dish was scraped with a cell scraper to resuspend the contents. The contents of the petri dish were immediately passed through a Sephadex column utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the CRFK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

Neutralization Control (TABLE 3)
A neutralization control was performed by inoculating a 100 µL aliquot of the 10^{-1} to 10^{-3} dilutions of the cytotoxicity control dilutions into the indicator cell cultures in quadruplicate. A 100 µL aliquot of low titer stock virus was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Infectivity Assays
The CRFK cell line, which exhibits cytopathic effect (CPE) in the presence of Feline Calicivirus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. Cultures are incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability.

Calculations
The average titer ($TCID_{50}$) was calculated for the test and dried virus control replicates. The average percent and log reductions in viral titer achieved by the GPS-2400-1 Cold Plasma Generator were calculated using the average titer ($TCID_{50}$) of the dried virus control.

Per Sponsor's direction, the study was not required to be conducted under US EPA 40 CFR Part 160 or US FDA 21 CFR Part 58.

CONCLUSION

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum organic soil load, the GPS-2400-1 Cold Plasma Generator **did not demonstrate complete inactivation** of Feline Calicivirus following a 30 minute exposure time at room temperature (22.0°C). A 93.5% average reduction in viral titer was demonstrated following a 30 minute exposure time, as compared to the average titer of the dried virus control. The average log reduction in viral titer was 1.19 $\log_{10}$.

STUDY RESULTS

TABLE 1: Virus Control Results

Input Virus Control and Dried Virus Controls Following 30 Minute Exposure Time

Dilution	Input Virus Control	Dried Virus Control	
		Replicate #1	Replicate #2
Cell Control	0 0	0 0 0 0	0 0 0 0
10^{-1}	+ +	+ + + +	+ + + +
10^{-2}	+ +	+ + + +	+ + + +
10^{-3}	+ +	+ + + +	+ + + +
10^{-4}	+ +	+ + + +	+ + + +
10^{-5}	+ +	+ + + +	+ + + +
10^{-6}	+ +	0 0 0 +	+ + + +
10^{-7}	+ +	0 0 0 0	+ + + 0
10^{-8}	+ +	0 0 0 0	0 + 0 0
10^{-9}	0 0	NT	NT
$TCID_{50}$/100 µL	$10^{8.50}$	$10^{5.75}$	$10^{7.50}$
Average $TCID_{50}$/100 µL	NA	$10^{7.21}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NT) = Not tested
(NA) = Not Applicable

TABLE 2: Test Substance Assay Results

Effects of GPS-2400-1 Cold Plasma Generator Following a 30 Minute Exposure to Feline Calicivirus Dried on an Inanimate Surface

Dilution	Feline Calicivirus + GPS-2400-1 Cold Plasma Generator	
	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{6.25}$	$10^{5.50}$
Average $TCID_{50}$/100 µL	$10^{6.02}$	
Average Log Reduction	1.19 Log_{10}	
Average Percent Reduction	93.5%	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

TABLE 3: Cytotoxicity Control and Neutralization Control

Dilution	Cytotoxicity Control	Neutralization Control
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	0 0 0 0	+ + + +
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
TCD_{50}/100 µL	$\leq 10^{0.50}$	See below

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present

Results of the neutralization control (non-virucidal level control) indicate that the test substance was neutralized at a $TCID_{50}$/100 µLof ≤ 0.50 $\log_{10}$.

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5-28-13
Date