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July 28, 2003

Rik J. Wijsenbeek
HBA Sa
62 Sixth Ave
San Francisco, CA 94118

Re: Evaluation of BioScreen C000C against Human Coronavirus

Dear Mr. Wijsenbeek,

Enclosed is the report for the evaluation of BioScreen C000C against Human Coronavirus, ATCC VR-740, Strain 229E. This strain was selected to serve as a surrogate for the SARS virus. A viral suspension assay was performed to assess the antiviral properties of the product. There are no EPA requirements for minimum viral reduction for this type of study. The BioScreen C000C product achieved > 99.97% (> 3.6 $\log_{10}$) inactivation of the virus within 10 minutes. The ratio of product to dilution buffer suspension was 1:10 (i.e. 0.5 g of BioScreen C000C was amended to 4.5 mL of Butterfield's buffer). All experimentation was performed in triplicate. Please do not hesitate to contact me if you have any questions.

Respectfully submitted,

Nancy Linde

Nancy Linde, M.S.
Senior Associate
Regulatory Affairs

789 N. Dixboro Rd. · Ann Arbor · Michigan · 48105 · USA
Toll-free (800) 673-6275 · Direct (734) 913-5756 · Fax (734) 827-7100

ATS LABS

NON-GLP STUDY REPORT

STUDY TITLE

Evaluation of Antiviral Properties of A Product Using A Virucidal Suspension Assay

Virus: Human Coronavirus

PRODUCT IDENTITY

S302109019 PAF96616001NL

TEST REQUEST FORM NUMBER

NSF01052703.COR

PROJECT NUMBER

A01488

AUTHOR

Karen M. Ramm
Technical Director

STUDY COMPLETION DATE

July 25, 2003

PERFORMING LABORATORY

ATS LABS
2540 Executive Drive
St. Paul, MN 55120

SPONSOR

NSF International
789 N. Dexboro Road
Ann Arbor, MI 48105

This study was not performed under
FDA Good Laboratory Practice Regulations
(21 CFR Part 58)

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of A Product Using A Virucidal Suspension Assay

Project Number: A01488

Test Request Form Number: NSF01052703.COR

TEST SUBSTANCE IDENTITY

Test Substance: S302109019 PAF96616001NL

STUDY DATES

Date Received: June 4, 2003
Study Initiation Date: June 13, 2003
Experimental Start Date: June 26, 2003
Experimental End Date: July 3, 2003
Study Completion Date: July 25, 2003

EXPERIMENTAL DESIGN

Dilution: 0.5 g/4.5 mL Butterfield's Buffer
Virus: Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time: 10 minutes and 24 hours
Exposure Temperature: Room temperature (23°C)
Organic Soil Load: 1% fetal bovine serum
Test Medium: Test medium used in this study was Eagles minimal essential medium (E-MEM) supplemented with 2% heat-inactivated FBS, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone.
Indicator Cell Cultures: MRC-5 (human embryonic lung)

EXPERIMENTAL SUMMARY

A 0.5 mL aliquot of the virus was exposed to a 4.5 mL aliquot of the use dilution of the product. At 10 minute and 24 hour exposure times, a 0.1 mL aliquot was removed, neutralized by serial dilution, and assayed for the presence of virus. The positive virus controls, cytotoxicity controls, and neutralization controls were assayed in parallel. Antiviral properties of the product were evaluated and compared at the specified concentrations and time intervals.

CONCLUSION

Under these test conditions, S302109019 PAF96616001NL **demonstrated** complete inactivation of Human Coronavirus. Taking the cytotoxicity and neutralization control results into consideration, an average reduction of ≥99.97% in viral titer was demonstrated for S302109019 PAF96616001NL following a 10 minute exposure time. The average log reduction was ≥3.6 $\log_{10}$. A ≥99.4% reduction in viral titer was demonstrated following a 24 hour exposure time. The average log reduction was ≥2.2 $\log_{10}$.

STUDY RESULTS

TABLE 1: Virus Control Results of Human Coronavirus in Suspension Following Ten and 24 Hour Exposure Periods

Dilution	Virus Control 10 Minute Exposure			Virus Control 24 Hour Exposure		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Cell Control	0000	0000	0000	0000	0000	0000
10^{-2}	++++	++++	++++	++++	++++	++++
10^{-3}	++++	++++	++++	+0+0	++++	++++
10^{-4}	++++	++++	++++	000+	++00	0000
10^{-5}	+00+	+00+	+00+	0000	0000	0000
10^{-6}	0000	00+0	0000	0000	0000	0000
$TCID_{50}$/0.1mL	$10^{5.0}$	$10^{5.25}$	$10^{5.0}$	$10^{3.25}$	$10^{4.0}$	$10^{3.5}$
Average $TCID_{50}$/0.1mL	$10^{5.10}$			$10^{3.70}$		

TABLE 2: Effects of S302109019 PAF96616001NL Against Human Coronavirus in Suspension Following Ten Minute and 24 Hour Exposure Periods

Dilution	Test: Human Coronavirus + S302109019 PAF96616001NL 10 Minute Exposure			Test: Human Coronavirus + S302109019 PAF96616001NL 24 Hour Exposure		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
Cell Control	0000	0000	0000	0000	0000	0000
10^{-2}	0000	0000	0000	0000	0000	0000
10^{-3}	0000	0000	0000	0000	0000	0000
10^{-4}	0000	0000	0000	0000	0000	0000
10^{-5}	0000	0000	0000	0000	0000	0000
10^{-6}	0000	0000	0000	0000	0000	0000
$TCID_{50}$/0.1mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$
Average Percent Reduction	≥99.97%			≥99.4%		
Average Log Reduction	≥3.6 $\log_{10}$			≥2.2 $\log_{10}$		

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

TABLE 3: Cytotoxicity and Neutralization Control Results of S302109019 PAF96616001NL

Dilution	Cytotoxicity Control: S302109019 PAF96616001NL	Neutralization Control: Human Coronavirus + S302109019 PAF96616001NL
Cell Control	0 0 0 0	0 0 0 0
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
10^{-4}	0 0 0 0	+ + + +
10^{-5}	0 0 0 0	+ + + +
10^{-6}	0 0 0 0	+ + + +
TCD_{50}/0.1mL	$\leq 10^{1.5}$	Neutralized at a $TCID_{50}$/0.1mL of ≤ 1.5 Log_{10}

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

PROFESSIONAL PERSONNEL INVOLVED:

Douglas G. Anderson, Ph.D.	- President
Karen M. Ramm, B.A.	- Technical Director
Mary J. Miller, M.T.	- Research Scientist II
Katherine A. Paulson, M.L.T.	- Research Assistant II
Tonia Lewandowski	- Research Assistant I

PREPARED BY:

Karen M. Ramm — 7/25/03
Karen M. Ramm, B.A. — Date
Technical Director

REVIEWED BY:

Rachelle L. Iverson — 07/25/03
Quality Assurance Auditor — Date