ANTIMICROBIAL TEST LABORATORIES

Study Report NG4423: Modified AOAC Germicidal Spray Products as Disinfectants

Custom Device Test Against Human Coronavirus 229E

Client Information

Company Name:	Advanced Vapor Technologies	Sponsor:	Rick Hoverson
Sponsor's Phone:	(800) 997-6584	E-mail:	rick@advap.com

Test Information

Test Performed:	AOAC Germicidal Spray Test (with Modifications for Viruses) Custom Device Study
Performed by:	Luisa Ikner, Ph.D.

Device Information

Official Device Name:	VaporJet Professional 2400R

Test Parameters

Virus, Strain:	**Human coronavirus, 229E**	Soil Load:	5% FBS (Heat-Inactivated)
Host Cell Line:	Human Lung Fibroblast (MRC-5)	Cell Passage Number:	37
Type of Carrier:	Clay Quarry Tile	# of Replicates:	Singlet per Exposure Time
Exposure Conditions:	25.7 °C, 44% R.H.	**ExposureTimes:**	**2 Total: 3 Seconds, 5 Seconds**
Incubation Period:	7 Days	Incubation Temp:	34.0 ± 1 °C

Application: After preparation of the device per the Study Sponsor's instructions, steam flow was applied at the 'low volume' setting to test carriers for the exposure period of either 3 seconds or 5 seconds.

Neutralizer Used: Test/Assay Medium (2% FBS EMEM + Antibiotics)

Control and Test Results (All Log10 Values are per Carrier.)

Virus Control Titer: 3.98 log10 TCID50

T_{3Sec} Log10 Reduction: ≥ 3.25 log10

T_{5Sec} Log10 Reduction: ≥ 3.25 log10

Notes: The Test/Assay medium used during the study was comprised of the following: EMEM supplemented with fetal bovine serum to 2% (v/v), plus antibiotics. The cell culture virucidal efficacy assay was performed according to EPA-approved methodology. Viral and cytotoxicity titers were determined using the Spearman-Karber Method.

Test Completed: 08-Aug-2013 **Report Sent:** 21-Aug-2013

1304 W. Industrial Blvd.
Round Rock, Texas 78681

Phone: (512) 310-TEST
Web site: www.AntimicrobialTestLabs.com

Results

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 1. AOAC Germicidal Spray Test (with Modifications for Viruses) Custom Device Study Against Human Coronavirus 229E: Log10 and and Percent Reduction Data

Test Virus	Test Device	Virus Control Titer (Log_{10})	Contact Time	Virus Titer Post-Exposure (Log_{10})	Log_{10} Reduction	Percent Reduction
Human coronavirus 229E	VaporJet Professional 2400R	5.05	3 Seconds	≤ 1.80	≥ 3.25	≥ 99.94%
			5 Seconds	≤ 1.80	≥ 3.25	≥ 99.94%

Table 2. AOAC Germicidal Spray Test (with Modifications for Viruses) Custom Device Study Against Human Coronavirus 229E: Virus Control and Test Replicate Data

Dilution	Dried Virus Control[a]	Dried Virus Test Carriers	
		T = 3 Seconds	T = 5 Seconds
10^{-1}	+ + + +	0 0 0 0	0 0 0 0
10^{-2}	+ + + +	0 0 0 0	0 0 0 0
10^{-3}	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 +	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
Per 0.1 ml	**3.75 log_{10} $TCID_{50}$**	**≤ 0.50 log_{10} $TCLD_{50}$**	**≤ 0.50 log_{10} $TCLD_{50}$**
Per Carrier (2 ml)	**5.05 log_{10} $TCID_{50}$**	**≤ 1.80 log_{10} $TCLD_{50}$**	**≤ 1.80 log_{10} $TCLD_{50}$**

[a] $TCID_{50}$: Tissue Culture Infectivity Dose at the 50% Endpoint Dilution

[b] $TCLD_{50}$: Tissue Culture Lethal Dose at the 50% Endpoint Dilution

Results, Continued.

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Toxicity observed

Table 3. AOAC Germicidal Spray Test (with Modifications for Viruses) Custom Device Study Against Human Coronavirus 229E: Cytotoxicity and Neutralization Data

Dilution	Cytotoxicity Control[a]	Neutralization Control (Low Titer HcoV)[b]
10^{-1}	0 0 0 0	+ + + +
10^{-2}	0 0 0 0	+ + + +
10^{-3}	0 0 0 0	+ + + +
Per 0.1 ml	**≤ 0.50 $\log_{10}$ $TCCD_{50}$**	
Per Carrier (2 ml)	**≤ 1.80 $\log_{10}$ $TCCD_{50}$**	

[a] $TCCD_{50}$: Tissue Culture Cytotoxic Dose at the 50% Endpoint Dilution

[b] HcoV: Human coronavirus 229E

Table 4. Sterility Control Data, Human Coronavirus Test Assay

Set	Results
1	0 0 0 0
2	0 0 0 0
3	0 0 0 0

Summarized Test Procedure

Preparation and Inoculation of Carriers

- The glass quarry tile carriers and stainless steel mounts were wiped with a moist cotton cloth, and autoclaved.
- Laundered terry cloth towels (cut to 16" x 17") were autoclaved and dried under laminar flow conditions prior to use in testing.
- The carriers were placed into stainless steel mounts using flame-sterilized forceps.
- Aliquots of stock human coronavirus 229E (0.02 ml) were aseptically spread over each carrier surface, except for the outer ~3/8" perimeter area.
- A total of three virus films were prepared [1-Virus Control, 2-Test Carriers (1 per Time Point)].
- Drying time and conditions: 19 minutes, 25.7 °C, 44% relative humidity.

Treatment of Test Carriers

- The test device was prepared according to the "Protocol, Test Use, Instruction" manual issued by the Study Sponsor.
- The dried virus test films were treated using the device (one per contact time), followed by a ~10 second hold time.
- Test/Assay Medium (2.0 ml) was pipetted over each carrier surface to harvest remaining infectious viruses. Sterile cell scrapers (one per carrier) were also used to facilitate mechanical detachment.
- The virus suspensions were serially diluted (1:10), and plated in quadruplicate per dilution through 10^{-6} onto MRC-5 host cell monolayers prepared to the appropriate confluency (70 to 80%).

Processing of the Virus Control Carrier

- The virus control was processed by pipetting 2.0 ml of sterile, 2% FBS EMEM over the carrier surface.
- A sterile cell scraper was used to mechanically detach the virus film from carrier surface.
- The control virus suspension was serially diluted (1:10), and plated in quadruplicate per dilution through 10^{-6} onto MRC-5 host cell monolayers prepared to the appropriate confluency (70 to 80%).

Processing of the Cytotoxicity/Neutralization Control Carrier

- One clay quarry tile (with no virus film) was treated with the device in the same manner as the test carrier exposed for the longest contact time (5 seconds), and harvested as previously described.
- For the cytotoxicity control, an aliquot of the neutralized carrier suspension was serially diluted (1:10), and plated in quadruplicate through 10^{-3} onto MRC-5 host cell monolayers prepared to the appropriate confluency (70 to 80%).
- For the neutralization control, an aliquot of each cytotoxicity control filtrate was serially diluted (1:10) through 10^{-3}. A low titer inoculum of stock test virus was added to each neutralization control dilution tube, and held for the longest study contact time (5 seconds). Aliquots from each dilution (0.1 ml) were plated in quadruplicate per dilution onto MRC-5 host cell monolayers prepared to the appropriate confluency (70 to 80%).

Summarized Test Procedure, Continued.

Incubation Assay and Data Analysis

- Cell culture assay trays were incubated at 35 °C on an orbital rotator (set to 60 r/min) for 30 minutes to facilitate virus-host cell adsorption.
- The trays were removed from incubation, and cell culture assay medium (2% FBS EMEM plus antibiotics) was pipetted into each well (~1.0 ml). The trays were incubated for the designated study assay period of 7 days (35 °C, 5% CO_2).
- The assay trays were observed regularly for the presence of cytotoxicity, viral cytopathic effects, and contamination. At the close of the assay, the plates were scored accordingly. The Spearman-Karber Method was used to compute viral titers and levels of cytotoxicity.

Study References

Official Methods of Analysis of the AOAC International, Chapter 6, Disinfectants, Official Method 961.02 Germicidal Spray Products as Disinfectants, Current Edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

Study Photos

Photo 1. Prepared device attachment.

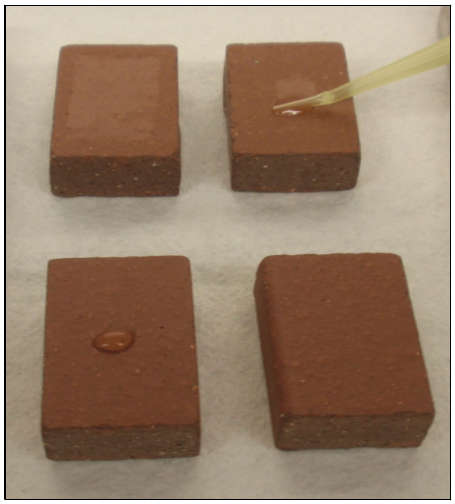

Photo 2. Inoculation of clay quarry tiles with viral inoculum.

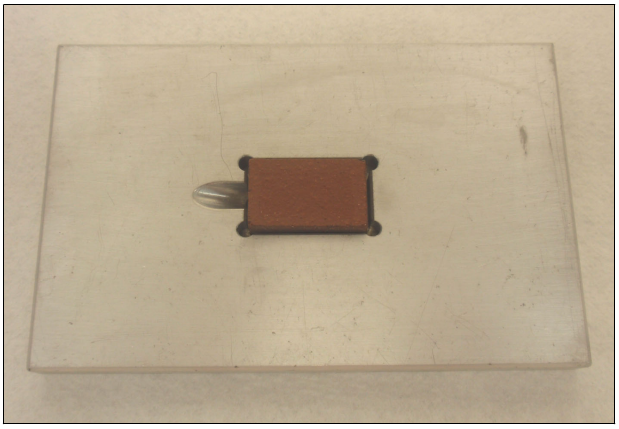

Photo 3. Mounted carrier in stainless steel holder.

Study Photos, Continued.

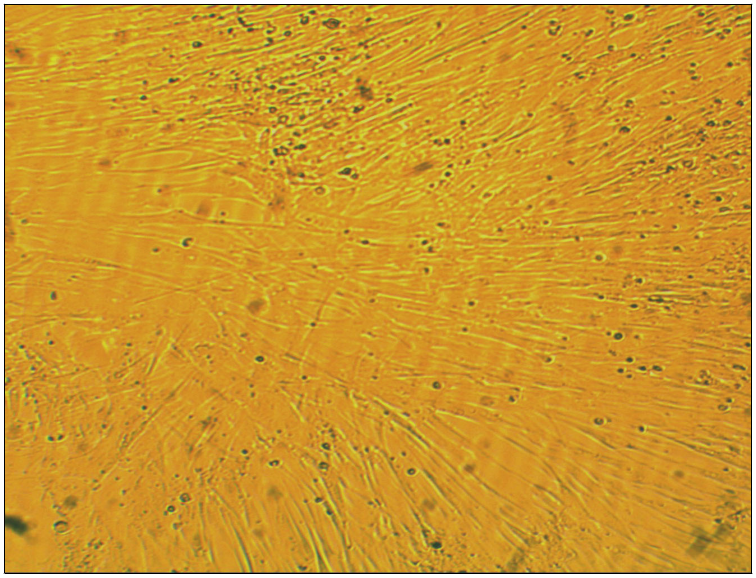

Photo 4. Healthy MRC-5 host cell monolayer.

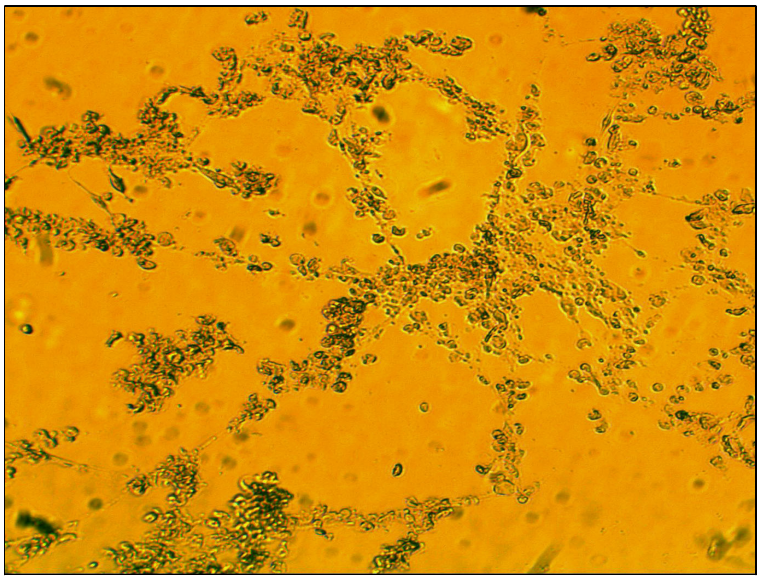

Photo 5. Advanced viral cytopathic effects due to human coronavirus 229E infection of MRC-5's.