

CONFIDENTIAL FINAL REPORT SENT VIA E: MAIL

SPONSOR: Advanced Vapor Technologies, Inc.

SPONSOR'S REPRESENTATIVE: Rick Hoverson

STUDY TITLE: Virucidal Effectiveness Test – Feline calicivirus (Surrogate for Norwalk and Norwalk-like viruses) on Unglazed, Clay Tiles – R&D

STUDY IDENTIFICATION: MICROBIOTEST Project No. 567-102 (refer to signed protocol)

<u>TEST AGENT SYSTEM</u>	<u>DATE RECEIVED</u>	<u>DS NO.</u>
Dry Steam Vapor System, 2400 Series (TANCS equipped)	06/10/05	NA
Unglazed, clay tiles	03/28/05	7564

APPLICATION: Using the Dry Steam Vapor System, 2400 Series with the nozzle brush and towel placed over the brush head the steam was applied to the carrier using back and forth motion for 7 and 10 seconds. Duplicate carriers were treated individually at each time point.

ACTIVE INGREDIENT: “Dry steam”

NEUTRALIZER USED: Fetal bovine serum

CHALLENGE VIRUS: Feline calicivirus, University of Ottawa

HOST: Crandell feline kidney (CrFK) cells, American BioResearch Laboratories

EXPOSURE TIMES: 7 seconds and 10 seconds

COMMENTS: The system was brought to the laboratory on the assay start date and operated by a representative of the sponsor.

CARRIER: Unglazed clay tiles.

RESULTS:

Results are presented in Tables 1 – 3. A titration was performed to determine the titer of the viral stock. The cell culture infective dose 50% per mL (CCID₅₀/mL) was determined from the virus stock, test, and tile recovery data using the method of Reed and Muench, 1938. The cell viability control demonstrated CrFK cell viability and media sterility. Virus was not recovered in the cell viability control. Neither the steam-treated tile nor tile alone was cytotoxic to the assay system. The lower limit of viral detection for this test system is at 10^{1.50} infectious units per mL.

MICROBIOTEST

RESULTS (continued)Table 1a
Test Results

Dilutions (log ₁₀)	Virus Tile Recovery Control	Contact Time			
		7 seconds		10 seconds	
		Tile 1	Tile 2	Tile 1	Tile 2
10 ⁻²	++++	0000	0000	0000	0000
10 ⁻³	++++	0000	0000	0000	0000
10 ⁻⁴	++++	0000	0000	0000	0000
10 ⁻⁵	0+0+	0000	0000	0000	0000
10 ⁻⁶	0++0	0000	0000	0000	0000
10 ⁻⁷	0000	0000	0000	0000	0000
CCID₅₀/mL	10^{5.50}	≤10^{1.50}	≤10^{1.50}	≤10^{1.50}	≤10^{1.50}

Table 1b
Log Reduction Summary

Contact Time	Tile Replicate	CCID ₅₀ /mL	Log ₁₀ Reduction from Virus Tile Recovery Control
7 seconds	Tile 1	≤ 10 ^{1.50}	≥4.00
	Tile 2	≤ 10 ^{1.50}	≥4.00
10 seconds	Tile 1	≤ 10 ^{1.50}	≥4.00
	Tile 2	≤ 10 ^{1.50}	≥4.00

Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls

Dilution	Dry Steam Vapor System, 2400 Series		
	Neutralizer Effectiveness	Cytotoxicity Control	Cytotoxicity-related Viral Interference Control
10 ⁻²	++++	0000	++++
10 ⁻³	++++	0000	++++
10 ⁻⁴	++++	0000	++++

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed
 0 = Feline calicivirus infected cells not detected, no cytopathic effects observed; no cytotoxicity observed.

RESULTS (continued)Table 3
Control Results

Dilution	Tile Carrier Cytotoxicity Control	Dilution	Cell Viability/Media Control
10^{-1}	0 0 0 0	10^0	0 0 0 0
10^{-2}	0 0 0 0		
10^{-3}	0 0 0 0		
CCID ₅₀ /mL	$\leq 10^{0.50}$		

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed
 0 = Feline calicivirus infected cells not detected, no cytopathic effects observed; no cytotoxicity observed.
 C = Cytotoxicity observed

CONCLUSIONS

When tested as described, Dry Steam Vapor System, 2400 Series passed the Virucidal Effectiveness Test when Feline calicivirus surrogate for Norwalk Virus, containing at least a 5% organic load, was exposed to the test agent for 7 seconds or for 10 seconds. These conclusions are based on observed data.

 Study Director

Date

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