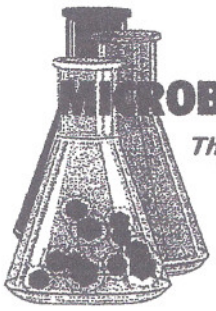

MICROBIOTEST, INC

The Microbiology and Virology Laboratory

Volume _____

FINAL REPORT

CONFIRMATORY VIRUCIDAL EFFECTIVENESS TEST
Using Feline calicivirus
(Surrogate for Norwalk virus)

TEST AGENT:
San-A-Safe

Data Requirements
EPA Guidelines 810.2100 (g)

Author
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Study Completion Date
May 15, 2006

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
557-106

TEST SUMMARY

TITLE: Confirmatory Virucidal Effectiveness Test Using Feline calicivirus (Surrogate for Norwalk virus)

STUDY DESIGN: This study was performed according to the signed protocol and project sheets issued by the Study Director.

See Project Sheets (Appendix I)
See signed protocol (Appendix II)

TEST MATERIALS:

San-A-Safe, Lot No. 33106D, received at MICROBIOTEST, 04/04/06, and assigned DS No. 8194.

SPONSOR:

TEST CONDITIONS

Challenge virus:

Feline calicivirus, University of Ottawa (CREM)

Host:

CrFK cells, American BioResearch Laboratories

Active ingredient in test product:

Citric acid

Neutralizer used:

Fluid thioglycollate medium + 20% Newborn calf serum + 1% Polysorbate 80

Dilution:

Ready to use

Spray distance

6 inches

Contact time:

1 minute

Contact temperature:

22 C

Organic load:

Viral stock contained at least 5% organic load

Media and reagents:

Newborn calf serum (NCS)
RPMI 1640 containing 10% NCS
Fluid thioglycollate medium
Polysorbate 80

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 04/26/06 to 05/02/06. The study director signed the protocol 04/25/06. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

RESULTS

Results are presented in Tables 1 – 4. A titration was performed to determine the titer of the viral stock. The log_{10} reduction (LR) of infectious virus observed as cytopathic effects of Feline calicivirus was determined using the Most Probable Number (MPN) method as described in EPA-Statistic Primer (EPA-SP). The cell viability control demonstrated CrFK cell viability and media sterility. Virus was not recovered in the cell viability control. The log_{10} reduction was calculated in the following manner:

Log_{10} reduction = Infectious virus titer recovered from plate recovery control - Infectious virus titer recovered from test

RESULTS (continued)

Table 1
Test Results

Dilution	San-A-Safe	
	Lot No. 33106D	
	Replicate 1	Replicate 2
10^{-2}	C C C C	C C C C
10^{-3}	C C C C	C C C C
10^{-4}	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
$\log_{10}$MPN/mL	3.37985	3.37985
Mean ($\log_{10}$ MPN/mL)	3.37985	

Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls
Lot No. 33106D

Dilution	Neutralizer Effectiveness Control	Cytotoxicity Control	Cytotoxicity-related Viral Interference Control
10^{-2}	C C C C	C C C C	C C C C
10^{-3}	C C C C	C C C C	C C C C
10^{-4}	+ + + +	0 0 0 0	+ + + +

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

RESULTS (continued)

Table 3
Control Results

Dilution	Feline calicivirus Plate Recovery Control	
	Replicate 1	Replicate 2
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +
$\log_{10}$MPN/mL	≥ 7.37983	≥ 7.37983
Mean ($\log_{10}$ MPN/mL)	≥ 7.37983	

Cell Viability Control
0 0 0 0

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

Table 4
Log_{10} Reduction

San-A-Safe
Lot No.33106D
3.99998

CONCLUSIONS

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titier must be demonstrated beyond the cytotoxic level. When tested as described, San-A-Safe passed the Virucidal Effectiveness Test when Feline calicivirus was exposed to the test agent for 1 minute at 22C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.