



MICROBIOTEST, INC

*The Microbiology and
Virology Laboratory*

Vol. _____

FINAL REPORT

Virucidal Efficacy Test Avian Influenza virus

**Test Agent:
San-A-Safe**

**Data Requirements
EPA Guidelines 810.2100 (g)**

**Author
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**Study Completion Date
05/30/06**

**Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164**

**Laboratory Project Identification Number
557-105**

TEST SUMMARY

TITLE: Virucidal Efficacy Test – Avian Influenza 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 SUPPLIED BY THE SPONSOR OF THE STUDY:

Final Report: Virucidal Efficacy Test- Avian Influenza virus
Project 557-105

TEST CONDITIONS

Challenge virus:

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TEST CONDITIONS

Challenge virus:

Avian Influenza virus, Type A, (H9N2), Turkey/Wis/66, SPAFAS

Host:

Embryonated chicken eggs; BE eggs

Organic load:

Viral stock contained $\geq 5\%$ organic load

Active ingredient in test product:

Citric acid

Neutralizer used:

Fluid thioglycollate medium + 20% Newborn calf serum (NCS) + 0.1%
Polysorbate 80

Contact time:

1 minute

Contact temperature:

Ambient temperature (22C)

Dilution:

Ready to use

Test agent application:

Test carriers were sprayed at a distance of 6 inches until thoroughly wet

Carrier Inoculation:

Test carriers were inoculated with 0.2mL viral stock and dried for 30 minutes at 22C.

Media and reagents:

Fluid thioglycollate medium + 20% NCS + 0.1% Polysorbate 80
RPMI 1640 containing 20% NCS
Phosphate buffered saline
Chicken red blood cells

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RESULTS (continued)

Table 1
Test Results

Dilution	San-A-Safe	
	Lot No. 33106D	Lot No. 21506D
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
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
EID/ELD ₅₀ /mL	≤10 ^{1.50}	≤10 ^{1.50}

San-A-Safe **Table 2**
Neutralizer Effectiveness and Cytotoxicity Related Controls

Dilution	San-A-Safe Lot No. 21506D		
	Neutralizer Effectiveness Control	Toxicity Control	Toxicity-related Viral Interference Control
10 ⁻²	++++	0 0* 0 0	++++
10 ⁻³	++++	0 0 0 0	++++
10 ⁻⁴	++++	0 0 0 0	++++

Table 3
Control results

Dilution	Avian Influenza Virus Plate Recovery Control
10 ⁻²	++++
10 ⁻³	++++
10 ⁻⁴	++++
10 ⁻⁵	++++
10 ⁻⁶	++++
10 ⁻⁷	++++
EID/ELD ₅₀ /mL	≥10 ^{7.50}

Host Viability Control
0 0 0 0

Key: + = Avian Influenza virus infected embryos were detected, hemagglutination observed.
0 = Avian Influenza virus infected embryos not detected, no hemagglutination observed;
no toxicity
0* = Non viable chicken embryo. No hemagglutination observed.

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RESULTS (continued)

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Test Results

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Table 2
Neutralizer Effectiveness and Cytotoxicity Related Controls

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RESULTS (continued)

Table 4
Log₁₀ Reduction

San-A-Safe	
Lot No. 33106D	Lot No. 21506D
≥6.00	≥6.00

CONCLUSIONS

When tested as described, San-A-Safe passed the Virucidal Efficacy Test when Avian Influenza virus, containing a 5% organic load, 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.