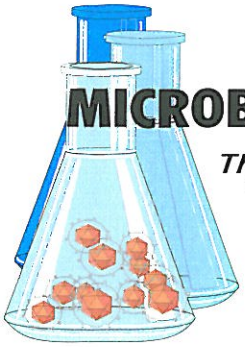


MICROBIOTEST, INC

The Microbiology and Virology Laboratory

Volume _____

AOAC USE DILUTION TEST HEALTHCARE

Test Agent: Gluquat 2

Data Requirements
EPA Guidelines 810.2100 (c), (d), (e)

Author
Morgan E. Johnson

Study Completion Date
August 29, 2007

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
601-105

Sponsor:
WEST PENETONE, INC.
10900 Secant
Anjou (Montreal), Quebec, H1J 1S5

TEST SUMMARY

TITLE: AOAC USE DILUTION TEST - HEALTHCARE

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:

1. Gluquat 2, Lot No. 706013, ≥ 60 days old, received at MICROBIOTEST on 05/10/07, and assigned DS No. 8699.

2. Gluquat 2, Lot No. 706031, ≥ 60 days old, received at MICROBIOTEST on 05/10/07, and assigned DS No. 8700.

3. Gluquat 2, Lot No. 708052, received at MICROBIOTEST on 08/03/07, and assigned DS No. 8820.

SPONSOR: WEST PENETONE, INC.
10900 Secant
Anjou (Montreal), Quebec, H1J 1S5

TEST CONDITIONS

Challenge microorganisms:

Staphylococcus aureus, ATCC 6538
Pseudomonas aeruginosa, ATCC 15442
Salmonella enterica, ATCC 10708

Active ingredient in test product:

Glutaraldehyde, Quaternary Ammonium Compound(s)

Neutralizer:

Letheen Broth containing 7% Polysorbate 80 + 1% Lecithin + 0.2% Glycine

Contact time:

10 minutes

Contact temperature:

Ambient Room Temperature (20-22C)

Carriers:

Stainless steel penicylinders

Dilution:

1:250 (4 mL product into 1,000 mL diluent)

Diluent:

300 ppm ± 2.9% AOAC hard water

Organic load:

Heat-inactivated horse serum added to the inoculum to achieve 5% final concentration

RESULTS

Results are presented in Tables 1 - 2. The challenge microorganisms were confirmed by colony morphology and Gram stain to be consistent with *S. aureus, P. aeruginosa,* and *S. enterica.* The sterility control exhibited no growth. The viability and neutralizer effectiveness controls exhibited growth. An average of 17 colony-forming units (CFU) of *S. aureus,* 14 CFU of *P. aeruginosa*, and 12 CFU of *S. enterica* were added to the neutralizer effectiveness controls. An evaluation of bacteriostasis was not applicable for *S. aureus* and *P. aeruginosa*; all bacteriostasis streaks for *S. enterica* exhibited no growth.

Table 1
Test Results
Results Expressed as Number of Tubes Exhibiting Growth / Total Number of Tubes

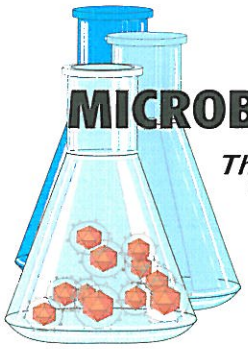
Microorganism	Lot No. 706013	Lot No. 706031	Lot No. 708052
S. aureus	1/60	1/60	0/60
P. aeruginosa	0/60	1/60	1/60
S. enterica	0/60	0/60	0/60

Table 2
Carrier Counts
Results Expressed as Average Colony Forming Units (CFU) per carrier

Microorganism	Average CFU/carrier
S. aureus	6.4×10^5
P. aeruginosa	1.4×10^6
S. enterica	9.7×10^5

CONCLUSIONS

When tested as described, Gluquat 2 passed the AOAC Use Dilution Test - Healthcare when *S. aureus, P. aeruginosa,* and *S. enterica*, each containing 5% organic load, were exposed to the test agent for 10 minutes at 20-22C. All of the controls met the criteria established for a valid test. This conclusion is based on observed data.



MICROBIOTEST, INC

The Microbiology and Virology Laboratory

Volume _____

FINAL REPORT

Assessment of Virucidal Effectiveness of Gluquat 2 Using Porcine Circovirus Type 2 (PCV-2)

Test Agent
Gluquat 2

Data Requirements
EPA Guidelines 810.2100 (g)

Author
S. Steve Zhou, Ph.D.

Study Completion Date
June 29, 2007

Performing Laboratory
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105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
601-103

Submitted to:
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TEST CONDITIONS

Challenge virus:

Porcine Circovirus Type 2 (PCV-2), American BioReserach Laboratories (initial assay)

Porcine Circovirus Type 2 (PCV-2), Iowa State University (retest assay)

Host:

PT-1 cells, American BioResearch Laboratories

Organic load:

Viral stock contained ≥5% organic load

Active ingredient in test product:

Glutaraldehyde, Quaternary Ammonium compounds

Neutralizer used:

Fetal bovine serum containing 0.3% Glycine

Contact time:

10 minutes

Contact temperature:

23±2C [24C] – (initial assay)

[25C] – (retest assay)

Dilution:

1:256 (1 mL test agent + 255 mL diluent)

Diluent:

1000 ppm ± 2.9% AOAC hard water

Carrier Inoculation:

Test carriers were inoculated with 0.4mL viral stock and dried for 45 minutes at 24C (initial assay)

Test carriers were inoculated with 0.4mL viral stock and dried for 45 minutes at 23C - 25C (retest assay)

Media and reagents:

RPMI 1640 containing 5% fetal bovine serum

Phosphate buffered saline

Fetal bovine serum containing 0.3% glycine

Newborn calf serum containing 0.3% glycine

Acetone containing 20% deionized water

1000 ppm ± 2.9% AOAC hard water

Anti-PCV conjugate

Anti-PCV dilution buffer

RESULTS (continued)

Table 11
Neutralizer Effectiveness Control (retest assay)

Dilution (log_{10})	Gluquat 2 Lot No. 706013
	Neutralizer Effectiveness Control
10^{-2}	C C C C
10^{-3}	C C C C
10^{-4}	+ + + +
10^{-5}	+ + + +

Table 12
Plate Recovery Control results (retest assay)

Dilution (log_{10})	Plate Recovery Control
10^{-2}	+ + + +
10^{-3}	+ + + +
10^{-4}	+ 0 + +
10^{-5}	0 + 0 +
10^{-6}	0 0 0 0
10^{-7}	0 0 0 0
$FFFUD_{50}$/mL	4.75

Table 13
Cell Viability Control results (retest assay)

Cell Viability Control
0 0 0 0

Key: + = Porcine circovirus type 2 infected cells were detected, fluorescence observed.
0 = Porcine circovirus type 2 infected cells were not detected, no fluorescence observed; no cytotoxicity observed
C = Cytotoxicity observed

Table 14
Log_{10} Reduction (retest assay)

	Gluquat 2	
	Lot No. 706013	Lot No. 706031
Log_{10} reduction	≥ 3.40	≥ 3.40

CONCLUSIONS

According to the regulatory agencies, the test agent passes the Virucidal Effectiveness Test if there is complete inactivation of the challenge virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level. When tested as described, Gluquat 2 passed the Assessment of Virucidal Effectiveness of Gluquat when Porcine circovirus type 2, containing at least 5% organic load, was exposed to the test agent for 10 minutes at 25C. These conclusions are based on observed data.

ATS LABS

FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Porcine Respiratory & Reproductive Syndrome virus

PRODUCT IDENTITY

Gluquat 2
Lot # 708052 and Lot # 706031

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158,
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2(f)

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

February 26, 2008

PERFORMING LABORATORY

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SPONSOR

West Penetone, Inc.
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CANADA

PROJECT NUMBER

A05876

SUMMARY OF RESULTS

Test Substance:	Gluquat 2, Lot # 708052 and Lot # 706031
Dilution:	1:250 in 300 ppm AOAC Synthetic Hard Water
Virus:	Porcine Respiratory & Reproductive Syndrome virus
Exposure Time:	Ten minutes
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	Two lots of Gluquat 2 (Lot # 708052 and Lot # 706031) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Porcine Respiratory & Reproductive Syndrome virus under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

1. Virus
The NVSL strain of Porcine Respiratory & Reproductive Syndrome virus (PRRS) used for this study was obtained from the University of Kentucky. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤ -70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot PRR-23) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Porcine Respiratory & Reproductive Syndrome virus on MARC-145 cells.

2. Test Cell Cultures
Cultures of MARC-145 cells were originally obtained from National Veterinary Services Laboratories, Ames, Iowa. The cells were propagated by ATS Labs personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2.

3. Test Medium
The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

STUDY RESULTS

Results of tests with two lots of Gluquat 2 (Lot # 708052 and Lot # 706031), diluted 1:250 in 300 ppm AOAC Synthetic Hard Water, exposed to Porcine Respiratory & Reproductive Syndrome virus in the presence of a 5% fetal bovine serum soil load at room temperature (20.0°C) for ten minutes are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the dried virus control was 5.5 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ($\leq$2.5 $\log_{10}$). Test substance cytotoxicity was observed in both lots at 2.5 $\log_{10}$. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq$2.5 $\log_{10}$ for both lots. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq$3.0 $\log_{10}$ for both lots.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, Gluquat 2 (Lot # 708052 and Lot # 706031), diluted 1:250 in 300 ppm AOAC Synthetic Hard Water, demonstrated complete inactivation of Porcine Respiratory & Reproductive Syndrome virus following a ten minute exposure time at room temperature (20.0°C) as required by the U.S. EPA for virucidal label claims.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.