

FINAL STUDY REPORT

STUDY TITLE

Evaluation of Antiviral Properties of a Product
Using a Virucidal Suspension Assay

Virus: Coxsackievirus type A16

PRODUCT IDENTITY

The Germ Killer
Lot BN100296 and Lot BNL100001

AUTHOR

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Study Director

STUDY COMPLETION DATE

March 2, 2011

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Vance Chemicals Pte. Ltd.
No. 24 Gul Lane
Singapore 629418

PROJECT NUMBER

A10971

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Evaluation of Antiviral Properties of a Product Using a Virucidal Suspension Assay

Project Number: A10971

Protocol Number: VBS01020711.COX

Sponsor: Vance Chemicals Pte. Ltd.
No. 24 Gul Lane
Singapore 629418

Testing Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance: The Germ Killer

Lot/Batch(s): BN100296 and BNL100001

Test Substance Characterization

Test substance characterization as to content, stability, solubility, storage, etc., (21 CFR, Part 58, Subpart F [58.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: July 14, 2010 (Lot BN100296) July 15, 2010 (Lot BNL100001)

Study Initiation Date: February 15, 2011

Experimental Start Date: February 16, 2011

Experimental End Date: February 23, 2011

Study Completion Date: March 2, 2011

OBJECTIVE

The objective of this study was to evaluate the antiviral properties of a product against Coxsackievirus type A16 when exposed (in suspension) for a specified exposure period(s). The protocol is a modification of the Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension (ASTM E 1052).

SUMMARY OF RESULTS

Test Substance:	The Germ Killer, Lot BN100296 and Lot BNL100001
Dilution Tested:	Ready to use (RTU)
Virus:	Coxsackievirus type A16, Strain G10, ATCC VR-174
Exposure Time:	5 minutes
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	1% fetal bovine serum
Efficacy Result:	Under these test conditions, The Germ Killer (BN100296) demonstrated a 90% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The Germ Killer (BNL100001) demonstrated a 98.2% reduction in the stock virus titer as compared to the titer of the corresponding virus control. The log reductions in viral titer were 1.0 log ₁₀ and 1.75 log ₁₀ , respectively.

TEST SYSTEM

- Virus
The G10 strain of Coxsackievirus type A16 used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-174). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% 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 CX16-36) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Coxsackievirus on LLC-MK₂ (Rhesus monkey kidney) cells.
- Indicator Cell Cultures
LLC-MK₂ (Rhesus monkey kidney) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-7.1). 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% CO₂. On the day of testing, cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study. On the day of testing, cells were observed as having proper cell integrity and confluency and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

STUDY RESULTS

Cytotoxicity and Neutralization Controls

Test substance cytotoxicity was observed at 3.5 log₁₀. The neutralization control demonstrated that the test substance was neutralized at ≤3.5 log₁₀.

5 Minute Exposure Time

The titer of the virus control was 5.5 log₁₀. Following exposure, test virus infectivity was detected in the virus-test substance mixture at 4.5 log₁₀ for Lot BN100296 and 3.75 log₁₀ for Lot BNL100001. Lot BN100296 demonstrated a log reduction of 1.0 log₁₀. Lot BNL100001 demonstrated a log reduction of 1.75 log₁₀.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, The Germ Killer (Lot BN100296), ready to use, demonstrated a 90.0% reduction in viral titer following a 5 minute exposure time to Cocksackievirus type A16 as compared to the titer of the corresponding virus control. Lot BN100296 demonstrated a log reduction of 1.0 log₁₀.

Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, The Germ Killer (Lot BNL100001), ready to use, demonstrated a 98.2% reduction in viral titer following a 5 minute exposure time to Cocksackievirus type A16 as compared to the titer of the corresponding virus control. Lot BNL100001 demonstrated a log reduction of 1.75 log₁₀.

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

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