

FINAL STUDY REPORT

PROTOCOL TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Human Immunodeficiency Virus Type 1

DATA REQUIREMENT

U.S. EPA 40 CFR Part 158,
"Data Requirements for Registration"
Subdivision G: Product Performance, 91-2(f)

PRODUCT IDENTITY

Axen (EPA # 72977-2), the 30 ppm use dilution of Axenohl
(EPA # 72977-1), a 2400 ppm concentrate, Lot# 2001-042-001 and Lot# 2001-005-001

PROTOCOL NUMBER

IMS99111501.HIV

PROJECT NUMBER

12305

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

December 20, 2001

PERFORMING LABORATORY

AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

SPONSOR

Innovative Medical Services
1725 Gillespie Way
El Cajon, CA 92020

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: INNOVATIVE MEDICAL SERVICES

Company Agent: Dale A. [Signature]

Date: 12-24-2001

CERTIFICATION OF GOOD LABORATORY PRACTICE

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR part 160.

The studies not performed by or under the direction of AppTec Laboratory Services are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

Study Director:	<u>Mary J. Miller</u> Mary J. Miller, M.T.	<u>12-20-01</u> Date
Submitter:	<u>Dulan BSA</u>	<u>12-24-01</u> Date
Sponsor Representative:	<u>Dulan BSA</u>	<u>12-24-01</u> Date

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director Review	Management Review
Critical Phase	November 29, 2001	December 2, 2001	December 19, 2001
Final Report	December 18, 2001	December 19, 2001	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: *Scott W. Alvarado*

Date: 12-19-01

Professional personnel involved:

- William D. Smith, B.S. - President
- Karen M. Ramm, B.A. - Division Director
- Mary J. Miller, M.T. - Research Scientist II
- Katherine A. Paulson, M.L.T. - Research Assistant II

REPORT

VIRUCIDAL EFFICACY OF A DISINFECTANT FOR USE ON INANIMATE ENVIRONMENTAL SURFACES

TEST OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against Human Immunodeficiency Virus type 1 according to test criteria and methods approved by the U.S. Environmental Protection Agency for registration of a product as a virucide.

TESTING FACILITY: AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

SPONSOR: Innovative Medical Services
1725 Gillespie Way
El Cajon, CA 92020

SAMPLE NAME OR CODE: Axen (EPA # 72977-2), the 30 ppm use dilution of Axenohl (EPA # 72977-1), a 2400 ppm concentrate, Lot# 2001-042-001 and Lot# 2001-005-001

DATE SAMPLES RECEIVED BY APPTEC: November 20, 2001

APPTEC PROTOCOL NUMBER: IMS99111501.HIV
APPTEC PROJECT NUMBER: 12305

STUDY INITIATION DATE: November 26, 2001
EXPERIMENTAL START DATE: November 29, 2001
EXPERIMENTAL COMPLETION DATE: December 7, 2001
STUDY COMPLETION DATE: December 20, 2001

TEST SUBSTANCE CHARACTERIZATION

The identity, strength, purity, stability, solubility, and chemical composition of the test material are the responsibility of the Sponsor.

DATA AND TEST SUBSTANCE RETENTION

A certified copy of this report as well as all raw data pertinent to this study will be stored at AppTec Laboratory Services, 2540 Executive Drive, St. Paul, MN 55120. As stated in the study protocol, test substance retention is the responsibility of the Sponsor. Unused test substances will be discarded following study completion.

SUMMARY OF RESULTS:

Test Substance: Axen (EPA # 72977-2), the 30 ppm use dilution of Axenohl (EPA # 72977-1), a 2400 ppm concentrate, Lot# 2001-042-001 and Lot# 2001-005-001

Dilution: No dilution required, used as received from Sponsor

Virus: Human Immunodeficiency Virus type 1, Strain HTLV-III_B

Exposure Time: 30 seconds

Exposure Temperature: Room temperature

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Two lots of Axen (EPA #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Human Immunodeficiency Virus type 1 under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

- Virus
The HTLV-III_B strain of Human Immunodeficiency Virus type 1 (HIV-1) used for this study was obtained from Advanced Biotechnologies, Inc., Columbia, Maryland. Stock virus was prepared by collecting the supernatant culture fluid from infected culture cells as determined by an indirect immunofluorescence assay specific for the HIV-1 antigen. The cells were disrupted and cell debris removed by centrifugation at approximately 1200 RPM for 10 minutes. The supernatant was removed, aliquoted and stored at $\leq -60^{\circ}\text{C}$ until the day of use. On the day of use an aliquot of stock virus (ViroMed Laboratories, Inc. Lot HT-111B-2C) was removed, thawed and refrigerated until use 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 HIV on MT-2 cells.
- Test Cell Cultures
MT-2 cells (human CD4+ lymphocytes) were originally obtained from the National Cancer Institute, Frederick, MD. Cultures were grown and propagated in-house and used in suspension in disposable tissue culture labware. On the day of testing, cells were observed as having proper cell integrity and therefore, were acceptable for use in this study.
- Test Medium
The test medium used in this study was RPMI 1640 supplemented with 15% (v/v) heat-inactivated fetal bovine serum (FBS). The medium was also supplemented with 2 mM L-glutamine and 50 $\mu\text{g}/\text{mL}$ gentamicin.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures used. See text for a more detailed explanation.

NUMBER OF DILUTIONS AND CULTURES FOR VIRUCIDAL EFFICACY STUDY			
Test or Control Group	Dilutions Assayed (log₁₀)	Cultures per dilution	Total Cultures
Cell Control	N/A	4	4/group
Dried Virus Control (Group A)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample lot #1 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample lot #2 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #1 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #2 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #1 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #2 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28

METHODS

- Preparation of Test Substance
 Axen (EPA #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate (Lot #2001-042-001 and Lot #2001-005-001) was used, undiluted, as received from the Sponsor. The test substance was in solution as determined by visual observation.
- Preparation of Virus Films
 Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 X 15mm sterile glass petri dishes. The virus films were air-dried at room temperature (17°C) until visibly dry (20 minutes) and then incubated at 36-38°C for an additional 30 minutes to increase the level of dryness.
- Sephadex Gel Filtration
 To reduce the cytotoxic level of the virus-disinfectant mixture prior to assay of virus and/or to reduce the virucidal level of the disinfectant, virus was separated from disinfectant by filtration through Sephadex gel. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin, centrifuged for three minutes to clear the void volume, loaded with 2.0 mL of virus-disinfectant mixture and immediately passed through the column utilizing the syringe plunger.

4. Treatment of Virus Films with Test Substance (GROUP B, TABLE 1)
For each lot of disinfectant separate dried virus films were exposed to 2.0 mL of the use dilution for 30 seconds at room temperature (17°C). Following the exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-disinfectant mixture was immediately passed through a Sephadex column utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by serial dilution and assayed for infectivity.
5. Treatment of Virus Control Films (GROUP A, TABLE 1)
A virus film was prepared as previously described (paragraph 2). The control film was exposed to 2.0 mL of test medium for the same amount of time as the test film was exposed to the disinfectant. The virus was then scraped and passed through a Sephadex column in the same manner as the test virus and the filtrate (10^{-1} dilution) was then titered by serial dilution and assayed for infectivity (paragraph 4).
6. Cytotoxicity Assay (GROUP C, TABLE 2)
A 2.0 mL aliquot of each lot of the disinfectant was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into MT-2 cell cultures. Cytotoxicity of the MT-2 cell cultures was scored at the same time as the virus-disinfectant and virus control cultures.
7. Assay of Non-Virucidal Level of Test Substance (GROUP D, TABLE 3)
Each dilution of the Sephadex-filtered disinfectant (disinfectant control for cytotoxicity assay) was mixed with an aliquot of low titer stock virus, and the resulting mixtures of dilutions were assayed for infectivity in order to determine the dilution(s) of disinfectant at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining the reduction in infectivity by the test substance.
8. Infectivity Assays
The MT-2 cell line, which exhibits CPE in the presence of HIV-1, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ in sterile disposable cell culture labware. The cultures were scored periodically for eight days for the absence or presence of CPE, cytotoxicity, and for viability.
9. Statistical Methods: N/A

PROTOCOL CHANGES

Protocol Amendments: No protocol amendments were required for this study.

Protocol Deviations: No protocol deviations occurred during this study.

CALCULATION OF TITERS

Viral and cytotoxicity titers are expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity (TCID_{50}) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

$$-1- \left[\frac{(\text{Sum of \% mortality at each dilution}) - 0.5 \times (\text{logarithm of dilution})}{100} \right]$$

ANALYSIS AND CONCLUSIONS

Results of tests with two lots of Axen (EPA #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate (Lot# 2001-042-001 and Lot# 2001-005-001) exposed to HIV-1 for 30 seconds are shown in Tables 1-3. The titer of the virus control was $5.25 \log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ($\leq 1.5 \log_{10}$). Test substance cytotoxicity was observed for both lots at $1.5 \log_{10}$. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq 1.5 \log_{10}$ for both lots. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq 3.75 \log_{10}$ for both lots. Under these test conditions, two lots of Axen (EPA #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate **demonstrated complete inactivation** of the HIV-1 as required by the U.S. EPA for virucidal claims.

TABLE 1: Effects of Axen (EPA #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate (Lot# 2001-042-001 and Lot# 2001-005-001) Following a 30 Second Exposure to HIV-1 Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)	HIV-1 + Lot# 2001-042-001 (GROUP B)	HIV-1 + Lot# 2001-005-001 (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻¹	+ + + +	T T T T	T T T T
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 0 0
10 ⁻⁶	0 + 0 0	0 0 0 0	0 0 0 0
10 ⁻⁷	0 0 0 0	0 0 0 0	0 0 0 0
TCID ₅₀ /0.2 mL	10 ^{5.25}	≤10 ^{1.5}	≤10 ^{1.5}

TABLE 2: Cytotoxicity of Axen (EPA Reg #72977-2), the 30 ppm use dilution of Axenohl (EPA #72977-1), a 2400 ppm concentrate on MT-2 Cell Cultures

Dilution	Cytotoxicity Control Lot# 2001-042-001 (GROUP C)	Cytotoxicity Control Lot# 2001-005-001 (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10 ⁻¹	T T T T	T T T T
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
TCD ₅₀ /0.2 mL	10 ^{1.5}	10 ^{1.5}

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

(T) = Cytotoxicity present

TABLE 3: Non-Virucidal Level of Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control Lot# 2001-042-001 (GROUP D)	Test Virus + Cytotoxicity Control Lot# 2001-005-001 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10 ⁻¹	T T T T	T T T T
10 ⁻²	+ + + +	+ + + +
10 ⁻³	+ + + +	+ + + +
10 ⁻⁴	+ + + +	+ + + +
10 ⁻⁵	+ + + +	+ + + +
10 ⁻⁶	+ + + +	+ + + +
10 ⁻⁷	+ + + +	+ + + +

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)
 (0) = No test virus recovered and/or no cytotoxicity present
 (T) = Cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at TCID₅₀ of $\leq 1.5 \log_{10}$.

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

REFERENCES

1. Annual Book of ASTM Standards 2000, Section 11 Water and Environmental Technology Volume 11.05 Biological Effects and Environmental Fate: Biotechnology; Pesticides, E1053-97.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G: Product Performance, 91-2(f) November 1982.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Schmidt, N.J. and Emmons, R.W. editors. Sixth edition, 1989. p. 18-20.
5. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
6. Techniques in HIV Research, A. Aldovini and B. Walker, 1990.

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