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MicroBioTest, Inc.

Study Title

Virucidal Effectiveness Test

Final Report

Data Requirements

40 CFR 158.160, Guideline 91-2

Author

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Study Completed On

August 24, 1991

Performing Laboratory

MicroBioTest, Inc. (MBT)
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Laboratory Project ID

Project No: 178-102

Submitted to: A. H. ROBBINS COMPANY
1407 Cummings Drive
Richmond, Virginia 23261-6609

September 6, 1991

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

AH ROBBINS COMPANY

Company Agent _____

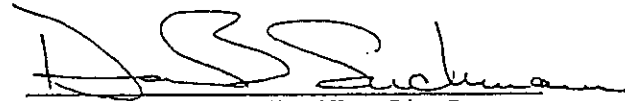
Date

QUALITY ASSURANCE STATEMENT

TITLE OF STUDY: Virucidal Efficacy Test

In compliance with the Good Laboratory Practice Regulations, this study was inspected by MBT's Director of Quality Assurance. The data presented in the final report were compared with the data collected during the conduct of the study. Dates of inspection, phase inspected and reporting dates to the study director and to the management are as follows:

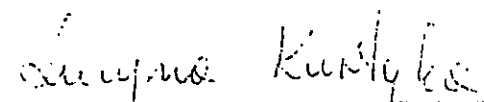
<u>PHASE INSPECTED</u>	<u>DATE(S) OF INSPECTION</u>	<u>DATE REPORTED</u>
FINAL REPORT	8/30/91	8/30/91

for 
 JOHN M. VENDITTI, Ph.D.
 Director, Quality Assurance

COMPLIANCE STATEMENT

To the best of my knowledge, this study was conducted in compliance with The Good Laboratory Practice regulation (FFDCA)/21 CFR 58, June 1979.

I hereby declare that the work was performed under my direction according to the procedures herein described.

for 
 GREGORY R. DENNIS, M.S.
 Study Director

11/11/91
 Date

SPONSOR: A. H. ROBBINS COMPANY

SUBJECT: Virucidal Efficacy
Interim Report

OBJECTIVE:

This study is designed to determine the disinfectant efficacy of an EPA registered agent when faced with a viral challenge. The test is designed to simulate consumer use and conforms to EPA Guidelines DIS/TSS-7, November 1981. The following pass/fail guidelines apply: There must be a minimum of a three-log reduction between the cytotoxicity level and the plate recovery control with no surviving virus particles in order to pass.

MATERIALS:

Information on the methods of synthesis, stability, purity, as well as data on composition and other characteristics which define the test material reside with the sponsor.

The test material was tested as supplied and all operations performed on the test agent are addressed in this final report. The testing laboratory assumed the test compound remained stable under the conditions of the test.

- A. Supplied by the Sponsor (received at the testing laboratory 7/13/90):
1. Virkon S, Lot number: 3133, DS #: 673.
 2. Virkon S, Lot number: 3134, DS #: 674.
- B. General materials and supplies provided by MicroBioTest, Inc. including, but not limited to:

Challenge viruses:

1. Calf rotavirus, ATCC VR-452.
2. Transmissible gastroenteritis virus, ATCC VR-763.
3. Parvovirus, ATCC VR-742.
4. Parainfluenza virus, ATCC VR-281.
5. Duck adenovirus, ATCC VR-921, (egg drop syndrome),
6. Infectious bovine rhinotracheitis virus, ATCC VR-188.
7. Bovine viral diarrhea virus, ATCC VR-534.
8. Pseudorabies virus, ATCC VR-135.
9. Turkey herpes virus, ATCC VR-584-C (TRT).
10. Equine arteritis virus, ATCC VR-796.

2. Host Cell Lines:

- a. Chick embryo fibroblasts, ATCC CRL-1590.
- b. African green monkey kidney cells, ATCC CCL-81.
- c. Embryonic swine kidney cells, ATCC CCL-184.
- d. HeLa cells, ATCC CCL-2.
- e. Duck embryonic cells, ATCC CCL-141.
- f. HeLa cells, ATCC CCL-2.

3. Reagents and media:

- a. Minimum Essential Medium (MEM).
- b. M199 Medium (M199).
- c. Newborn calf serum (NBCS).
- d. Fetal Bovine Serum (FBS).
- e. Sterile Phosphate Buffered Saline (PBS).
- f. Neutralizer.
- g. Laboratory equipment and instruments.

EXPERIMENTAL DESIGN:

A. Virus titration:

The virus stock cultures were titrated and adjusted to contain approximately 10^6 50% cell culture infectious doses per milliliter (CCID₅₀/ml), frozen and stored in liquid nitrogen.

B. Test Material Preparation:

The test material was prepared immediately prior to testing in DI water at a 1% concentration.

C. Test:

A quantity of the frozen virus was thawed and mixed with organic soil (heat inactivated NBCS) at 5% concentration in cell culture medium.

A 0.2 ml aliquot of this inoculum was spread over the surface of a sterile Petri dish (60-mm) with a sterile bent glass spreader and allowed to air dry for 20 minutes at ambient temperature. After the inoculum dried, two (2) ml of the disinfectant was added to each plate. The plates remained at 20C for ten minutes.

After the contact period, disinfectant mixture was scraped from the surface of the dish with a rubber policeman and mixed 1:1 with neutralizer.

C. Test (continued):

The eluate was collected and diluted ten-fold in the appropriate medium. Dilutions were added to monolayers of cells (four wells per dilution), and incubated at $37\pm 1^{\circ}\text{C}$, $5\pm 1\%$ CO_2 for viral adsorption.

Following adsorption, the fluids were aspirated, the monolayers washed with PBS and refed with the appropriate medium. The cultures were incubated as above and observed for virus-specific cytopathogenic effect (CPE) at suitable times. The observations were recorded.

D. Controls:

The viral inoculum was titered at the time of the test to confirm the infectivity and titer of the virus. The stock was diluted ten-fold in the appropriate medium and a 1.0 ml quantity of the appropriate dilutions inoculated onto four host cell monolayers and adsorbed as described above (stock titer).

One sterile Petri dish was inoculated with 0.2 ml of the viral inoculum. The inoculum was spread over the surface of the dish with a sterile bent glass rod and dried at ambient temperature after the drying period, 2 ml of PBS was added, the mixture scraped from the surface of the dish with a rubber policeman, collected, neutralized and titered as described previously. This control confirms the infectivity of each virus after drying on the dish and that the neutralization procedure did not effect the infectivity of the virus (plate recovery).

E. Calculations:

The average $\text{CCID}_{50}/\text{ml}$ for each test and control set was determined from the dilutions plated.

ASSAY ACCEPTANCE CRITERIA:

The assay will be acceptable for evaluation of the test results if all of the criteria listed below are satisfied. This listing does not encompass all test situations; therefore, the study director may exercise scientific judgment in modifying the criteria or considering other causes which may affect assay reliability and acceptance.

- The $\text{CCID}_{50}/\text{ml}$ of the PBS recovery must be in the range to allow a demonstration of a three log reduction above cytotoxicity titer.

ASSAY ACCEPTANCE CRITERIA (continued):

- Viral-induced CPE must be distinguishable from test compound-induced cytotoxic effects. This may be accomplished several ways and is at the discretion of the study director.
- The neutralization process may not significantly reduce the CCID₅₀/ml of the virus.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test material records and correspondence between MBT and the sponsor will be stored in the archives at MicroBioTest, Inc., 14280 Sullyfield Circle, Chantilly, Virginia 22021 where this study was conducted. All changes or revisions of the approved protocol were documented, signed by the study director, dated and maintained with the protocol.

COMMENTS:

All operations, except incubation, were performed in a vertical laminar flow hood.

RESULTS:

The results are given in the accompanying tables.

This study was initiated 9/7/90 and completed 8/24/91.

CONCLUSIONS:

When tested as described, Powder Detergent/Disinfectant, lot numbers 1468-56 and 1468-57, support label claims for virucidal efficacy against Calf rotavirus, ATCC VR-452, Transmissible gastroenteritis virus, ATCC VR-763, Parvovirus, ATCC VR-742, Parainfluenza virus, ATCC VR-281, Duck adenovirus, ATCC VR-921, (egg drop syndrome), Infectious bovine rhinotracheitis virus, ATCC VR-188, Bovine viral diarrhea virus, ATCC VR-534, Equine arteritis virus, ATCC VR-796, Pseudorabies virus, ATCC VR-135 and Turkey herpes virus, ATCC VR-584-C.

TABLE I
VIRAL TITERS at Day 5

Calf rotavirus

DILU- TION	STOCK TITER	PLATE RECOVERY.	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	+---	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	1.0x10 ⁵	4.7x10 ³	<10	<10

TABLE II

VIRAL TITERS at Day 5

Transmissible gastroenteritis virus

DILU- TION	STOCK TITER	PLATE RECOVERY	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	+---	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	2.1x10 ⁵	4.7x10 ³	<10	<10

- * = 50% Cell Culture Infective Dose per Milliliter
Reed and Muench, American Journal of Hygiene, 1938.
- = NO CPE Observed.
+ = CPE Observed.
<10 = no viruses recovered.

TABLE III
VIRAL TITERS at Day 5

Parvovirus

DILU- TION	STOCK TITER	PLATE RECOVERY	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	----	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	1.0x10 ⁵	3.2x10 ³	<10	<10

TABLE IV
VIRAL TITERS at Day 5

Parainfluenza virus

DILU- TION	STOCK TITER	PLATE RECOVERY	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	++++	----	----
10 ⁻⁵	++++	----+	----	----
10 ⁻⁶	-+--	----	----	----
CCID ₅₀ /ml*	4.7x10 ⁵	4.7x10 ⁴	<10	<10

* = 50% Cell Culture Infective Dose per Milliliter
Reed and Muench, American Journal of Hygiene, 1938.
- = NO CPE Observed.
+ = CPE Observed.
<10 = no viruses recovered.

TABLE V
VIRAL TITERS at Day 5

Duck adenovirus
(egg drop syndrome)

<u>DILU- TION</u>	<u>STOCK TITER</u>	<u>PLATE RECOVERY</u>	<u>TEST MATERIAL LOTS</u>	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	----	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	4.7x10 ⁴	3.2x10 ³	<10	<10

TABLE VI

VIRAL TITERS at Day 5

Infectious bovine rhinotracheitis virus

<u>DILU- TION</u>	<u>STOCK TITER</u>	<u>PLATE RECOVERY</u>	<u>TEST MATERIAL LOTS</u>	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	----+	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	1.0x10 ⁵	4.7x10 ³	<10	<10

* = 50% Cell Culture Infective Dose per Milliliter
Reed and Muench, American Journal of Hygiene, 1938.
- = NO CPE Observed.
+ = CPE Observed.
<10 = no viruses recovered.

TABLE VII

VIRAL TITERS at Day 5

Bovine viral diarrhea virus

DILU- TION	STOCK TITER	PLATE RECOVERY	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	+--+	----	----
10 ⁻⁵	++++	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	3.2x10 ⁵	1.0x10 ⁴	<10	<10

TABLE VIII

VIRAL TITERS at Day 5

Pseudorabies virus

DILU- TION	STOCK TITER	PLATE RECOVERY	TEST MATERIAL LOTS	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	+--+	----	----
10 ⁻⁵	+--+	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml*	1.0x10 ⁵	1.0x10 ⁴	<10	<10

* = 50% Cell Culture Infective Dose per Milliliter
 Reed and Muench, American Journal of Hygiene, 1938.
 - = NO CPE Observed.
 + = CPE Observed.
 <10 = no viruses recovered.

TABLE IX

VIRAL TITERS at Day 7
Turkey herpes virus

<u>DILU- TION</u>	<u>STOCK TITER</u>	<u>PLATE RECOVERY</u>	<u>TEST MATERIAL LOTS</u>	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	+++-	----	----
10 ⁻⁴	++++	----	----	----
10 ⁻⁵	+---	----	----	----
10 ⁻⁶	----	----	----	----
CCID ₅₀ /ml	3.2x10 ⁵	1.0x10 ⁴	<10	<10

TABLE X

VIRAL TITERS at Day 5

Equine arteritis virus

<u>DILU- TION</u>	<u>STOCK TITER</u>	<u>PLATE RECOVERY</u>	<u>TEST MATERIAL LOTS</u>	
			<u>3313</u>	<u>3314</u>
10 ⁻¹	++++	++++	----	----
10 ⁻²	++++	++++	----	----
10 ⁻³	++++	++++	----	----
10 ⁻⁴	++++	+--	----	----
10 ⁻⁵	++++	----	----	----
10 ⁻⁶	--+-	----	----	----
10 ⁻⁷	----	----	----	----
10 ⁻⁸	----	----	----	----
CCID ₅₀ /ml	1.0x10 ⁶	1.0x10 ⁴	<10	<10