

Antimicrobial Program Branch
EFFICACY REVIEW-EETMS Section

IN 07-29-91 OUT 08-07-91

EPA Reg. No. or File Symbol # 10492-4

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Type Product (s) Hospital Disinfectant(Saturated Towellete)

Data MRID NO. 414914-02, 414914-01, 415215-02, 415215-01,

Product Manager 31

Prod.Name Isotex 70 disinfecting Towelettes

Company Name Palmero Sales Company, Inc.,

Submission Purpose An amendment to add claims against S.aureus,
S. choleraesuis, Ps. aeruginosa, T. mentagrophyes, M. tuberculosis,
HIV-1(AIDS Virus), Poliovirus-Type 1, and Herpes simplex Type II.

Chemical & Formulation Single-use disposable towelette saturated
with ready-to-use liquid enclosed in a dispenser

<u>Active Ingredients</u>	<u>%</u>
n-Alkyl (C14, 60%; C16, 30%; C12, 5%; C18, 5%) dimethyl benzyl ammonium chlorides	0.12
n-alkyl (C12, 50%; C14, 30%; C16, 17%, C18, 3%) dimethyl ethylbenzyl ammonium chlorides	0.12
Isopropyl alcohol	63.00

09/04/91
Reviewed by: Bruce H. Clear
J. Santiago 9/6/91

200.0 Introduction

200.1 Use(s)

Refer to the subject product proposed label "Isotex 70 Disinfecting Towellete".

200.2 Background Information

The current submission received 07-11-91 is an amendment which in include efficacy data generated against S. aureus, S. choleraesuis, Ps. aeruginosa, T.mentagrophytes, M. tuberculosis, HIV-1(AIDS virus), Poliovirus Type I and Herpes simplex Type II in support of the product proposed label claims. A proposed label was also included with the proposed label claims.

201.0 Data Summary(MRID Nos.) 414914-02, 414914-01, 415215-02, and 415215-01.

201.2 Brief Description of Tests

- A. Bactericidal Studies, Shaladra Biotest Inc, W. Bethesda, MD, by Robin A. Smith & Kyle Sibinovich.
The Data for Required Test Organisms developed against Ps. aeruginosa, S. aureus and S. choleraesuis by the Official Methods of the AOAC, 14th ed., 1984. (MRID #414914-01)
- B. Fungicidal Tests, Shaladra Biotest, Inc, W. Bethesda, MD, by Robin A. Smith, data developed by the AOAC Fungicidal Test, 14th Ed., 1984, against Tri. mentagrophytes (MRID # 414914-01).
- C. Tuberculocidal Test(Quantitative Logarithmic Reduction TB Test). Shaladra Biotest, Inc. W. Bethesda, MD 20817.
Official Methods of the AOAC, chapter 4, 15th ed., 1990 (Basic Data Reports)(MRID #414914-01). Tester: Robin Smith.
- D. Tuberculocidal Test(Quantitative Log., Reduction Method). Reports performed by Nelson Laboratories, Inc, 4535 South 2300, Salt Lake City, UT 84117. (MRID #415215-01).
(Validation Data). Reports by Dennis Ransom & Jerry R. Nelson.
- E. Virucidal Test (AIDS Virus) USEPA Pesticide Assessment Guidelines (Sub-Div. G, Product Performance, 1982, Section 91-30, pp 72-76). Testing performed by Southern Research Institute, Birmingham, AL 35255, SRI Project #7042. (MRID # 415215-02). Reports by: Bonnie J. Bowdon.
- F. Virucidal Tests(EPA Test Method, H. simplex II & Poliovirus I) Reports by Integrity Bioservices, Rockville, MD 20852, and followed the guidelines specified in DIS/TSS-7, Nov., 12, 1981. (MRID # 414914-02) Reports by: Philip R. Roane.

201.2 Test Summaries:

A. Disinfection(Microbiological Reports):

1. Method: AOAC Use-Dilution Test, 14th ed., 1984
2. Modifications: Tested with a 5% horse serum(DIS/TSS-2, item 4)

3. Samples:

Efficacy studies were reported to be conducted on the same product whether identified as Isotex 70, as Ideal Disinfecting Towelette, and/or as Decide. All samples were reported to be manufactured on the same day and reported to have the same lot number.

	<u>Mfd date</u>	<u>Test date</u>	<u>Completion Date</u>
Decide (A199-1)	01-19-90	02-12-90	02-14-90
Decide (B280-1)	02-28-90	03-07-90	03-09-90
Decide (D139-1)	04-13-89	02-14-90	02-16-90

4. Dilution Tested: Tested undiluted (as provided by the mfd.).

5. Test Bacteria:

<u>Pseudomonas aeruginosa</u>	<u>Staphylococcus aureus</u>
(ATCC 15442)(Phen. Res=1:90)	(ATCC 6538)(Phen. Res=1:60)

<u>Salmonella choleraesuis</u>
(ATCC 10708) (Phen. Res=1:90)

6. Exposure Time: Four(4) minutes; Exposure Temp. = 20°C.
Neutralizer Time: 10 minutes at 20°C.
Types of Carriers: Stainless Steel Penicylinders(SS)
7. Subculture Medium: Synthetic broth(as per chapter 4, Section 4.001)
The synthetic broth is not an acceptable subculture medium.
8. Incubation: 48 hours at 37°C.
9. Results:

<u>Test Organism</u>	<u>Test Sample</u>	<u>Exposed</u>	<u>No. of Pos./Tested</u>		<u>Avg CFU Recovered per cylinder</u>
			<u>Pri</u>	<u>Sec</u>	
<u>Ps. aeruginosa</u>	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)
<u>S. choleraesuis</u>	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)
<u>S. aureus</u>	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)

Neutralization data:

No data were submitted to support the residual effects of the active ingredient as per item 7 of DIS/TSS-2 enclosure.

Control survival studies:

No data were submitted to support the recovery of surviving organisms after drying as specified in DIS/TSS-2, item #4 & #6.

10. Conclusions: Unsatisfactory performance for all three test organisms in the presence of 5% horse serum; no carrier drying conditions or times submitted (this information is critical to determine efficacy level); also the incorrect subculture medium was utilized. The applicant needs to provide secondary cultures as specified in item 7 of DIS/TSS-2 enclosure and control survival studies as indicated in items 4 & 6 of DIS/TSS-2. Also, the generated data must utilize the applicable subculture media as listed in the Official Methods of Analysis of the AOAC(14th or 15th editions).

B. Disinfection: (Pathogenic fungi)

Shaladra Biotest, Inc. By Robin A. Smith.

1. Method: AOAC Fungicidal Test, 14th ed., 1984.
2. Modifications: Tested in 5% horse serum as per DIS/TSS-2, item 4.
3. Dilution tested: Undiluted(not diluted).
4. Samples: Decide (A199-1) & Decide (D139-1).
5. Test Organism: Trichophyton mentagrophytes, ATCC 9533,
(Phenol Resistance =1:70 at 10 min. at 20°C...
... Phenol Sensitivity=1:60 at 10 min. at 20°C.)
6. Subculture Medium: Glucose Broth 2% (as per chapter 4, Section 4.019).
Type of Carriers: Stainless Steel Penicylinders(SS).
7. Exposure: 4 minutes at 20°C. *See next page*
8. Neutralizer: Lethen Broth with Tween 80
9. Incubation: 10 days at 25°C to 30°C.
10. Results

Organism	Test Sample	No. Exposed	No growth (0)/Growth(+) at 10 min. with 5% horse serum
<u>Tri. mentagrophytes</u>	A199-1		
	(Subculture 1)	10	0/10
	(Subculture 2)	10	0/10
<u>Tri. mentagrophytes</u>	D139-1		
	(Subculture 1)	10	0/10
	(Subculture 2)	10	0/10

Viability control in neutralizer		Exposed	No showed growth
		2	2/2
Phenol:	5 min*	10 min.*	15 min*
1:60	5/5	0/5	0/5
1:70	5/5	4/5	3/5

*Number of carriers showing growth/Total Number of Carriers.
Conidia count: 6.0×10^6 /ml.

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11. Conclusions: Raw data need to be submitted for review to determine satisfactory performance for the undiluted test product as a fungicide against Trichophyton mentagrophytes for a 4 minute exposure time.

C. Disinfection(Tuberculocidal Tests):

(Basic Tuberculocidal Data).

(MRID 414914-01) Shaladra Biotest, Inc., By Kyle H. Sibinovic.

1. Method: AOAC Use-Dilution Test Method, 15th ed., 1990.

(EPA Logarithmic Reduction Method)

The Quantitative Logarithmic Reduction Test Method was used to generate the tuberculocidal data and to determine product effectiveness as a tuberculocide.

2. Modifications: None reported.

3. Test Samples:
- | | mfd | test date |
|--------------|----------|-----------|
| Decide A199, | 01-19-89 | 03-09-90 |
| Decide D139, | 04-13-89 | 03-09-90 |

4. Dilution: product tested undiluted.

5. Exposure time: 0.50, 1.0, 1.5, 2.0 and 3.0 minutes.

6. Test Organism: Mycobacterium bovis ATCC 35743 (1028); see the results under attachment #1 for phenol resistance.

7. Test carriers: None, a bacterial test suspension was used.

8. Subculture Media/Neutralizer: Middlebrook 7H11 Agar.

Neutralizer: Lethen broth with Tween 80

9. Incubation: 21 days at 37°C.

10. Exposure Temp: 20°C.

11. Results:

Test Sample	Time (minutes)	Dilution Tested				
		10 ⁻¹ ,	10 ⁻² ,	10 ⁻³ ,	10 ⁻⁴ ,	10 ⁻⁵
Decide (A199)	0.5	TNTC*	64**	7	Not done	Not done(ND)
"	1.0	36	1	0	ND	ND
"	1.5	3	0	0	ND	ND
"	2.0	0	0	0	ND	ND
"	3.0	0	0	0	ND	ND
(D139)	0.5	TNTC	34	4	Not done(ND)	ND
"	1.0	69	2	0	ND	ND
"	1.5	10	0	0	ND	ND
"	2.0	0	0	0	ND	ND
"	3.0	0	0	0	ND	ND

* TNTC= Too numerous to count

** Counts are average of duplicates:

Positive Control	10 ⁻¹ , 10 ⁻² , 10 ⁻³ , 10 ⁻⁴ , 10 ⁻⁵ , 10 ⁻⁶															
Static Control	<table><tr><td></td><td>TNTC</td><td>TNTC</td><td>36</td><td>7</td></tr><tr><td>10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶</td><td></td><td></td><td></td><td></td></tr><tr><td></td><td>TNTC</td><td>52</td><td>2</td><td>0</td></tr></table>		TNTC	TNTC	36	7	10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶						TNTC	52	2	0
	TNTC	TNTC	36	7												
10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶																
	TNTC	52	2	0												

		Time in Minutes		
		10	20	30
		TNTC	TNTC	53/48
Phenol	10 ⁻⁴	62/51	22/17	7/4
Control	10 ⁻⁵	7/4	3/1	1/0
CFU/ml	10 ⁻⁶	5.5x 10 ⁻⁶	2.0x10 ⁻⁶	5.5x10 ⁻⁵

Phenol control appear to meet the criteria of no less 0.5 log₁₀ and no more than 1.0 log₁₀ kill or reduction in 20 minutes.

12. Conclusions: The submitted tuberculocidal data developed by the logarithmic quantitative reduction method appear to show adequate performance for an exposure period of 5 minutes at 20°C for 2 batches of product; however, the tuberculocidal data reports are deficient. The submitted data are reflective only of one study per exposed test period. The reports are not reflective of an average of 4 separate independent studies for each exposed test period and for each test lot as per protocol requirements. This deficiency makes the conclusions invalid. In addition, it is not clear what neutralizer was used for the test. The neutralizer used as per the submitted protocol (2% sodium bisulfite) is inappropriate. Also, the submitted; summary data are not sufficient for review.

D. Disinfection(Tuberculocidal Tests):

(Validation data).

(MRID 415215-01) Nelson Labs., Performed by Dennis Ransom.

1. Method: AOAC Use-Dilution Test Method, data were generated by the logarithmic reduction test method.

2. Modifications: None reported.

3. Test samples: Lot #119-91(Ideal disinfecting towelletes)
(60 day sample).

Lot #510-91(Isotex 70 disinfecting towelletes)

4. Exposure time: 0.5, 1.0, 1.5, and 2.0 minutes.

5. Dilution tested: Product tested undiluted

6. Test organism: Mycobacterium bovis BCG.

7. Test carriers: None, bacterial suspension was used.

8. Subculture Media/Neutralizer: Lethen Broth

9. Exposure time: 20°C

10. Results:

Test Samples	Time (minutes)	Dilution Tested (tested undiluted)	
		Colony Forming Units(CFUs) (Duplicate 1)	(Duplicate 2)
Lot #510-91	0.50	0	0
"	1.00	*	0
"	1.50	0	0
"	2.00	0	0

*Contaminated

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		(Duplicate 1)	(Duplicate 2)	
Lot #119-91	0.50	0	0	
"	1.00	0	0	
"	1.50	0	0	
"	2.00	0	0	
				Plate Count Data
				<u>10⁴,</u> <u>10⁻⁵,</u> <u>10⁻⁶</u>
Initial Titer: Organism is 9.27 x 10 ⁶ cfu/ml				----- TNTC** 85 13
				----- TNTC 95 8
**Too numerous to count.				----- TNTC 98 9
Phenol Exposure:	<u>10⁴,</u>	<u>10⁵,</u>	<u>10⁶</u>	
	115,	12,	0	
	110,	10,	0	
	118,	5,	0	

Average = 1.14 x 10⁶ cfu/ml

Log₁₀ reduction = 0.91 of initial titer.

12. Conclusions: The submitted validation data appear to be adequate and are supportive of the product effectiveness as a tuberculocide against M. tuberculosis when used the product is used undiluted; however, the acceptance is pending the clarification or submission of acceptable basic tuberculocidal efficacy data for an efficacy determination.

E. Disinfection: (Viruses)

1. Method: Virucidal Tests: Tests were conducted in accordance with the virucidal requirements specified in DIS/TSS-7 enclosure and EPA Modified(AOAC), dated 12 November 1981.
2. Modification: Tested in the presence with 10% FCS.
3. Test samples: Isotex (A189-1) and Isotex(A199-2).
4. Exposure time: 3 minutes at 20⁰C.
5. Virus: Herpes simplex Type 2
6. Cell line & hosts: Propagation cell line/media: Hep II/EME 90% & 10% FBS. Recovery cell line/media: Vero/EMEM 98% + 2% FBS.
7. Type of carrier: 60 mm petri dishes
8. Neutralizer: Letheen broth
9. Incubation time for virus: 7 days at 37⁰C.

General Test Procedure: Virus coated petri dishes were dried at 2 hrs. at 20⁰C. After drying, the petri dishes were treated with germicide solution, then exposed time, then with the neutralizer solution, and followed by incubating an aliquot of the mixture on the applicable cell line for evidence of CPE.

Virucidal activity was enumerated/determined by the cytopathic effect(CPE) produced in cell culture. All reports included virus-disinfectant control, cell control, toxicity control, and virus-neutralization control.

E. Disinfection -Poliovirus Type 1-

10. Results:

Herpes simplex 2

Herpes simplex 2

(A189-1)

(A199-2)

Virus + disinfectant TCID ₅₀	< 3.50	3.50
Virus control, TCID ₅₀	> 7.50	> 7.50
Virus toxicity, TTLD ₅₀	3.50	3.50
Log. reduction in titer	> 4.00	> 4.00

11. Satisfactory performance versus Herpes simplex Type 2 in the presence of 10% FCS for an exposure time of 3 minutes.

Except for the cell lines used to propagate and recover poliovirus type 1, the remaining test conditions are the same as indicated on the previous page for H. simplex 2.

For the sake of brevity, the same conditions, materials, methods, etc... will not be listed for Poliovirus Type 1.

The EPA Virucidal Test Method as per DIS/TSS-7 enclosure was used to generate the data for (Poliovirus Type 1)

- (a) Propagating cell line utilized was BGM/EMEM 90% & FBS 10%, and the recovery cell line was BGM/EMEM 90% & 2% FCS.
- (b) Virus: Poliovirus Type 1 (Brunhilde-VR-ATCC 58).
- (c) Sample: Isotex (A189-1) and Isotex (A199-2).
- (d) For "Materials", "Methods", "Test Procedure", refer to the comments made for Herpes simplex Type 2 under 201.2-Test Summary- Item E.

(e) Results:

	A189-1	(A199-2)
Virus + disinfectant, TCID ₅₀	< 3.50	< 3.50
Virus control, TCID ₅₀	> 7.50	> 7.50
Virus toxicity, TLTD ₅₀	3.50	3.50
Log* reduction in titer	4.00	4.00

- (f) Conclusions: The tests for herpes and polio viruses are inadequate because they were not inspected by a Quality Assurance unit and: the complete test procedure and data were not submitted; the volume placed on carrier (0.5 ml) should be 0.2 ml in order to achieve adequate drying, and the drying conditions (2 hr at 20 °C) are not in compliance with the approved protocol.

F.

201.1 Test Summary(cont'd)

201.1 Disinfection(AIDS virus)(MRID 415215-02):

"Virucidal Efficacy for Palmero Sales Company's Isotex-70
" Project No. 7042 by Bonnie J. Bowdon, performing laboratory
is Southern Research Institute, Birmingham, AL 35255. The report
was initiated on 03-29-90 and completed on April 5, 1990.

201.2 Test Summary

1. Method: EPA product Performance Guidelines and the specifics in
"Southern Research Institute Test Protocol approved by EETMS
Efficacy, APB, RD, dated 08-15-89.
2. Test Virus: Human immunodeficiency virus Type 1 (HTLV-III_{RF}).
Modifications: Tested in the presence of 5% serum.
3. Virus Inoculum & Drying Procedure: Two-tenths ml of virus pool in
RPMI-1640, containing 5% heat-inactivated fetal bovine serum, was
dried on the bottom surface of glass petri dishes (28 cm² area)
at 23⁰C until visibly (approx. 45 minutes), and then incubated
at 35-37⁰C in a dry-air oven for an additional 30 minutes.
4. Exposure: 2.0 ml of disinfectant was applied to dried virus film
for a total exposure period of 1.0 minute at approximate (23⁰C).
5. Test samples: Batch # A199-1 and # D139-1.
6. Use Dilution: Not diluted(undiluted).
7. Preparation of virus film, treatment of virus film with disinfect-
ant, treatment of virus control film, cytotoxicity control, and
cell control measures are indicated in attachment #1.
8. Refer to infectivity assays under the attached materials/methods
and note attachment #1.

Host Infection & Virus Assay:

MT2 T-cells were indicator cells for the infectivity assay. The
MT2 cells were treated with polybrene(2 μ g/ml) for 30 minutes
at 37⁰C, collected by centrifugation and plated in 96-well
culture plates at approximately 1×10^4 cells per well in 0.15ml
per well in 0.15ml of medium, inoculated and incubated for 7
7 days at 37⁰C for virus infection.

Primary detection of virus was scored by lytic cytopathic effect
(CPE) after 7 days of incubation at 37⁰C.

9. Results:

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<u>Test Sample</u>	<u>Disinfectant</u>	<u>Exposure</u>	<u>Organic Soil</u>	<u>Hard Water</u>	<u>ID-50/LD50 (-Log 10)</u>	
	<u>Temperature</u>	<u>Time</u>			<u>A199-1</u>	<u>D139-1</u>
Virus Control	 NA	NA	5% Serum	NA	7.00	7.00
Non-virucidal level of Disinfectant	 23 ⁰ C (RT)	1 min.	5% serum	NA	5.70	6.00
Virus + Disinfectant	 NA	1 min.	5% serum	NA	<3.5	< 3.5
Toxicity Control	... NA	NA	NA	NA	3.5	3.5
Log Reduction	 23 ⁰ C	1.0 Minutes	5% serum	NA	≥ 3.50	≥ 3.50

NA= Not applicable.

11. Conclusions: The data meet the requirements for demonstrating virucidal efficacy of the product against HIV-1 in the presence of a moderate amount of 5% fetal (bovine serum) when used undiluted for a contact contact time of 1 minute at room temperature (23⁰C).

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