

BIO SCIENCE
LABORATORIES-INC

June 2, 2000

FINAL REPORT #000302

**EVALUATION OF THREE TEST PRODUCTS FOR THEIR ANTIMICROBIAL PROPERTIES
WHEN CHALLENGED WITH VARIOUS MICROORGANISM STRAINS
USING AN IN-VITRO TIME-KILL METHOD**

Prepared for:

BECTON DICKINSON (SPONSOR)
9450 South State Street
Sandy, Utah 84070-3213

Prepared by:

BIO SCIENCE LABORATORIES, INC. (COMPANY)
P.O. Box 190
Bozeman, Montana 59771-0190
(406) 587-5735

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June 2, 2000

FINAL REPORT #000302

1.0 **TITLE:** Evaluation of Three Test Products For Their Antimicrobial Properties When Challenged With Various Microorganism Strains Using an In-Vitro Time-Kill Method

2.0 **SPONSOR:** BECTON DICKINSON
9450 South State Street
Sandy, Utah 84070-3213

3.0 **COMPANY:** BIOSCIENCE LABORATORIES, INC.
P.O. Box 190
Bozeman, Montana 59771-0190

4.0 **STUDY DIRECTORS:**

Terri Eastman - Principal Study Director
James McDowell - Associate Study Director

5.0 **PURPOSE:**

This study evaluated the antimicrobial efficacy of three (3) products when challenged with ten (10) different microorganism strains using an In-Vitro Time-Kill method. All testing was performed in accordance with Good Laboratory Practices as specified in 21 CFR, Part 58.

6.0 **SCOPE:**

This study determined by means of an In-Vitro Time-Kill method the antimicrobial efficacy of three (3) products when challenged with ten (10) different microorganism strains utilizing fifteen (15) second, thirty (30) second, one (1) minute, and five (5) minute exposure times. The Percent and Log₁₀ reductions from the initial populations were determined for each organism versus each product. The antibacterial properties of each test product were evaluated at a concentration of 99% (v/v). Responsibility for the identity, strength, purity, composition, and stability of the test products remained with Sponsor.

7.0 **TEST PRODUCTS:**

Product #1: 4% CHG Solution
Lot Number: 000323
Manufacture Date: 3/20/00

Product #2: 3% PCMX Solution
Lot Number: 000181
Expiration Date: 2002-01

Product #3 - Ultradex Solution (3% PCMX)
Lot Number: 000247
Expiration Date: 03/02

8.0 EQUIPMENT:

- 8.1 Steam Autoclaves: BSLI 91113 and BSLI 91127
- 8.2 Laminar Biological Flowhood (certified): BSLI 91119
- 8.3 Water Bath, 47° ± 2°C: BSLI 91123
- 8.4 Water Bath Thermometer: BSLI TI-971012
- 8.5 Continuously Adjustable Pipettors, 100µL - 1000µL Capacity: BSLI 961001 and BSLI 991001
- 8.6 Continuously Adjustable Pipetter, 20µL - 200µL Capacity: BSLI 981201
- 8.7 Microman® Positive Displacement Pipetter, 100µL - 1000µL Capacity: BSLI 971104
- 8.8 Environmental Chamber, 30° ± 2°C: BSLI 930214
- 8.9 Environmental Chamber Thermometers: BSLI TI-960111 and BSLI TI-960611
- 8.10 Incubators, 30° ± 2°C: BSLI 930712 and BSLI 930905
- 8.11 Incubator Thermometers: BSLI TI-930712A and BSLI TI-971003
- 8.12 Incubator, 35° ± 2°C: BSLI 91101
- 8.13 Incubator Thermometers: BSLI TI-960109 and BSLI TI-971006
- 8.14 Incubator, 55° - 60°C: BSLI 91059
- 8.15 Incubator Thermometer: BSLI TI-2064
- 8.16 Vortex Mixer: BSLI 980103
- 8.17 Calibrated Minute/Second Timer: BSLI 961010
- 8.18 Orion pH Meter Model 720: BSLI 931104
- 8.19 Mettler BB240 Balance: BSLI 930409
- 8.20 A & D Balance Model EK-2000G: BSLI 960801
- 8.21 Troemner Weights: BSLI 930408
- 8.22 Ohaus Weights: BSLI 961011
- 8.23 Hewlett-Packard HP-15C Hand Calculator
- 8.24 Texas Instruments TI-35X Hand Calculator
- 8.25 Texas Instruments TI-36X Hand Calculator
- 8.26 MiniTab® Statistical Software (PC Version, Release 8.2 and 10xtra)

9.0 SUPPLIES:

- 9.1 Sterile Disposable Pipettes
- 9.2 Inoculating Loops
- 9.3 Sterile Disposable Petri Dishes, 100 mm x 15 mm
- 9.4 Test Tubes, Sterilized
- 9.5 Universal 1.0 and 0.2 mL Pipette Tips, Sterilized
- 9.6 Sterile 1.0 mL Positive Displacement Tips: Gilson Batch Number B0025922S
- 9.7 Sterile 20 cc Syringes: Becton-Dickinson Lot Number 9281282
- 9.8 Hand-Tally Counters

10.0 MEDIA:

- 10.1 Tryptic Soy Broth (TSB): TSB000628E
- 10.2 Tryptic Soy Agar (TSA): TSA000711B
- 10.3 Sabouraud Dextrose Agar (SDA): SDA000727B
- 10.4 Tryptic Soy Agar with product neutralizers (TSA+): TSA+000524B, TSA+000718A, TSA+000719A, and TSA+000719B
- 10.5 Sabouraud Dextrose Agar with product neutralizers (SDA+): SDA+000517E
- 10.6 Phosphate Buffered Saline (PBS): PBS000608E and PBS000727C
- 10.7 Butterfield's Phosphate Buffer solution with product neutralizers (BBP++): BBP++000711D and BBP++000718D

11.0 NEUTRALIZATION STUDY:

A neutralization study (SOP L-2007) was performed using *Staphylococcus aureus* (ATCC #25923) to ensure that the neutralizing solution employed (BBP++) was effective in neutralizing the antimicrobial properties of each test product. This neutralization procedure followed guidelines set forth in ASTM E-1054-91, "Standard Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products."

12.0 METHODOLOGY:

Inoculum Preparation

- 12.1 Approximately forty-eight (48) to seventy-two (72) hours prior to initiating the study, sterile tubes of Tryptic Soy Broth were inoculated from stock cultures, cryogenic cultures, or lyophilized vials containing the challenge microorganisms. The broth cultures were incubated for the times and at the temperatures appropriate for each species (reference Table I) for approximately twenty-four (24) hours, or until sufficient growth was observed.
- 12.2 Approximately twenty-four (24) to forty-eight (48) hours prior to initiating the study, the broth cultures prepared as described in Section 12.1 were inoculated onto the surface of the solid medium appropriate for the microorganisms and incubated at the temperature appropriate for each species (reference Table I) until sufficient growth was observed. This produced a lawn of microorganisms on the surface of the agar which was used to prepare the challenge suspensions.

Challenge Suspensions

- 12.3 Immediately prior to initiating the test procedure, a challenge suspension of each microorganism was prepared in Phosphate Buffered Saline solution by suspending the challenge microorganisms from the solid media prepared as described in Section 12.2. Suspension concentrations of approximately 1.0×10^9 CFU/mL were prepared.

Initial Population Determinations

- 12.4 An initial population was determined for each challenge suspension by making ten-fold dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7}) in Butterfield's Phosphate Buffer solution with product neutralizers, mixing thoroughly using a vortex mixer between dilutions. 0.1 mL aliquots of the 10^{-5} , 10^{-6} , and 10^{-7} dilutions were pour-plated, in duplicate, producing final plated dilutions of 10^{-6} , 10^{-7} , and 10^{-8} in the appropriate agar with product neutralizers (reference Table I). These plates were incubated for the times and at the temperatures appropriate for each species (reference Table I).

Testing Procedure

- 12.5 A 0.1 mL aliquot of a challenge suspension containing approximately 1.0×10^9 CFU/mL of challenge suspension was inoculated into a sterile test tube containing 9.9 mL of test product to achieve the 99% (v/v) concentration of each product and mixed thoroughly using a vortex mixer and/or positive displacement pipetter. The microorganisms were exposed to each test product for fifteen (15) seconds, thirty (30) seconds, one (1) minute, and five (5) minutes, timed using a calibrated minute/second timer.

- 12.6 After each designated exposure time had elapsed, 1.0 mL was removed from each tube containing product and inoculum, placed into a sterile test tube containing 9.0 mL of Butterfield's Phosphate Buffer solution with product neutralizers (10^{-3}), and mixed thoroughly using a vortex mixer. Appropriate ten-fold dilutions (10^{-4} , 10^{-5} , and 10^{-6}) were made in Butterfield's Phosphate Buffer solution with product neutralizers, mixing thoroughly using a vortex mixer between dilutions.
- 12.7 1.0 mL aliquots of the 10^{-3} dilution and 0.1 mL aliquots of the 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} dilutions of the product/neutralizer/inoculum suspension were pour-plated, in duplicate, using the appropriate solid medium with product neutralizers (reference Table I). These plates were incubated for the times and at the temperatures appropriate for each species (reference Table I).

Data Collection

- 12.8 After incubation, the colonies on the plates were counted manually using a hand-tally counter. Counts in the thirty (30) to three-hundred (300) CFU range were preferentially used in the data calculations.

TABLE I

Microorganism Species	ATCC #	Incubation Time (Test Plates)	Incubation Temperature	Media
<i>Enterobacter cloacae</i>	23355	40 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Enterococcus faecalis</i>	29212	40 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Escherichia coli</i>	25922	40 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Klebsiella pneumoniae</i>	13883	40 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Proteus vulgaris</i>	13315	40 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Pseudomonas aeruginosa</i>	27853	42.25 - 42.75 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Serratia marcescens</i>	8100	42.25 Hours	30° ± 2°C	TSB/TSA/TSA+
<i>Staphylococcus aureus</i>	25923	42.25 - 42.75 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Staphylococcus epidermidis</i>	12228	42.25 - 42.75 Hours	35° ± 2°C	TSB/TSA/TSA+
<i>Candida albicans</i>	10231	42.25 Hours	30° ± 2°C	TSB/SDA/SDA+

13.0 CALCULATIONS:

13.1 The Log₁₀ Average and the CFU/mL of the average of the duplicate plate counts for the initial population and the population after exposure to each product were calculated as follows:

$$\text{Log}_{10} \text{ Average} = \text{Log}_{10} (C_i \times 10^{-D})$$

$$\text{CFU/mL} = (C_i \times 10^{-D})$$

Where:

C_i = Average of the Two (2) Plates Counted
D = Dilution Factor of the Plates Counted

13.2 The Log₁₀ Reduction was calculated for each product and each time exposure as follows:

$$\text{Log}_{10} \text{ Reduction} = \text{IP} - \text{P}_{\text{EX}}$$

Where:

IP = Log₁₀ of the Initial Population of Challenge Microorganism
P_{EX} = Log₁₀ of the Average Population after Exposure to each Product

13.3 The Percent Reduction was calculated for each product and each time exposure as follows:

$$\text{Percent Reduction} = \frac{\text{IP} - \text{P}_{\text{EX}}}{\text{IP}} \times 100$$

Where:

IP = Initial Population of Challenge Microorganism (CFU/mL)
P_{EX} = Average Population after Exposure to each-Product (CFU/mL)

14.0 RESULTS - TABLES II to IV:

Table II lists the Log₁₀ Reductions and Percent Reductions for Test Product #1 (4% CHG Solution - Lot Number: 000323) at a 99% (v/v) concentration versus each of the ten (10) microorganisms tested. Table III lists the Log₁₀ Reductions and Percent Reductions for Test Product #2 (3% PCMX Solution - Lot Number: 000181) at a 99% (v/v) concentration versus each of the ten (10) microorganisms tested. Table IV lists the Log₁₀ Reductions and Percent Reductions for Test Product #3 (Ultradex Solution [3% PCMX] - Lot Number: 000247) at a 99% (v/v) concentration versus each of the ten (10) microorganisms tested.

TABLE II
 Product #1 - 99% (v/v) concentration
 4% CHG Solution, Lot #000323

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Enterobacter cloacae</i>	23355	15 seconds	> 6.8893	> 99.9999%
		30 seconds	> 6.8893	> 99.9999%
		1 minute	> 6.8893	> 99.9999%
		5 minutes	> 6.8893	> 99.9999%
<i>Enterococcus faecalis</i>	29212	15 seconds	> 6.8228	> 99.9999%
		30 seconds	> 6.8228	> 99.9999%
		1 minute	> 6.8228	> 99.9999%
		5 minutes	> 6.8228	> 99.9999%
<i>Escherichia coli</i>	25922	15 seconds	> 6.6767	> 99.9999%
		30 seconds	> 6.6767	> 99.9999%
		1 minute	> 6.6767	> 99.9999%
		5 minutes	> 6.6767	> 99.9999%
<i>Klebsiella pneumoniae</i>	13883	15 seconds	> 6.5441	> 99.9999%
		30 seconds	> 6.5441	> 99.9999%
		1 minute	> 6.5441	> 99.9999%
		5 minutes	> 6.5441	> 99.9999%
<i>Proteus vulgaris</i>	13315	15 seconds	> 6.9191	> 99.9999%
		30 seconds	> 6.9191	> 99.9999%
		1 minute	> 6.9191	> 99.9999%
		5 minutes	> 6.9191	> 99.9999%

TABLE II - continued
 Product #1 - 99% (v/v) concentration
 4% CHG Solution, Lot #000323

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Pseudomonas aeruginosa</i>	27853	15 seconds	> 6.7202	> 99.9999%
		30 seconds	> 6.7202	> 99.9999%
		1 minute	> 6.7202	> 99.9999%
		5 minutes	> 6.7202	> 99.9999%
<i>Serratia marcescens</i>	8100	15 seconds	> 6.7404	> 99.9999%
		30 seconds	> 6.7404	> 99.9999%
		1 minute	> 6.7404	> 99.9999%
		5 minutes	> 6.7404	> 99.9999%
<i>Staphylococcus aureus</i>	25923	15 seconds	2.7853	99.8361%
		30 seconds	6.7853	> 99.9999%
		1 minute	> 6.9614	> 99.9999%
		5 minutes	> 6.9614	> 99.9999%
<i>Staphylococcus epidermidis</i>	12228	15 seconds	> 6.2418	> 99.9999%
		30 seconds	> 6.2418	> 99.9999%
		1 minute	> 6.2418	> 99.9999%
		5 minutes	> 6.2418	> 99.9999%
<i>Candida albicans</i>	10231	15 seconds	5.1680	99.9993%
		30 seconds	> 6.1222	> 99.9999%
		1 minute	> 6.1222	> 99.9999%
		5 minutes	> 6.1222	> 99.9999%

TABLE III
 Product #2 - 99% (v/v) concentration
 3% PCMX Solution, Lot #000181

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Enterobacter cloacae</i>	23355	15 seconds	> 6.8893	> 99.9999%
		30 seconds	> 6.8893	> 99.9999%
		1 minute	> 6.8893	> 99.9999%
		5 minutes	> 6.8893	> 99.9999%
<i>Enterococcus faecalis</i>	29212	15 seconds	0.3843	58.7218%
		30 seconds	1.0099	90.2256%
		1 minute	3.2100	99.9383%
		5 minutes	> 6.8228	> 99.9999%
<i>Escherichia coli</i>	25922	15 seconds	> 6.6767	> 99.9999%
		30 seconds	> 6.6767	> 99.9999%
		1 minute	> 6.6767	> 99.9999%
		5 minutes	> 6.6767	> 99.9999%
<i>Klebsiella pneumoniae</i>	13883	15 seconds	4.5507	99.9972%
		30 seconds	> 6.5441	> 99.9999%
		1 minute	> 6.5441	> 99.9999%
		5 minutes	> 6.5441	> 99.9999%
<i>Proteus vulgaris</i>	13315	15 seconds	> 6.9191	> 99.9999%
		30 seconds	> 6.9191	> 99.9999%
		1 minute	> 6.9191	> 99.9999%
		5 minutes	> 6.9191	> 99.9999%

TABLE III - continued
 Product #2 - 99% (v/v) concentration
 3% PCMX Solution, Lot #000181

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Pseudomonas aeruginosa</i>	27853	15 seconds	> 6.7202	> 99.9999%
		30 seconds	> 6.7202	> 99.9999%
		1 minute	> 6.7202	> 99.9999%
		5 minutes	> 6.7202	> 99.9999%
<i>Serratia marcescens</i>	8100	15 seconds	> 6.7404	> 99.9999%
		30 seconds	> 6.7404	> 99.9999%
		1 minute	> 6.7404	> 99.9999%
		5 minutes	> 6.7404	> 99.9999%
<i>Staphylococcus aureus</i>	25923	15 seconds	0.4422	63.8798%
		30 seconds	0.5557	72.1858%
		1 minute	0.7956	83.9891%
		5 minutes	1.9381	98.8470%
<i>Staphylococcus epidermidis</i>	12228	15 seconds	0.4898	67.6218%
		30 seconds	0.5472	71.6332%
		1 minute	0.8661	86.3897%
		5 minutes	1.4157	96.1605%
<i>Candida albicans</i>	10231	15 seconds	1.0540	91.1698%
		30 seconds	1.1179	92.3774%
		1 minute	1.4365	96.3396%
		5 minutes	2.4787	99.6679%

TABLE IV
 Product #3 - 99% (v/v) concentration
 Ultradex Solution (3% PCMX), Lot #000247

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Enterobacter cloacae</i>	23355	15 seconds	> 6.8893	> 99.9999%
		30 seconds	> 6.8893	> 99.9999%
		1 minute	> 6.8893	> 99.9999%
		5 minutes	> 6.8893	> 99.9999%
<i>Enterococcus faecalis</i>	29212	15 seconds	3.3109	99.9511%
		30 seconds	5.2605	99.9995%
		1 minute	> 6.8228	> 99.9999%
		5 minutes	> 6.8228	> 99.9999%
<i>Escherichia coli</i>	25922	15 seconds	> 6.6767	> 99.9999%
		30 seconds	> 6.6767	> 99.9999%
		1 minute	> 6.6767	> 99.9999%
		5 minutes	> 6.6767	> 99.9999%
<i>Klebsiella pneumoniae</i>	13883	15 seconds	2.5641	99.7271%
		30 seconds	> 6.5441	> 99.9999%
		1 minute	> 6.5441	> 99.9999%
		5 minutes	> 6.5441	> 99.9999%
<i>Proteus vulgaris</i>	13315	15 seconds	> 6.9191	> 99.9999%
		30 seconds	> 6.9191	> 99.9999%
		1 minute	> 6.9191	> 99.9999%
		5 minutes	> 6.9191	> 99.9999%

TABLE IV - continued
 Product #3 - 99% (v/v) concentration
 Ultradex Solution (3% PCMX), Lot #000247

Microorganism Species	ATCC #	Exposure Time	Log ₁₀ Reduction	Percent Reduction
<i>Pseudomonas aeruginosa</i>	27853	15 seconds	> 6.7202	> 99.9999%
		30 seconds	> 6.7202	> 99.9999%
		1 minute	> 6.7202	> 99.9999%
		5 minutes	> 6.7202	> 99.9999%
<i>Serratia marcescens</i>	8100	15 seconds	4.9275	99.9988%
		30 seconds	> 6.7404	> 99.9999%
		1 minute	> 6.7404	> 99.9999%
		5 minutes	> 6.7404	> 99.9999%
<i>Staphylococcus aureus</i>	25923	15 seconds	0.9702	89.2896%
		30 seconds	1.0749	91.5847%
		1 minute	1.4631	96.5574%
		5 minutes	3.1761	99.9333%
<i>Staphylococcus epidermidis</i>	12228	15 seconds	0.6186	75.9312%
		30 seconds	0.8202	84.8711%
		1 minute	1.2375	94.2120%
		5 minutes	2.8439	99.8567%
<i>Candida albicans</i>	10231	15 seconds	2.4410	99.6377%
		30 seconds	2.2273	99.4075%
		1 minute	2.4020	99.6038%
		5 minutes	2.6531	99.7777%


15.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC. (COMPANY)

P.O. Box 190

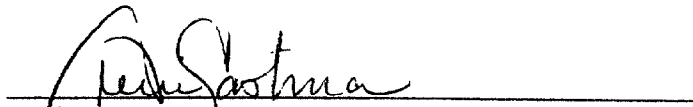
Bozeman, Montana 59771-0190

President
and CEO:


Daryl S. Paulson, Ph.D.

6-2-00
Date

Manager of
In-Vitro
Laboratory/
Principal
Study Director:


Terri Eastman

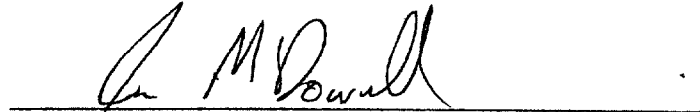
06/02/00
Date of Study Completion

Associate
Study Director:

Jayne Ballantyne*
* No longer in the employ of BioScience Laboratories, Inc.

Date

Associate
Study Director:


James McDowell

6-2-00
Date

QUALITY ASSURANCE STATEMENT:

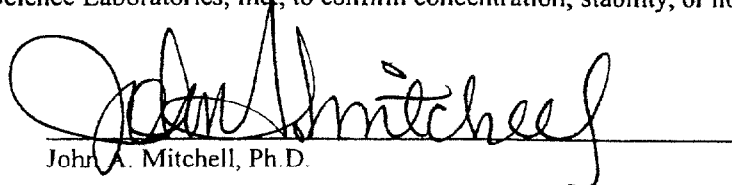
This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

<u>Phase</u>	<u>Date</u>
Product Testing	05/04/00
Data Audit	06/01/00
Final Report Review	06/01/00 & 06/02/00

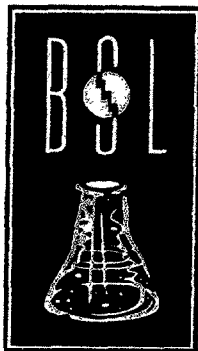
Reports to Study Director
and Management 05/04/00

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (21 CFR Part 58), with the following exception: test article preparations were not analyzed at BioScience Laboratories, Inc., to confirm concentration, stability, or homogeneity.

Director of
Quality
Assurance:


John A. Mitchell, Ph.D.

6/02/00
Date



BIOSCIENCE
LABORATORIES•INC

September 27, 2000

FINAL REPORT #000608

**DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC)
OF TWO PRODUCTS WHEN CHALLENGED WITH FIFTY MICROORGANISM STRAINS
USING THE MACRODILUTION BROTH METHOD**

Prepared for:

BECTON DICKINSON (SPONSOR)
9450 South State Street
Sandy, Utah 84070-3213

Prepared by:

BIOSCIENCE LABORATORIES, INC. (COMPANY)
P.O. Box 190
Bozeman, Montana 59771
(406) 587-5735

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September 27, 2000

FINAL REPORT #000608

1.0 **TITLE:** Determination of the Minimum Inhibitory Concentrations (MIC) of Two Products When Challenged with Fifty Microorganism Strains Using the Macrodilution Broth Method

2.0 **SPONSOR:** **BECTON DICKINSON**
9450 South State Street
Sandy, Utah 84070-3213

3.0 **COMPANY:** **BIOSCIENCE LABORATORIES, INC.**
P.O. Box 190
Bozeman, Montana 59771

4.0 **STUDY DIRECTORS:**

Terri Eastman - Principal Study Director
James McDowell - Associate Study Director

5.0 **PURPOSE:**

This study evaluated the Minimum Inhibitory Concentrations (MIC) of two (2) test products when challenged with fifty (50) different microorganism strains. All testing was performed in accordance with Good Laboratory Practices as specified in 21 CFR, Part 58.

6.0 **SCOPE:**

This study was a Minimum Inhibitory Concentration (MIC) evaluation for two (2) test products, performed following the methods outlined in NCCLS Document M7-A5, "*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*," 5th Edition. Each product was evaluated, in duplicate, against fifty (50) different microorganism strains – twenty-five (25) ATCC strains and twenty-five (25) Clinical Isolates of those same species – as specified in the Tentative Final Monograph, *Federal Register*, 17 June 1994, vol. 59:116, p. 31444.

7.0 **TEST PRODUCTS:**

The test products evaluated were provided to Company by Sponsor. Responsibility for the identity, strength, purity, composition, and stability of the test products remained with Sponsor.

Product 1 - 3% PCMX Solution, Formula 351-J9DF
Lot Number: 000181
Expiration Date: 01/02

Product 2 - 3% PCMX Solution (Ultradex)
Lot Number: 000247
Manufacture Date: 02/22/00
Expiration Date: 03/02

8.0 EQUIPMENT:

- 8.1 Steam Autoclaves: BSLI 91113 and BSLI 91127
- 8.2 Laminar Biological Flowhood (certified): BSLI 91119
- 8.3 Water Bath, $47^{\circ} \pm 2^{\circ}\text{C}$: BSLI 930611
- 8.4 Water Bath Thermometer: BSLI TI-971001
- 8.5 Continuously Adjustable Pipettors, 100 μL - 1000 μL Capacity: BSLI 970204, BSLI 991204, and BSLI 000504
- 8.6 Continuously Adjustable Pipetter, 20 μL - 200 μL Capacity: BSLI 991205
- 8.7 Microman[®] Positive Displacement Pipettors, 100 μL - 1000 μL Capacity: BSLI 970203, BSLI 971104, and BSLI 000503
- 8.8 Portable Pipettors: BSLI 971206 and BSLI 980902
- 8.9 Beckman Model TJ-6 Centrifuge, Serial Number 7408
- 8.10 Environmental Chamber, $30^{\circ} \pm 2^{\circ}\text{C}$: BSLI 930214
- 8.11 Environmental Chamber Thermometers: BSLI TI-960111 and BSLI TI-960611
- 8.12 Incubator, $30^{\circ} \pm 2^{\circ}\text{C}$: BSLI 930712
- 8.13 Incubator Thermometer: BSLI TI-930712A
- 8.14 Incubator, $35^{\circ} \pm 2^{\circ}\text{C}$: BSLI 91101
- 8.15 Incubator Thermometers: BSLI TI-960109 and BSLI TI-971006
- 8.16 Anaerobic Incubator, $35^{\circ} \pm 2^{\circ}\text{C}$: BSLI 960802
- 8.17 Anaerobic Incubator Thermometer: BSLI TI-960602
- 8.18 Incubator, 55° - 60°C : BSLI 91059
- 8.19 Incubator Thermometer: BSLI TI-2064
- 8.20 Refrigerators, 2° - 8°C : BSLI 91109 and BSLI 991201
- 8.21 Refrigerator Thermometers: BSLI TI-960303 and BSLI TI-971004
- 8.22 Vortex Mixers: BSLI 980103 and BSLI 991002
- 8.23 Orion pH Meter Model 720: BSLI 931104
- 8.24 Mettler BB240 Balance: BSLI 930409
- 8.25 A & D Balance Model EK-2000G: BSLI 960801
- 8.26 Troemner Weights: BSLI 930408
- 8.27 Ohaus Weights: BSLI 961011
- 8.28 Hewlett-Packard HP-15C Hand Calculator
- 8.29 Texas Instruments TI-35X Hand Calculator
- 8.30 Texas Instruments TI-36X Hand Calculator

9.0 SUPPLIES:

- 9.1 Sterile 5 mL Disposable Pipettes: Kimble Lot Number N00080C
- 9.2 Sterile 25 mL Disposable Pipettes: VWR Lot Number W00103C and Kimble Lot Number 23099021
- 9.3 Sterile 20 cc syringes: Becton-Dickinson Lot Number 9281282
- 9.4 Sterile Disposable Petri Plates, 100 mm x 15 mm: American Precision Plastics Lot Number 00268906
- 9.5 Test Tubes, Sterilized
- 9.6 Universal 1.0 and 0.2 mL Pipette Tips, Sterilized
- 9.7 Sterile 1.0 mL Positive Displacement Tips: Gilson Batch Number B0030922S
- 9.8 Hand-Tally Counters
- 9.9 125 mL Polypropylene Bottles, Sterilized
- 9.10 Inoculating Loops
- 9.11 GasPak[™] Anaerobic System
- 9.12 GasPak Plus[™] Hydrogen plus Carbon Dioxide Gas Generator Envelopes

10.0 MEDIA:

- 10.1 Tryptic Soy Broth (TSB): TSB001012A
- 10.2 Brain-Heart Infusion Broth (BHIB): BHIB001107A
- 10.3 Schaedler's Broth (SB): SB000914A and SB001020E
- 10.4 Mueller-Hinton Broth (MHB): MHB001109B
- 10.5 Mueller-Hinton Broth with Bacto Supplement B (MHB-VX): MHB001109B
Supplement VX: Bacto Control Number 140556KA, Expires 04/30/02
- 10.6 Cation-Adjusted Mueller-Hinton Broth with Lysed Horse Blood (CAMHB-B): CAMHB001020D
and CAMHB001201C
SP Blood Supplement: Difco Lot Numbers 143115KA, Expires 09/30/00 and 143812KA, Expires 10/31/00
- 10.7 Anaerobic MIC Broth (AMIC): AMIC001104E
- 10.8 Tryptic Soy Agar (TSA): TSA001013A, TSA001014A, TSA001017A, and TSA001122A
- 10.9 Brain-Heart Infusion Agar (BHIA): BHIA001020G and BHIA001104C
- 10.10 Sabouraud Dextrose Agar (SDA): SDA001020B
- 10.11 Schaedler's Agar with Lysed Horse Blood (SA-B): SA001020C
SP Blood Supplement: Difco Lot Number 143812KA, Expires 10/31/00
- 10.12 Tryptic Soy Agar with 5% Sheep Blood (SBA): PML Lot Numbers 66723-1, Expires 10/03/00
and 68375-1, Expires 10/25/00
- 10.13 Chocolate Agar with Enrichment (CAE): PML Lot Numbers 65383-1, Expires 08/29/00 and
68175-1, Expires 10/03/00
- 10.14 Mueller-Hinton Agar with Dextrose and Bacto Supplement B (MHAD-VX): MHAD000907B
Supplement VX: Bacto Control Number 140556KA, Expires 04/30/02
- 10.15 Phosphate Buffered Saline Solution (PBS): PBS001013D and PBS001116E

11.0 METHODOLOGY:

Inoculum Preparation - Approximately 48 - 96 hours prior to testing

- 11.1 Separate sterile tubes of the broth medium appropriate for each of the challenge microorganisms (except *Haemophilus influenzae* [ATCC #19418 and Clinical Isolate], *Streptococcus pneumoniae* [ATCC #6303 and Clinical Isolate], and *Streptococcus pyogenes* [ATCC #19615]) were inoculated from lyophilized vials or cryogenic cultures containing the microorganisms (reference Table I). The microorganism cultures were incubated at the temperatures and under the conditions appropriate for each species (reference Table I) for approximately twenty-four (24) hours, or until sufficient growth was observed.
- 11.2 For *Haemophilus influenzae* (ATCC #19418 and Clinical Isolate), *Streptococcus pneumoniae* (ATCC #6303 and Clinical Isolate), and *Streptococcus pyogenes* (ATCC #19615), plates of the appropriate solid media (reference Table I) were inoculated from lyophilized vials, cryogenic cultures, or stock cultures containing these microorganisms. These plates were incubated at the temperatures and under the conditions appropriate for these species (reference Table I) for twenty-four (24) to forty-eight (48) hours, or until sufficient growth was observed.

Inoculum Preparation - Approximately 24 - 48 hours prior to testing

- 11.3 The broth cultures prepared as described in Section 11.1 (except those for *Bacteroides fragilis* [ATCC #25285 and Clinical Isolate]) were inoculated onto the surface of the solid medium appropriate for each microorganism and incubated at the temperatures and under the conditions appropriate for each species (reference Table I) for twenty-four (24) hours, or until sufficient growth was observed. This produced lawns of the microorganisms on the surface of the agar plates which were used to prepare the challenge suspensions.
- 11.4 For *Bacteroides fragilis* (ATCC #25285 and Clinical Isolate), the broth cultures prepared as described in Section 11.1 were subcultured in additional tubes of Schaedler's Broth and incubated anaerobically at $35^{\circ} \pm 2^{\circ}\text{C}$ for twenty-four (24) to forty-eight (48) hours, or until sufficient growth was observed. Following incubation, the challenge suspensions were prepared by centrifuging the broth culture tubes, combining the resulting pellets, and resuspending them in Schaedler's Broth.
- 11.5 For *Haemophilus influenzae* (ATCC #19418 and Clinical Isolate), *Streptococcus pneumoniae* (ATCC #6303 and Clinical Isolate), and *Streptococcus pyogenes* (ATCC #19615), a suspension was prepared for each microorganism from the plates prepared as described in Section 11.2 by rinsing the plates with sterile Phosphate Buffered Saline. Aliquots of each suspension were then spread-plated onto the surface of additional plates of the solid medium appropriate for each microorganism (reference Table I). These plates were incubated at the temperature and under the conditions appropriate for these species (reference Table I) for twenty-four (24) to forty-eight (48) hours, or until sufficient growth was observed. This produced lawns of the microorganisms on the surface of the agar plates which were used to prepare the challenge suspensions.

Challenge Suspensions

- 11.6 Immediately prior to initiating the test procedure, an initial suspension of each microorganism (except *Bacteroides fragilis* [ATCC #25285 and Clinical Isolate]) was prepared by inoculating a test tube of Phosphate Buffered Saline with microorganisms taken from the plates of solid media prepared as described in Sections 11.3 and 11.5. Suspension concentrations of approximately 1.0×10^9 CFU/mL were prepared. The challenge suspensions of *Bacteroides fragilis* (ATCC #25285 and Clinical Isolate) were prepared as described in Section 11.4.
- 11.7 Final challenge suspensions containing approximately 1.0×10^6 CFU/mL were achieved for each microorganism by placing a 0.1 mL aliquot of the approximately 1.0×10^9 CFU/mL suspension into a sterile 125 mL polypropylene bottle containing 100 mL of the broth appropriate for each microorganism (reference Table I). The challenge suspensions were mixed thoroughly prior to use in testing.

Initial Population Determination

- 11.8 An initial population was determined for each challenge suspension by making ten-fold dilutions (10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) from the inoculum bottle into Phosphate Buffered Saline and pour- or spread-plating, in duplicate, 0.1 mL aliquots of the 10^{-2} , 10^{-3} , and 10^{-4} dilutions using the solid medium appropriate for each microorganism (reference Table I). Hence, the final plated dilutions were 10^{-3} , 10^{-4} , and 10^{-5} . These plates were incubated at the temperatures and under the conditions appropriate for each challenge microorganism (reference Table I) until sufficient growth was observed.

Testing Procedure

- 11.9 A series of 1:2 (v/v) dilutions of each test product were prepared using the broth appropriate for each challenge microorganism (reference Table I), resulting in product dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1,024, 1:2,048, 1:4,096, 1:8,192, 1:16,384, and 1:32,768.
- 11.10 1.0 mL aliquots of each product dilution prepared were transferred to separate sterile test tubes. A series of fifteen (15) tubes, each containing 1.0 mL of the appropriate product dilution (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1,024, 1:2,048, 1:4,096, 1:8,192, 1:16,384, and 1:32,768), were prepared for each microorganism evaluated (reference Table I).
- 11.11 A 1.0 mL aliquot of challenge suspension containing approximately 1.0×10^6 CFU/mL was introduced into each dilution tube in the series, thereby resulting in a final product dilution series of 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1,024, 1:2,048, 1:4,096, 1:8,192, 1:16,384, 1:32,768, and 1:65,536, with each dilution containing approximately 5.0×10^5 CFU/mL of the challenge microorganism.
- 11.12 The test procedure outlined in Sections 11.10 and 11.11 was performed, in duplicate, for each of the microorganism species tested (reference Table I).

Controls

- 11.13 A positive control tube (growth control) containing a 1.0 mL aliquot of the broth medium appropriate for the microorganism (reference Table I) and a 1.0 mL aliquot of the challenge suspension was prepared for each microorganism.
- 11.14 Negative (media) control tubes (no microbial inoculation) of the broth medium appropriate for each microorganism (reference Table I) were also prepared.

Incubation

- 11.15 The challenge suspension/product dilution tubes and the controls were incubated at $35^\circ \pm 2^\circ\text{C}$ for sixteen (16) to twenty-four (24) hours, or until good growth was apparent in the positive control tubes.

Determination of Results

- 11.16 Following incubation, the tubes were examined for growth of the microorganism, as indicated by turbidity.
- 11.17 The Minimum Inhibitory Concentration (MIC) for each product versus each challenge microorganism was recorded as the highest dilution of test product that completely inhibited growth of the microorganism, as detected by the unaided eye. The MIC was also calculated in parts per million (ppm) of the active ingredient of the test product present at this product dilution. The results of the duplicate runs for each test product versus each microorganism were averaged together to provide the final reported values.

TABLE I

No.	Microorganism Species	ATCC or BSLI #*	Incubation Time (MIC Tubes)	Incubation Temperature (Inoc. Prep. & IP Plates Only)	Media
1	<i>Acinetobacter baumannii</i>	19606	20 Hours	35° ± 2°C	BHIB/BHIA/MHB
2	<i>Acinetobacter baumannii</i>	061700Ab6*	20 Hours	35° ± 2°C	BHIB/BHIA/MHB
3	<i>Bacteroides fragilis</i>	25285	42.50 Hours	35° ± 2°C (Anaerobic)	SB/SA-B/AMIC
4	<i>Bacteroides fragilis</i>	060700Bf2*	42.50 Hours	35° ± 2°C (Anaerobic)	SB/SA-B/AMIC
5	<i>Enterobacter cloacae</i>	13047	20 Hours	35° ± 2°C	TSB/TSA/MHB
6	<i>Enterobacter cloacae</i>	121799Ecl1*	20 Hours	35° ± 2°C	TSB/TSA/MHB
7	<i>Enterococcus faecalis</i>	29212	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
8	<i>Enterococcus faecalis</i>	121699Efs1*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
9	<i>Enterococcus faecium</i>	19434	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
10	<i>Enterococcus faecium</i>	061700Efm1*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
11	<i>Escherichia coli</i>	11229	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
12	<i>Escherichia coli</i>	010500Ec8*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
13	<i>Escherichia coli</i>	25922	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
14	<i>Escherichia coli</i>	010500Ec6*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
15	<i>Haemophilus influenzae</i>	19418	20 Hours	35° ± 2°C	CAE/MHB-VX
16	<i>Haemophilus influenzae</i>	062900Hi9*	20 Hours	35° ± 2°C	CAE/MHB-VX
17	<i>Klebsiella oxytoca</i>	43165	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
18	<i>Klebsiella oxytoca</i>	060700Ko6*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
19	<i>Klebsiella pneumoniae</i>	11296	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
20	<i>Klebsiella pneumoniae</i>	040400Kpn12*	20.25 Hours	35° ± 2°C	TSB/TSA/MHB
21	<i>Micrococcus luteus</i>	7468	20 Hours	30° ± 2°C	TSB/TSA/MHB
22	<i>Micrococcus spp.</i>	060700Ms8*	20 Hours	30° ± 2°C	TSB/TSA/MHB
23	<i>Proteus mirabilis</i>	7002	20.75 Hours	35° ± 2°C	TSB/TSA/MHB
24	<i>Proteus mirabilis</i>	062900Pm1*	20 Hours	35° ± 2°C	TSB/TSA/MHB
25	<i>Pseudomonas aeruginosa</i>	15442	17.75 Hours	35° ± 2°C	TSB/TSA/MHB

* = Clinical Isolate

Inoc. Prep. = Inoculum Preparation

IP = Initial Population

TABLE I (continued)

No.	Microorganism Species	ATCC or BSLI #*	Incubation Time (MIC Tubes)	Incubation Temperature (Inoc. Prep. & IP Plates Only)	Media
26	<i>Pseudomonas aeruginosa</i>	040400Pa8*	20 Hours	35° ± 2°C	TSB/TSA/MHB
27	<i>Pseudomonas aeruginosa</i>	27853	20 Hours	35° ± 2°C	TSB/TSA/MHB
28	<i>Pseudomonas aeruginosa</i>	040400Pa9*	20 Hours	35° ± 2°C	TSB/TSA/MHB
29	<i>Serratia marcescens</i>	14756	20 Hours	30° ± 2°C	TSB/TSA/MHB
30	<i>Serratia marcescens</i>	060700Sm3*	20 Hours	30° ± 2°C	TSB/TSA/MHB
31	<i>Staphylococcus aureus</i>	6538	17.75 Hours	35° ± 2°C	TSB/TSA/MHB
32	<i>Staphylococcus aureus</i>	040400Sa4*	39.75 Hours	35° ± 2°C	TSB/TSA/MHB
33	<i>Staphylococcus aureus</i>	29213	20 Hours	35° ± 2°C	TSB/TSA/MHB
34	<i>Staphylococcus aureus</i>	040400Sa5*	20 Hours	35° ± 2°C	TSB/TSA/MHB
35	<i>Staphylococcus epidermidis</i>	12228	17.75 Hours	35° ± 2°C	TSB/TSA/MHB
36	<i>Staphylococcus epidermidis</i>	061700Se13*	20 Hours	35° ± 2°C	TSB/TSA/MHB
37	<i>Staphylococcus haemolyticus</i>	29970	20 Hours	35° ± 2°C	TSB/TSA/MHB
38	<i>Staphylococcus haemolyticus</i>	061700Sha5*	20 Hours	35° ± 2°C	TSB/TSA/MHB
39	<i>Staphylococcus hominis</i>	27844	39.75 Hours	35° ± 2°C	TSB/TSA/MHB
40	<i>Staphylococcus hominis</i>	060700Sho4*	20 Hours	35° ± 2°C	TSB/TSA/MHB
41	<i>Staphylococcus saprophyticus</i>	15305	39.75 Hours	35° ± 2°C	TSB/TSA/MHB
42	<i>Staphylococcus saprophyticus</i>	060700Ss3*	20 Hours	35° ± 2°C	TSB/TSA/MHB
43	<i>Streptococcus pneumoniae</i>	6303	20.25 Hours	35° ± 2°C	SBA/CAMHB-B
44	<i>Streptococcus pneumoniae</i>	062900Spn6*	20 Hours	35° ± 2°C	SBA/CAMHB-B
45	<i>Streptococcus pyogenes</i>	19615	20.25 Hours	35° ± 2°C	SBA/BHIA/CAMHB-B
46	<i>Streptococcus pyogenes</i>	040400Spy10*	20 Hours	35° ± 2°C	BHIB/BHIA/CAMHB-B
47	<i>Candida albicans</i>	10231	20 Hours	30° ± 2°C	TSB/SDA/MHB
48	<i>Candida albicans</i>	040400Ca1*	20 Hours	30° ± 2°C	TSB/SDA/MHB
49	<i>Candida tropicalis</i>	750	20 Hours	30° ± 2°C	TSB/SDA/MHB
50	<i>Candida tropicalis</i>	121799Ct*	20 Hours	30° ± 2°C	TSB/SDA/MHB

* = Clinical Isolate

Inoc. Prep. = Inoculum Preparation

IP = Initial Population

TABLE II
Origin of Clinical Isolates Supplied by Company

Organism	Date Isolated	Specimen	Patient Age/Sex	Source	BSLI ID No.
<i>Acinetobacter baumannii</i>	Unknown	Sputum	Unknown	MRL	061700Ab6
<i>Bacteroides fragilis</i>	Unknown	Unknown	Unknown	MRL	060700Bf2
<i>Enterobacter cloacae</i>	12/03/99	Wound	47/M	UW/HMC	121799Ec11
<i>Enterococcus faecalis</i>	12/06/99	Blood	45/M	UW/HMC	121699Efs1
<i>Enterococcus faecium</i>	Unknown	Rectal Swab	Unknown	MRL	061700Efm1
<i>Escherichia coli</i>	12/23/99	Unknown	Unknown	WMC	010500Ec8
<i>Escherichia coli</i>	12/22/99	Unknown	Unknown	WMC	010500Ec6
<i>Haemophilus influenzae</i>	Unknown	Eye	Unknown	MRL	062900Hi9
<i>Klebsiella oxytoca</i>	Unknown	Nares	Unknown	MRL	060700Ko6
<i>Klebsiella pneumoniae</i>	01/28/00	Sputum	60/M	U of U	040400Kpn12
<i>Micrococcus spp.</i>	Unknown	Skin	Unknown	MRL	060700Ms8
<i>Proteus mirabilis</i>	Unknown	Nares	Unknown	MRL	062900Pm1
<i>Pseudomonas aeruginosa</i>	01/23/00	Sputum	35/M	U of U	040400Pa8
<i>Pseudomonas aeruginosa</i>	01/22/00	Urine	33/M	U of U	040400Pa9
<i>Serratia marcescens</i>	Unknown	Nares	Unknown	MRL	060700Sm3
<i>Staphylococcus aureus</i>	01/16/00	Blood	50/M	U of U	040400Sa4
<i>Staphylococcus aureus</i>	01/15/00	Blood	71/M	U of U	040400Sa5
<i>Staphylococcus epidermidis</i>	Unknown	Eye	Unknown	MRL	061700Se13
<i>Staphylococcus haemolyticus</i>	Unknown	Eye	Unknown	MRL	061700Sha5
<i>Staphylococcus hominis</i>	Unknown	Unknown	Unknown	MRL	060700Sho4
<i>Staphylococcus saprophyticus</i>	Unknown	Unknown	Unknown	MRL	060700Ss3
<i>Streptococcus pneumoniae</i>	Unknown	Sputum	Unknown	MRL	062900Spn6
<i>Streptococcus pyogenes</i>	Unknown	Throat	Unknown	U of U	040400Spy10
<i>Candida albicans</i>	02/19/00	Sputum	33/M	U of U	040400Ca1
<i>Candida tropicalis</i>	10/21/99	Subhepatic Fluid	47/M	UW/HMC	121799Ct

MRL = MRL Research Laboratory in Cypress, CA

WMC = Western Montana Clinic in Missoula, MT

U of U = University of Utah Hospital and Clinics in Salt Lake City, UT

UW/HMC = University of Washington, WA / Harborview Medical Center

12.0 **RESULTS - TABLES III & IV:**

Table III presents the Minimum Inhibitory Concentration, in dilution and parts per million (ppm), of the active ingredient for Product 1 (3% PCMX Solution, Formula 351-J9DF - Lot Number: 000181) versus each of the fifty (50) microorganisms tested. Table IV presents the Minimum Inhibitory Concentration, in dilution and parts per million (ppm), of the active ingredient for Product 2 (3% PCMX Solution, Ultradex - Lot Number: 000247) versus each of the fifty (50) microorganisms tested.

TABLE III
Formula 351-J9DF - Lot Number: 000181
(3% [30,000 ppm] PCMX)

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million (ppm)
<i>Acinetobacter baumannii</i>	19606	1 : 256	117.1875
<i>Acinetobacter baumannii</i>	061700Ab6*	1 : 512	58.5938
<i>Bacteroides fragilis</i>	25285	1 : 2,048	14.6484
<i>Bacteroides fragilis</i>	060700Bf2*	1 : 8,192	3.6621
<i>Enterobacter cloacae</i>	13047	1 : 128	234.3750
<i>Enterobacter cloacae</i>	121799Ec11*	1 : 128	234.3750
<i>Enterococcus faecalis</i>	29212	1 : 128	234.3750
<i>Enterococcus faecalis</i>	121699Efs1*	1 : 128	234.3750
<i>Enterococcus faecium</i>	19434	1 : 4	7,500.0000
<i>Enterococcus faecium</i>	061700Efm1*	1 : 128	234.3750
<i>Escherichia coli</i>	11229	1 : 128	234.3750
<i>Escherichia coli</i>	010500Ec8*	1 : 256	117.1875
<i>Escherichia coli</i>	25922	1 : 256	117.1875
<i>Escherichia coli</i>	010500Ec6*	1 : 192	156.2500
<i>Haemophilus influenzae</i>	19418	1 : 768	39.0625
<i>Haemophilus influenzae</i>	062900Hi9*	1 : 1,536	19.5313
<i>Klebsiella oxytoca</i>	43165	1 : 128	234.3750
<i>Klebsiella oxytoca</i>	060700Ko6*	1 : 128	234.3750
<i>Klebsiella pneumoniae</i>	11296	1 : 256	117.1875
<i>Klebsiella pneumoniae</i>	040400Kpn12*	1 : 64	468.7500
<i>Micrococcus luteus</i>	7468	1 : 128	234.3750
<i>Micrococcus spp.</i>	060700Ms8*	≤ 1 : 4	≥ 7,500.0000
<i>Proteus mirabilis</i>	7002	1 : 128	234.3750
<i>Proteus mirabilis</i>	062900Pm1*	1 : 128	234.3750
<i>Pseudomonas aeruginosa</i>	15442	1 : 12	2,500.0000
<i>Pseudomonas aeruginosa</i>	040400Pa8*	1 : 4	7,500.0000

* = Clinical Isolate

TABLE III (continued)
 Formula 351-J9DF - Lot Number: 000181
 (3% [30,000 ppm] PCMX)

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million
<i>Pseudomonas aeruginosa</i>	27853	1 : 6	5,000.0000
<i>Pseudomonas aeruginosa</i>	040400Pa9*	1 : 4	7,500.0000
<i>Serratia marcescens</i>	14756	1 : 96	312.5000
<i>Serratia marcescens</i>	060700Sm3*	1 : 128	234.3750
<i>Staphylococcus aureus</i>	6538	1 : 384	78.1250
<i>Staphylococcus aureus</i>	040400Sa4*	1 : 6,144	4.8828
<i>Staphylococcus aureus</i>	29213	1 : 512	58.5938
<i>Staphylococcus aureus</i>	040400Sa5*	1 : 256	117.1875
<i>Staphylococcus epidermidis</i>	12228	1 : 256	117.1875
<i>Staphylococcus epidermidis</i>	061700Se13*	1 : 256	117.1875
<i>Staphylococcus haemolyticus</i>	29970	1 : 512	58.5938
<i>Staphylococcus haemolyticus</i>	061700Sha5*	1 : 128	234.3750
<i>Staphylococcus hominis</i>	27844	1 : 512	58.5938
<i>Staphylococcus hominis</i>	060700Sho4*	1 : 512	58.5938
<i>Staphylococcus saprophyticus</i>	15305	1 : 512	58.5938
<i>Staphylococcus saprophyticus</i>	060700Ss3*	1 : 1,024	29.2969
<i>Streptococcus pneumoniae</i>	6303	1 : 8,192	3.6621
<i>Streptococcus pneumoniae</i>	062900Spn6*	1 : 512	58.5938
<i>Streptococcus pyogenes</i>	19615	1 : 2,048	14.6484
<i>Streptococcus pyogenes</i>	040400Spy10*	1 : 256	117.1875
<i>Candida albicans</i>	10231	< 1 : 4	> 7,500.0000
<i>Candida albicans</i>	040400Ca1*	< 1 : 4	> 7,500.0000
<i>Candida tropicalis</i>	750	< 1 : 4	> 7,500.0000
<i>Candida tropicalis</i>	121799Ct*	< 1 : 4	> 7,500.0000

* = Clinical Isolate

TABLE IV
 Ultradex - Lot Number: 000247
 (3% [30,000 ppm] PCMX)

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million (ppm)
<i>Acinetobacter baumannii</i>	19606	1 : 256	117.1875
<i>Acinetobacter baumannii</i>	061700Ab6*	1 : 512	58.5938
<i>Bacteroides fragilis</i>	25285	1 : 8,192	3.6621
<i>Bacteroides fragilis</i>	060700Bf2*	1 : 12,288	2.4414
<i>Enterobacter cloacae</i>	13047	1 : 192	156.2500
<i>Enterobacter cloacae</i>	121799Ecl1*	1 : 128	234.3750
<i>Enterococcus faecalis</i>	29212	1 : 192	156.2500
<i>Enterococcus faecalis</i>	121699Efs1*	≤ 1 : 128	≥ 234.3750
<i>Enterococcus faecium</i>	19434	< 1 : 128	> 234.3750
<i>Enterococcus faecium</i>	061700Efm1*	< 1 : 128	> 234.3750
<i>Escherichia coli</i>	11229	1 : 256	117.1875
<i>Escherichia coli</i>	010500Ec8*	1 : 128	234.3750
<i>Escherichia coli</i>	25922	1 : 384	78.1250
<i>Escherichia coli</i>	010500Ec6*	1 : 128	234.3750
<i>Haemophilus influenzae</i>	19418	1 : 2,048	14.6484
<i>Haemophilus influenzae</i>	062900Hi9*	1 : 4,096	7.3242
<i>Klebsiella oxytoca</i>	43165	≤ 1 : 128	≥ 234.3750
<i>Klebsiella oxytoca</i>	060700Ko6*	≤ 1 : 128	≥ 234.3750
<i>Klebsiella pneumoniae</i>	11296	1 : 512	58.5938
<i>Klebsiella pneumoniae</i>	040400Kpn12*	< 1 : 128	> 234.3750
<i>Micrococcus luteus</i>	7468	1 : 192	156.2500
<i>Micrococcus spp.</i>	060700Ms8*	1 : 512	58.5938
<i>Proteus mirabilis</i>	7002	1 : 256	117.1875
<i>Proteus mirabilis</i>	062900Pm1*	1 : 128	234.3750
<i>Pseudomonas aeruginosa</i>	15442	< 1 : 128	> 234.3750
<i>Pseudomonas aeruginosa</i>	040400Pa8*	< 1 : 128	> 234.3750

* - Clinical Isolate

TABLE IV (continued)
 Ultradex - Lot Number: 000247
 (3% [30,000 ppm] PCMX)

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million
<i>Pseudomonas aeruginosa</i>	27853	< 1 : 128	> 234.3750
<i>Pseudomonas aeruginosa</i>	040400Pa9*	< 1 : 128	> 234.3750
<i>Serratia marcescens</i>	14756	< 1 : 64	> 468.7500
<i>Serratia marcescens</i>	060700Sm3*	1 : 64	468.7500
<i>Staphylococcus aureus</i>	6538	1 : 1,024	29.2969
<i>Staphylococcus aureus</i>	040400Sa4*	1 : 256	117.1875
<i>Staphylococcus aureus</i>	29213	1 : 512	58.5938
<i>Staphylococcus aureus</i>	040400Sa5*	1 : 256	117.1875
<i>Staphylococcus epidermidis</i>	12228	1 : 512	58.5938
<i>Staphylococcus epidermidis</i>	061700Se13*	1 : 256	117.1875
<i>Staphylococcus haemolyticus</i>	29970	1 : 512	58.5938
<i>Staphylococcus haemolyticus</i>	061700Sha5*	1 : 512	58.5938
<i>Staphylococcus hominis</i>	27844	1 : 384	78.1250
<i>Staphylococcus hominis</i>	060700Sho4*	1 : 4,096	7.3242
<i>Staphylococcus saprophyticus</i>	15305	1 : 512	58.5938
<i>Staphylococcus saprophyticus</i>	060700Ss3*	1 : 1,024	29.2969
<i>Streptococcus pneumoniae</i>	6303	1 : 6,144	4.8828
<i>Streptococcus pneumoniae</i>	062900Spn6*	1 : 256	117.1875
<i>Streptococcus pyogenes</i>	19615	1 : 2,048	14.6484
<i>Streptococcus pyogenes</i>	040400Spy10*	1 : 256	117.1875
<i>Candida albicans</i>	10231	< 1 : 64	> 468.7500
<i>Candida albicans</i>	040400Ca1*	< 1 : 64	> 468.7500
<i>Candida tropicalis</i>	750	< 1 : 128	> 234.3750
<i>Candida tropicalis</i>	121799Ct*	< 1 : 64	> 468.7500

* - Clinical Isolate


13.0 REFERENCE:

NCCLS Document M7-A5, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically," 5th Edition.

14.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC.
P.O. Box 190
Bozeman, Montana 59771-0190


President
and CEO:



Daryl S. Paulson, Ph.D.

9-27-00
Date

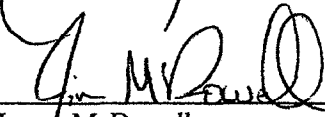
Manager of
In-Vitro
Laboratory/
Principal
Study Director:



Terri Eastman

09/27/00
Study Completion Date

Associate
Study Director:



James McDowell

9-27-00
Date

QUALITY ASSURANCE STATEMENT:

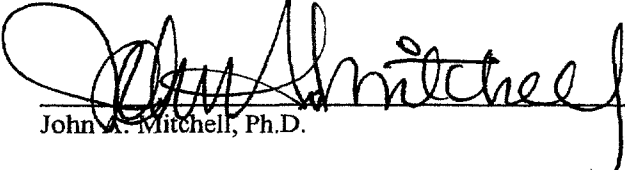
This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

<u>Phase</u>	<u>Date</u>
Product Testing	08/15/00 & 08/31/00
Data Audit	09/26/00
Final Report Review	09/26/00

Reports to Study Director
and Management 08/15/00, 08/31/00 & 09/26/00

This study was conducted in compliance with Good Laboratory Practices standards, as described by the FDA (21 CFR Part 58), with the following exception: test article preparations were not analyzed at BioScience Laboratories, Inc., to confirm concentration, stability, or homogeneity.

Director of
Quality
Assurance:



John A. Mitchell, Ph.D.

9/27/00
Date

MicroBioTest, Inc.

Study Title

**MINIMUM INHIBITORY CONCENTRATIONS FOR
ULTRADEX® 3% PCMX
AS SURGICAL HAND SCRUB SOLUTION**

Data Requirements

**This report is designed to be
used internally by the sponsor of the study**

Author

Sherry C. Conlin

Final Report Written

1/23/95

Performing Laboratory

**MicroBioTest, Inc. (MBT)
14280 Sullyfield Circle, #200
Chantilly, Virginia 22021**

Laboratory Project Identification

361-101

**Submitted to: BECTON-DICKINSON
9450 South State Street
Sandy, Utah 80470**

Page 1 of 8

MICROBIOTEST, INC. COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160 with the exception that information on the synthesis, purity analysis, composition and other characteristics of the test product remain with the sponsor.

Study Director: MICROBIOTEST, INC.

Angela M. Staples 11/31/95

ANGELA M. STAPLES

MICROBIOTEST QUALITY ASSURANCE UNIT STATEMENT

The Quality Assurance Unit of MicroBioTest, Inc. has inspected the final report of Project Number 361-101 entitled "MINIMUM INHIBITORY CONCENTRATIONS FOR ULTRADEX® 3% PCMX AS SURGICAL HAND SCRUB SOLUTION", in compliance with current Good Laboratory Practice regulations, (40 CFR Part 160).

<u>DATE OF INSPECTION</u>	<u>DATE REPORTED TO STUDY DIRECTOR</u>	<u>DATE REPORTED TO MANAGEMENT</u>
10/05/94	10/06/94	10/06/94
01/18/95	01/18/95	01/25/95
01/30/95	01/30/95	01/30/95

Judithann R. Harvey 1/30/95

JUDITHANN R. HARVEY Date
QAU Auditor

OBJECTIVE:

The test procedure was designed to supply basic antimicrobial data before specific clinical testing is undertaken. The testing procedure conforms to the requirements of Federal Register, Vol. 59, No. 116 §333.470, June 17, 1994.

MATERIALS:**A. supplied by the sponsor**

1. PCMX, lot M53509, received 09/20/94, assigned DS No. 1990.
2. ULTRADEX Solution without PCMX, lot M061240A, received 09/20/94, assigned DS NO. 1991, and lot M063350A, received 10/19/94, assigned DS No. 1997.
3. ULTRADEX Solution with 3% PCMX, lot M061240, received 09/20/94, assigned DS NO. 1992, and lot M063350, received 10/19/94, assigned DS NO. 1998.

The sponsor assures MicroBioTest, Inc. testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable. All unused test materials will be retained by MBT for a period of three months after completion of the test then discarded in a manner which meets the approval of the safety officer.

B. Materials supplied by MicroBioTest, Inc., including, but not limited to:

1. Media and reagents:
 - a. Trypticase Soya Agar.
 - b. Trypticase Soya Broth.
 - c. Sheep Blood Agar.
 - d. Sabouraud Dextrose Agar
 - e. Yeast Maltose Agar.
 - f. Sterile Saline solution blanks (SS).
 - g. Dimethyl Sulfoxide (DMSO).
2. Challenge organisms

All ATCC strains were acquired directly from the ATCC. All fresh clinical isolates (CI) were acquired from the Howard University Hospital, or MicroBioTest, Inc.

3. Laboratory equipment and supplies.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

Bacteria and yeast were subcultured from stock cultures on agar, incubated at $37 \pm 2C$ in ambient air. Anaerobic bacteria were subcultured on pre-reduced media, incubating at $37 \pm 2C$ under anaerobic conditions. A 20-24 hr culture was used for the test procedure for all organisms except, *Propionibacterium* was five days old, the *Candida* and *Bacteroides* were 48 ± 1 hr old. On the day of the test, bacteria were harvested by swabbing the surface of the agar with a cotton-tipped swab previously dipped in sterile SS and dispersed in approximately 10 ml of SS. The suspension of the challenge organisms were adjusted with SS to contain approximately 1×10^8 cfu/ml by using spectrophotometric methods extant in the laboratory.

B. Test material preparation:

The ULTRADEX with and without PCMX were tested as sent by the sponsor. The PCMX was prepared every test date by adding 1.5 g of the PCMX to 50 ml of filter sterilized DMSO or 0.75 g of the PCMX to 25 ml of DMSO.

C. Test :

1. Nine tubes containing two ml of broth medium each were prepared in duplicate for each challenge microorganism.
2. The first tube contained four-ml test material.
3. Doubling dilutions were performed for each set of ten tubes by transferring two ml from the first tube to the second tube, mixing thoroughly, then transferring two ml to the next tube up to the tenth tube. From the tenth tube two ml of mixture were discarded.
4. Each tube was inoculated with 0.05 ml of one of the challenge organisms. Tubes were incubated at $37 \pm 2C$ for the time appropriate for each organism, then scored for growth (+) or no growth (-).
5. After the incubation period, one loopful from each tube was streaked onto an agar plate. Plates were incubated and observed for growth (+) or no growth (-).

D. Controls:**1. Negative controls:**

The plates and aliquots of broth media used in the test were incubated with the test.

2. Positive controls:

For each organism, duplicate tubes containing two ml of appropriate broth medium were inoculated with 0.05 ml of the appropriate challenge organism and incubated with the test tubes.

TEST ACCEPTANCE CRITERIA:

The test is acceptable for evaluation of the test results if the criteria listed below are satisfied.

- The positive control must exhibit growth of the challenge organisms.
- The negative control must exhibit no growth of any microorganism.

TESTING FACILITIES AND STUDY DATES:

The study was conducted at MicroBioTest, Inc, 14280 Sullyfield Circle, Suite 200, Chantilly, Virginia 22021. The Laboratory phase was conducted in the Applied Microbiology Laboratory at MicroBioTest, Inc. between the dates 10/12/94 and 1/27/95. The study director signed the protocol on 10/03/94.

RECORDS:

All raw data, protocol, protocol modifications, test material records, final report, and correspondence relevant to this study, between MBT and the sponsor will be stored in the archives at MicroBioTest, Inc., 14280 Sullyfield Circle, Suite 200, Chantilly, VA 22021.

All changes or revisions of the approved protocol were documented, signed by the study director, dated and maintained with the protocol. The sponsor was notified of the change, resolution, and impact on the study as soon as practical.

INTERPRETATION OF RESULTS:

The endpoint or minimum inhibitory concentration (MIC) is defined as the concentration of test compound that completely inhibits growth of the challenge organism. In order to arrive at an MIC for the test compound, the growth control tubes must exhibit growth and the solvent used must show little or no effect upon the growth of the organisms. The titer for each test compound was determined.

CALCULATION:

The test compound concentrations (ppm) or dilution for each tube resulting from a two-fold dilution are shown below:

Tube	3% PCMX	ULTRADEX	ULTRADEX + 3% PCMX
1	30,000	neat	30,000
2	15,000	1:2	15,000
3	7,500	1:4	7,500
4	3,750	1:8	3,750
5	1,875	1:16	1,875
6	937.5	1:32	937.5
7	468.75	1:64	468.75
8	234.38	1:128	234.38
9	117.19	1:256	117.19
10	58.59	1:512	58.59

RESULTS:

The results for all sterility controls were negative , and all organisms demonstrated growth for the positive control. The results are on pages seven and eight which describe the minimum inhibitory concentration, the 3% PCMX, and the ULTRADEX + 3% PCMX are described as the concentration of the active ingredient in parts per million (ppm). The ULTRADEX without PCMX is described as a dilution of the product.

RESULTS MIC ATCC STRAINS

Organism	3% PCMX DS No. 1990	ULTRADEX DS No. 1991,1997	ULTRADEX + 3% PCMX DS No. 1992, 1998
<i>Acinetobacter sp.</i> ATCC 15308	234.38	1:2	234.38
<i>Bacteroides fragilis</i> , ATCC 25285	468.75	1:2	937.5
<i>Candida albicans</i> , ATCC 10231	234.38	neat	234.38
<i>Candida tropicalis</i> , ATCC 750	175.78	neat	117.19
<i>Enterobacter aerogenes</i> , ATCC 13048	468.75	1:2	468.75
<i>Enterococcus faecalis</i> , ATCC 29212	937.5	neat	468.75
<i>Enterococcus faecium</i> , ATCC 6569	234.38	1:2	117.19
<i>Escherichia coli</i> , ATCC 11229	175.58	neat	468.75
<i>Escherichia coli</i> , ATCC 25922	468.75	1:2	234.38
<i>Haemophilus influenzae</i> , ATCC 19418	234.38	1:4	468.75
<i>Klebsiella pneumoniae</i> , ATCC 29995	468.75	neat	234.38
<i>Micrococcus luteus</i> , ATCC 7468	234.38	UTR	117.19
<i>Micrococcus roseus</i> , ATCC 186	117.19	1:2	117.19
<i>Proteus mirabilis</i> , ATCC 7002	468.75	UTR	468.75
<i>Pseudomonas aeruginosa</i> , ATCC 15442	468.75	1:2	468.75
<i>Pseudomonas aeruginosa</i> , ATCC 27853	937.5	neat	937.5
<i>Serratia marcescens</i> , ATCC 14756	234.38	1:2	234.38
<i>Staphylococcus aureus</i> , ATCC 6538	175.78	1:2	58.59
<i>Staphylococcus aureus</i> , ATCC 29213	234.38	UTR	117.19
<i>Staphylococcus epidermidis</i> , ATCC 12228	58.59	neat	58.59
<i>Staphylococcus hominis</i> , ATCC 29885	468.75	UTR	234.38
<i>Staphylococcus haemolyticus</i> , ATCC 29970	468.75	neat	234.38
<i>Staphylococcus saprophyticus</i> , ATCC 15305	468.75	neat	468.75
<i>Streptococcus pyogenes</i> , ATCC 19615	468.75	0.75	468.75
<i>Streptococcus pneumoniae</i> , ATCC 6303	468.75	1:2	937.5

UTR - unable to read the first tube

RESULTS MIC CLINICAL ISOLATES

Organism	3% PCMX DS No. 1990	ULTRADEX DS No. 1991,1997	ULTRADEX + 3% PCMX DS No. 1992, 1998
Bacillus sp. CI 232	468.75	neat	937.5
Candida albicans CI 224	3,750	1:2	1,875
Enterobacter cloacae CI 207	58.59	1:2	937.5
Enterobacter cloacae CI 208	468.75	1:2	937.5
Enterococcus faecalis CI 216	468.75	1:2	468.75
Enterococcus faecalis CI 217	468.75	1:2	937.5
Escherichia coli CI 215	468.75	1:2	15,000
Klebsiella pneumoniae CI 210	117.19	1:2	1,875
Micrococcus citreus CI 230	234.38	1:2	468.75
Propionibacterium acnes CI 221	234.38	1:2	468.75
Propionibacterium acnes CI 222	234.38	1:2	468.75
Pseudomonas aeruginosa CI 204	468.75	1:2	30,000
Pseudomonas aeruginosa CI 205	351.56	1:2	1,406.25
Pseudomonas aeruginosa CI 206	234.38	1:2	3,750
Salmonella group D CI 209	58.59	1:2	234.38
Staphylococcus sp (coagulase negative) CI 211	468.75	1:2	15,000
Staphylococcus aureus CI 214	234.38	1:2	468.75
Staphylococcus aureus CI 225	468.75	1:2	937.5
Staphylococcus epidermidis CI 212	468.75	1:2	15,000
Staphylococcus epidermidis CI 213	468.75	1:2	15,000
Staphylococcus epidermidis CI 226	468.75	1:2	703.12
Staphylococcus epidermidis CI 227	468.75	1:2	2,812.5
Staphylococcus epidermidis CI 228	1,406.25	1:2	937.5
Streptococcus pneumoniae CI 220	468.75	1:2	937.5
Staphylococcus pyogenes CI 218	117.19	1:2	58.59