

LEWIS & HARRISON

Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

0892 '00 OCT 12 P12:21

October 10, 2000

Documents Management Branch (HFA-305)
Division of OTC Products
Center for Drug Evaluation and Research
Food and Drug Administration
5630 Fishers Lane, Room #1061
Rockville, MD 20857

re: **Topical Antimicrobial Products for Over-the-Counter Human Use;
Tentative Final Monograph (TFM) for Health-Care Antiseptic Products
Docket Number 75N-183H
Benzethonium Chloride**

Dear Sir or Madam:

On behalf of Lonza Inc., I am submitting dermal absorption, pharmacokinetic and efficacy studies in support of Category I status for the active ingredient benzethonium chloride, for all the product types listed in the above TFM (patient preoperative skin preparation; surgical hand-scrub, antiseptic handwash; hand sanitizer/dip for food handlers).

Lonza previously submitted to FDA an extensive database of safety studies conducted with benzethonium chloride. After reviewing these studies, FDA scientists (at feedback meetings with Lonza in April and August, 1998) asked Lonza to provide dermal absorption and pharmacokinetic data with aqueous and ethanol formulations of benzethonium chloride. The specific studies dermal absorption and pharmacokinetic studies and related studies included with this submission are:

- Overview of Benzethonium Chloride Studies.
- The *In Vitro* Percutaneous Absorption of [¹⁴C]-Benzethonium Chloride Through Human and Rat Skin.
- Preliminary Pharmacokinetics Study of Dermal Applied ¹⁴C-Benzethonium Chloride in Rats
- Dermal Irritation of Benzethonium Chloride in Rats

75N-183H

RPT 4

As noted in the "Overview" document, dermal absorption of benzethonium chloride through human skin is negligible (less than 1% of the applied dose). The preliminary pharmacokinetic study did not detect quantifiable levels of radiolabeled benzethonium chloride in the blood at any of the sampling intervals. Accordingly, the dermal absorption and pharmacokinetic studies show that the safety margins for benzethonium chloride are large.

The following efficacy study is also being submitted:

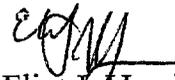
- Determination of the Minimum Inhibitory Concentration (MIC) of One Product When Challenged with Fifty Microorganism Strains Using the Macrodilution Broth Method.

The efficacy study determined MIC values for benzethonium chloride against twenty-five (25) ATCC strains and twenty-five clinical isolates of those same species, as specified in the TFM. Lonza believes that the efficacy study provides sufficient presumptive evidence to show that benzethonium chloride is efficacious for the monograph uses.

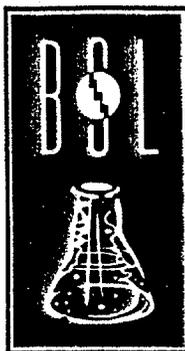
In separate correspondence, Lonza will be requesting a meeting with FDA to discuss this submission and the time-line for evaluating the submission. In particular, Lonza would like to discuss the accelerated finalization of the monograph status of benzethonium chloride. We strongly believe that such a step is in the public-interest since it would publicly clarify the status of this important ingredient and provide a clear alternative to existing Category I actives.

If you have any questions about this submission, please contact me at (202) 393-3903.

Sincerely,



Eliot K. Harrison
Agent for Lonza



0854 '00 OCT 12 P12:21

BIO SCIENCE
LABORATORIES • INC

September 21, 2000

FINAL REPORT #000622
(TS# 00-1096)

**DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)
OF ONE PRODUCT WHEN CHALLENGED WITH FIFTY MICROORGANISM STRAINS
USING THE MACRODILUTION BROTH METHOD**

Prepared for:

LONZA, INC. (SPONSOR)
79 Route 22 East
Annandale, New Jersey 08801

Prepared by:

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

Copy # _____

TABLE OF CONTENTS

TITLE 3

SPONSOR 3

COMPANY 3

STUDY DIRECTORS 3

PURPOSE 3

SCOPE 3

TEST PRODUCT 3

EQUIPMENT 4

SUPPLIES 4

MEDIA 5

METHODOLOGY 5

TABLE I 8

TABLE II 10

RESULTS 11

REFERENCE 12

ACCEPTANCE 13

INDEX OF ADDENDA 14

September 21, 2000

FINAL REPORT #000622
(TS# 00-1096)

1.0 **TITLE:** Determination of the Minimum Inhibitory Concentration (MIC) of One Product when Challenged with Fifty Microorganism Strains using the Macrodilution Broth Method

2.0 **SPONSOR:** LONZA, INC.
79 Route 22 East
Annandale, New Jersey 08801

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 Concentration (MIC) of one (1) test product 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 one (1) test product, performed following the methods outlined in NCCLS Document M7-A5, "*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*," 5th Edition. The test 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 PRODUCT:**

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

Product 1 - Benzethonium Chloride, USP
Lot Number: 100037 (4022-S)
Expiration Date: 07/17/01

On each test day, a 5% (w/v) solution (50,000 ppm) of the test product was prepared by weighing 5 g of test product into a sterile glass beaker and adding 100 mL of Sterile Water-for-Irrigation.

Sterile Water-for-Irrigation, Abbott Laboratories Lot Number: 58-842-4B-2, Expires 11/01/02

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 Pipetters, 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 Pipetters, 100 μL - 1000 μL Capacity: BSLI 970203, BSLI 971104, and BSLI 000503
- 8.8 Portable Pipetters: BSLI 971206, BSLI 980602, 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-91109 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 23099021
- 9.3 Sterile 50 mL Disposable Pipettes: Kimble Lot Number 01000008
- 9.4 Sterile 20 cc Syringes: Becton-Dickinson Lot Number 9281282
- 9.5 Sterile Disposable Petri Plates, 100 mm x 15 mm: American Precision Plastics Lot Number 00268906
- 9.6 Test Tubes, Sterilized
- 9.7 Universal 1.0 and 0.2 mL Pipette Tips, Sterilized
- 9.8 Sterile 1.0 mL Positive Displacement Tips: Gilson Batch Number B0030922S
- 9.9 Hand-Tally Counters
- 9.10 125 mL Polypropylene Bottles, Sterilized
- 9.11 Glass Beakers, Sterilized
- 9.12 Inoculating Loops
- 9.13 Graduated Cylinders, Sterilized
- 9.14 GasPak[™] Anaerobic System
- 9.15 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 143812KA, Expires 10/31/00 and 143115KA, Expires
09/30/00
- 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 An inoculating loop was used to inoculate 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]) 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 the *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 the *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 at the temperature and under the conditions appropriate for this species (reference Table I) 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 the *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 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 testing, 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 temperature 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 the 5% (w/v) solution of the test product (reference Section 7.0) 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 were prepared for each microorganism (reference Table I).
- 11.14 A negative (media) control tube (no microbial inoculation) of the broth medium appropriate for the microorganism (reference Table I) was 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 microorganisms, as indicated by turbidity.
- 11.17 The Minimum Inhibitory Concentration (MIC) for the test 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 the 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>	121799Ec11*	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
 U of U = University of Utah Hospital and Clinics in Salt Lake City, UT
 UW/HMC = University of Washington, WA / Harborview Medical Center

WMC = Western Montana Clinic in Missoula, MT

12.0 **RESULTS - TABLE III:**

Table III presents the Minimum Inhibitory Concentration, in dilution and parts per million (ppm), of the active ingredient for the product versus each of the fifty (50) microorganisms tested.

TABLE III
Benzethonium Chloride, USP - Lot Number: 100037 (4022S)
5% (w/v) Solution in Sterile Water-for-Irrigation - 50,000 ppm

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million (ppm)
<i>Acinetobacter baumannii</i>	19606	1 : 2,048	24.4141
<i>Acinetobacter baumannii</i>	061700Ab6*	1 : 8,192	6.1035
<i>Bacteroides fragilis</i>	25285	1 : 2,048	24.4141
<i>Bacteroides fragilis</i>	060700Bf2*	1 : 6,144	8.1380
<i>Enterobacter cloacae</i>	13047	1 : 1,024	48.8281
<i>Enterobacter cloacae</i>	121799Ecl1*	1 : 2,048	24.4141
<i>Enterococcus faecalis</i>	29212	1 : 8,192	6.1035
<i>Enterococcus faecalis</i>	121699Efs1*	1 : 8,192	6.1035
<i>Enterococcus faecium</i>	19434	1 : 8,192	6.1035
<i>Enterococcus faecium</i>	061700Efm1*	1 : 8,192	6.1035
<i>Escherichia coli</i>	11229	1 : 1,024	48.8281
<i>Escherichia coli</i>	010500Ec8*	1 : 2,048	24.4141
<i>Escherichia coli</i>	25922	1 : 2,048	24.4141
<i>Escherichia coli</i>	010500Ec6*	1 : 2,048	24.4141
<i>Haemophilus influenzae</i>	19418	1 : 6,144	8.1380
<i>Haemophilus influenzae</i>	062900Hi9*	1 : 8,192	6.1035
<i>Klebsiella oxytoca</i>	43165	1 : 2,048	24.4141
<i>Klebsiella oxytoca</i>	060700Ko6*	1 : 2,048	24.4141
<i>Klebsiella pneumoniae</i>	11296	1 : 4,096	12.2070
<i>Klebsiella pneumoniae</i>	040400Kpn12*	1 : 1,024	48.8281
<i>Micrococcus luteus</i>	7468	> 1 : 65,536	< 0.7629
<i>Micrococcus spp.</i>	060700Ms8*	1 : 32,768	1.5259
<i>Proteus mirabilis</i>	7002	1 : 512	97.6563
<i>Proteus mirabilis</i>	062900Pm1*	1 : 512	97.6563
<i>Pseudomonas aeruginosa</i>	15442	1 : 1,024	48.8281

* = Clinical Isolate

TABLE III (continued)
Benzethonium Chloride, USP - Lot Number: 100037 (4022S)
5% (w/v) Solution in Sterile Water-for-Irrigation - 50,000 ppm

Microorganism Species	ATCC or BSLI #*	Minimum Inhibitory Concentration	
		Product Dilution	Parts per Million (ppm)
<i>Pseudomonas aeruginosa</i>	040400Pa8*	1 : 1,024	48.8281
<i>Pseudomonas aeruginosa</i>	27853	1 : 1,024	48.8281
<i>Pseudomonas aeruginosa</i>	040400Pa9*	1 : 1,024	48.8281
<i>Serratia marcescens</i>	14756	1 : 512	97.6563
<i>Serratia marcescens</i>	060700Sm3*	1 : 512	97.6563
<i>Staphylococcus aureus</i>	6538	> 1 : 65,536	< 0.7629
<i>Staphylococcus aureus</i>	040400Sa4*	> 1 : 65,536	< 0.7629
<i>Staphylococcus aureus</i>	29213	1 : 32,768	1.5259
<i>Staphylococcus aureus</i>	040400Sa5*	> 1 : 65,536	< 0.7629
<i>Staphylococcus epidermidis</i>	12228	> 1 : 65,536	< 0.7629
<i>Staphylococcus epidermidis</i>	061700Se13*	> 1 : 65,536	< 0.7629
<i>Staphylococcus haemolyticus</i>	29970	> 1 : 65,536	< 0.7629
<i>Staphylococcus haemolyticus</i>	061700Sha5*	> 1 : 65,536	< 0.7629
<i>Staphylococcus hominis</i>	27844	> 1 : 65,536	< 0.7629
<i>Staphylococcus hominis</i>	060700Sho4*	> 1 : 65,536	< 0.7629
<i>Staphylococcus saprophyticus</i>	15305	> 1 : 65,536	< 0.7629
<i>Staphylococcus saprophyticus</i>	060700Ss3*	> 1 : 65,536	< 0.7629
<i>Streptococcus pneumoniae</i>	6303	1 : 8,192	6.1035
<i>Streptococcus pneumoniae</i>	062900Spn6*	1 : 8,192	6.1035
<i>Streptococcus pyogenes</i>	19615	1 : 16,384	3.0518
<i>Streptococcus pyogenes</i>	040400Spy10*	1 : 4,096	12.2070
<i>Candida albicans</i>	10231	< 1 : 256	> 195.3125
<i>Candida albicans</i>	040400Ca1*	< 1 : 256	> 195.3125
<i>Candida tropicalis</i>	750	< 1 : 256	> 195.3125
<i>Candida tropicalis</i>	121799Ct*	< 1 : 256	> 195.3125

* = Clinical Isolate

13.0 REFERENCE:

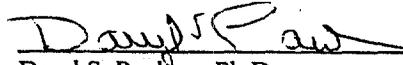
NCCLS Document M7-A5, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically," 5th Edition.

14.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC.

P.O. Box 190
Bozeman, Montana 59771-0190

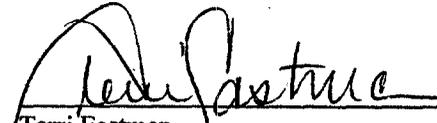
President
and CEO:



Daryl S. Paulson, Ph.D.

9-21-00
Date

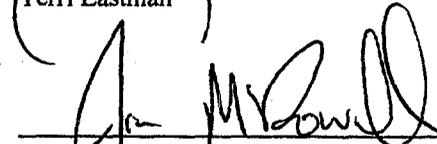
Manager of
In-Vitro
Laboratory/
Principal
Study Director:



Terri Eastman

09/21/00
Study Completion Date

Associate
Study Director:



James McDowell

9-21-00
Date

QUALITY ASSURANCE STATEMENT:

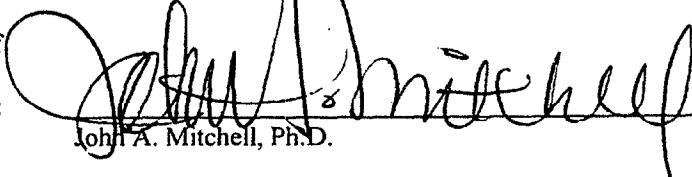
This study was inspected by the Quality Assurance Unit, and reports were submitted to the Study Director and Management in accordance with Standard Operating Procedures, as follows:

<u>Phase</u>	<u>Date</u>
Product Testing	08/15/00 & 08/31/00
Data Audit	09/20/00
Draft Report Review	09/20/00
Final Report Review	09/21/00

Reports to Study Director
and Management 08/15/00, 08/31/00 & 09/20/00

This study was conducted in compliance with Good Laboratory Practices standards, as described by then 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.

9/21/00
Date

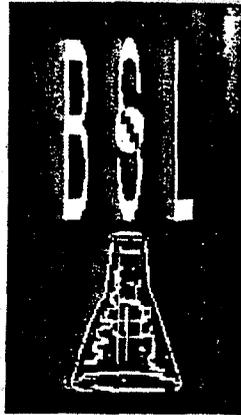
INDEX OF ADDENDA

- I Protocol #000622 and Protocol and/or SOP Deviation Recording Forms (Form No. 99-QA-004)
- II Log of Samples (Form No. 92-L-023), Sample Tracking Forms (Form No. 93-L-029), Certificate of Analysis, and MSDS
- III Media/Diluent Tracking Forms (Form No. 97-L-007) and Media Production and Growth Testing Data Sheets (Form No. 91-L-003)
- IV Minimum Inhibitory Concentration Evaluation
 - Inoculum Population Data Sheets for MIC (Form No. 96-L-019)
 - Minimum Inhibitory Concentration Evaluation Forms (Form No. 93-L-030)
- V General Data Gathering (Form No. 91-L-002)
- VI Equipment Logs
 - Equipment Tracking Forms (Form No. 98-L-007)
 - Water Bath Temperature Recording Form (Form No. 95-L-007)
 - Incubator Log Forms (Form No. 96-L-008)
 - Refrigerator Log Forms (Form No. 96-L-015)

Addendum 1

ADDENDUM I

Protocol #000622
Protocol and/or SOP Deviation Recording Forms (Form No. 99-QA-004)



**BIOSCIENCE
LABORATORIES • INC.**

July 11, 2000

PROTOCOL #000622

**DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)
OF ONE PRODUCT WHEN CHALLENGED WITH FIFTY MICROORGANISM STRAINS
USING THE MACRODILUTION BROTH METHOD**

Prepared for:

LONZA, INC. (SPONSOR)
79 Route 22 East
Annandale, New Jersey 08801

Prepared by:

BIOSCIENCE LABORATORIES, INC. (COMPANY)
300 North Willson Avenue
Bozeman, Montana 59715
(406) 587-5735

Copy # _____

CONFIDENTIAL

This Document has been copyrighted by BioScience Laboratories, Inc., and is considered confidential between BioScience Laboratories, Inc. (Company) and Lonza, Inc. (Sponsor). This document is not to be shown, given to, or used by anyone except Lonza, Inc. (Sponsor) without written permission from BioScience Laboratories, Inc. (Company).

TABLE OF CONTENTS

TITLE 4
SPONSOR 4
COMPANY 4
STUDY DIRECTORS 4
PURPOSE 4
SCOPE 4
TEST PRODUCT 4
EQUIPMENT 4
SUPPLIES 5
MEDIA 5
INOCULUM PREPARATION 5
TESTING PROCEDURE 7
TABLE I 8
REFERENCE 11
FINAL REPORT 11
EXCEPTIONAL CONDITIONS 11
DOCUMENTATION AND RECORD-KEEPING 11
ACCEPTANCE 12

July 11, 2000

PROTOCOL #000622

1.0 **TITLE:** **DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF ONE PRODUCT WHEN CHALLENGED WITH FIFTY MICROORGANISM STRAINS USING THE MACRODILUTION BROTH METHOD**

2.0 **SPONSOR:** **LONZA, INC.**
79 Route 22 East
Annandale, New Jersey 08801

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 will evaluate the Minimum Inhibitory Concentrations (MIC) of one (1) test product when challenged with fifty (50) different microorganism strains. All testing will be performed in accordance with Good Laboratory Practices as specified in 21 CFR, Part 58.

6.0 **SCOPE:**

This study, a Minimum Inhibitory Concentration (MIC) evaluation for one (1) test product, will be performed following the methods outlined in NCCLS Document M7-A5, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically," Fifth Edition. The test product will be 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 PRODUCT:**

The test product to be evaluated will be provided to Company by Sponsor. Responsibility for the identity, strength, purity, composition and stability of the test product rests with Sponsor.

Product 1:
Lot Number:
Label Information:

8.0 **EQUIPMENT:**

- 8.1 Steam Autoclaves
- 8.2 Laminar Biological Flowhood
- 8.3 Water Bath, 47° ± 2°C
- 8.4 Water Bath Thermometer
- 8.5 Continuously Adjustable Pipettors, 100 µl - 1000 µl Capacity

- 8.6 Continuously Adjustable Pipetters, 20 μ l - 200 μ l Capacity
- 8.7 Microman® Positive Displacement Pipetters, 100 μ l - 1000 μ l Capacity
- 8.8 Refrigerators, Temperature Range 2° - 8°C
- 8.9 Refrigerator Thermometers
- 8.10 Incubator, Temperature Range 35° \pm 2°C
- 8.11 Incubator, Temperature Range 30° \pm 2°C
- 8.12 Incubator Thermometers
- 8.13 Drummond Pipette Aid
- 8.14 Vortex Mixers
- 8.15 Beckman Model TJ-6 Centrifuge

9.0 SUPPLIES:

- 9.1 Sterile Disposable Pipettes
- 9.2 Sterile Disposable Petri Plates, 100 mm x 15 mm
- 9.3 Test Tubes, Sterilized
- 9.4 Universal 1.0 and 0.2 mL Pipette Tips, Sterilized
- 9.5 Sterile 1.0 mL Positive Displacement Tips
- 9.6 Hand-Tally Counters
- 9.7 125 mL Polypropylene Bottles or Glass Beakers, Sterilized
- 9.8 Inoculating Loops
- 9.9 GasPak™ Anaerobic System
- 9.10 GasPak Plus™ Hydrogen plus Carbon Dioxide Gas Generator Envelopes

10.0 MEDIA:

- 10.1 Tryptic Soy Broth (TSB)
- 10.2 Brain-Heart Infusion Broth (BHIB)
- 10.3 Schaedler's Broth (SB)
- 10.4 Mueller-Hinton Broth (MHB)
- 10.5 Mueller-Hinton Broth with Bacto Supplement B (MHB-XV)
- 10.6 Cation-Adjusted Mueller-Hinton Broth with Lysed Horse Blood (CAMHB-B)
- 10.7 Anaerobic MIC Broth (AMIC)
- 10.8 Tryptic Soy Agar (TSA)
- 10.9 Brain-Heart Infusion Agar (BHIA)
- 10.10 Sabouraud Dextrose Agar (SDA)
- 10.11 Schaedler's Agar with Lysed Horse Blood (SA-B)
- 10.12 Tryptic Soy Agar with 5% Sheep Blood (SBA)
- 10.13 Chocolate Agar with Enrichment (CAE)
- 10.14 Mueller-Hinton Agar with Dextrose and Bacto Supplement B (MHA-XV)
- 10.15 Phosphate Buffered Saline Solution (PBS)

11.0 INOCULUM PREPARATION:

All species except for *Bacteroides fragilis* (ATCC #25285 and Clinical Isolate), *Haemophilus influenzae* (ATCC #19418 and Clinical Isolate), and *Streptococcus pneumoniae* (ATCC #6303 and Clinical Isolate)

- 11.1 Approximately forty-eight (48) hours prior to initiating the study, separate sterile tubes of the appropriate broth (reference Table I) will be inoculated from lyophilized vials or cryogenic cultures containing the challenge microorganisms. The microorganism cultures will be incubated at the temperatures and under the conditions appropriate for each species for approximately twenty-four (24) hours, or until sufficient growth is observed (reference Table I).

- 11.2 Approximately twenty-four (24) hours prior to initiating the study, the broth cultures prepared as described in Section 11.1 will be inoculated onto the surface of the appropriate solid media contained in Petri plates and incubated appropriately (reference Table I). This will produce a lawn of bacteria or yeast on the surface of the agar which will be used to prepare the challenge suspensions.

Bacteroides fragilis (ATCC #25285 and Clinical Isolate)

- 11.3 Approximately forty-eight (48) to ninety-six (96) hours prior to initiating the study, separate sterile tubes of Schaedler's Broth will be inoculated from lyophilized vials or cryogenic cultures containing these microorganisms. These broth cultures will be incubated anaerobically at $35^{\circ} \pm 2^{\circ}$ C for twenty-four (24) to forty-eight (48) hours, or until sufficient growth is observed (reference Table I).
- 11.4 Approximately twenty-four (24) to forty-eight (48) hours prior to initiating the study, the broth cultures prepared as described in Section 11.3 will be subcultured into additional tubes of Schaedler's Broth and incubated appropriately (reference Table I). Following incubation, the challenge suspensions will be prepared by centrifuging the broth culture tubes, combining the resulting pellets, and resuspending them in Schaedler's Broth.

Haemophilus influenzae (ATCC #19418 and Clinical Isolate) and *Streptococcus pneumoniae* (ATCC #6303 and Clinical Isolate)

- 11.5 Approximately forty-eight (48) to ninety-six (96) hours prior to initiating the study, plates of the appropriate solid medium will be inoculated from lyophilized vials or cryogenic cultures containing these microorganisms. These cultures will be incubated at the temperatures and under the conditions appropriate for each species (reference Table I) for twenty-four (24) to forty-eight (48) hours, or until sufficient growth is observed.
- 11.6 Approximately twenty-four (24) to forty-eight (48) hours prior to initiating the study, a suspension will be prepared for each microorganism by rinsing the plates of solid media prepared in Section 11.5 with sterile Phosphate Buffered Saline solution. Aliquots of each suspension will then be spread-plated onto the surface of additional plates of the appropriate solid media and incubated appropriately (reference Table I). This will produce a lawn of microorganisms on the surface of the agar which will be used to prepare the challenge suspensions.

Challenge Suspensions

- 11.7 Immediately prior to initiating the test procedure, a challenge suspension of each microorganism will be prepared by inoculating a test tube of Phosphate Buffered Saline solution with microorganisms taken from the plates of solid media prepared earlier. Suspension concentrations of approximately 1.0×10^9 CFU/mL will be prepared. The challenge suspensions of *Bacteroides fragilis* (ATCC #25285 and Clinical Isolate) will be prepared as described in Section 11.4.
- 11.8 Final challenge suspensions containing approximately 1.0×10^6 CFU/mL will be prepared for each microorganism species by placing aliquots of the approximately 1.0×10^9 CFU/mL suspension into a sterile 125 mL polypropylene bottle or a sterile glass beaker containing a sufficient volume of the appropriate broth to complete the study (reference Table I).
- 11.9 The final challenge suspensions will be mixed thoroughly prior to use in testing.

Initial Population Determination

11.10 An initial population for each challenge microorganism will be determined from the inoculum bottle used for testing by making ten-fold dilutions (e.g., 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) out of the bottle in Phosphate Buffered Saline solution. Using the appropriate solid media (reference Table I), pour spread plates will be prepared, in duplicate, from the inoculum dilutions by plating 0.1 mL of the three (3) final dilutions, e.g., 10^{-2} , 10^{-3} , and 10^{-4} , to achieve plated dilutions of, e.g., 10^{-3} , 10^{-4} , and 10^{-5} . These plates will be incubated for twenty-four (24) to forty-eight (48) hours at the temperature appropriate for each challenge microorganism (reference Table I).

12.0 TESTING PROCEDURE:

12.1 A series of 1:2 (v/v) dilutions of the test product will be prepared using the appropriate broth (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.

12.2 1.0 mL aliquots of each product dilution prepared will be 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), will be prepared for each microorganism to be evaluated against the product (reference Table I).

12.3 A 1.0 mL aliquot of a challenge suspension containing approximately 1.0×10^6 CFU/mL will be 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 organism.

12.4 The test procedure outlined in Sections 12.2 and 12.3 will be performed, in duplicate, for each of the microorganism species tested (reference Table I).

Controls

12.5 A positive control tube (growth control) containing a 1.0 mL aliquot of the appropriate broth medium and a 1.0 mL aliquot of the challenge suspension will be prepared for each microorganism (reference Table I).

12.6 A negative (media) control tube (no microbial inoculation) of each broth medium will also be prepared (reference Table I).

Incubation

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

Determination of Results

12.8 Following incubation, the tubes will be examined for growth of the microorganism, as indicated by turbidity.

12.9 The Minimum Inhibitory Concentration (MIC) for the test product versus each challenge microorganism strain will be recorded as the highest dilution of test product that completely inhibits growth of the microorganism, as detected by the unaided eye. The results of the duplicate runs for the test product versus each microorganism will be averaged together to provide the final reported values.

TABLE I

No.	Microorganism Species	Incubation Time (Inoculum Only)	Incubation Temperature (Inoculum Only)	Media
1	<i>Acinetobacter baumannii</i> (ATCC# 19606)	24 - 48 Hours	35° ± 2°C	BHIB/BHIA/ MHB
2	<i>Acinetobacter</i> species (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	BHIB/BHIA/ MHB
3	<i>Bacteroides fragilis</i> (ATCC# 25285)	24 - 96 Hours	35° ± 2°C (Anaerobic)	SB/SA-B/AMIC
4	<i>Bacteroides</i> species (Clinical Isolate)	24 - 96 Hours	35° ± 2°C (Anaerobic)	SB/SA-B/AMIC
5	<i>Enterobacter cloacae</i> (ATCC# 13047)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
6	<i>Enterobacter</i> species (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
7	<i>Enterococcus faecalis</i> (ATCC# 29212)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
8	<i>Enterococcus faecalis</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
9	<i>Enterococcus faecium</i> (ATCC# 19434)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
10	<i>Enterococcus faecium</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
11	<i>Escherichia coli</i> (ATCC# 11229)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
12	<i>Escherichia coli</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
13	<i>Escherichia coli</i> (ATCC# 25922)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
14	<i>Escherichia coli</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB

TABLE I (continued)

No.	Microorganism Species	Incubation Time (Inoculum Only)	Incubation Temperature (Inoculum Only)	Media
15	<i>Haemophilus influenzae</i> (ATCC# 19418)	24 - 96 Hours	35° ± 2°C	CAE/MHAD-XV/MHB-XV
16	<i>Haemophilus influenzae</i> (Clinical Isolate)	24 - 96 Hours	35° ± 2°C	CAE/MHAD-XV/MHB-XV
17	<i>Klebsiella oxytoca</i> (ATCC# 43165)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
18	<i>Klebsiella</i> species (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
19	<i>Klebsiella pneumoniae</i> (ATCC# 11296)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
20	<i>Klebsiella pneumoniae</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
21	<i>Micrococcus luteus</i> (ATCC# 7468)	24 - 48 Hours	30° ± 2°C	TSB/TSA/MHB
22	<i>Micrococcus</i> species (Clinical Isolate)	24 - 48 Hours	30° ± 2°C	TSB/TSA/MHB
23	<i>Proteus mirabilis</i> (ATCC# 7002)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
24	<i>Proteus mirabilis</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
25	<i>Pseudomonas aeruginosa</i> (ATCC# 15442)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
26	<i>Pseudomonas aeruginosa</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
27	<i>Pseudomonas aeruginosa</i> (ATCC# 27853)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
28	<i>Pseudomonas aeruginosa</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
29	<i>Serratia marcescens</i> (ATCC# 14756)	24 - 48 Hours	30° ± 2°C	TSB/TSA/MHB
30	<i>Serratia marcescens</i> (Clinical Isolate)	24 - 48 Hours	30° ± 2°C	TSB/TSA/MHB

TABLE I (continued)

No.	Microorganism Species	Incubation Time (Inoculum Only)	Incubation Temperature (Inoculum Only)	Media
31	<i>Staphylococcus aureus</i> (ATCC# 6538)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
32	<i>Staphylococcus aureus</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
33	<i>Staphylococcus aureus</i> (ATCC# 29213)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
34	<i>Staphylococcus aureus</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
35	<i>Staphylococcus epidermidis</i> (ATCC# 12228)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
36	<i>Staphylococcus epidermidis</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
37	<i>Staphylococcus haemolyticus</i> (ATCC# 29970)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
38	<i>Staphylococcus haemolyticus</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
39	<i>Staphylococcus hominis</i> (ATCC# 27844)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
40	<i>Staphylococcus hominis</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
41	<i>Staphylococcus saprophyticus</i> (ATCC# 15303)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
42	<i>Staphylococcus saprophyticus</i> (Clinical Isolate)	24 - 48 Hours	35° ± 2°C	TSB/TSA/MHB
43	<i>Streptococcus pneumoniae</i> (ATCC# 6303)	24 - 96 Hours	35° ± 2°C	SBA/CAMHB-B
44	<i>Streptococcus pneumoniae</i> (Clinical Isolate)	24 - 96 Hours	35° ± 2°C	SBA/CAMHB-B
45	<i>Streptococcus pyogenes</i> (ATCC# 19615)	24 - 48 Hours	35° ± 2°C	BHIB/BHIA/ CAMHB-B

TABLE I (continued)

No.	Microorganism Species	Incubation Time (Inoculum Only)	Incubation Temperature (Inoculum Only)	Media
47	<i>Candida albicans</i> (ATCC# 10231)	48 - 72 Hours	30° ± 2°C	TSB/SDA/MHB
48	<i>Candida albicans</i> (Clinical Isolate)	48 - 72 Hours	30° ± 2°C	TSB/SDA/MHB
49	<i>Candida tropicalis</i> (ATCC# 750)	48 - 72 Hours	30° ± 2°C	TSB/SDA/MHB
50	<i>Candida tropicalis</i> (Clinical Isolate)	48 - 72 Hours	30° ± 2°C	TSB/SDA/MHB

Note: Incubation times are nominal, but in practice, incubation will continue until good growth is observed.

13.0 REFERENCE:

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

14.0 FINAL REPORT:

A Final Report will be issued and will include the following: Purpose and Scope of Study, Test Materials, Equipment, Supplies, Media, Test Methods, Dates of Initiation and Completion of Testing, Exceptional Conditions, Results, and Data Sheets.

15.0 EXCEPTIONAL CONDITIONS:

Sponsor will be notified immediately by telephone and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (See Proposal/Contract).

16.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Inc. at its laboratory facility in Bozeman, Montana. All raw data for this program will be retained in safe storage by Company for a period of three (3) years.

17.0 ACCEPTANCE:

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF ONE PRODUCT WHEN CHALLENGED WITH FIFTY MICROORGANISM STRAINS USING THE MACRODILUTION BROTH METHOD

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

President
and CEO:

Daryl S. Paulson
Daryl S. Paulson, Ph.D.

7-12-00

Date

Manager of
In-Vitro
Laboratory/
Principal
Study Director:

Teri Eastman
Terri Eastman

07-12-00

Date of Study Initiation

Associate
Study Director:

James McDowell
James McDowell

7.12.00

Date

Director of
Quality
Assurance:

John A. Mitchell
John A. Mitchell, Ph.D.

7/11/00
Date

LONZA, INC. (SPONSOR)
79 Route 22 East
Annandale, New Jersey 08801

Mike Conway
Representative

7/19/00

Date

Team Leader, Disinfection
Title

PROTOCOL AND/OR SOP DEVIATION RECORDING FORM

PROTOCOL NUMBER: 000622 / 000608 09/21/00

SOP NUMBER: N/A

RECORDED BY: [Signature]

DATE: 08/28/00

PROCEDURE AS OUTLINED IN THE PROTOCOL AND/OR SOP: Protocol Table I states that Staphylococcus saprophyticus (ATCC # 15303) would be evaluated.

DEVIATION FROM PROCEDURE: Staphylococcus saprophyticus (ATCC # 15305) was evaluated, not ATCC # 15303.

REASON FOR DEVIATION AND EFFECT ON STUDY OUTCOME: Staphylococcus saprophyticus (ATCC # 15305) does not exist. This was included in the Protocol due to a typographical error.

The use of ATCC # 15305 does not have an adverse effect on the study outcome. No specific ATCC strain for this species is required by the Tentative Tentative Final Monograph, Federal Register, Vol. 59, No. 116, 08/15/94 June 17, 1994, Proposed Rules, Page 31444.

ACKNOWLEDGMENTS:

[Signature]
STUDY DIRECTOR

08/28/00
DATE

[Signature]
STUDY MANAGEMENT

8/28/00
DATE

[Signature]
QUALITY ASSURANCE

08/28/00
DATE

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1999 by BioScience Laboratories, Inc.

Form No. 99-OA-004
Rev. 0 11/99

EFFECTIVE
11/11/99 [Signature]

PROTOCOL AND/OR SOP DEVIATION RECORDING FORM

PROTOCOL NUMBER: 000622 / 000622

SOP NUMBER: N/A

RECORDED BY: [Signature]

DATE: 08/28/00

PROCEDURE AS OUTLINED IN THE PROTOCOL AND/OR SOP: Protocol Table I
Specifies that Mueller-Hinton Agar with Dextrose and Bacto Supplement B
(MHAD-XV) would be used as a growth medium for the population recoveries
for Haemophilus influenzae (ATCC #19418 and Clinical Isolate).

DEVIATION FROM PROCEDURE: Chocolate Agar with Enrichment (CAE) was
used as the growth medium for the population recoveries for
Haemophilus influenzae (ATCC #19418 and Clinical Isolate).

REASON FOR DEVIATION AND EFFECT ON STUDY OUTCOME: The Mueller-Hinton Agar
with Dextrose and Bacto Supplement B did not exhibit acceptable growth
of these species.

Chocolate Agar with enrichment is used to prepare the inocula for these
species, and acceptable growth was observed for the inocula as
well as for the population recoveries.

Therefore, there is no adverse effect on the Study outcome due to this deviation.

ACKNOWLEDGMENTS:

[Signature]
STUDY DIRECTOR

08/28/00
DATE

[Signature]
STUDY MANAGEMENT

8/28/00
DATE

[Signature]
QUALITY ASSURANCE

08/28/00
DATE

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

© Copyright 1999 by BioScience Laboratories, Inc.

Form No. 99-0A-004
Rev. 0 11/99

EFFECTIVE

PROTOCOL AND/OR SOP DEVIATION RECORDING FORM

PROTOCOL NUMBER: 000622

SOP NUMBER: _____

RECORDED BY: [Signature]

DATE: 8/28/00

PROCEDURE AS OUTLINED IN THE PROTOCOL AND/OR SOP: Protocol Table I, pages 10 and 11,
does not list Microorganism Species No. 46, Streptococcus pyogenes
(Clinical Isolate).

(100) 16 8-28-00

DEVIATION FROM PROCEDURE: Streptococcus pyogenes (Clinical Isolate),
Should be included in Table I, as Microorganism Species No. 46.
It is included in the Scope of the testing, as detailed in Section 6.0, page 4.

REASON FOR DEVIATION AND EFFECT ON STUDY OUTCOME: Typographical error --
inadvertent deletion from Table I only. There is no adverse effect
on the study, since this microorganism was evaluated as intended
by the scope of the study.

ACKNOWLEDGMENTS:

[Signature]
STUDY DIRECTOR

8/28/00
DATE

[Signature]
STUDY MANAGEMENT

8/28/00
DATE

[Signature]
QUALITY ASSURANCE

8/28/00
DATE

EFFECTIVE
11/11/00 [Signature]

PROTOCOL AND/OR SOP DEVIATION RECORDING FORM

PROTOCOL NUMBER: 000622/000608 @mm 9/19/00

SOP NUMBER: NA

RECORDED BY: [Signature]

DATE: 09/15/00

PROCEDURE AS OUTLINED IN THE PROTOCOL AND/OR SOP: Protocol Table I states that BHIB and BHIA (Brain Heart Infusion Broth and Brain Heart Infusion Agar) will be used for inoculum preparation of Streptococcus pyogenes (ATCC #19615).

DEVIATION FROM PROCEDURE: TSA w/ 5% Sheep Blood (SBA) was used for the preparation of the inoculum for Streptococcus pyogenes (ATCC #19615) for the testing performed on 09/05/00.

REASON FOR DEVIATION AND EFFECT ON STUDY OUTCOME: To ensure culture purity by visual determination of beta-hemolysis, the preparation of Streptococcus pyogenes (ATCC #19615) and inoculum into TSA-15-00 w/ 5% sheep blood was performed using Tryptic Soy Agar w/ 5% Sheep Blood. Since a pure culture with adequate growth for testing was obtained, this deviation has no adverse effect on the outcome of the study.

ACKNOWLEDGMENTS:

[Signature]
STUDY DIRECTOR

09/15/00
DATE

[Signature]
STUDY MANAGEMENT

9/15/00
DATE

[Signature]
QUALITY ASSURANCE

9/15/00
DATE

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1999 by BioScience Laboratories, Inc.

EFFECTIVE
11/19/99 [Signature]

ADDENDUM II

Log of Samples (Form No. 92-L-023)
Sample Tracking Forms (Form No. 93-L-029)
Certificate of Analysis
MSDS

Protocol No. 600622

LOG OF SAMPLES

Sponsor: LONZA INC Date Received: 7/19/00 Received By: [Signature]

Shipping Information: FED EX Number of Products Received: 1 Condition Received: Intact

Sponsor Sample Submission Form: Yes No

SDS Form: Yes No Precautions: None Unknown See Below

Storage Conditions: Room Temp

Quantity	Product Lot Number	Expiration Date	Product Description	Initial Weight
1	100037 (4022-S)	7/17/00 7/17/01	BENZETHONIUM CHLORIDE, USP	166.6g
N/A 9-15-00				

Reviewed by: [Signature]

Date: 9-15-00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

© Copyright 1996 by BioScience Laboratories, Inc.

Form No. 92-L-023
 Rev. 4 07/98

EFFECTIVE

SAMPLE TRACKING FORM

Protocol No. 00022

Date Received: 7/19/00 Received By: [Signature] Sponsor: LORETTA PEE

In Test Date: 8/15/00 Out of Test Date: 9-15-00 Product No.: 1 * Sample Lot No.: 100037 (40225)

Product Name/Description: BENZOTITANIUM CHLORIDE

Sample Number	Date/Time Out/ Initials	Purpose	Initial Quantity	Quantity Used	Quantity Returned	Date/Time In/ Initials
1	TZ 8/15/00 8:25 AM	Testing (MIC)	166.6g	4.8g	161.8g	9-2:00p 8:15:00
1	TZ 8/16/00 8:10 AM	Testing (MIC)	161.8g	5.7g	156.1g	cm 8/16/00 4:36 pm
1	TZ 8-17-00 7:45 AM	Testing (MIC)	156.1g	5.0g	151.1g	cm 8/17/00 11:32 PM
1	TZ 8-18-00 8:07 AM	Testing (MIC)	151.1g	5.0g	146.1g	cm 8/18/00 2:02 PM
1	9-8:300 8:20m	Testing mic	146.1g	5.0g	141.1g	BU 4:30pm 8/18/00 8/20/00 8/20/00 (M) 9/1/00
1	9-8:450 9:4:00	Testing mic	141.1g	5.0g	136.1g	cm 9/8/00 3:35 pm
N/A TZ 9-15-00						

Reviewed By: [Signature]

Date: 9/19/00

* Product No. (if any), as listed in Protocol

EFFECTIVE
4/30/97 [Signature]

Mike Douglas
BioScience Laboratories Inc.
300 North Willson
Bozeman Montana, 59771-0190
406-587-5735

July 18, 2000

Dear Mike,

Enclosed is the paperwork for a sample of Lonzagroup Benzethonium Chloride USP to be tested for minimum inhibitory concentration (MIC). The test conditions are summarized below:

Regulatory Status	GLP (40CFR 160)
Technical Service Number	00-1096
Study Protocol	#000622
Purchase Order Number	213853
Test Substance Name	Benzethonium Chloride USP
TRCS # (Lot #)	100037 (4022-S)
Dilution	Macrodilution broth method
Diluent	DI water
Contact Time	Per protocol
Test Organisms	50 microorganism strains, [see BioScience Lab study protocol #000622]

The Technical Service Number will be used to track the study, so please include these as identifiers in the report. If you should have any questions, please fax me at 908-730-1575.

Sincerely,



Joe Scheblein
Study Monitor

cc: Lonza QAU

Lonza Inc
Clinton
79 Route 22 East Tel 908 730 1500
PO Box 993 Fax 908 730 1546
Annandale, NJ 08801, USA www.lonzagroup.com

Five Chemicals and Cosmetics

Lonzagroup

Addendum 4 Date 9/21/00
Page 4 of 2
Protocol # 000622

Mike Douglas
BioScience Laboratories Inc.
300 North Willson
Bozeman Montana, 59771-0190
406-587-5735

July 18, 2000

000622

RE: Sample Shipment/Chain of Custody Documentation

Dear Mike,

The following sample was shipped on, 7/18/00 Lonzagroup Benzethonium Chloride USP (TS# 00-1096):

Formulation	TRCS #	Gross Weight
Benzethonium Chloride USP	100037	165.9g

Samples will be used for experimental use only in the following testing program:

**Minimum Inhibitory Concentration (MIC) Test against 50 microorganisms
using the Macrodilution Broth Method.**

The materials scheduled for testing were characterized at the sponsor's facility under a program complying with Good Laboratory Practices as set forth in 40 CFR 160. The documentation and records supporting the characterization are archived at the Sponsor's facilities.

In order to maintain records of sample custody please complete the bottom of this letter and return a signed copy to me at the above address upon receipt of the samples.

Should you have any problems with the shipment please call Lonza immediately at 908-730-1500.

Sincerely,



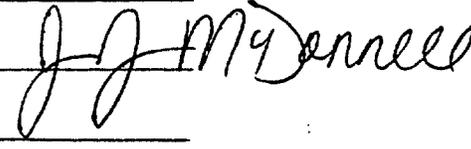
Joe Scheblein
Study Monitor

cc: Lonzagroup QAU, Lonza Archives

Chain of Custody Section

The sample(s) cited above were received on (date): 7/19/00

In good/poor (describe) condition: Intact

Name of recipient (print/sign): Jessica J McDonnell 

Title: manager of Project Quality

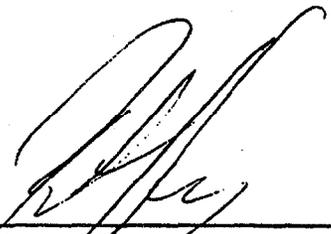
Lonza Inc
Clinton
79 Route 22 East Tel 908 730 1500
PO Box 993 Fax 908 730 1546
Annandale, NJ 08801, USA www.lonzagroup.com

CERTIFICATE OF ANALYSIS

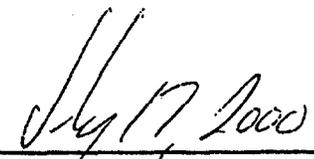
Benzethonium Chloride, USP

Identification Number: 100037

<u>ANALYSIS</u>	<u>RESULTS</u>
Quaternary Ammonium Chloride (EW 448.1)	99.0%
Appearance	White Powder
Expiration Date:	July 17, 2001



 Robert J. Sloan
 Research Associate / Study Director, Technical Services
 Specialty Chemicals Research & Development



 Date

Reference: Product Registration Study SP-00061-A

This testing was performed according to the requirements of FIFRA Good Laboratory Practice Standards (40 CFR Part 160). Data supporting this document will be maintained in the archive at the Annandale, New Jersey, facility of Lonzagroup.

C:\Current Work\Documentation\Certificates Of Analysis\100037.Doc

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

Health : 5
Flammability : 1
Reactivity : 0

95972 Benzethonium chloride USP

PAGE 1 OF 7

MATERIAL	DATE ISSUED	DOT HAZARD CLASSIFICATION
Benzethonium chloride USP	04/17/00 - Rev.	Non-hazardous
CAS NO. 121-54-0	SUPERSEDES	DOT SHIPPING NAME
	06/26/89	Not regulated
FORMULA C ₂₇ H ₄₂ ClNO ₂ ·H ₂ O		DOT LABEL None

CHEMICAL NAME Diisobutylphenoxyethoxyethyl dimethylbenzyl ammonium chloride monohydrate

***** I - INGREDIENTS *****

	APPROXIMATE WEIGHT %	TWA/TLV
Diisobutylphenoxyethoxyethyl dimethylbenzyl ammonium chloride monohydrate (CAS No. 121-54-0)	100	None established

***** II - PHYSICAL AND CHEMICAL PROPERTIES *****

APPEARANCE White free-flowing powder	pH 8-10 (5% aqueous solution)
VISCOSITY Not applicable	ODOR Odorless
BOILING POINT Not known	MELTING OR FREEZING POINT 329°F
VAPOR DENSITY (Air=1) Not known	VAPOR PRESSURE (mm Hg) Not applicable
PERCENT VOLATILE (by weight) 4% (water)	SOLUBILITY IN WATER Soluble
EVAPORATION RATE (Butyl Acetate=1) Not known	BULK DENSITY 27.5 lb/ft ³

***** III - FIRE AND EXPLOSION INFORMATION *****

FLASH POINT Not known	AUTO IGNITION TEMPERATURE 716°F
LOWER EXPLOSION LIMIT (%) Not applicable	UPPER EXPLOSION LIMIT (%) Not applicable
EXTINGUISHING MEDIA FOAM	ALCOHOL FOAM CO ₂ X
DRY CHEMICAL X	WATER X OTHER

SPECIAL FIRE FIGHTING PROCEDURES:

Must wear NIOSH/MSHA approved self-contained breathing apparatus and protective clothing. Cool fire-exposed containers with water spray.

UNUSUAL FIRE AND EXPLOSION HAZARDS:

Products of combustion are toxic. As with most powdered organic compounds, dust explosions may be possible.

***** IV - HEALTH EFFECTS INFORMATION *****

ROUTES OF ENTRY - SKIN CONTACT X	EYE CONTACT X
INHALATION X	INGESTION

The information provided herein is compiled from internal reports and data from professional publications. IT IS FURNISHED WITHOUT WARRANTY OF ANY KIND, EXPRESSED OR IMPLIED. It is intended to assist in evaluating the suitability and proper use of the material in manufacturing and in the development and implementation of safety precautions and procedures.

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

Health : 3
 Flammability : 1
 Reactivity : 0

5972 Benzethonium chloride USP

PAGE 2 OF 7

***** IV - HEALTH EFFECTS INFORMATION (continued) *****

EFFECTS OF OVEREXPOSURE

Based upon the results of animal toxicity studies, it is anticipated that this material will be toxic if ingested, and direct contact will produce severe skin and eye irritation, which, upon prolonged exposure, may produce chemical burns with possible irreversible damage.

OVEREXPOSURE MAY AGGRAVATE EXISTING CONDITIONS:

No effects indicated.

EMERGENCY AND FIRST AID PROCEDURES:

Eyes: Flush eyes with large amounts of running water for at least 15 minutes. Hold eyelids apart to ensure rinsing of the entire surface of the eye and lids with water. Get immediate medical attention. If physician not available, flush for additional 15 minutes and then transport victim to medical care.

Skin: Wash with large amounts of running water, and soap if available, for 15 minutes. Remove contaminated clothing and shoes. Get immediate medical attention. Wash clothing and decontaminate shoes before reuse.

Ingestion: If swallowed, immediately give 3-4 glasses of milk (if unavailable, give water). DO NOT induce vomiting. If vomiting occurs, give fluids again. Get immediate medical attention. Have physician determine if patient's condition allows for induction of vomiting or evacuation of the stomach. Do not give anything by mouth to a convulsing or unconscious person.

Inhalation: Remove from area to fresh air. If not breathing, clear airway and start artificial respiration. If victim is having trouble breathing, give supplemental oxygen, if available. Get immediate medical attention.

NOTE TO PHYSICIAN:

Acute effects may include mucosal damage, severe laryngeal edema and shock associated with corrosive agents. Alcohol can increase toxic effects. Delayed effects may include life threatening respiratory paralysis and convulsions.

CHEMICALS LISTED AS CARCINOGEN BY:

NATIONAL TOXICOLOGY PROGRAM - No
 I.A.R.C. MONOGRAPHS - No
 OSHA - No

***** V - REACTIVITY INFORMATION *****

STABILITY: STABLE X CONDITIONS TO AVOID
 UNSTABLE None known

HAZARDOUS DECOMPOSITION PRODUCTS

Thermal decomposition may produce toxic vapors/fumes of amines and other organic materials, and oxides of carbon and nitrogen.

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Health : 3
Flammability : 1
Reactivity : 0

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

95972 Benzethonium chloride USP

PAGE 3 OF 7

***** V - REACTIVITY INFORMATION (continued) *****

HAZARDOUS POLYMERIZATION

MAY OCCUR	WILL NOT X	CONDITIONS TO AVOID
	OCCUR	None known

INCOMPATIBILITY (MATERIALS TO AVOID)

WATER	OTHER X	Strong oxidizing or reducing agents, anionic surfactants
-------	---------	--

***** VI - SPILL AND DISPOSAL INFORMATION *****

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED

Warning! Dusts may be explosive. If wet, floors may become slippery. Wear appropriate protective gear and respiratory protection where dusts or airborne particulates of unknown concentrations may be generated (self-contained breathing apparatus preferred for large spills).

Carefully shovel spills into appropriate containers for disposal. Avoid generation of dust. To remove residue, wet with water, absorb with sand or vermiculite and place in compatible container for disposal. Keep out of sewers and open bodies of water.

WASTE DISPOSAL METHODS

Dispose of in compliance with all Federal, state and local laws and regulations. Incineration is the preferred method.

CONTAINER DISPOSAL

Completely empty liner by shaking and tapping sides and bottom to loosen clinging particles. Empty residue into application equipment. Then dispose of liner in a sanitary landfill or by incineration if allowed by State and local authorities. If drum is contaminated and cannot be reused, dispose of in the same manner.

***** VII - PERSONAL PROTECTION INFORMATION *****

VENTILATION TYPE

In processes where dusts or airborne particulates may be generated, proper ventilation must be provided in accordance with good ventilation practices.

RESPIRATORY PROTECTION

In processes where dusts or airborne particulates may be generated, a NIOSH/MSHA jointly approved respirator is advised in the absence of proper environmental controls.

PROTECTIVE GLOVES

Rubber or neoprene, to prevent skin contact.

EYE PROTECTION

Wear chemical goggles where there is a potential for eye contact. Use safety glasses with side shields under normal use conditions.

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtree)

Health : 3

Flammability : 1

Reactivity : 0

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

5972 Benzethonium chloride USP

PAGE 4 OF 7

***** VII - PERSONAL PROTECTION INFORMATION (continued) *****

OTHER PROTECTIVE EQUIPMENT

Eye wash; safety shower; protective clothing (long sleeves, coveralls or other, as appropriate), to prevent skin contact.

***** VIII - STORAGE AND HANDLING *****

PRECAUTIONS FOR STORAGE AND HANDLING:

Maximum storage temperature: 140°F. Do not contaminate drinking water, food or feed by storage or disposal. Keep containers closed until used.

***** IX - TOXICOLOGY INFORMATION *****

TOXICITY**ACUTE**

- oral LD₅₀ (rat): 295 mg/kg; 420 mg/kg (2 tests)
- dermal LD₅₀ (rabbit): 3000 mg/kg
- eye irritation (rabbit): Mild to moderate irritant (1% active)
- skin irritation (rabbit): Severe irritant
- skin corrosivity (rabbit - DOT method): Not corrosive
- skin irritation (human): Application of 5 % solution to the skin for 48 hours under occlusion caused irritation characterized by redness. (CTFA CIR Report)
- skin irritation (human): 0.1 to 0.2% as a tincture, aqueous solution or aerosol, was non-irritating (CTFA CIR Report)
- contact sensitization (mouse): Application of 1, 3 or 10% in ethanol for induction followed by a challenge with a 20 % solution. No sensitization.
- delayed contact sensitization - (guinea pig - Magnusson-Kligman Maximization test): A 1% aqueous solution found to be the maximum non-irritating concentration under these test conditions. Intra-dermal injection of 1% aqueous solution alone or with Freund' Adjuvant as challenge followed 1 week later by topical application of the 1% aqueous solution did not produce delayed contact sensitization.

GENOTOXICITY/MUTAGENICITY

- mutagenicity - (Salmonella - Ames test): Not mutagenic in various strains of Salmonella with or without metabolic activation (CTFA CIR Report).
- mutagenicity - (CHO cells - Sister Chromatid Exchange) Not mutagenic/clastogenic with or without metabolic activation (NTP Report).
- mutagenicity - (CHO cells - Chromosomal Aberration) Not mutagenic/clastogenic with or without metabolic activation (NTP Report)

SUBACUTE

- 28 Day feeding study (rat): Rats were given feed containing either 0, 20, 100, 500 or 2500 ppm active. The NOAEL was 100 ppm. No histological changes were observed at any exposure level. Increased levels of SGPT and SGOT seen in high dose exposure group. Increased levels of plasma inorganic P noted in the 500 and 2500 ppm dose groups.
- 4 Week dermal toxicity (rabbit): Daily application of 0.1%, five days/week for 4 weeks did not produce systemic effects (CTFA CIR Report).

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Page 12 of 12
Health Protocol # 3 000622
Flammability: 1
Reactivity: 0

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

95972 Benzethonium chloride U.S.P.

PAGE 5 OF 7

***** IX - TOXICOLOGY INFORMATION (continued) *****

TOXICITY (continued):

SUBCHRONIC

- 13 Week dermal toxicity (rat & mouse): Rats and mice were topically dosed with either 0, 1.56, 3.13, 6.25, 12.5 or 25 mg/kg 5-days/week for 13-weeks. Mean body weight gains for high dose level males (rats & mice) were significantly lower than controls. Clinical findings included: epithelial hyperplasia at the site of contact for all groups (rats & mice) receiving the active material; irritation and inflammation was noted at the site of application for all groups of mice and those groups of rats administered 3.13 mg/kg or greater; ulceration was noted in male and female rats administered 3.13 mg/kg or greater and thickening of the skin, scales and/or discoloration was noted in male mice administered 6.25 mg/kg or greater and female mice administered 12.5 mg/kg or greater (NTP Report).

REPRODUCTIVE/DEVELOPMENTAL

- Developmental toxicity (rat): Rats were orally gavaged with exposure doses of either 0, 10, 30, 100 or 170 mg/kg/day from day 6 through 15 of gestation. Some adverse reproductive/developmental effects were noted, including decreased body weight gains. The NOAEL for developmental effects was = 170 mg/kg/day. The NOAEL for maternal effects was = 100 mg/kg/day.

CHRONIC

- 2 Year dermal study (rat & mouse): Not carcinogenic when topically administered at exposure doses of 0, 0.15, 0.5 and 1.5 mg/kg for 5-Days/Week for 104-Weeks. Exposure of rats and mice by dermal application for 2-Years resulted in epithelial hyperplasia in male and female rats and mice and sebaceous gland hyperplasia and ulcers in female rats at the site of application (NTP Report).
- 2 Year dietary study (rat): Not carcinogenic in rats when administered at levels of 0, 50, 200, 1000, 2000 and 5000 ppm. With the exception of a significant increase in mortality in the 5000 ppm exposure group, no significant clinical findings were noted. (CTFA CIR Report)

***** X - MISCELLANEOUS AND REGULATORY INFORMATION *****

FEDERAL LEVEL REGULATIONS:

TOXIC SUBSTANCES CONTROL ACT (TSCA INVENTORY) STATUS:

This product is currently listed on U.S. EPA TSCA 8(b) inventory list.

TSCA Section 12(b) Export Notification

Components present in this product which, if exported, could require either annual or one-time reporting under this regulation are as follows:

ONE-TIME REPORTING REQUIREMENT

Chemical Name

CAS Number

Typical Maximum Concentration

None known

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

5972 Benzethonium chloride USP

PAGE 6 OF 7

Page 11 of 12
Health Protocol # 3 000622
Flammability: 1
Reactivity: 0

***** X - MISCELLANEOUS AND REGULATORY INFORMATION *****

FEDERAL LEVEL REGULATIONS (continued):

US FDA Regulations on Pharmaceuticals:

This product is regulated by the FDA as a pharmaceutical ingredient. This Product meets the specifications of the United States Pharmacopeia (USP).

US EPA Regulations on Pesticides:

This product is an EPA FIFRA registered pesticide. EPA Registration No. 6836-97. This product can be used commercially only in the EPA registered application(s) noted on the product label.

CERCLA (Comprehensive Environmental Response, Compensation and Liability Act of 1980 requires notification of the National Response Center (Telephone 800-424-8802) in the event of a release of quantities of the following hazardous materials contained in this product, if the release is equal to or greater than the Reportable Quantities (RQs) listed in 40 CFR 302.4:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
None known		

SARA Title III, Sections 302/304 (Superfund Amendments and Reauthorization act of 1986) - This act requires emergency planning, including agency notification, for possible release of the following components of this material, based upon the Threshold Planning Quantities (TPQs) and release Reportable Quantities (RQs) listed for the Components in 40 CFR 355:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
None known		

SARA Title III Sections 311/312 - This act requires reporting under the Community Right-to-Know provisions due to the inclusion of the following components of this material in one or more of the five hazard categories listed in 40 CFR 370:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Hazard *) Categories</u>
Diisobutylphenoxyethoxyethyl dimethylbenzyl ammonium chloride monohydrate	121-54-0	A

*) The five hazard categories are as follows: F= FIRE HAZARD; S= SUDDEN RELEASE OF PRESSURE; R= REACTIVE; A= IMMEDIATE (ACUTE) HEALTH HAZARD; C= DELAYED (CHRONIC) HEALTH HAZARD

SARA Title III Section 313 - This act requires submission of annual reports of releases of the following components of this material if the threshold reporting quantities, as listed in 40 CFR 372, are met or exceeded:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
None known		

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONE: 800-424-9300 (Chemtrec)

Lonza Inc. Fine Chemicals and Specialties

17-17 Route 208 Fair Lawn, NJ 07410 800-777-1875 (9am - 5pm) 309-697-7200 (After 5pm)

95972 Benzethonium chloride USP

PAGE 7 OF 7

***** X - MISCELLANEOUS AND REGULATORY INFORMATION (continued) *****

Health : 3
 Flammability : 1
 Reactivity : 0

STATE RIGHT-TO-KNOW REGULATIONS:

CALIFORNIA PROPOSITION 65 - Components present in this material which the State of California has found to cause cancer, birth defects or other reproductive harm are as follows:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
Benzene	71-43-2	2 ppm
Benzyl chloride	100-44-7	10 ppm
Bis(2-chloroethyl) ether	111-44-4	10 ppm
N-Nitrosodiethanolamine	1116-54-7	1 ppm
Propylene oxide	75-56-9	10 ppm
Toluene	108-88-3	Trace

MASSACHUSETTS Right-to-Know - The following components of this material are included in the Massachusetts Substance List and are present at or above reportable levels:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
Benzene	71-43-2	2 ppm
Benzyl chloride	100-44-7	10 ppm
Bis(2-chloroethyl) ether	111-44-4	10 ppm
N-Nitrosodiethanolamine	1116-54-7	1 ppm
Propylene oxide	75-56-9	10 ppm

MICHIGAN Critical Materials - The following components of this material are included in the Michigan Critical Materials List:

<u>Chemical Name</u>	<u>CAS Number</u>
None known	

NEW JERSEY Right-to-Know - The following components of this material are included in the New Jersey Hazardous Substance List and are present at or above reportable levels:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
None known		

PENNSYLVANIA Right-to-Know - The following components of this material are included in the Pennsylvania Hazardous Substance List and are present at or above reportable levels:

<u>Chemical Name</u>	<u>CAS Number</u>	<u>Typical Maximum Concentration</u>
None known		

ADDENDUM III

Media/Diluent Tracking Forms (Form No. 97-L-007)
Media Production and Growth Testing Data Sheets (Form No. 91-L-003)

Protocol No. 000622

MEDIA/DILUENT TRACKING FORM

Test Date: 8/15/00 Procedure: Testing - MIC (15 bugs)
 (Unoc. Prep: BH18001107A; BH1A001020G; TSB001012A; TSA001014A; TSA w/Sheep Blood; SDA001020B; PML lot # 66723-1 Exp. Oct 03, 2000)
 Media/Diluent Lot Numbers: PBS001013D; MHB001109B; CaMHB001020D
TSA001014A; SDA001020B; TSA001013A; BH1A001020G; BH1A001104C; TSA w/Sheep Blood PML lot # 66723-1 Exp. 10-03-00
 Supplement Lot Numbers (if any): SP Blood Supplement Difco lot # 143812KA Exp. 10-31-00
 Negative Controls Checked (Y/N): y-neg Recorded by: Initials TR Date 8/15/00

Test Date: 8/16/00 Procedure: Testing - MIC (16 bugs)
 (Unoc. Prep: TSA w/Sheep Blood; PML lot # 66723-1 Exp. 10-03-00; SB000914A; SB001020E; TSB001012A; TSA001014A)
 Media/Diluent Lot Numbers: PBS001013D; CaMHB001020D; AMIC001104E
MHB001109B; TSA001013A; TSA001014A; SA001020C; TSA w/Sheep Blood PML lot # 66723-1 Exp. 10-03-00
 Supplement Lot Numbers (if any): SP Blood Supplement (Difco) lot # 143812KA Exp. 10-31-00
 Negative Controls Checked (Y/N): y-neg Recorded by: Initials TR Date 8/16/00

Test Date: 8-17-00 Procedure: Testing - (MIC - 16 bugs)
 (Unoc. Prep: CAE, PML lot # 68175-1, Exp. 10-3-2000, TSB001012A, TSA001014A)
 Media/Diluent Lot Numbers: MHB001109B; PBS001013D; MHB001109B; CAE 65533-1 Exp. 4/25/00
CAE 68175-1 Exp. 10/3/00; TSB001012A; TSA001014A; TSA001017A
 Supplement Lot Numbers (if any): Supplement VX (Bacto) Control # 140556KA Exp. 30 APR 02
 Negative Controls Checked (Y/N): y-neg Recorded by: Initials cm TR Date 8/17/00

Reviewed By: [Signature] Date: 9/15/00

Approved By: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 07-1-007
New 05/97

MEDIA/DILUENT TRACKING FORM

Test Date: 8/26/00 Procedure: MIC - 3 bugs
 (Inoc. Prep: TSA001012A, TSA001014A) ^{TS 8/25/00}
 Media/Diluent Lot Numbers: TSA001014A, TSB00012A, DBS10TSD, PBS001013D
MHB001109B ^{TS 8/25/00}
 Supplement Lot Numbers (if any): N/A
 Negative Controls Checked (Y/N): Y Recorded by: Initials cm Date 8/26/00

Test Date: 8-31-00 Procedure: Retest - P.mirabilis #7002 and S.pyogenes #19615
^{BH1A 001104C}
 Media/Diluent Lot Numbers: MHB 001109B, CaMHB 001020D, PBS 001013D, TSA 001017A
^{TS 8/25/00}
 Inoc prep: TSA 001122A, TSB 001012A, BH1B 001103A, BH1A 001104C
 Supplement Lot Numbers (if any): Sp Blood Supplement Difco lot 143115KA Exp. 30 Sep 00
 Negative Controls Checked (Y/N): Y (-) Recorded by: Initials J Date 8-31-00
^{TS 8-31-00}

Test Date: 8-9 9-8-00 Procedure: Testing (MIC) (retest S.pyogenes 19615)
⁹⁻⁸⁻⁰⁰
 Media/Diluent Lot Numbers: CaMHB 001201C, PBS 001116E, (Inoc. Prep. Only PML lot 68375-1 TSA w/5% Sheep Blood Exp Oct 25 2000)
^{BH1B 001102A 9-18-00 BH1B 001104C 9-18-00}
 Supplement Lot Numbers (if any): SP Blood Supplement Difco lot 143115KA Exp. 30 SEP 00
 Negative Controls Checked (Y/N): Y (-) Recorded by: Initials J Date 9-8-00

Reviewed By: _____ Date: 9/15/00

Approved By: J J Donnell Date: 9/19/00

Notice: Proprietary Information - Not for Publication. Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

original
in 000725

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/12/00 Media Type: Tryp Soy Broth

Lot Number: TSB 001012A Expiration Date: 10/12/00 Prepared by:

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioReo	Tryp Soy Broth	DCM98030m	12/9/02	195g/6.5L
N/A				
7/12/00				
 				

Deionized H₂O Volume: 6.5L

Dispensed In: 6-2LF/flasks 1-1LF/flask

Volume per Container: 1000mls, 500mls

Date Autoclaved: 7/12/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
<u>7/12/00</u>	<i>Escherichia coli</i>	11229	+1+	930712	30±2°C	<u>7/14/00</u>
<u>7/12/00</u>	<i>Staphylococcus aureus</i>	6538	+1+	930712	30±2°C	<u>7/14/00</u>
<u>7/12/00</u>	<i>Serratia marcescens</i>	14756	+1+	930712	30±2°C	<u>7/14/00</u>
<u>7/12/00</u>	<i>Klebsiella pneumoniae</i>	10031	+1+	930712	30±2°C	<u>7/14/00</u>
<u>7/12/00</u>	<i>Pseudomonas aeruginosa</i>	9027	+1+	930712	30±2°C	<u>7/14/00</u>
<u>7/12/00</u>	<i>Staphylococcus epidermidis</i>	12228	+1+	930712	30±2°C	<u>7/14/00</u>
N/A			1	930712	30±2°C	7/14/00
<u>7/12/00</u>	Negative Controls	N/A	-1-	930712	30±2°C	<u>7/14/00</u>

pH of Media: 7.1 Autoclave No.: 91127 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: Date Read: 7/17/00 Results:

Rejected By/Reason: N/A Date:

Accepted By: Jalany Brann Date: 7/18/00

Reviewed By: J. J. McDonnell Date: 7/18/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003
Rev. 10 05/99

5/12/00 JAM

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/7/00 Media Type: BHIB
Lot Number: BHIB001107A Expiration Date: 11/7/00 Prepared by: ---

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioPro	Brain Heart Infusion Broth	9708-128	01 Sept 00	27.75g / 750ml
NA - 8/7/00				

Deionized H₂O Volume: 750mls Dispensed In: 1 - 1L Flask
Volume per Container: 750mls Date Autoclaved: 8/7/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
NA 9/20/00						
	<i>Escherichia coli</i>	11229	/			
	<i>Staphylococcus aureus</i>	6538	/			
	<i>Serratia marcescens</i>	14756	/			
	<i>Klebsiella pneumoniae</i>	10031	/			
	<i>Pseudomonas aeruginosa</i>	9027	/			
	<i>Staphylococcus epidermidis</i>	12228	/			
			/			
			/			
8/13/00 Tz	<i>A. baumannii</i>	19606	+ +	91101	35±2°C	8/14/00 Tz
8/13/00 Tz	Negative Controls	N/A	- -	91101	*35±2°C	8/13/00 Tz

pH of Media: 7.2 * Negative Controls were incubated at room temperature with the clamp of 4.5. 7/21/00 (W) Tz 9-20-00 (C) Tz 9/20/00
Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: ccc Date Read: 8/9/00 Results: ---
Rejected By/Reason: NA Date: NA
Accepted By: J J McDonnell Date: 9/20/00
Reviewed By: [Signature] Date: 9-20-00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc. **EFFECTIVE** Form No. 91-L-003 Rev. 10 05/99

56410a [Signature]

COPY

original in 000426

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 6/14/00

Media Type: Schaedler Broth

Lot Number: SB000914A*

Expiration Date: 9/14/00 Prepared by: _____

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
Difco	Schaedler Broth	130229XA	Jul 01	14.2g/500mls
NA - 6/14/00				

Deionized H₂O Volume: 500mls

Dispensed In: 1 - 1 LF/ask

Volume per Container: 500mls

Date Autoclaved: 6/14/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
 						
 						
 						
 						
 						
 						
 						
 						
 						
 						
 						
6/20/00 RBN	<i>B. fragilis</i> *	25285	+ + +	91101**	35±2°C	6/22/00 RBN
6/20/00 RBN	Negative Controls *	N/A	- - -	91101**	35±2°C	6/22/00 RBN

pH of Media: 7.5 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: _____ Date Read: 6/16/00 Results: _____

Rejected By/Reason: NA Date: NA

Accepted By: Tanya Brann Date: 6/29/00

Reviewed By: J.D. McDonnell Date: 6/29/00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

* incubated anaerobically. JMB/29/00
... was added to the

EFFECTIVE

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/20/00 Media Type: Schaedler Broth
Lot Number: SB001020E Expiration Date: 10/20/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
Difco	Schaedler Broth	130229XA	5-1-01	42.6g/1.5L
N/A - 7/20/00				

Deionized H₂O Volume: 1.5L Dispensed In: 2 - 1L flasks
Volume per Container: ~750mls Date Autoclaved: 7/20/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
 						
	<i>Escherichia coli</i>	11229	/			
	<i>Staphylococcus aureus</i>	6538	/			
	<i>Serratia marcescens</i>	14756	/			
	<i>Klebsiella pneumoniae</i>	10031	/			
	<i>Pseudomonas aeruginosa</i>	9027	/			
	<i>Staphylococcus epidermidis</i>	12228	/	NA SKM		
			/			
			/			
			/			
8/15/00 TR	<i>B. fragilis</i>	25285 [®]	+ / N/A	91101	Anaerobic 35 ± 2°C	8/15/00 TR
8/15/00 TR	Negative Controls	N/A	- / N/A	91101	Anaerobic 35 ± 2°C	8/15/00 TR

pH of Media: 7.5 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: St. Murphy Date Read: 7/22/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: J J M Donnell Date: 9/20/00

Reviewed By: Pedrick Date: 9/20/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE
5/06/99 AM

Form No. 91-L-003
Rev. 10 05/99

Ⓢ only one (1) positive and negative control were prepared. TR 9/20/00

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/9/00 Media Type: Mueller Hinton Broth
Lot Number: M43001109B Expiration Date: 11/9/00 Prepared by: _____

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
<u>Difco</u>	<u>Mueller Hinton Broth</u>	<u>141023</u>	<u>May 03</u>	<u>252g / 12L</u>
<u>N/A</u> <u>8/9/00</u>				

Deionized H₂O Volume: 12L Dispensed In: 15-16 flasks
Volume per Container: ~800ml Date Autoclaved: 8/9/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
<u>N/A</u>						
	<u>Escherichia coli</u>	<u>11229</u>	<u>1</u>			
	<u>Staphylococcus aureus</u>	<u>6538</u>	<u>1</u>			
	<u>Serratia marcescens</u>	<u>14756</u>	<u>1</u>			
	<u>Klebsiella pneumoniae</u>	<u>10031</u>	<u>1</u>	<u>N/A</u>		
	<u>Pseudomonas aeruginosa</u>	<u>9027</u>	<u>1</u>	<u>9120100</u>		
	<u>Staphylococcus epidermidis</u>	<u>12228</u>	<u>1 (w)</u>	<u>Tr 9-20-00</u>		
<u>8/15/00 R</u>	<u>SA Neg. Controls</u>	<u>N/A</u>	<u>⊕ - 1 +</u>	<u>930214</u>	<u>30 ± 2°C</u>	<u>8/16/00 TR</u>
<u>8/15/00 R</u>	<u>S. marcescens</u>	<u>14756</u>	<u>⊕ + 1 +</u>	<u>930214</u>	<u>30 ± 2°C</u>	<u>8/16/00 TR</u>
<u>8/15/00 TR</u>	<u>E. cloacae</u>	<u>13047</u>	<u>⊕ + 1 +</u>	<u>91101</u>	<u>35 ± 2°C</u>	<u>8/16/00 TR</u>
<u>8/15/00 TR</u>	<u>Negative Controls</u>	<u>N/A</u>	<u>⊕ - 1 +</u>	<u>91101</u>	<u>35 ± 2°C</u>	<u>8/16/00 TR</u>

3 Only one (+) positive and negative control were prepared. Tr 9-20-00
pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-3/0

BI Read By: _____ Date Read: 8/11/00 Results: _____

Rejected By/Reason: N/A Date: N/A

Accepted By: J J McDonnell Date: 9/20/00

Reviewed By: J J McDonnell Date: 9-20-00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003
Rev. 10 05/99

5/1/00 JSM

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/20/00 Media Type: CaMHB
 Lot Number: CaMHB001020* Expiration Date: 10/20/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BBL	Mueller-Hinton II Broth Cation Adjusted	0174002	7/1/04	132g/6L
N/A 7/20/00				

Deionized H₂O Volume: 6L Dispensed In: 8- 1L Flasks
 Volume per Container: ~750mls Date Autoclaved: 7/20/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
—						
	<i>Escherichia coli</i>	11229	1			
	<i>Staphylococcus aureus</i>	6538	1			
	<i>Serratia marcescens</i>	14756	1			
	<i>Klebsiella pneumoniae</i>	10031	1			
	<i>Pseudomonas aeruginosa</i>	9027	1	N/A		
	<i>Staphylococcus epidermidis</i>	12228	1	912000		
			1 (GC)	912000		
8/15/00 T2	<i>S. pyogenes</i>	CI	+1*	91101	35±20C	8/16/00 T2
			1 (GC)	912000		8/16/00
8/15/00 T2	Negative Controls	N/A	-1*	91101	35±20C	8/16/00 T2

Only one (1) Positive and negative control were prepared. 7/29/00
 pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: St. Murphy Date Read: 7/22/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: J. J. McDonnell Date: 9/20/00

Reviewed By: [Signature] Date: 09/20/00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc. Form No. 91-L-003 Rev. 10 05/99

* Blood supplement added to broth prior to growth testing. JM 9/20/00
EFFECTIVE
5/06/99 SAM

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 9/1/00 Media Type: Ca MHB
 Lot Number: CaMHB001201c* Expiration Date: 12/1/00 Prepared by: _____

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BBL	Mueller-Hinton II Broth Lactin adjusted	0179002	7/1/07	16.5g/750ml
N/A - 9/1/00				

Deionized H₂O Volume: 750mls Dispensed In: 1-1 L F/ask
 Volume per Container: 750mls Date Autoclaved: 9/1/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read

	<i>Escherichia coli</i>	11229	1			
	<i>Staphylococcus aureus</i>	6538	1			
	<i>Serratia marcescens</i>	14756	1			
	<i>Klebsiella pneumoniae</i>	10031	1			
	<i>Pseudomonas aeruginosa</i>	9027	1			
	<i>Staphylococcus epidermidis</i>	12228	1			
			1			
9/8/00 TR	<i>S. pyogenes</i>	19615	⊕ + 1 +	91101	35°±2°C	9/9/00 TR
9/8/00 TR	Negative Controls	N/A	⊕ - 1 +	91101	35°±2°C	9/9/00 TR

Only one (+) positive and negative control were prepared. TR 9/20/00
 pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: _____ Date Read: 9/9/00 Results: _____

Rejected By/Reason: NA Date: NA

Accepted By: J J McDannell Date: 9/20/00

Reviewed By: _____ Date: 9/20/00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc.

* Blood supplement added to broth prior to growth testing. JM 9/20/00
5/06/99 JAM

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/4/00 Media Type: AMIC
 Lot Number: AMIC 001104E Expiration Date: 11/4/00 Prepared by: _____

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
Difco	Anaerobe Broth MIC	1111255F	Jun 01	99g / 3L
N/A - 8/4/00				

Deionized H₂O Volume: 3L Dispensed In: 4-16 flasks
 Volume per Container: ~750mls Date Autoclaved: 8/4/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read

	<i>Escherichia coli</i>	11229	1			
	<i>Staphylococcus aureus</i>	6538	1			
	<i>Serratia marcescens</i>	14756	1			
	<i>Klebsiella pneumoniae</i>	10031	1			
	<i>Pseudomonas aeruginosa</i>	9027	1	N/A		
	<i>Staphylococcus epidermidis</i>	12228	1	9/20/00		
			1 (GOD)	R9-20-00		
B116100 Tz	<i>B. fragilis</i>	25285	⊕ + 1 X	960802	Anaerobic 35±2°C	B116100 Tz
			1 (GOD)	R9-20-00		
B116100 Tz	Negative Controls	N/A	⊖ - 1 f	960802	Anaerobic 35±2°C	B116100 Tz

Only one (1) positive and negative control were prepared. Tz 9-20-00
 pH of Media: 7.0 Autoclave No.: 9/113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: _____ Date Read: 8/6/00 Results: _____
 Rejected By/Reason: NA Date: NA
 Accepted By: [Signature] Date: 9/20/00
 Reviewed By: [Signature] Date: 09/20/00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE
 5-16-100 AM

COPY

original

with 000627

Protocol No.

000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/13/00

Media Type: Tryp Soy Agar

Lot Number: TSA001013A

Expiration Date: 10/13/00 Prepared by: JS

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioPro	Tryp Soy Agar	DCM00041	3/10/04	600g/15L
N/A ~ 7/13/00				

Deionized H₂O Volume: 15L

Dispensed In: 18-1L Flasks

Volume per Container: ~833ml

Date Autoclaved: 7/13/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
~ 7/13/00	<i>Escherichia coli</i>	11229	+ +	930712	30±2°C	~ 7/15/00
~ 7/13/00	<i>Staphylococcus aureus</i>	6538	+ +	930712	30±2°C	~ 7/15/00
~ 7/13/00	<i>Serratia marcescens</i>	14756	+ +	930712	30±2°C	~ 7/15/00
~ 7/13/00	<i>Klebsiella pneumoniae</i>	10031	+ +	930712	30±2°C	~ 7/15/00
~ 7/13/00	<i>Pseudomonas aeruginosa</i>	9027	+ +	930712	30±2°C	~ 7/15/00
~ 7/13/00	<i>Staphylococcus epidermidis</i>	12228	+ +	930712	30±2°C	~ 7/15/00
N/A ~ 7/15/00						
~ 7/13/00	Negative Controls	N/A	- -	930712	30±2°C	~ 7/15/00

pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: _____ Date Read: 7/15/00 Results: -

Rejected By/Reason: NA Date: NA

Accepted By: [Signature] Date: 7/18/00

Reviewed By: [Signature] Date: 7/18/00

Notice: Proprietary Information - Not for Publication

Confidential/proprietary information of BioScience Laboratories, Inc. is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom. © Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003 Rev. 10 05/99

[Handwritten initials]

COPY

original in 000725

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/14/00 Media Type: TSA
Lot Number: TSA001014A Expiration Date: 10/14/00 Prepared by:

Table with 5 columns: VENDOR, PRODUCT, LOT NUMBER, EXP. DATE, WEIGHT/VOLUME. Contains handwritten entries for Biolco, Trypt Soy Agar, and lot number DCM 00041.

Deionized H2O Volume: 15L Dispensed In: 18-16F/flasks
Volume per Container: ~833mls Date Autoclaved: 7/19/00

Table with 7 columns: Initials/Date Incubated, Organism, ATCC #, Results, Incubator #, Temperature °C, Initials/Date Read. Lists various bacterial strains like Escherichia coli, Staphylococcus aureus, etc.

pH of Media: 7.2 Autoclave No.: 91112 Biological Indicator (BI) - Lot No.: SA-310
BI Read By: [Signature] Date Read: 7/16/00 Results: -
Rejected By/Reason: N/A Date:
Accepted By: Julian Brown Date: 7/18/00
Reviewed By: [Signature] Date: 7/18/00

Notice: Proprietary Information - Not for Publication. Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent.

EFFECTIVE

5/1/00 DM

COPY

original in 600419

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/17/00

Media Type: Tryp Soy Agar

Lot Number: TSA001017A

Expiration Date: 10/17/00

Prepared by: MB

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioPro	Tryp Soy Agar	DCM00041	3/10/04	600g/15L
N/A ~ 7/17/00				

Deionized H₂O Volume: 15L

Dispensed In: 18-1L flasks

Volume per Container: ~833ml

Date Autoclaved: 7/17/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
~ 7/17/00	<i>Escherichia coli</i>	11229	+ + +	930712	30 ± 2 °C	~ 7/19/00
~ 7/17/00	<i>Staphylococcus aureus</i>	6538	+ + +	930712	30 ± 2 °C	~ 7/19/00
~ 7/17/00	<i>Serratia marcescens</i>	14756	+ + +	930712	30 ± 2 °C	~ 7/19/00
~ 7/17/00	<i>Klebsiella pneumoniae</i>	10031	+ + +	930712	30 ± 2 °C	~ 7/19/00
~ 7/17/00	<i>Pseudomonas aeruginosa</i>	9027	+ + +	930712	30 ± 2 °C	~ 7/19/00
~ 7/17/00	<i>Staphylococcus epidermidis</i>	12228	+ + +	930712	30 ± 2 °C	~ 7/19/00
N/A						
~ 7/17/00	Negative Controls	N/A	- - -	930712	30 ± 2 °C	~ 7/19/00

pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: [Signature] Date Read: 7/19/00 Results: -

Rejected By/Reason: N/A Date: N/A

Accepted By: [Signature] Date: 7/21/00

Reviewed By: [Signature] Date: 7/25/00

Notice: Proprietary Information - Not for Publication
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003 Rev. 10 05/99

5/10/00 [Signature]

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/22/00 Media Type: TSA

Lot Number: TSA00122A Expiration Date: 11/22/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
<u>BioPro</u>	<u>Trypt Soy Agar</u>	<u>DCM 00041</u>	<u>3/10/04</u>	<u>600g/15L</u>
—				

Deionized H₂O Volume: 15L Dispensed In: 18-1CF/45L

Volume per Container: ~833mls Date Autoclaved: 8/22/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
<u>— 8/22/00</u>	<u>Escherichia coli</u>	<u>11229</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
<u>— 8/22/00</u>	<u>Staphylococcus aureus</u>	<u>6538</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
<u>— 8/22/00</u>	<u>Serratia marcescens</u>	<u>14756</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
<u>— 8/22/00</u>	<u>Klebsiella pneumoniae</u>	<u>10031</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
<u>— 8/22/00</u>	<u>Pseudomonas aeruginosa</u>	<u>9027</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
<u>— 8/22/00</u>	<u>Staphylococcus epidermidis</u>	<u>12228</u>	<u>+ 1+</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>
—						
—						
—						
<u>— 8/22/00</u>	<u>Negative Controls</u>	<u>N/A</u>	<u>- 1 -</u>	<u>930712</u>	<u>30±2°C</u>	<u>— 8/24/00</u>

pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: 5A-310

BI Read By: — Date Read: 8/24/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: [Signature] Date: 8/25/00

Reviewed By: [Signature] Date: 9-06-00

Notice: Proprietary Information - Not for Publication.
 Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE
5/06/99 [Signature]

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/20/00 Media Type: BHIA
Lot Number: BHIA0010206 Expiration Date: 10/20/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioPro	Brain Heart Infusion Agar	9906-116	7/1/02	104g/2L
N/A ~ 7/20/00				

Deionized H₂O Volume: 2L Dispensed In: 3-1 CF flasks
Volume per Container: ~667mls Date Autoclaved: 7/20/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
 						
	<i>Escherichia coli</i>	11229	/			
	<i>Staphylococcus aureus</i>	6538	/			
	<i>Serratia marcescens</i>	14756	/			
	<i>Klebsiella pneumoniae</i>	10031	/			
	<i>Pseudomonas aeruginosa</i>	9027	/			
	<i>Staphylococcus epidermidis</i>	12228	/			
			/			
8/15/00 TB	<i>S. pyogenes</i>	CI	+ / +	91101	35±2°C	8/17/00 TB
~7/20/00	Negative Controls	N/A	- / -	930712	30±2°C	8m 7/22/00

pH of Media: 7.4 Autoclave No.: 9/113 Biological Indicator (BI) - Lot No.: 5A-310

BI Read By: St. Anthony Date Read: 7/22/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: MW O'Neill Date: 9/20/00

Reviewed By: Devi Datta Date: 09-20-00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE
5/06/99 AM

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/4/00 Media Type: Brain Heart Infusion Agar
Lot Number: BHIA 001104 C Expiration Date: 11/4/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
<u>BioPro</u>	<u>Brain Heart Infusion Agar</u>	<u>9906-116</u>	<u>7/1/02</u>	<u>78g / 1.5L</u>
N/A				
8/4/00				
—				

Deionized H₂O Volume: 1500 mL Dispensed In: 2 - 1L Flasks
Volume per Container: ~750 mL Date Autoclaved: 8/4/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
—						
Escherichia coli						
11229						
1						
Staphylococcus aureus						
6538						
1						
Serratia marcescens						
14756						
1						
Klebsiella pneumoniae						
10031						
1						
Pseudomonas aeruginosa						
9027						
1						
Staphylococcus epidermidis						
12228						
1						
<u>2/15/00 T2</u>	<u>A. baumannii</u>	<u>19606</u>	<u>+ +</u>	<u>91101</u>	<u>35 ± 2°C</u>	<u>8/17/00 T2</u>
<u>8/4/00</u>	<u>Negative Controls</u>	<u>N/A</u>	<u>- -</u>	<u>930712</u>	<u>30 ± 2°C</u>	<u>8/6/00</u>

pH of Media: 7.4 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: 5A-310

BI Read By: — Date Read: 8/6/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: J. McDonnell Date: 9/20/00

Reviewed By: [Signature] Date: 9-20-00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003
Rev. 10 05/99

5/10/00 [Signature]

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/20/00 Media Type: Sabouraud Dextrose Agar
Lot Number: SDA001020B Expiration Date: 10/20/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BioPro	Sabouraud Dextrose Agar	DCM99114M	8/19/03	455g/7L
NA ~ 7/20/00				

Deionized H₂O Volume: 7L Dispensed In: 9-16 flasks
Volume per Container: ~778 mls Date Autoclaved: 7/20/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
NA ~ 7/20/00						
	<i>Escherichia coli</i>	11229	/			
	<i>Staphylococcus aureus</i>	6538	/			
	<i>Serratia marcescens</i>	14756	/			
	<i>Klebsiella pneumoniae</i>	10031	/			
	<i>Pseudomonas aeruginosa</i>	9027	/	NA JM		
	<i>Staphylococcus epidermidis</i>	12228	/	9/20/00		
			/			
8/15/00 TR	<i>C. albicans</i>	10231	+ +	930214	30°±2°C	8/17/00 TR
8/15/00 TR	<i>C. tropicalis</i>	750	+ +	930214	30°±2°C	8/17/00 TR
~ 7/20/00	Negative Controls	N/A	- -	930712	30±2°C	8m 7/27/00

pH of Media: 5.6 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: M. Murphy Date Read: 7/22/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: J. McDonnell Date: 9/20/00

Reviewed By: [Signature] Date: 09-20-00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003
Rev. 10 05/99

5/06/99 [Signature]

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/20/00 Media Type: Schaedler Agar
 Lot Number: SA001020C* Expiration Date: 10/20/00 Prepared by: —

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
<u>Difco</u>	<u>Schaedler Agar</u>	<u>1301495B</u>	<u>5/1/01</u>	<u>293.3g/7L</u>
NA				
7/20/00				

Deionized H₂O Volume: 7L Dispensed In: 9- 1 CF/flasks
 Volume per Container: ~778mls Date Autoclaved: 7/20/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
—						
	<u>Escherichia coli</u>	<u>11229</u>	<u>1</u>			
	<u>Staphylococcus aureus</u>	<u>6538</u>	<u>1</u>			
	<u>Serratia marcescens</u>	<u>14756</u>	<u>1</u>			
	<u>Klebsiella pneumoniae</u>	<u>10031</u>	<u>1</u>			
	<u>Pseudomonas aeruginosa</u>	<u>9027</u>	<u>1</u>	<u>NA JM</u>		
	<u>Staphylococcus epidermidis</u>	<u>12228</u>	<u>1</u>	<u>9/20/00</u>		
			<u>1</u>			
<u>8/16/00 TR</u>	<u>B. fragilis</u>	<u>25285</u>	<u>+ +</u>	<u>900802/91101</u>	<u>(Anaerobic) 35±2°C</u>	<u>8/21/00 TR</u>
<u>7/20/00</u>	<u>Negative Controls</u>	<u>N/A</u>	<u>- -</u>	<u>930712</u>	<u>30±2°C</u>	<u>SM 7/20/00</u>

pH of Media: 7.4 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: At Murphy Date Read: 7/27/00 Results: —

Rejected By/Reason: NA Date: NA

Accepted By: JM Donnell Date: 9/20/00

Reviewed By: Reifast Date: 09-20-00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc.

* Blood supplement added to agar prior to growth testing. JM 9/20/00

EFFECTIVE
5/06/99 SM

COPY original

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 6/7/00 Media Type: Mueller Hinton Agar and Dextrose
Lot Number: MHAD000907B* Expiration Date: 6/7/00 Prepared by:

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
Difco	Mueller Hinton Agar	142697KA	8/31/04	570g / 15L
BioPro	Dextrose	1694A73	8/19/04	7.5g / 15L
NA ~6/7/00				

Deionized H₂O Volume: 15L Dispensed In: 18 - 1L Flasks
Volume per Container: ~833-15 Date Autoclaved: 6/7/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
 						
	<i>Escherichia coli</i>	11229	1			
	<i>Staphylococcus aureus</i>	6538	1			
	<i>Serratia marcescens</i>	14756	1			
	<i>Klebsiella pneumoniae</i>	10031	1			
	<i>Pseudomonas aeruginosa</i>	9027	1	NA (M)		
	<i>Staphylococcus epidermidis</i>	12228	1		6/29/00	
			1			
			1			
Cellulose from ~6/7/00	Influenza*	33533	+ / +	91101	35 ± 2°C	6/16/00 BSM
	Negative Controls	N/A	- / -	930712	30 ± 2°C	~6/9/00

pH of Media: 7.2 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: Date Read: 6/9/00 Results:

Rejected By/Reason: NA Date: NA

Accepted By: J. J. McDonnell Date: 6/29/00

Reviewed By: J. J. McDonnell Date: 6/29/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc. **EFFECTIVE** Form No. 91-L-003 Rev. 10 05/99
*Supplement B was added to the agar

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 7/13/00 Media Type: PBS
 Lot Number: PBS001013D Expiration Date: 10/13/00 Prepared by: ---

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
BSCI	PBS stock	PBS0010 ^{stock} ₁₃	10/13/00	1L/10L
BSCI	Deionized water	N/A	N/A	9L/10L
N/A - 7/13/00				

Deionized H₂O Volume: 9L Dispensed In: 12-1C flasks
 Volume per Container: ~833mls Date Autoclaved: 7/13/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read

	<i>Escherichia coli</i>	11229	/			
	<i>Staphylococcus aureus</i>	6538	/			
	<i>Serratia marcescens</i>	14756	/			
	<i>Klebsiella pneumoniae</i>	10031	/			
	<i>Pseudomonas aeruginosa</i>	9027	N/A			
	<i>Staphylococcus epidermidis</i>	12228	/			
			/			
			/			
			/			
	Negative Controls	N/A	/			

pH of Media: 7.3 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: 5A-310

BI Read By: --- Date Read: 7/15/00 Results: ---

Rejected By/Reason: N/A Date: ---

Accepted By: Johannmann Date: 7/18/00

Reviewed By: J. J. McDonnell Date: 7/18/00

Notice: Proprietary Information - Not for Publication.
 Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
 © Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE
 5/26/00 JAM

Protocol No. 000622

MEDIA PRODUCTION AND GROWTH TESTING DATA SHEET

Date: 8/16/00 Media Type: PBS
Lot Number: PBS00116E Expiration Date: 11/16/00 Prepared by: _____

VENDOR	PRODUCT	LOT NUMBER	EXP. DATE	WEIGHT/VOLUME
<u>BSLI</u>	<u>PBS stock solution</u>	<u>PBS00116 stock</u>	<u>11/16/00</u>	<u>1L / 10L</u>
<u>BSLF</u>	<u>Deionized water</u>	<u>N/A</u>	<u>N/A</u>	<u>9L / 10L</u>
<u>N/A - 8/16/00</u>				

Deionized H₂O Volume: 9L Dispensed In: 12-100 flasks
Volume per Container: ~833mls Date Autoclaved: 8/16/00

Initials/Date Incubated	Organism	ATCC #	Results	Incubator #	Temperature °C	Initials/Date Read
	<u>Escherichia coli</u>	<u>11229</u>	<u>/</u>			
	<u>Staphylococcus aureus</u>	<u>6538</u>	<u>/</u>			
	<u>Serratia marcescens</u>	<u>14756</u>	<u>/</u>			
	<u>Klebsiella pneumoniae</u>	<u>19031</u>	<u>/</u>			
	<u>Pseudomonas aeruginosa</u>	<u>9027</u>	<u>/</u>			
	<u>Staphylococcus epidermidis</u>	<u>12228</u>	<u>/</u>			
			<u>/</u>			
			<u>/</u>			
			<u>/</u>			
	<u>Negative Controls</u>	<u>N/A</u>	<u>/</u>			

pH of Media: 7.4 Autoclave No.: 91113 Biological Indicator (BI) - Lot No.: SA-310

BI Read By: _____ Date Read: 8/18/00 Results: _____

Rejected By/Reason: _____ Date: _____

Accepted By: _____ Date: 8/18/00

Reviewed By: _____ Date: 8/22/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1996 by BioScience Laboratories, Inc.

EFFECTIVE

Form No. 91-L-003
Rev. 10 05/99

ADDENDUM IV

Minimum Inhibitory Concentration Evaluation

- Inoculum Population Data Sheets for MIC (Form No. 96-L-019)
- Minimum Inhibitory Concentration Evaluation Forms (Form No. 93-L-030)

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: Jim M. Powell Date: 08/15/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>A. baumannii</i>	-3	TNR	TNR	N/A			Counted By: h Date: 8-17-00	
ATCC #19606	-4	TNR	TNR	↓				
	-5	37	45	↓	41.00	4.10x10 ⁶ 2.050x10 ⁶		
<i>Acinetobacter</i> spp.	-3	TNR	TNR	N/A			Counted By: h Date: 8-17-00	
BSLI #061700Ab6	-4	TNR	TNR	↓				
	-5	53	77	↓	65.00	6.50x10 ⁶ 3.250x10 ⁶		
<i>S. pyogenes</i> ^Δ	-3	X	X	N/A	N/A	N/A	Counted By: h Date: 8-17-00	
ATCC #19615	-4	X	X	↓				
	-5	X	X	↓				
<i>S. pyogenes</i>	-3	TNR	TNR	N/A			Counted By: h Date: 8-17-00	
BSLI #040400Spy10	-4	222	202	↓	212.00	2.120x10 ⁶ 1.060x10 ⁶		
	-5	16	27	↓				
<i>S. pneumoniae</i>	-3	TNR	TNR	N/A			Counted By: h Date: 8-17-00	
BSLI #062900Spn6	-4	TNR	TNR	↓				
	-5	74	74	↓	74.00	7.40x10 ⁶ 3.70x10 ⁶		
<i>C. albicans</i>	-3	TNR	TNR	N/A			Counted By: h Date: 8-17-00	
ATCC #10231	-4	TNR	TNR	↓				
	-5	40	37	↓	38.50	3.850x10 ⁶ 1.9250x10 ⁶		

Δ Organism will be retested. Jim 8/21/00
 Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$ * contaminated with organism other than the challenge Microorganism 8-18-00

Recorded By: [Signature] Date: 8-18-00
 Calculated By: [Signature] Date: 8/18/00
 Reviewed By: [Signature] Date: 8/21/00
 Approved By: [Signature] Date: 9/15/00

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: J. M. Powell Date: 08/15/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>C. albicans</i>	-3	TNR	TNR	N/A			Counted By: <u>h</u> Date: <u>8/17/00</u>	
BSLI #040400Ca1	-4	TNR	TNR	↓				
	-5	46	47	↓	46.50	4.650x10 ⁶ 2.3250x10 ⁶		
<i>C. tropicalis</i>	-3	TNR	TNR	N/A			Counted By: <u>Om</u> Date: <u>8/17/00</u>	
ATCC #750	-4	248	254	↓	251.00	2.510x10 ⁶ 1.2550x10 ⁶		
	-5	18	23	↓				
<i>C. tropicalis</i>	-3	TNR	TNR	N/A			Counted By: <u>h</u> Date: <u>8/17/00</u>	
BSLI #121799Ct	-4	TNR	TNR	↓				
	-5	46	54	↓	50.00	5.00x10 ⁶ 2.50x10 ⁶		
<i>S. marcescens</i>	-3	TNR	TNR	N/A			Counted By: <u>h</u> Date: <u>8/17/00</u>	
ATCC #14756	-4	TNR	TNR	↓				
	-5	32	37	↓	34.50	3.450x10 ⁶ 1.7250x10 ⁶		
<i>S. marcescens</i>	-3	TNR	TNR	N/A			Counted By: <u>h</u> Date: <u>8/17/00</u>	
BSLI #060700Sm3	-4	146	135	↓	140.50	1.4050x10 ⁶ 7.0250x10 ⁵		
	-5	18	14	↓				
<i>M. luteus</i>	-3	TNR	TNR	N/A			Counted By: <u>Om</u> Date: <u>8/17/00</u>	
ATCC #7468	-4	152	142	↓	147.00	1.470x10 ⁶ 7.350x10 ⁵		
	-5	18	15	↓				

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature] Date: 8-18-00
 Calculated By: [Signature] Date: 8/18/00
 Reviewed By: [Signature] Date: 8/21/00
 Approved By: [Signature] Date: 9/15/00

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: [Signature] Date: 08/15/00

Organism/ATCC#	Dilution 10 ^D	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>Micrococcus</i> spp.	-3	TNR	TNR	N/A			Counted By: Am Date: 8/17/00	
BSLI #060700Ms8	-4	TNR	TNR	↓		Ⓡ 1.75x10 ⁶		
	-5	268	282	↓	275.00	2.75x10 ⁷ 1.375x10 ⁷		
<i>E. cloacae</i>	-3	TNR	TNR	N/A			Counted By: L Date: 8-17-00	
ATCC #13047	-4	TNR	TNR	↓				
	-5	34	36	↓	35.00	3.50x10 ⁶ 1.75x10 ⁶		
<i>Enterobacter</i> spp.	-3	TNR	TNR	N/A			Counted By: Am Date: 8/17/00	
BSLI #121799Ecl1	-4	TNR	TNR	↓				
	-5	35	33	↓	34.00	3.40x10 ⁶ 1.70x10 ⁶		
							Counted By:	
							Date:	
							Counted By:	
							Date:	
							Counted By:	
							Date:	

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature]

Date: 8-18-00

Calculated By: [Signature]

Date: 8/18/00

Reviewed By: [Signature]

Date: 8/21/00

Approved By: [Signature]

Date: 9/15/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc.

Performed By: Reinfach

Date: 08/16/00

Organism/ATCC#	Dilution 10 ^D	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>B. fragilis</i>	-3	TNTZ	TNTZ	N/A			Counted By: RZ Date: 8/21/00	
ATCC #25285	-4	216	163		189.50	1.8950x10 ⁶		
	-5	40	28			9.475x10 ⁵		
<i>B. fragilis</i>	-3	TNTZ	TNTZ				Counted By: TZ Date: 8/21/00	
BSLI #060700Bf2	-4	199	159		179.00	1.790x10 ⁶		
	-5	23	25			8.950x10 ⁵		
<i>E. faecalis</i>	-3	TNTZ	TNTZ				Counted By: h Date: 8-18-00	
ATCC #29212	-4	356	315					
	-5	39	25		32.00	3.20x10 ⁶		
<i>E. faecalis</i>	-3	TNTZ	TNTZ				Counted By: h Date: 8-18-00	
BSLI #121699Efs1	-4	TNTZ	TNTZ					
	-5	65	55		60.00	6.00x10 ⁶		
<i>E. faecium</i>	-3	TNTZ	TNTZ				Counted By: h Date: 8-18-00	
ATCC #19434	-4	TNTZ	TNTZ					
	-5	289	303		296.00	2.960x10 ⁷		
<i>E. faecium</i>	-3	TNTZ	TNTZ				Counted By: h Date: 8/18/00	
BSLI #061700Efm1	-4	194	167		180.50	1.8050x10 ⁶		
	-5	19	15	↓		9.0250x10 ⁵		

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: Reinfach Reinfach
 Calculated By: Reinfach Reinfach
 Reviewed By: JJ McDonnell
 Approved By: [Signature]

Date: 8/18/00 8/21/00
 Date: 8/18/00 8/21/00
 Date: 8/22/00
 Date: 9/15/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc.

Performed By: [Signature]

Date: 08 / 16 / 00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>E. coli</i>	-3	TNTZ	TNTZ	N/A			Counted By: ✓ Date: 8-18-00	
ATCC #11229	-4	TNTZ	TNTZ					
	-5	117	104		110.50	1.105 x 10 ⁷ 5.5250 x 10 ⁶		
<i>E. coli</i>	-3	TNTZ	TNTZ				Counted By: ✓ Date: 8-18-00	
BSLI #010500Ec8	-4	115	129		122.00	1.220 x 10 ⁶ 6.10 x 10 ⁵		
	-5	13	19					
<i>E. coli</i>	-3	TNTZ	TNTZ				Counted By: ✓ Date: 8-18-00	
ATCC #25922	-4	207	195		201.00	2.010 x 10 ⁶ 1.005 x 10 ⁶		
	-5	23	27					
<i>E. coli</i>	-3	TNTZ	TNTZ				Counted By: ✓ Date: 8-18-00	
BSLI #010500Ec6	-4	163	170		166.50	1.665 x 10 ⁶ 8.325 x 10 ⁵		
	-5	15	22					
<i>K. oxytoca</i>	-3	TNTZ	TNTZ			8.175 x 10 ⁵	Counted By: ✓ Date: 8-18-00	
ATCC #43165	-4	167	160		163.50	1.6350 x 10 ⁶ 8.325 x 10⁵		
	-5	18	15		(100) 78 8-18-00	(CE) 17811100		
<i>K. oxytoca</i>	-3	TNTZ	TNTZ				Counted By: ✓ Date: 8-18-00	
BSLI #060700Ko6	-4	118	125		121.50	1.2150 x 10 ⁶ 6.075 x 10 ⁵		
	-5	23	19	↓				

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature]
 Calculated By: [Signature]
 Reviewed By: [Signature]
 Approved By: [Signature]

Date: 8/18/00
 Date: 8/18/00
 Date: 8/22/00
 Date: 9/15/00

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc.

Performed By: [Signature]

Date: 08/16/00

Organism/ATCC#	Dilution 10 ^D	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>K. pneumoniae</i>	-3	TNTZ	TNTZ	N/A				Counted By: L Date: 8-18-00
ATCC #11296	-4	219	220		219.50	2.1950x10 ⁶	1.0975x10 ⁶	
	-5	35	35					
<i>K. pneumoniae</i>	-3	TNTZ	TNTZ					Counted By: L Date: 8-18-00
BSLI #040400Kpn12	-4	130	134		132.00	1.320x10 ⁶	6.60x10 ⁵	
	-5	17	19					
<i>P. mirabilis</i> *	-3	0	0		*	*	*	Counted By: L Date: 8-18-00
ATCC #7002	-4	0	0					
	-5	0	0					
<i>S. pneumoniae</i>	-3	6	17		11.50	1.150x10 ⁴	5.750x10 ³	Counted By: L Date: 8-18-00
ATCC #6303	-4	2	0					
	-5	1	0	↓				
N/A 7/28/18/00								Counted By:
								Date:
								Counted By:
								Date:

* Will be retested due to no growth (not inoculated). Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$
7/28/18/00

Recorded By: [Signature]
Calculated By: [Signature]
Reviewed By: [Signature]
Approved By: [Signature]

Date: 8/18/00
Date: 8/18/00
Date: 8/22/00
Date: 9/15/00

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: [Signature] Date: 08/17/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>P. mirabilis</i>	-3	TNTC	TNTC	N/A			Counted By: <u>BU</u> Date: <u>8/22/00</u>	
BSLI #062900Pm1	-4	TNTC	TNTC					
	-5	42	40		41.00	4,100 x 10 ⁶ 2,050 x 10 ⁶		
<i>P. aeruginosa</i>	-3	TNTC	TNTC				Counted By: <u>BU</u> Date: <u>8/22/00</u>	
ATCC #27853	-4	TNTC	TNTC					
	-5	35	34		34.50	3,450 x 10 ⁶ 1,725 x 10 ⁶		
<i>P. aeruginosa</i>	-3	TNTC	TNTC				Counted By: <u>BU</u> Date: <u>8/22/00</u>	
BSLI #040400Pa8	-4	58	59		58.50	5,850 x 10 ⁶ 2,925 x 10 ⁶		
	-5	6	7					
<i>P. aeruginosa</i>	-3	TNTC	TNTC				Counted By: <u>BU</u> Date: <u>8/22/00</u>	
BSLI #040400Pa9	-4	143	119		131.00	1,310 x 10 ⁶ 6,550 x 10 ⁵		
	-5	17	11					
<i>S. aureus</i>	-3	TNTC	TNTC				Counted By: <u>BU</u> Date: <u>8/22/00</u>	
ATCC #29213	-4	213	216		214.50	2,145 x 10 ⁶ 1,072.5 x 10 ⁶		
	-5	23	33					
<i>S. aureus</i>	-3	TNTC	TNTC				Counted By: <u>BU</u> Date: <u>8/22/00</u>	
BSLI #040400Sa4	-4	182	163		172.50	1,725 x 10 ⁶ 8,625 x 10 ⁵		
	-5	13	14					

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature] Date: 8/22/00
 Calculated By: [Signature] Date: 8/23/00
 Reviewed By: [Signature] Date: 8/25/00
 Approved By: [Signature] Date: 08/28/00

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: [Signature] Date: 08/17/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>S. aureus</i>	-3	TNTC	TNTC	N/A			Counted By: BJ Date: 8/22/00	
BSLI #040400Sa5	-4	TNTC	TNTC					
	-5	41	45		43.00	4.300x10 ⁶ 2.150x10 ⁶		
<i>S. epidermidis</i>	-3	TNTC	TNTC				Counted By: BJ Date: 8/22/00	
BSLI #061700Se13	-4	60	74		67.00	6.700x10 ⁵ 3.350x10 ⁵		
	-5	6	5					
<i>S. haemolyticus</i>	-3	317	269				Counted By: BJ Date: 8/22/00	
ATCC #29970	-4	43	62		52.50	5.250x10 ⁵ 2.625x10 ⁵		
	-5	0	5					
<i>S. haemolyticus</i>	-3	TNTC	TNTC				Counted By: BJ Date: 8/22/00	
BSLI #061700Sha5	-4	79	77		78.00	7.800x10 ⁵ 3.900x10 ⁵		
	-5	12	8					
<i>S. hominis</i>	-3	147	164		155.50	1.555x10 ⁵ 7.775x10 ⁴	Counted By: BJ Date: 8/22/00	
ATCC #27844	-4	22	17					
	-5	1	2					
<i>S. hominis</i>	-3	TNTC	TNTC				Counted By: BJ Date: 8/22/00	
BSLI #060700Sho4	-4	233	255		244.00	2.440x10 ⁶ 1.220x10 ⁶		
	-5	27	22					

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature] Date: 8/22/00
 Calculated By: [Signature] Date: 8/23/00
 Reviewed By: [Signature] Date: 8/25/00
 Approved By: [Signature] Date: 8/28/00

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonaz, Inc. Performed By: J. M. Donnell Date: 08/17/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>S. saprophyticus</i>	-3	TNTC	TNTC	N/A			Counted By: BV Date: 8/22/00	
ATCC #15305	-4	91	84		87.500	8.750x10 ⁵		
	-5	5	9					
<i>S. saprophyticus</i>	-3	239	241		240.00	2.400x10 ⁵	Counted By: BV Date: 8/22/00	
BSLI #060700Ss3	-4	22	35					
	-5	8	4					
<i>H. influenzae</i> (*)	-3	TNTC	TNTC				Counted By: BV Date: 8/22/00	
ATCC #19418	-4	TNTC	TNTC					
(CAE)	-5	441	391		416.00	4.160x10 ⁷		
<i>H. influenzae</i> (*)	-3	TNTC	TNTC				Counted By: BV Date: 8/22/00	
BSLI #062900Hi9	-4	TNTC	TNTC					
(CAE)	-5	299	335		317.00	3.170x10 ⁷		
N/A BV 8/22/00							Counted By: Date:	
N/A BV 8/22/00								Counted By: Date:

(*) Organisms were also plated on MHAD. No growth was observed for either organism on any of the dilutions plated (-3, -4, or -5). Tz 8/22/00
 Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: B. Valer

Date: 8/22/00

Calculated By: B. Valer

Date: 8/23/00

Reviewed By: J. M. Donnell

Date: 8/25/00

Approved By: [Signature]

Date: 8/28/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc.

Performed By: [Signature]

Date: 08/18/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>S. aureus</i>	-3	TNTC	TNR	N/A			Counted By: TZ Date: 8/21/00	
ATCC #6538	-4	171	171		171.00	1.710x10 ⁶		
	-5	13	19			8.950x10 ⁵		
<i>P. aeruginosa</i>	-3	TNTC	TNR				Counted By: TZ Date: 8/21/00	
ATCC #15442	-4	75	76		75.50	7.550x10 ⁵		
	-5	6	4			3.7750x10 ⁵		
<i>S. epidermidis</i>	-3	TNTC	TNR				Counted By: TZ Date: 8/21/00	
ATCC #12228	-4	260	267		263.50	2.635x10 ⁶		
	-5	23	24			1.3175x10 ⁶		
(US) 78-21-00								
Counted By: Date:								
Counted By: Date:								
Counted By: Date:								
Counted By: Date:								

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: [Signature]
 Calculated By: [Signature]
 Reviewed By: [Signature]
 Approved By: [Signature]

Date: 8/21/00
 Date: 8/21/00
 Date: 8/22/00
 Date: 9/15/00

Notice: Proprietary Information - Not for Publication.

INOCULUM POPULATION DATA SHEET FOR MIC

Sponsor: Lonza, Inc. Performed By: Jim Maxwell Date: 09/08/00

Organism/ATCC#	Dilution 10 ^{-D}	Plate Counts			Average Colony Count	Average CFUs/ml	Population per Tubes CFUs/ml	Initials/Date
		A	B	C				
<i>S. pyogenes</i>	-3	tntc	tntc	N/A			Counted By: <u>JM</u> Date: <u>9.12.00</u>	
ATCC # 19615	-4	tntc	tntc	↓				
(retest data) ^R 9/15/00	-5	41	40	↓	40.50	4.0500010 ⁶ 7.0250010 ⁶		
	-3						Counted By: Date:	
	-4							
	-5							
	-3						Counted By: Date:	
	-4							
	-5							
	-3						Counted By: Date:	
	-4							
	-5							
	-3						Counted By: Date:	
	-4							
	-5							
	-3						Counted By: Date:	
	-4							
	-5							

Population per Tube (CFUs/ml) = $\frac{\text{Average CFUs/ml}}{2}$

Recorded By: Jim Maxwell Date: 9.12.00
 Calculated By: Jim Maxwell Date: 9.12.00
 Reviewed By: [Signature] Date: 09/15/00
 Approved By: [Signature] Date: 9/15/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: A. baumannii ATCC # 19606 Population per Tube(CFU's/mL): 2.5 x 10⁶

Date Inoculated/Initials: 8/15/00 Cm Date Read/Initials: TE 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2,048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done TE 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: Acinetobacter spp. (baumannii) BSLI # 061700Ab6 Population per Tube(CFU's/mL): 3.250 x 10⁶
 Date Inoculated/Initials: 8/15/00 Gm Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0
 Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: B. fragilis ATCC# 25285 Population per Tube(CFU's/mL): 9.4150 x 10⁵

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/18/00 Incubation time (hrs): 42.50

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done 8/17/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: B. fragilis BSLI# 060700Bf2 Population per Tube(CFU's/mL): 8.450 x 10⁵
 Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/18/00 Incubation time (hrs): 42.50
 Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1: 6,144		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8/17/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E. cloacae ATCC# 13047 Population per Tube(CFU's/mL): 1.750 x 10⁶

Date Inoculated/Initials: 8/15/00 Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
<u>(w)</u> <u>8/15/00</u> 1:2	<u>ND</u> *	<u>ND</u> *	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1,024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: Enterobacter spp. BSLI# 121799Ecl1 Population per Tube(CFU's/mL): 1.70 x 10⁶
(Cloacae)
 Date Inoculated/Initials: 8/15/00 UM Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0
 Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done T2 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: *E. faecalis* ATCC# 29212 Population per Tube(CFU's/mL): 1.60×10^6

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{(\text{Run 1} + \text{Run 2})}{2}$

ND = Not Done To 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E. faecalis BSLI# 121699Efs1 Population per Tube(CFU's/mL): 3.00 x 10⁶

Date Inoculated/Initials: 8/16/00 GWA Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E. faecium ATCC# 19434 Population per Tube(CFU's/mL): 1.480 x 10⁷

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/17/00 Incubation time (hrs): 20.75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TS 8-17-00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E. faecium BSLI# 061700Efm1 Population per Tube(CFU's/mL): 9.0250×10^5

Date Inoculated/Initials: 8/16/00 Am Date Read/Initials: TZ 8-17-00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{(\text{Run 1} + \text{Run 2})}{2}$
 ND = Not Done TZ 8/17/00

Recorded By: Quifast Date: 8/17/00

Approved By: J J M Donnell Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E.coli ATCC# 11229 Population per Tube(CFU's/mL): 5.5250 x 10⁶

Date Inoculated/Initials: 8/16/00 GA Date Read/Initials: TE 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1,024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TE 8-17-00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E.coli BSLI# 010500Ec8 Population per Tube(CFU's/mL): 6.10×10^5

Date Inoculated/Initials: 8/16/00 GA Date Read/Initials: TR 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = $\frac{(\text{Run 1} + \text{Run 2})}{2}$

ND = Not Done TR 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E. coli ATCC# 25922 Population per Tube(CFU's/mL): 1.0050 x 10⁶

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done Tz 8-17-00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: E.coli BSLI# 010500Ec6 Population per Tube(CFU's/mL): 9.3250 x 10⁴ ⁵ 8/18/00

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	^{WL 8/17/00} *	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2
 ND = Not Done 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: H. influenzae ATCC# 19418 Population per Tube(CFU's/mL): 2.08 x 10⁷

Date Inoculated/Initials: 8/17/00 Date Read/Initials: TE 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:6,144		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2)

2

ND = Not Done TE 8/18/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: H. influenzae BSL# 062900Hi9 Population per Tube(CFU's/mL): 1.585x10⁷

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2)
 ND = Not Done T2 8/18/00 2

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: K. oxytoca ATCC# 43165 Population per Tube(CFU's/mL): 8.1750 x 10⁵

Date Inoculated/Initials: 8/16/00 Date Read/Initials: PE 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done PE 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: K. oxytoca BSLI# 060700Ko6 Population per Tube(CFU's/mL): 6.0750x10⁵

Date Inoculated/Initials: 8/16/00 MM Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:2048		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8-17-00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: K. pneumoniae ATCC# 11296 Population per Tube(CFU's/mL): 1.0975 x 10⁶

Date Inoculated/Initials: 8/16/00 Date Read/Initials: 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:4096		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8-17-00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: M. luteus ATCC# 7468 Population per Tube(CFU's/mL): 7.350×10^5

Date Inoculated/Initials: 8/15/00 WM Date Read/Initials: TE 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = NOT DONE TE 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: Micrococcus spp. BSLI# 060700Ms8 Population per Tube(CFU's/mL): 1.3750 x 10⁷

Date Inoculated/Initials: 8/15/00^{UM} Date Read/Initials: TZ 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:32,768		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to-product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$
 ND = Not Done TZ 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: *P. mirabilis* ^(*) ATCC#: 7002 Population per Tube(CFU's/mL): 1.70×10^6
 Date Inoculated/Initials: 8-31-00 Date Read/Initials: 9-1-00 Incubation time (hrs): 20.75
 Positive Control: + Negative Control: - ^(*) Retest Data 7-9-15-00

Product Dilution	Product Description							
	Product:#1,5% (w/v) Lot#: TRCS # 100037		Product: Lot#:		Product: Lot#:		Product: Lot#:	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	<u>9-1-00</u> <u>1:512</u>		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

Recorded By: [Signature] Date: 9-1-00
 Approved By: [Signature] Date: 09-15-00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: *P. mirabilis* BSLI# 062900Pm1 Population per Tube(CFU's/mL): 2.05×10^6

Date Inoculated/Initials: 8/17/00 *Ch* Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:512		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read, due to product turbidity

Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done 8/18/00

Recorded By: *[Signature]* Date: 8/18/00

Approved By: *[Signature]* Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: *P. aeruginosa* ATCC# 15442 Population per Tube(CFU's/mL): 3.775 x 10⁵

Date Inoculated/Initials: 8/18/00/um Date Read/Initials: 7/8/19/00 Incubation time (hrs): 17.75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$
 ND = Not Done T2 8-21-00

Recorded By: [Signature] Date: 8/21/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: *P. aeruginosa* BSLI# 040400Pa8 Population per Tube(CFU's/mL): 2.925×10^5

Date Inoculated/Initials: 8/17/00 UM Date Read/Initials: TE 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
^{(WD) TE 8/18/00} 1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{(\text{Run 1} + \text{Run 2})}{2}$

ND = Not Done TE 8/18/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: P. aeruginosa ATCC# 27853 Population per Tube(CFU's/mL): 1.725x10⁶

Date Inoculated/Initials: 8/17/00/um Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8/18/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: P. aeruginosa BSLI# 040400Pa9 Population per Tube(CFU's/mL): 6.55x10⁵

Date Inoculated/Initials: 8/17/00/44 Date Read/Initials: TE 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:1024		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TE 8/18/00
 Recorded By: [Signature] Date: 8/18/00
 Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. marcescens ATCC# 14756 Population per Tube(CFU's/mL): 1.7250 x 10⁶

Date Inoculated/Initials: 5/15/00 VM Date Read/Initials: 7/28/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:512		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = NOT DONE 7/28/00

Recorded By: [Signature] Date: 8/16/00
 Approved By: [Signature] Date: 8/21/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. marcescens BSLI# 060700Sm3 Population per Tube(CFU's/mL): 7.0250 x 10⁵

Date Inoculated/Initials: 8/15/00 Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:512		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. aureus ATCC# 6538 Population per Tube(CFU's/mL): 8.550×10^5

Date Inoculated/Initials: 8-18-00 CM Date Read/Initials: 8/19/00 Incubation time (hrs): 17.75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	<u>> 1:65,536</u>		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done T28-21-00

Recorded By: [Signature] Date: 8/21/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. aureus BSLI# 040400Sa4 Population per Tube(CFU's/mL): 89.8.625 x 10⁵

Date Inoculated/Initials: 8/17/00 TR 8/18/00 Date Read/Initials: 8/18/00 TR 8/18/00 Incubation time (hrs): 20.0 39.75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	<u>> 1:65,536</u>		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TR 8/18/00

Recorded By: [Signature] Date: 8/18/00 8-21-00
 Approved By: [Signature] Date: 8/20/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. aureus ATCC # 29213
BSL# 040400Sa5 Population per Tube (CFU's/mL): 1.0725 x 10⁶
 Date Inoculated/Initials: 8/17/00 cm Date Read/Initials: 8/18/00 Tz Incubation time (hrs): 20.0
 Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:32,768		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity
 ND = Not Done Tz 8/18/00
 Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

Recorded By: [Signature] Date: 8/18/00
 Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. aureus BSLI # 040400Sa5
 ATCC# 29213 Population per Tube(CFU's/mL): 2.15 x 10⁶
© 1996 TE 8-18-00

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 8/19/00 Incubation time (hrs): 26.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2)
 ND = Not Done TE 8/19/00 2

Recorded By: [Signature] Date: 8/19/00
 Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. epidermidis ATCC# 12228 Population per Tube(CFU's/mL): 1.3175x10⁶

Date Inoculated/Initials: 8-18-00 Date Read/Initials: T&S/19/00 Incubation time (hrs): 17.75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	71:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2)

ND = Not Done T&S 8/21/00

2

Recorded By: [Signature] Date: 8/21/00

Approved By: [Signature] Date: 8/22/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. epidermidis BSLI# 06070053 Population per Tube(CFU's/mL): 3.35 x 10⁵
(WB) 72818100 (WB) 72818100

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 72818100 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	7:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done T2 8-18-00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. haemolyticus ATCC# 29970 Population per Tube(CFU's/mL): 2.625 x 10⁵

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 8-18-00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done T2 8-18-00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. haemolyticus BSLI# 061700Sha5 Population per Tube(CFU's/mL): 3.90 x 10⁵

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 728-18-00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. hominis BSLI# 27844 Population per Tube(CFU's/mL): 7.775 x 10⁴

Date Inoculated/Initials: 8/17/00 AM Date Read/Initials: TZ 8-19-00 Incubation time (hrs): 39.75
TC 8/18/00 TC 8/19/00 TC 8/18/00

Positive Control: + Negative Control: -
WD 8/19/00

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	7:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TZ 8/18/00

Recorded By: [Signature]

Date: 8/18/00 8/21/00

Approved By: [Signature]

Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. hominis BSLI# 060700Sho4 Population per Tube(CFU's/mL): 1.22 x 10⁶

Date Inoculated/Initials: 8/17/00 cm Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done TEG-18-00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. saprophyticus ATCC# 15305 Population per Tube(CFU's/mL): 4.375x10⁵

Date Inoculated/Initials: 8/17/00 Date Read/Initials: 8-19-00 Incubation time (hrs): 39-75

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	71:65536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2
 ND = Not Done 8/18/00

Recorded By: [Signature] Date: 8/18/00 8-21-00
 Approved By: [Signature] Date: 8/25/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. saprophyticus S. epidermidis BSLI# 061700Sc13 Population per Tube(CFU's/mL): 1.20 x 10⁵
 Date Inoculated/Initials: 8/17/00 cm Date Read/Initials: 8/18/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	-	-	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	> 1:65,536		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$
 ND = Not Done 8/18/00

Recorded By: [Signature] Date: 8/18/00

Approved By: [Signature] Date: 8/25/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. pneumoniae ATCC# 6303 Population per Tube(CFU's/mL): 5.5750x10³

Date Inoculated/Initials: 8/16/00 Date Read/Initials: TE 8/17/00 Incubation time (hrs): 20.25

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{(\text{Run 1} + \text{Run 2})}{2}$

ND = Not Done TE 8/17/00

Recorded By: [Signature] Date: 8/17/00

Approved By: [Signature] Date: 8/22/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. pneumoniae BSLI# 062900Spn6 Population per Tube(CFU's/mL): 3.70 x 10⁶

Date Inoculated/Initials: 8/15/10 Date Read/Initials: 8/16/10 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:8,192		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done 8/16/10

Recorded By: [Signature] Date: 8/16/10

Approved By: [Signature] Date: 8/21/10

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. pyogenes ATCC#: 19615 Population per Tube(CFU's/mL): 2.025 x 10⁶
 Date Inoculated/Initials: 9.8.00 Date Read/Initials: 9/19/00 Incubation time (hrs): 20-167 20-25
 Positive Control: + Negative Control: - Retest Data. 9.12.00
729-15-00

Product Dilution	Product Description							
	Product #1, 5% (w/v) Lot#: TRCS # 100037		Product: N/A Lot#:N/A		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:16384		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

Recorded By: [Signature] Date: 9.12.00
 Approved By: [Signature] Date: 9-15-00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: S. pyogenes BSLI # 040400Spy10 Population per Tube(CFU's/mL): 1.060 x 10⁶

Date Inoculated/Initials: 8/15/00 Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:512	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	-	-	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	1:4,096		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2)

ND = Not Done T2 8/16/00

Recorded By: [Signature] Date: 8/16/00
 Approved By: [Signature] Date: 8/21/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: C. albicans ATCC# 10231 Population per Tube(CFU's/mL): 1.9250x10⁶

Date Inoculated/Initials: 8/15/00 CM Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:512	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	<u>< 1:256</u>		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$
 ND = Not Done 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: C. albicans BSLI# 040400Ca1 Population per Tube(CFU's/mL): 2.3250 x 10⁶

Date Inoculated/Initials: 8/15/00 Date Read/Initials: TE 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:512	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	<u>< 1:256</u> <u>1:128</u>		N/A <u>(ND) TE 8/16/00</u>		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity

Average Endpoint = $\frac{\text{Run 1} + \text{Run 2}}{2}$

ND = Not Done TE 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: C. tropicalis ATCC# 750 Population per Tube(CFU's/mL): 1.2550 x 10⁶

Date Inoculated/Initials: 8/15/00 Date Read/Initials: TZ 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:512	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	< 1:256		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2) / 2

ND = Not Done TZ 8/16/00

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

Protocol No. 000622

MINIMUM INHIBITORY CONCENTRATION EVALUATION

Microorganism: C. tropicalis BSLI# 121799Ct Population per Tube(CFU's/mL): 2.50 x 10⁶

Date Inoculated/Initials: 8/15/00 CM Date Read/Initials: 8/16/00 Incubation time (hrs): 20.0

Positive Control: + Negative Control: -

Product Dilution	Product Description							
	Product: #1, 5% (w/v) Lot#: TRCS #100037		Product: N/A Lot#:N/A		Product: N/A Lot#: N/A		Product: N/A Lot#: N/A	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1:2	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A
1:4	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:8	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:16	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:32	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:64	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:128	*	*	N/A	N/A	N/A	N/A	N/A	N/A
1:256	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:512	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:1024	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:2,048	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:4,096	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:8,192	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:16,384	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:32,768	+	+	N/A	N/A	N/A	N/A	N/A	N/A
1:65,536	+	+	N/A	N/A	N/A	N/A	N/A	N/A
Average Endpoint	< 1:256		N/A		N/A		N/A	

(+) Growth (-) No Growth * Not able to read due to product turbidity Average Endpoint = (Run 1 + Run 2)

ND = Not Done 8/16/00 ^{TS/11/00} _{TS/11/00} ^{TS/11/00} _{TS/11/00} 2

Recorded By: [Signature] Date: 8/16/00

Approved By: [Signature] Date: 8/21/00

Notice: Proprietary Information - Not for Publication.

ADDENDUM V

General Data Gathering (Form No. 91-L-002)

Protocol No. 000622

GENERAL DATA GATHERING

Sponsor: Lonza, Inc Subject: Preparation of test product

On each test day, a 5% w/v solution of the test product was prepared by weighing 5.0 grams of test product in a sterile glass beaker, and adding 100 ml of sterile Water for Irrigation.

(Test Days : 08/15/00, 08/16/00, 08/17/00, 08/18/00, and 08/31/00
and 09/08/00
T2 9/20/00 T2 8/28/00

Conducted By: [Signature] T2 T2 Date: 8/15/00 8/28/00 9/08/00

Reviewed By: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.

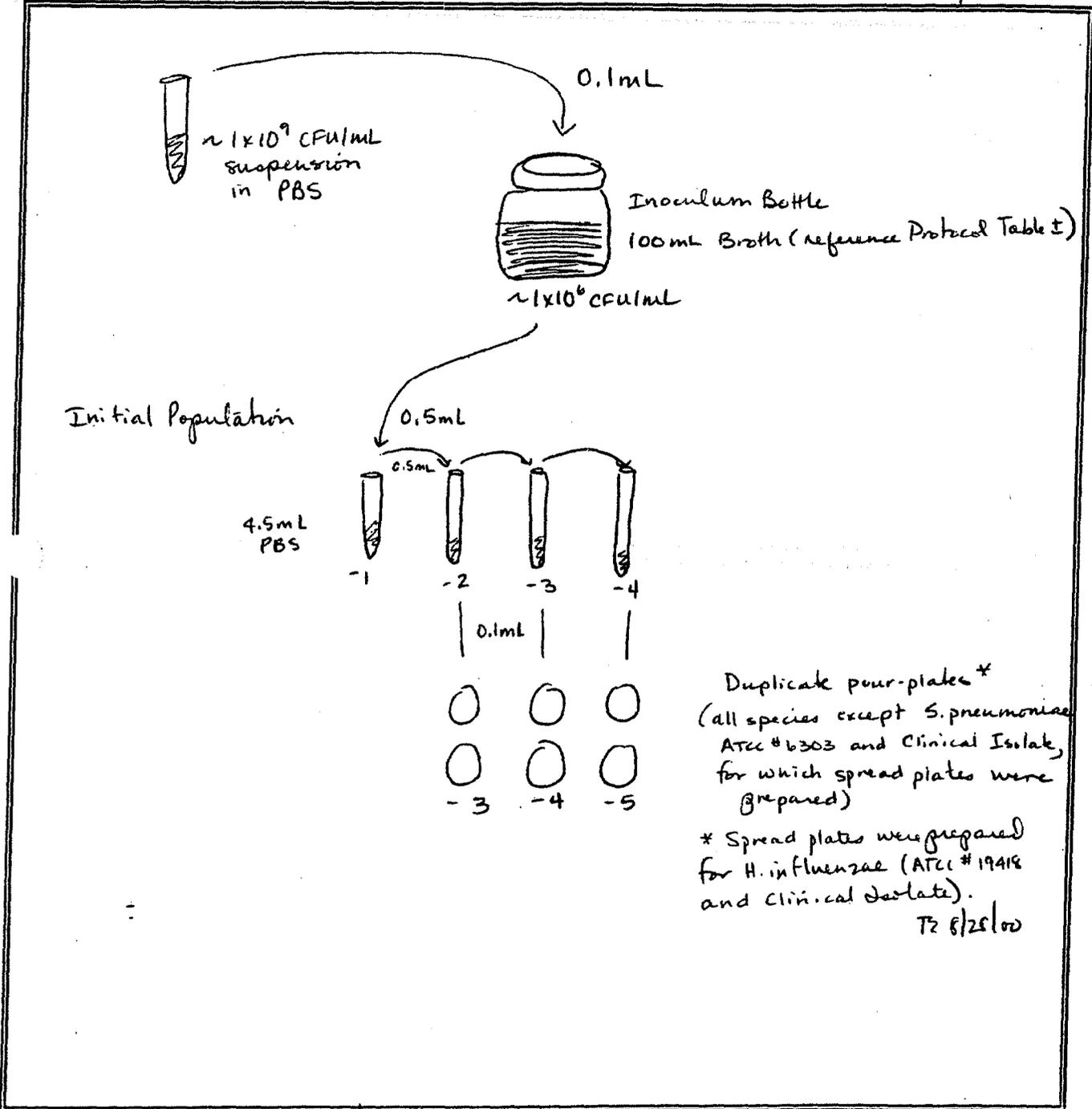
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

Protocol No. 000622

GENERAL DATA GATHERING

Sponsor: Lonza, Inc.

Subject: Preparation of Challenge Suspensions and Initial Population Determination



Conducted By: [Signature] T2 Date: 8/15/00 8/28/00
Reviewed By: [Signature] Date: 9/10/00 9/19/00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

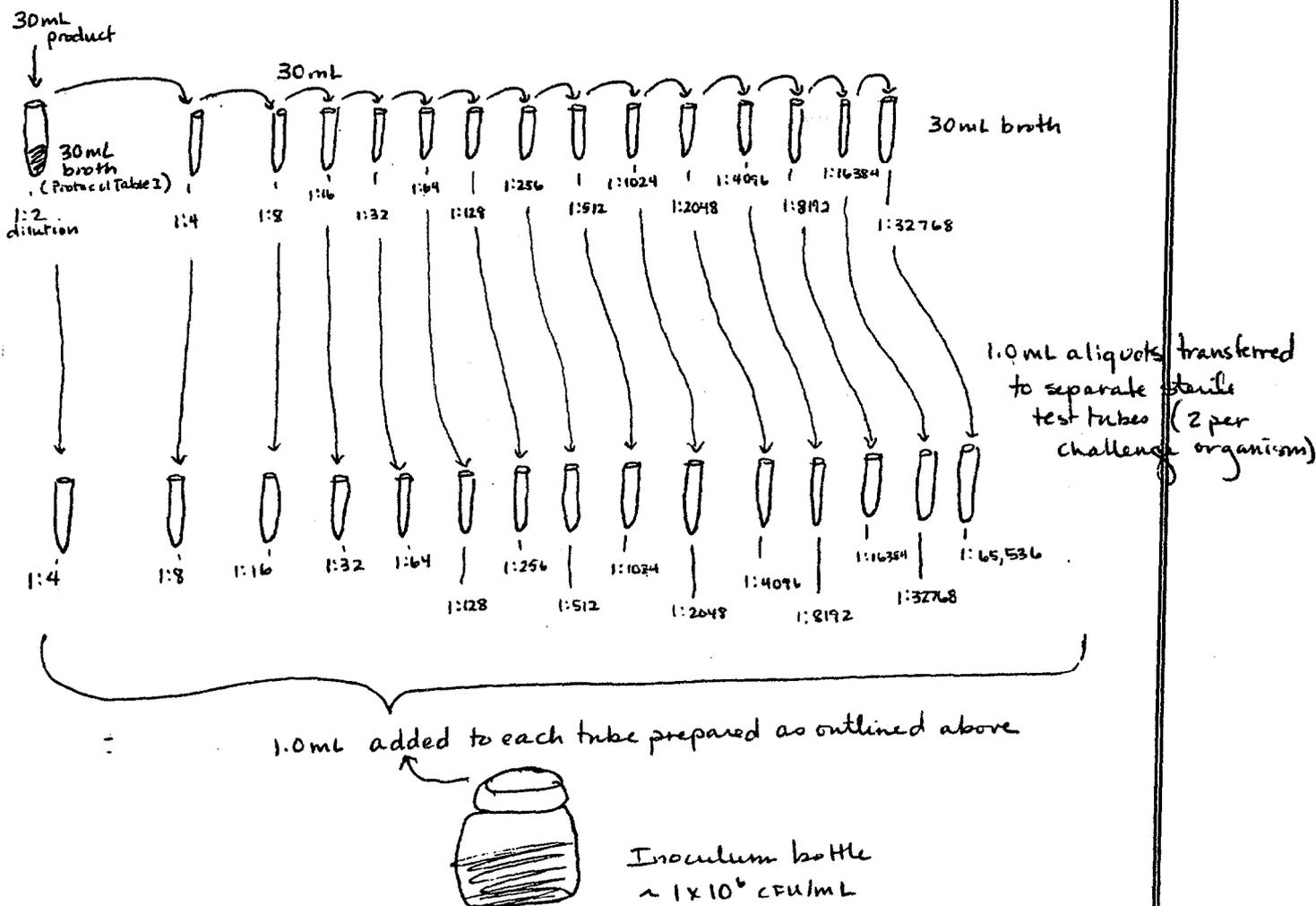
EFFECTIVE

GENERAL DATA GATHERING

Sponsor: Lonza, Inc

Subject: Testing Dilution Scheme

For all species except *Streptococcus pneumoniae* (ATCC # 6303 and Clinical Isolate), *Streptococcus pyogenes* (ATCC # 19615 and Clinical Isolate), *Bacteroides fragilis* (ATCC # 25285 and Clinical Isolate), *Haemophilus influenzae* (ATCC # 19418 and Clinical Isolate), and *Proteus mirabilis* (ATCC # 7002) tested on 08-31-00 only:



Conducted By: [Signature]

[Signature]

T2 T2

T2
T2

Date: 8/15/00 8/16/00 8/17/00 8/31/00

8/18/00

Reviewed By: [Signature]

[Signature]

Date: 9/19/00

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.

EFFECTIVE

GENERAL DATA GATHERING

Sponsor: Lonza, Inc Subject: Testing Dilution Scheme

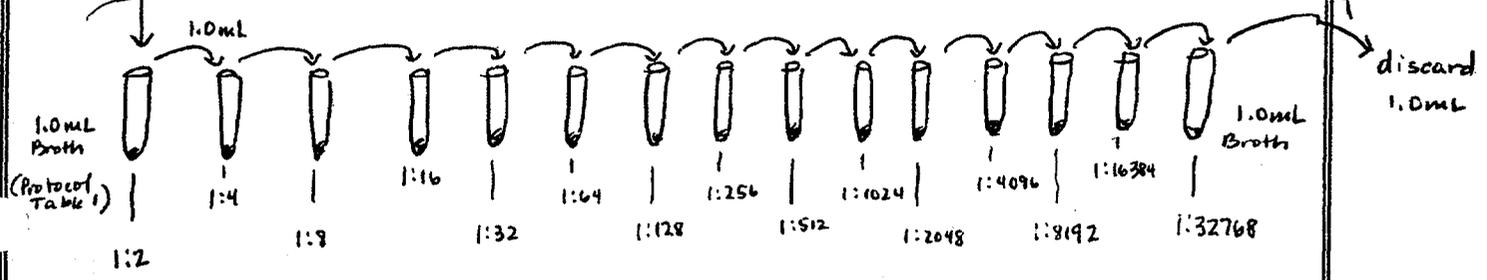
For *Streptococcus pneumoniae* (ATCC # 6303 and Clinical Isolate),
Streptococcus pyogenes (ATCC # 19615 and Clinical Isolate),
Bacteroides fragilis (ATCC # 25285 and Clinical Isolate)
Haemophilus influenzae (ATCC # 19418 and Clinical Isolate)
Proteus mirabilis (ATCC # 7002) tested on 08/31/00, only.



Inoculum Bottle
 $\sim 1 \times 10^6$ CFU/mL

1.0 mL Product

Add 1.0 mL to each tube, resulting in final product dilutions of 1:4 thru 1:65,536.



Performed in duplicate for each organism versus each product

Conducted By: [Signature] Date: 8/15/00 8/16/00 8/17/00 8/31/00
Reviewed By: [Signature] Date: 9/10/00 9/19/00

ADDENDUM VI

Equipment Logs

- Equipment Tracking Forms (Form No. 98-L-007)
- Water Bath Temperature Recording Form
(Form No. 95-L-007)
- Incubator Log Forms (Form No. 96-L-008)
- Refrigerator Log Forms (Form No. 96-L-015)

EQUIPMENT TRACKING FORM

Test Date: 8/15/00 Procedure: Testing (MIC - 15 mins)

Continuously Adjustable Pipette(s), 20µl - 200 µl Capacity: 991205 (tips: DG L1 9113 7-)

Continuously Adjustable Pipette(s), 100µl - 1000µl Capacity: 000504 991204 (tips: DG L1 9113 7-)

Positive Displacement Pipette(s): 970203, 971104, 000503 (Tips: Gilson Batch # B00309205)

Portable Pipetter(s): 971206

Vortex Mixer(s): 991002, 990103

Electronic Timers: N/A

Autoplaters: N/A

(Other) 20 cc Syringes : Becton Dickinson lot # 9281282

(Other) 25ml Pipettes : (VWR) # 23099021

(Other) Bottles : DG L1 91127 7-20-00

(Other) Test tubes DG L1 91127 8/8/00, DG L2 91127 8/7/00; DG L1 91127 71

(Other) Petri Dishes (American Precision Plastics) lot # 00268906

(Other) N/A 7/8/15/00 :

(Other) _____ :

(Other) _____ :

(Other) _____ :

(Other) sterile Graduated Cylinder : DG L2 91127 8/7/00

(Other) sterile Beaker : DG L1 91127 8/02/00

(Other) Mettler Balance : # 930409 (WL) 7/8/15/00

(Other) sterile Water for Irrigation : Abbott lot # 58-842-4B-2 Exp. 11-01-02

Recorded by: [Signature] Date: 8/15/00

Reviewed by: [Signature] Date: 9/15/00

Approved by: [Signature] Date: 9/19/00

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1998 by BioScience Laboratories, Inc.

EFFECTIVE
12/2/98 [Signature]

EQUIPMENT TRACKING FORM

Test Date: 8/16/00 Procedure: MIC (Testing - 16 bugs)

Continuously Adjustable Pipette(s), 20µl - 200 µl Capacity: 991205 (tips: DG LI 91127 7-26-00)

Continuously Adjustable Pipette(s), 100µl - 1000µl Capacity: 991204, 000504 (tips: DG LI 91113 7-21-00)

Positive Displacement Pipette(s): 000503 971104 970203 (tips: Gilson Batch # B00309225)

Portable Pipetter(s): 971206

Vortex Mixer(s): 991002 980103

Electronic Timers: N/A

Autoplate: N/A

(Other) ^{sterile} 5 mL pipettes: (KIMBLE) lot # N000800

(Other) Petri Dishes (American Precision Plastics) lot # 00268906

(Other) N/A

(Other) ↓

(Other) ↓ TZ 8/16/00

(Other) ↓

(Other) sterile water for irrigation (Abbott # 58-842-48-2 Exp. 1 Nov 2002)

(Other) test tubes (sterile): DG LI 91127 8/9/00 ; DG LI 91127 8/8/00

(Other) ^(sterile) 25 mL Pipettes (VWR) lot # 23099021

(Other) Bottles, sterile DG : LI 91127 7-20-00

(Other) Mettler Balance : # 930409

(Other) Graduated Cylinder, sterile DG LI 91127 8-11-00

(Other) Beaker, sterile: DG LI 91127 7-28-00

Recorded by: [Signature] Date: 8/16/00

Reviewed by: [Signature] Date: 9/15/00

Approved by: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1998 by BioScience Laboratories, Inc.

EFFECTIVE
12/2/98 [Signature]

EQUIPMENT TRACKING FORM

Test Date: 8/17/00 Procedure: Testing (MIC - 16 bugs)

Continuously Adjustable Pipette(s), 20µl - 200 µl Capacity: 991205 (tips: DG LI 91127 7-26-00)

Continuously Adjustable Pipette(s), 100µl - 1000µl Capacity: 000504, 991204 (tips: DG LI 91113 7-21-00)

Positive Displacement Pipette(s): 971104, 000503, 970203 (tips: Gilson Batch # 8003 9225
DG LI 8117100)

Portable Pipetter(s): 971206

Vortex Mixer(s): 991002 980103

Electronic Timers: N/A

Autoplater: N/A

(Other) _____ : _____

(Other) 729-17-00 : _____

(Other) _____ : _____

(Other) _____ : _____

(Other) 5 mL Pipettes (sterile) KIMBLE lot # N 00080C

(Other) Petri Dishes (sterile) American Precision Plastics lot # 00268906

(Other) test tubes (sterile) DG LI 91127 8-14-00 / DG LI 91127 8-11-00 / DG LI 2 91127 8-09-00

(Other) Bottles, Sterile : DG L2 91127 7-24-00

(Other) 25 mL Pipettes (sterile) VWR lot # 23099021

(Other) sterile Water for Injection (Abbott) lot # 58-842-⁴⁸2 Exp. 1 Nov 2002

(Other) Mettler balance: # 930409 (DG LI 9/20/00)

(Other) Graduated Cylinder, sterile DG LI 91127 8/15/00

(Other) beakers, sterile: DG LI 91127 7/28/00

Recorded by: [Signature] Date: 8/17/00

Reviewed by: [Signature] Date: 9/13/00

Approved by: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1998 by BioScience Laboratories, Inc.

EFFECTIVE
12/2/98 [Signature]

EQUIPMENT TRACKING FORM

Test Date: 8/18/00 Procedure: Testing (MIC - 3 bugs)

Continuously Adjustable Pipette(s), 20µl - 200 µl Capacity: 991205 (TIPS D6 LI 91127 7/21/00)

Continuously Adjustable Pipette(s), 100µl - 1000µl Capacity: 991204, 000504 (TIPS D6 LI 91127 7/21/00)

Positive Displacement Pipette(s): 971104, 970203, 000503 (GILSON 200201225 + 103) 728128100

Portable Pipetter(s): 980902

Vortex Mixer(s): 991002, 980103

Electronic Timers: NA

Autoplate: NA

(Other) SL Dishes : KIMBLE BUTZ Necessoac (N/L) 729120100

(Other) Petri Dishes : American 3M 9119100
PRECISION PLASTICS #1 00265706

(Other) T. Tubes : D6 LI 91127 8/14/00

(Other) 125mL Bottles : D6 12 91127 7/24/00

(Other) 25mL Pipet : VWR 23099021

(Other) NA : _____

(Other) 20µl : _____

(Other) 8/11/00 : _____

(Other) sterile Water for Irrigation, Abbott lot # 58-842-48-2

(Other) Mettler Balance 930409 Exp. 1 Nov 2002

(Other) Graduated Cylinder, sterile D6 LI 91127 8-17-00

(Other) Beaker, Sterile: D6 LI 91127 8-15-00

Recorded by: [Signature] [Signature] Date: 8/18/00 8/18/00

Reviewed by: [Signature] Date: 9/15/00

Approved by: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1998 by BioScience Laboratories, Inc.

EFFECTIVE
12/2/98 [Signature]

EQUIPMENT TRACKING FORM

Test Date: 9-8-00 Procedure: Testing (M.C.)

Continuously Adjustable Pipette(s), 20µl - 200 µl Capacity: 991205 (tips: Dg LZ 91127 8-9-00)

Continuously Adjustable Pipette(s), 100µl - 1000µl Capacity: 991204, 970204 (Dg LI 91127 8-18-00)

Positive Displacement Pipette(s): 971104 (tips: Gilson Batch # B00309225)

Portable Pipetter(s): 4 ⁹⁸⁰⁰ N/A 980602

Vortex Mixer(s): 991002

Electronic Timers: N/A

Autoplate: N/A

(Other) Bottles : Dg LZ 91127 8-17-00

(Other) Test tubes : Dg LI 91127 8-18-00, Dg LZ 91127

(Other) Petri Dishes : American Precision Plastics lot # 00268906

(Other) Sterile Beaker : Dg LI 91127 8-18-00

(Other) 50 ml Pipette : Kimble lot # 015 01000008
004-9-8-00

(Other) Mettler Balance : 930409

(Other) Sterile Water for Irrigation : Abbott lot # 58-842-48-2 Exp 1 NOV 2002

(Other) N/A :

(Other) 19-8-00 :

(Other) :

(Other) :

(Other) :

(Other) :

Recorded by: A. McSwell Date: 9-8-00

Reviewed by: [Signature] Date: 9/15/00

Approved by: [Signature] Date: 9/19/00

Notice: Proprietary Information - Not for Publication.
Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom.
© Copyright 1998 by BioScience Laboratories, Inc.

EFFECTIVE
12/2/98 [Signature]

EFFECTIVE
6/04/98 *AM*

Addendum VI Date 9/21/00
 Page 7 of 13

Protocol No. 000622

WATER BATH TEMPERATURE RECORDING FORM

Laboratory: In-vitro

Water Bath Serial Number: 930611

Water Bath Operating Temperature Range: 47° ± 2°C

Thermometer Number: JJ-971001

Date (Month/Day/Year)	Time (am/pm)	Temperature of Water Bath °C	Recorded By (To Be Initialed)
8.15.00 8.15.00	1:35 p-	47°C	<i>[Signature]</i>
8.16.00	2:30 p-	48°C	<i>[Signature]</i>
8/17/00	4:05 PM	47°C	<i>CM</i>
8.18.00	1:35 p-	48°C	<i>[Signature]</i>
8.31.00	1:35 p-	47°C 47°C (circled) 8.31.00	<i>[Signature]</i>
9/8/00	2:45 pm	47°C	<i>AM</i>
N/A Tz 9/18/00			

Project Notes (Initial and Date After Note): N/A Tz 9-18-00

Reviewed By: *[Signature]* Date: 09/18/00
 Approved By: *J J M'Donnell* Date: 9/19/00

Protocol No. 000622

INCUBATOR LOG FORM

BSLI No. 91101

Description	Date/Time In & Initials	Temperature In	Date/Time Out & Initials	Temperature Out
8 inoc plates/tubes	J - 8.13.00 11:00	36°C	J - 8.14.00 2:00	36°C
7 bags inoc plates	J - 8.14.00 2:20P	36°C	J - 8.15.00 8:20am	36°C
Sony 6303 Inoc Plates	On 8/14/00 3:40pm	36°C	J - 8.15.00 9:45	36°C
15 Inoc tubes	J - 8.14.00 12:00	36°C	J - 8.15.00 9:45	36°C
16 Inoc Tubes/Plates	J - 8.15.00 10:10am	36°C	J - 8.16.00 8:20am	36°C
16 Inoc tubes	J - 8.15.00 11:30P	36°C	J - 8.16.00 1:00P	36°C
15 MIC'S	On 8/15/00 2:30pm	36°C	TZ 8/16/00 10:30AM	35°C
16 Bags Inoc Plates	J - 8.16.00 1:35P	36°C	J - 8.17.00 8:20am	36°C
16 MIC'S 14 MIC'S *	On 8/16/00 4:20pm	36°C	TZ 8-17-00 12:40pm	36°C
IP PLATES 1CRATE	On 8/16/00 4:40pm	36°C	J - 8.18.00 8:40am	36°C
3 Inoc Tubes	J - 8.16.00 5:10P	36°C	J - 8.17.00 3:50P	36°C
IP PLATES	On 8/15/00 4:00pm	36°C	TZ 8/17/00 12:00pm	36°C
Sa, Se, Pa Inoc Plates	J - 8.17.00 3:20P	36°C	J - 8.18.00 8:20am	36°C
* Pm 7002 tubes back in	TZ 8/17/00 3:45pm	36°C	TZ 8/17/00 10:30 AM	36°C
MIC Tubes (16) ⊕	On 8/17/00 3:50pm	36°C	TZ 8-18-00 11:50AM	36°C
IP PLATES	On 8/17/00 5:00pm	36°C	TZ 8/19/00 8:40AM	36°C
⊕ Sa 4 tubes back in Sho 27844 SS15305	TZ 8/19/00 1:00pm	36°C	TZ 8/19/00 8:40AM	36°C
Sa, Se, Pa Inoc Plates	J - 8.18.00 3:00P	36°C	TZ 8/20/00 1:00pm	36°C
Sa, Se, Pa MIC Tubes	J - 8.18.00 3:00P	36°C	TZ 8/19/00 8:45AM	36°C
BF IP Plates Transferred from 960802 (retest)	TZ 8/18/00 2:00pm	36°C	TZ 8/21/00 9:00AM	36°C
Spy, Pm Inoc tubes (retest)	On 8/27/00 at 11:50	36°C	TZ 8/30/00 11:50 AM	36°C
Spy Pm Inoc. plates (retest)	TZ 8/30/00 12:00pm	36°C	J - 8.31.00 9:00am	35°C
MIC Tubes Spy, P- (retest)	J - 8.31.00 1:45P	36°C	J - 9.1.00 10:30am	35°C
IP Plates Spy, P- (retest)	J - 8.31.00 3:35P	36°C	J - 9.5.00 9:00am	36°C
(1) Spy lawn	On 9/7/00 9:00am	36°C	On 9/8/00 8:30am	36°C
MIC TUBES Spy	On 9/8/00 12:30pm	36°C	On 9/9/00 7:40am	36°C

TZ 8/20/00 Inactive. Leaky. Logged in.

Notice: Proprietary Information - Not for Publication.

Confidential/proprietary information of BioScience Laboratories, Inc., is contained here in and may not be disclosed, used, made available, duplicated, or distributed without its prior written consent. Failure to observe this may result in Liability for any damages and Losses resulting therefrom. © Copyright 1996 by BioScience Laboratories, Inc.

0853 '00 OCT 12 P12:21

Overview of Benzethonium Chloride (BZC) Studies

Following its review of the BZC safety studies submitted by Lonza Inc., FDA scientists asked Lonza to conduct a rat pharmacokinetic study in which BZC was applied dermally in the form of an aqueous solution. This study was requested since BZC was administered dermally as a solution in ethanol in the National Toxicology Program (NTP) studies but will be used in water based formulations in commercial products. Lonza agreed to attempt such a study by the dermal route of administration but stated its concern with being able to achieve quantifiable levels of radioactivity in the blood because BZC is not a good candidate for skin absorption due to its cationic nature and because the skin irritation properties of BZC limits the total dose that can be applied. To address this issue, Lonza also volunteered to conduct a set of *in vitro* dermal penetration studies to define the potential for BZC to penetrate rat and human skin either as a solution in water or 95% ethanol.

In both sets of studies, a 1% (w/v) concentration of BZC was evaluated. This concentration was selected because (i) it represented the "average" concentration evaluated in the NTP studies; (ii) it represented a typical concentration for commercial products and (iii) it represented the maximum concentration of BZC that could be applied on a repeated basis to the skin of the Fisher 344 rat without producing more than slight skin irritation. Also, in order to maximize the sensitivity of the analytical results, ¹⁴C-BZC of high specific activity (40.16 mCi/mmole) was used in both sets of studies.

75N-183H

RPT 4

The skin penetration studies were successfully completed and showed that less than 1% of the applied ^{14}C -BZC was absorbed through human skin as either a water or ethanol solution (Inveresk Study No. 18751). The results of the studies conducted with rat skin showed that 5.05 and 4.73% of the applied dose of ^{14}C -BZC was absorbed in the water and alcohol based dosing solutions, respectively.

Two preliminary studies were completed in an attempt to conduct the rat pharmacokinetic study. In the first preliminary study, it was confirmed that a 1% aqueous concentration of BZC represented the maximum concentration that could be applied without producing more than slight skin irritation (Baxter Study No. 10797). The second preliminary study was designed to determine the time course of appearance and disappearance of radioactivity in the blood of Fisher 344 rats following dermal application of a 1% aqueous concentration of ^{14}C -BZC (Baxter Study No. 10936). In this study, approximately 8% of the applied radioactivity was recovered in the urine and feces (0.37% in urine and 7.3% in feces) over the 48-hour study period; however, quantifiable levels of radioactivity were not found in the blood at any of the sampling intervals. Since the design of the preliminary study represented a worse-case exposure scenario in terms of BZC concentration, dose volume and total amount of radioactivity that could be applied, it was unlikely that the design of the definitive toxicokinetic study could have been modified from that used in the preliminary toxicokinetic study such that quantifiable levels of radioactivity could have been observed. Therefore, the definitive study was not undertaken.

While the definitive study was not undertaken, the results of the preliminary pharmacokinetic study along with the findings from the *in vitro* skin penetration studies clearly show that administration of BZC in the form of an aqueous solution does not represent a situation where BZC has a greater potential to penetrate the skin of rats or humans when it is administered as a solution in water versus a solution in ethanol. The results of the skin penetration studies also show that potential systemic exposure in humans to dermally applied BZC in the form in which it is intended to be marketed is very low.

TABLE OF CONTENTS

<u>Study Title</u>	<u>Tab</u>
The In Vitro Percutaneous Absorption of [¹⁴ C]- Benzethonium Chloride Through Human and Rat Skin	A
Preliminary Pharmacokinetics Study of Dermally Applied ¹⁴ C-Benzethonium Chloride in Rats	B
Dermal Irritation of Benzethonium Chloride in Rats	C

A



Inveresk Report Number 18751

The In Vitro Percutaneous Absorption of [¹⁴C]-Benzethonium Chloride Through Human and Rat Skin

Data Requirements:

None

Study Completion Date:

04 October 2000

Authors

L Gedik
C S Roper

Sponsor:

Lonzagroup
17-17 Route 208
Fairlawn
NJ 07410
USA

Performing Laboratory:

Inveresk Research
Tranent
EH33 2NE
Scotland



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 § 10(d) (1) (A), (B) or (C).

These data are the property of Lonzagroup and as such, are considered to be confidential for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any statute or in any other country.

Company: Lonza Group (Lonza Inc.)

Company Agent: Joseph R. Robinson (R)



Compliance with Good Laboratory Practice Standards

The study described in this report was conducted in accordance with the OECD Principles of Good Laboratory Practice as acceptable to the United States of America (EPA) as per 40 CFR 160 and Japan (MHW, MAFF, MITI) under the memorandum of understanding with the UK GLP Monitoring Authority. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.



C S Roper BSc PhD
Study Director
Inveresk Research

Date: 04 October 2000



Sponsor/Submitter

Date: 06 October 2000



Contents

	Title Page.....	1
	Statement of No Data Confidentiality Claims.....	2
	Compliance with Good Laboratory Practice Standards.....	3
	Contents.....	4
	Quality Assurance Statement.....	8
	Personnel Involved.....	9
1	Summary.....	10
2	Introduction	12
3	Experimental Procedure.....	13
	3.1 Materials.....	13
	3.1.1 Human Skin Samples.....	13
	3.1.2 Rat Skin Samples.....	13
	3.1.3 Preparation of Dermatome Skin Membranes.....	14
	3.2 Analytical Procedures.....	14
	3.2.1 Radiochemical Purity of [¹⁴ C]-Benzethonium Chloride	14
	3.2.2 Preparation of Dose Formulations	15
	3.2.2.1 Preparation of Stock Solution of [¹⁴ C]-Benzethonium Chloride in Ethanol	15
	3.2.2.2 Preparation of [¹⁴ C]-Benzethonium Chloride in Water Formulation	15
	3.2.2.3 Preparation of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation.....	15
	3.3 Flow Through Diffusion Cell Apparatus.....	15
	3.4 Barrier Integrity Assessment.....	16
	3.5 Application Information	17
	3.6 Sampling Information.....	17
	3.6.1 Receptor Fluid	17
	3.6.2 Terminal Procedures	17
	3.6.3 Re-analysis of Skin Wash + Swabs	18
	3.7 Determination of Radioactivity	18
	3.8 Limit of Reliable Measurement	18
	3.9 Calculations.....	19
	3.9.1 Permeability Coefficient (Kp) of Water	19
	3.9.2 Cumulative Flux of [¹⁴ C]-Benzethonium Chloride.....	19
	3.9.3 Definitions	20
	3.10 Protocol Deviations.....	20
4	Results.....	21
	4.1 Human Skin.....	21



4.1.1	[¹⁴ C]-Benzethonium Chloride in Water Formulation	21
4.1.2	[¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation	21
4.2	Rat Skin.....	22
4.2.1	[¹⁴ C]-Benzethonium Chloride in Water Formulation	22
4.2.2	[¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation	22
5	Discussion.....	23
6	Conclusions	24
7	Tables	25
Table 1	Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation to Human Dermatome Skin	25
Table 2	Cumulative Penetration (% Applied Dose) of [¹⁴ C]-Benzethonium Chloride in Water Formulation Through Human Dermatome Skin into Receptor Fluid.....	26
Table 3	Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [¹⁴ C]-Benzethonium Chloride in Water Formulation Through Human Dermatome Skin into Receptor Fluid.....	27
Table 4	Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Human Dermatome Skin	28
Table 5	Cumulative Penetration (% Applied Dose) of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Human Dermatome Skin into Receptor Fluid	29
Table 6	Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Human Dermatome Skin into Receptor Fluid.....	30
Table 7	Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation to Rat Dermatome Skin	31
Table 8	Cumulative Penetration (% Applied Dose) of [¹⁴ C]-Benzethonium Chloride in Water Formulation Through Rat Dermatome Skin into Receptor Fluid	32
Table 9	Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [¹⁴ C]-Benzethonium Chloride in Water Formulation Through Rat Dermatome Skin into Receptor Fluid.....	33
Table 10	Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Rat Dermatome Skin.....	34



Table 11	Cumulative Penetration (% Applied Dose) of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Rat Dermatome Skin into Receptor Fluid	35
Table 12	Cumulative Flux (µg equiv.cm ⁻²) of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Rat Dermatome Skin into Receptor Fluid.....	36
8	Figures	37
Figure 1	Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation to Human Skin (Mean ± SD, n = 10).....	37
Figure 2	Cumulative Flux (µg equiv.cm ⁻²) of Benzethonium Chloride Through Human Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation (Mean ± SD, n = 10)	38
Figure 3	Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Human Skin (Mean ± SD, n = 10).....	39
Figure 4	Cumulative Flux (µg equiv.cm ⁻²) of Benzethonium Chloride Through Human Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation (Mean ± SD, n = 10).....	40
Figure 5	Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation to Rat Skin (Mean ± SD, n = 13).....	41
Figure 6	Cumulative Flux (µg equiv.cm ⁻²) of Benzethonium Chloride Through Rat Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation (Mean ± SD, n = 13)	42
Figure 7	Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Rat Skin (Mean ± SD, n = 11).....	43
Figure 8	Cumulative Flux (µg equiv.cm ⁻²) of Benzethonium Chloride Through Rat Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation (Mean ± SD, n = 11).....	44



Figure 9	A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water (Mean ± SD, n = 10) and Ethanol:Water (95:5, v/v; Mean ± SD, n = 10) Formulations.....	45
Figure 10	A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Rat Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water (Mean ± SD, n = 12) and Ethanol:Water (95:5, v/v; Mean ± SD, n = 11) Formulations	46
Figure 11	A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human (Mean ± SD, n = 10) and Rat (Mean ± SD, n = 13) Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water Formulation	47
Figure 12	A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human (Mean ± SD, n = 10) and Rat (Mean ± SD, n = 11) Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation	48
Figure 13	A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human and Rat Skin Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water and Ethanol:Water (95:5, v/v) Formulations (Mean)	49
9	Appendices	50
Appendix 1	Product Information for [¹⁴ C]-Benzethonium Chloride	50
Appendix 2	Certificate of Analysis for Benzethonium Chloride	51
Appendix 3	Human Skin Donor Details	52
Appendix 4	Thickness of Dermatome Skin Membranes	53
Appendix 5	Radiochemical Purity of [¹⁴ C]-Benzethonium Chloride by HPLC.....	54
Appendix 6	Temperature and Relative Humidity of the Laboratory.....	55
Appendix 7	Cross Reference of Skin Sample Number with Skin Donor and Tritiated Water Permeability Coefficient (Kp).....	56
Appendix 8	A Comparison of the Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴ C]-Benzethonium Chloride in Water and Ethanol:Water (95:5, v/v) Formulations to Human and Rat Dermatome Skins.....	58
	Final Page of Report	58



Quality Assurance Statement

The conduct of this study has been subjected to periodic inspections by the Inveresk Research Quality Assurance Unit. The dates of inspection are given below.

<u>Date of QA Inspection</u>	<u>Phase</u>	<u>Date of Report To Management/SD</u>
10 April 2000	Protocol Review	12 April 2000
17 April 2000	Dose Preparation Review/Dosing/ Protocol Compliance	19 April 2000
25 April 2000	Barrier Integrity (Rat)	27 April 2000
26 April 2000	Skin Washes	27 April 2000
03 July 2000	Audit of Draft Report	04 July 2000
04 October 2000	Audit of Final Report	04 October 2000

The report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure(s). The report is considered to describe accurately the methods and procedures used in the study. The reported results accurately reflect the original data generated during the study.

Signed: Jane P Dunrie
(Quality Assurance)

Date: 04 October 2000



Personnel Involved

Study Director:

C S Roper BSc PhD

Head, Metabolism Chemistry:

M Phillips BSc PhD

Report Compilation:

L Gedik BSc MSc
C S Roper BSc PhD

Scientific Staff:

J Lauckner BSc
S Madden BSc PhD
L Oliver BSc
A G Simpson BSc
G Holmes BSc

Quality Assurance:

J P Dunsire CBIol MIBiol



1 Summary

[¹⁴C]-Benzethonium Chloride was applied at a concentration of 1.0% (w/v) in water and ethanol:water (95:5, v/v) vehicles, to human and rat dermatomed skin membranes *in vitro* using a flow through diffusion cell system. The formulations were applied to skins at 10 $\mu\text{l.cm}^{-2}$ giving the nominal dose levels shown in the table below. Receptor fluid (tissue culture medium with ca 4% bovine serum albumin maintained with 5% CO₂ in O₂) was collected hourly for 0-6 h post dose and every other hour from 6-24 h post dose. The underside of the membrane was washed with receptor fluid to remove absorbed material that had not been collected into the receptor chamber. The unabsorbed material was collected using ca 10 ml 2% soap solution. The donor and receptor chambers were also rinsed with receptor fluid. The skin surface was dried with tissue swabs to remove any residual material. These swabs were added to the soap prior to analysis. The swabs were later removed, dried and analysed by combustion. The stratum corneum was removed by a minimum of 5 successive tape strips. Radiolabelled material was extracted from the tape by mixing with Soluene-350 tissue solubiliser and methanol. The non dose site skin immediately adjacent to the dose site was collected to determine the amount of radioactivity that diffused laterally after penetration through the stratum corneum. The dose site skin was sealed in cling film and divided into epidermis and dermis by heat separation and all skin samples solubilised in Soluene-350 tissue solubiliser. Total unabsorbed material was the sum of the skin wash, cell wash, stratum corneum, cling wrap and epidermis (the epidermis is assumed to contain material associated with the stratum corneum which was not removed by tape stripping). Total absorbed material was the sum of the dermis, non dose site skin, receptor rinse and 0-24 h receptor fluid.

The cumulative flux, total penetration into receptor fluid and total absorbed material for both species are presented in the following table.

Formulation	Water		Ethanol:water (95:5, v/v)	
	100		100	
Nominal Dose ($\mu\text{g.cm}^{-2}$)				
Species	Human	Rat	Human	Rat
Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$)	0.03	0.15	0.03	0.31
Receptor Fluid (%)	0.03	0.15	0.03	0.31
Penetration (%)	0.03	0.16	0.29	0.32
Dermal Delivery(%)	0.64	5.05	0.22	4.73

For both formulations applied to both human and rat skin, penetration was very low.

The penetration and dermal delivery of [¹⁴C]-Benzethonium chloride was higher in the rat than human for both formulations. Compared to the human, total absorption was 7.8-fold greater in the rat for the water formulation and 10.1-fold greater for the ethanol:water (95:5, v/v) formulation. Dermal delivery was 7.9-fold greater in the rat for the water formulation and 22-fold greater for the ethanol:water (95:5, v/v) formulation. Penetration into the receptor fluid



was 5-fold greater in the rat for the water formulation and 10-fold greater for the ethanol:water (95:5, v/v) formulation than for the human skin. This was the same for penetration (receptor fluid + receptor rinse for the water formulation) but there was no difference in penetration (receptor fluid + receptor rinse) between human and rat in the ethanol:water (95:5, v/v) formulation.

In conclusion, [¹⁴C]-Benzethonium Chloride poorly penetrates human and rat skin in both test formulations; water and ethanol:water (95:5, v/v). Total absorption also was less than 1.0% in both test formulations with human skin. However, total absorption increased to approximately 5% of the applied dose in both test formulations with rat skin.



2 Introduction

Benzethonium Chloride is currently under development as a topical preparation. As part of the safety evaluation of the product, a study was conducted to assess the rate and extent of absorption of Benzethonium Chloride following topical application to rat and human skin at a concentration of 1.0% (w/v) using water and ethanol:water (95:5, v/v) as vehicles.

The study was conducted at Inveresk Research according to Protocol No. 167879.

Key dates in the conduct of this study were as follows:

Study Initiation:	04 April 2000
Experimental Start Date:	07 April 2000
Experimental Completion Date:	03 June 2000
Study Completion Date:	See Authentication page for date of Study Director's signature (page 3)

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk Research for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the transfer, disposal or continued storage of raw data.

This study was conducted to comply with

- (a) COLIPA. Cosmetic ingredients: guidelines for percutaneous absorption/ penetration. COLIPA, Brussels, 1995.
- (b) OECD Guideline for testing of chemicals. Draft OECD guideline, dermal delivery and percutaneous absorption: *in vitro* method. Draft document, June 1996.



3 Experimental Procedure

3.1 Materials

Wizard Laboratories, West Sacramento, CA, USA, supplied the [¹⁴C]-Benzethonium Chloride, Batch No. 000407 (1 mCi) with a stated specific activity of 40.16 mCi.mmol⁻¹ and stated radiochemical purity of 97.54%. The supplied material was stored at Inveresk at ca -20°C in the dark. A copy of the supplied product information sheet is given in Appendix 1.

Lonzagroup supplied the non-radiolabelled Benzethonium Chloride (Hyamine 1622), Batch No. 80102, Lot No. 8K0909 (ca 25 g) was stored at Inveresk at room temperature in the dark. A copy of the supplied certificate of analysis is given in Appendix 2.

Carbo-Sorb[®] CO₂ absorbing fluid and Permafluor[®]E⁺ scintillation fluid were used in conjunction with the Packard Tri-Carb 306 automatic sample oxidiser and were supplied by Canberra Packard Limited, Pangbourne, UK.

Spec-Chec[™]¹⁴C, used to estimate efficiencies of combustion, was supplied by Canberra Packard Limited, Pangbourne, UK.

Quickzint liquid scintillation fluid was obtained from Zinsser Analytic, Maidenhead, UK.

All other materials were supplied by Inveresk Research.

3.1.1 Human Skin Samples

Human breast and abdominal skin samples were obtained from the Plastic Surgery Unit, St Johns Hospital NHS Trust, Livingston, UK. Full thickness skin was obtained, cleaned of subcutaneous fat and connective tissue using scalpel blades, scissors and "blue roll" tissue paper. The skins were washed in cold running water, dried, cut into smaller pieces (where appropriate), wrapped in aluminium foil, put into self sealing plastic bags and stored at ca -20°C until required. The age and sex of the donor and site from which the skin was taken were recorded. The sample details are as shown in Appendix 3.

3.1.2 Rat Skin Samples

Adult male and female Fisher F344 rats were obtained from Charles River, UK. The male and female rats weighed 200-203 g and 146-153 g, respectively, on arrival. The animals were maintained in the laboratory environment to acclimatise for 2 days prior to sacrifice by CO₂ narcosis and cervical dislocation. The animals were weighed immediately after killing (male 208-232 g, female 153-164 g). The fur on the dorsal skin was clipped using hair clippers (Wella) and the skin excised using scissors. The skins were laid



out on aluminium foil (stratum corneum uppermost) and the skin divided into two down the mid-lateral line. Each piece was wrapped in aluminium foil and stored at ca -20°C until use.

3.1.3 Preparation of Dermatome Skin Membranes

When required, human or rat skin samples were removed from ca -20°C storage and allowed to thaw at room temperature. The full thickness skin thickness was measured using a micrometer. Dermatome membranes were prepared by pinning the full thickness skin, stratum corneum uppermost, onto a raised cork board and cutting at a setting equivalent to ca 400 µm depth using a Zimmer electric dermatome. The membranes were then laid out onto aluminium foil and the thickness of the membranes measured using a micrometer. The full thickness and dermatome membrane thickness of membranes are given in Appendix 4.

3.2 Analytical Procedures

3.2.1 Radiochemical Purity of [¹⁴C]-Benzethonium Chloride

The radiochemical purity of [¹⁴C]-Benzethonium Chloride was determined by HPLC prior to dose preparation using the following equipment and conditions. The chemical authenticity of the radiolabelled test material was confirmed by co-chromatography with the non-radiolabelled material.

Equipment

HP 1050 Series HPLC System
Gilson 231 Sample Injector
Canberra Packard Radiomatic™ Flo-one® Beta, Flow Scintillation Analyser (Model 150TR).

Conditions

Column: Alltima C18
(250 mm x 4.6 mm, 5 µm)
Mobile Phase: Solvent A = Acetonitrile:water (7:3, v/v) with 0.5% TFA

Solvent System (Isocratic)	Time (min)	Solvent A (%)
	0	100
	30	100

Flow rate: 1 ml.min⁻¹
U.V. Detection: 254 nm
Column Temp.: Ambient
Scintillant: Ultima-Flo™ M
Data Collection: LabSystems Vax Multichrom Version 2.30b



A representative HPLC trace is presented in Appendix 5. The radiochemical purity of [^{14}C]-Benzethonium Chloride was determined to be 97.6%.

3.2.2 Preparation of Dose Formulations

3.2.2.1 Preparation of Stock Solution of [^{14}C]-Benzethonium Chloride in Ethanol

The [^{14}C]-Benzethonium Chloride was mixed with 1 ml ethanol to prepare a ca 1 mCi.ml⁻¹ solution. The radioactive content was confirmed to be 117% of the target concentration.

3.2.2.2 Preparation of [^{14}C]-Benzethonium Chloride in Water Formulation

An aliquot (156 μl) of the stock ethanolic solution of [^{14}C]-Benzethonium Chloride was transferred into the dose vial and the solvent removed under nitrogen gas. Benzethonium chloride (8.39 mg) was weighed into the dose vial and 1.014 ml reverse osmosis water added. The dose vial was mixed for ca 10 seconds.

Seven 6.4 μl aliquots were taken and the radioactivity content determined by LSC. The concentration of this dose formulation was calculated to be 10.21 mg.ml⁻¹ (1.02% w/v), which was 102% of the target dose concentration.

3.2.2.3 Preparation of [^{14}C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation

A 95% ethanol:water solvent was prepared by mixing 9.5 ml ethanol with 0.5 ml reverse osmosis water.

An aliquot (156 μl) of the stock ethanolic solution of [^{14}C]-Benzethonium Chloride was transferred into the dose vial and the solvent removed under nitrogen gas. Benzethonium chloride (8.57 mg) was weighed into the dose vial and 1.032 ml of the ethanol:water (95:5, v/v) added. The dose vial was mixed for ca 10 seconds.

Seven 6.4 μl aliquots were analysed by LSC. The concentration of this dose formulation was calculated to be 10.21 mg.ml⁻¹ (1.02% w/v), which was also 102% of the target dose concentration.

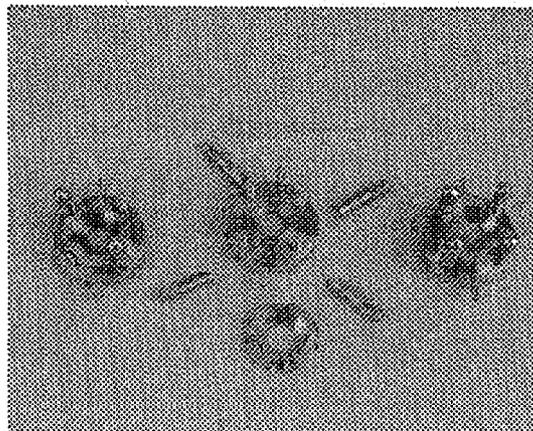
3.3 Flow Through Diffusion Cell Apparatus

An automated flow-through diffusion cell apparatus (Scott/Dick, University of Newcastle, UK) was used (see photograph on p 15). The flow-through cells were placed in a steel manifold heated *via* a circulating water bath. The skin surface temperature was 29.4-31.6°C. The cells were connected to multi-channel peristaltic pumps from their afferent ports, with the receptor fluid effluent dropping *via* fine bore tubing into scintillation vials on a fraction collector. The surface area of exposed skin within the cells was 0.64 cm². The receptor chamber volume was 0.25 ml. The peristaltic pumps were



adjusted to maintain flow rate ranging from 1.37 to 1.57 ml.h⁻¹. The temperature and relative humidity of the laboratory was measure throughout the study. Details can be found in Appendix 5.

A Photograph of the Flow Through Diffusion Cell



The receptor fluid used for the water and test material permeability measurements contained bovine serum albumin (ca 4%, w/v), glucose (ca 1%, w/v), penicillin G (100 units/ml) and streptomycin sulphate (ca 75 units/ml) in modified Earles medium. The atmosphere above the receptor fluid in the receptor reservoir was maintained with Carbogen (5% CO₂ in O₂) in order to maintain the pH and oxygen content of the receptor fluid.

3.4 Barrier Integrity Assessment

Sections of dermatomed skin membrane, ca 1.5 x 1.5 cm, were cut out, positioned in the receptor chamber of the diffusion cells and the donor chamber tightened into place with screws. The cells were then placed in the heated manifold and connected to the peristaltic pump. An equilibration period of ca 15 min was allowed while receptor fluid was pumped through the receptor chambers at ca 1.5 ml.h⁻¹. The effluent was then collected for ca 30 min and retained for LSC analysis for use as a blank sample.

Tritiated water (250 µl, equivalent to ca 300,000 d.p.m.) was applied to the surface of each skin sample and the donor chamber occluded using a cap. Penetration of tritiated water was assessed by collecting hourly fractions for 2 h and analysing the fractions by LSC. Permeability coefficients (Kp) were calculated for each skin sample. Any human or rat skin sample exhibiting a Kp greater than 2.5 x 10⁻³ cm.h⁻¹ was excluded from subsequent absorption measurements. Appendix 7 gives the cross reference of skin sample number and donor and its corresponding tritiated water permeability coefficient (Kp). At the end of the 2 h period, residual tritiated water on the skin surface was removed using a tissue swab and the skin rinsed with ca 2 ml water.



3.5 Application Information

An equilibration period of ca 2 h was allowed prior to collection of a predose sample from each cell for a period of ca 30 min. Each formulation was applied over the stratum corneum surface using an M25 Gilson Microman positive displacement pipette set to deliver ca 6.4 μ l. To accurately quantify the radioactivity applied to the skin samples, 7 aliquots of test formulation were dispensed directly into scintillation vials for mock dose determination. The donor chambers were left open to the atmosphere.

3.6 Sampling Information

3.6.1 Receptor Fluid

Receptor fluid was collected in hourly fractions from 0-6 h post dose, then 2 hourly fractions from 6-24 h post dose.

3.6.2 Terminal Procedures

At the end of the 24 h period, each cell was disconnected from the receptor fluid pump lines. The underside of the skin was washed (receptor rinse) with ca 1-2 ml receptor fluid, which was mixed with ca 10 ml scintillant and analysed by LSC. The receptor rinse represented absorbed material which was in the receptor chamber, but which had not been collected into the 24 h receptor fluid fraction.

The skin surface was washed (skin wash + swab) with ca 4 washes of a ca 2% soap solution (SC Johnson Professional General Purpose Neutral Cleaner, Johnson Wax Limited, UK) using a Gilson pipette P5000 set to deliver 2.5 ml. Each wash was aspirated with a 1 ml disposable pipette. This wash was collected into a pre-weighed pot. The disposable pipette was transferred to a pot and retained as part of the cell wash. The receptor chamber was removed from the donor chamber and the skin taken and laid onto a piece of tissue paper. The donor and receptor chambers were transferred to the cell wash pot. Ethanol:water (50:50, v/v), ca 40 ml, was added to the cell wash pot, weighed and gently mixed. Duplicate ca 1 ml weighed aliquots were taken for LSC analysis. The skin was dried with small pieces of tissue paper, which were retained along with the skin wash. A further ca 40 ml ethanol:water (50:50, v/v) was added and the total mass of skin wash was determined and duplicate ca 1 ml aliquots weighed into scintillation vials for LSC analysis.

The stratum corneum was removed by a minimum of 5 successive tape strips (Guilbert tape). These tapes were collected into a scintillation vial and mixed with ca 3 ml Soluene-350 tissue solubilising fluid. After a minimum of 2 days, ca 15 ml methanol was added, the pot was weighed and the sample mixed. Duplicate ca 1 ml weighed aliquots were taken and mixed with ca 10 ml scintillant for LSC analysis. The remaining skin was divided into dose site and non dose site skin. The dose site epidermis and dermis were heat separated.



A ca 200 g weight was heated to ca 60-70°C in a water bath. The skin was placed onto a piece of cling film epidermis uppermost and the cling film folded over to cover the epidermal surface. The heated weight was applied, with moderate pressure, for ca 60 seconds. The epidermis was peeled away from the dermis using forceps and the pieces of cling film collected. Material was extracted from the cling film by mixing with ca 40 ml ethanol:water (50:50, v/v) and duplicate ca 1 ml weighed aliquots taken for LSC analysis. The non dose site skin and dose site dermis were transferred to scintillation vials and ca 2 ml Soluene-350 was added. The samples were left to solubilise at room temperature prior to addition of ca 1 ml methanol and ca 10 ml scintillant. The dose site epidermis was analysed similarly, except that only ca 1 ml Soluene-350 and methanol were added.

All bulk samples were stored at ca -20°C after analysis.

3.6.3 Re-analysis of Skin Wash + Swabs

Initial analysis of the skin wash + swabs fraction produced low and variable recoveries of radioactivity. Binding of test material radioactivity to the tissue swabs was proposed as a potential reason of this. Each of the samples was, therefore, re-analysed using the procedure detailed below.

The tissue paper was removed from the solvent and placed on another piece of tissue paper to dry overnight. The remaining pot and contents were weighed and duplicate weighed ca 1 ml aliquots of the solvent collected for LSC analysis. The dried tissue swabs were weighed (separately of the new tissue) and cut into small pieces along with the new tissue and placed into combusticones[®] for combustion analysis. The tissue paper aliquots were combusted using a Packard Tri-Carb 306 or 307 Automatic Sample Oxidiser. The resultant ¹⁴CO₂ generated was collected by absorption in Carb-Sorb CO₂ absorbing fluid (8 ml) and Permafluor[®]E⁺ scintillation fluid (10 ml).

3.7 Determination of Radioactivity

All samples, except for the tritiated water samples, prepared in scintillation fluid were subject to liquid scintillation counting for 5 min, together with representative blank samples, using a Liquid Scintillation Analyser with automatic quench correction by an internal standard method. Prior to analysis, samples were allowed to stabilise with regard to light and temperature. The tritiated water samples were treated as above, except that they were subject to liquid scintillation counting for 1 min.

3.8 Limit of Reliable Measurement

A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories. Where results were below the limit of reliable measurement, the fact was noted in the Results section.



3.9 Calculations

The following calculations were performed.

3.9.1 Permeability Coefficient (Kp) of Water

Cumulative penetration of tritiated water was calculated for each cell by summing the net d.p.m. for each hourly fraction from 0 to 2 h. The slope of the penetration *versus* time curve from 0-2 h (*i.e.* 3 data points) was calculated by linear regression to give a penetration rate (d.p.m.cm⁻²h⁻¹).

$$\text{Penetration rate (d.p.m.cm}^{-2}\text{h}^{-1}) = \frac{\text{slope (d.p.m.h}^{-1})}{\text{area (0.64 cm}^2\text{)}}$$

This is converted to the permeability coefficient as follows:

$$Kp \text{ (cm.h}^{-1}\text{)} = \frac{\text{penetration rate (d.p.m.cm}^{-2}\text{h}^{-1})}{\text{}^3\text{H}_2\text{O dose rate (d.p.m.cm}^{-3}\text{)}}$$

3.9.2 Cumulative Flux of [¹⁴C]-Benzethonium Chloride

The individual d.p.m. in each receptor fluid fraction was summed from each timepoint to give a cumulative d.p.m. The cumulative µg equivalents were then calculated and the specific activity (S.A.) of the dose (d.p.m.µg⁻¹) calculated as follows:

$$\text{Cumulative } \mu\text{g equiv.} = \frac{\text{Cumulative d.p.m.}}{\text{Dose S.A. (d.p.m.}\mu\text{g}^{-1}\text{)}}$$

The cumulative flux of [¹⁴C]-TBC1269 was calculated from this and the area dosed (0.64 cm²) calculated as follows:

$$\text{Cumulative Flux (}\mu\text{g equiv.cm}^{-2}\text{)} = \frac{\text{Cumulative } \mu\text{g equiv. (}\mu\text{g)}}{\text{Dose Area (cm}^2\text{)}}$$



3.9.3 Definitions

The unabsorbed material was defined as material recovered in the skin wash + wipes, cell wash, cling wrap, dose site epidermis and material associated with the stratum corneum. The material associated with the dose site epidermis is assumed to be mainly stratum corneum bound material which has not been removed by tape stripping.

Dermal delivery was defined as test material recovered in the dose site dermis and non-dose site skin.

Penetration of material was defined as the test material that had permeated through the skin into the receptor fluid (*ie* receptor fluid plus receptor rinse). This material is equivalent to that which would be systemically available *in vivo*.

The absorbed material was defined as the penetrated material (*i.e.* receptor fluid + receptor rinse) and material recovered in the dose site dermis and non dose site skin. Dose site epidermis was assumed to contain unremoved stratum corneum, hence it was included in the unabsorbed material.

3.10 Protocol Deviations

Protocol section 7.1.1. One of the cells had a skin surface temperature of 29.4°C when checked. The protocol required a temperature of cells 30-32°C.

Protocol section 9.3.1. The tritiated water barrier integrity assessment used tritiated water containing ca 300,000 dpm in 250 µl. The protocol required tritiated water containing ca 100,000 dpm in 250 µl.

Protocol section 9.4.2. The heated block was placed on skin samples for ca 60 seconds. The protocol required that the block was in contact with the skin for ca 90 seconds.

Protocol section 9.4.2. The dose site epidermis was not separated from the dermis by heat separation for cells 43-54 and cell 56. However, for these samples, the tape stripping removed the epidermis from the dermis. The epidermis was then removed from the tape.

The above deviations did not affect the integrity of the study.



4 Results

4.1 Human Skin

4.1.1 [¹⁴C]-Benzethonium Chloride in Water Formulation

A total of 10 samples of human skin, taken from 5 different donors, were dosed topically with [¹⁴C]-Benzethonium Chloride in water formulation (Tables 1-3, Figures 1-2).

Table 1 shows the distribution of radioactivity at 24 h post dose. Mean mass balance was 94.72% of the applied dose. The mean total unabsorbed material was 94.06% which was made up of skin wash + swabs (77.40%), cell wash (3.18%), cling wrap (0.11%), stratum comeum (9.90%) and dose site epidermis (3.47%). Mean total [¹⁴C]-Benzethonium Chloride absorbed was 0.67%, consisting of dose site dermis (0.60%), non dose site skin (0.04%), receptor fluid (0.03%) and receptor rinse (<0.01%).

Dermal delivery was 0.64%. Total penetration (receptor fluid + receptor rinse) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was minimal (0.03% at 24 h). The mean cumulative recovery of radioactivity in the receptor fluid only at the end of the 24 h collection period was 0.03% of the applied dose (Table 2 and Figure 1). The mean cumulative flux of [¹⁴C]-Benzethonium Chloride through human skin was 0.03 µg equiv.cm⁻² at 24 h post dose (Table 3 and Figure 2).

4.1.2 [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation

A total of 10 samples of human skin, taken from 5 different donors, were dosed topically with [¹⁴C]-Benzethonium Chloride in ethanol:water (95:5, v/v) formulation (Tables 4-6, Figures 3-4).

Table 4 shows the distribution of radioactivity at 24 h post dose. Mean mass balance was 97.60% of the applied dose. The mean total unabsorbed material was 97.09% which was made up of skin wash + swabs (90.82%), cell wash (1.62%), cling wrap (0.03%), stratum comeum (3.16%) and dose site epidermis (1.47%). Mean total [¹⁴C]-Benzethonium Chloride absorbed was 0.50%, consisting of dose site dermis (0.19%), non dose site skin (0.03%), receptor fluid (0.03%) and receptor rinse (0.26%).

Dermal delivery was 0.22%. Total penetration (receptor fluid + receptor rinse) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was minimal (0.29% at 24 h). The mean cumulative recovery of radioactivity in the receptor fluid only at the end of the 24 h collection period was 0.03% of the applied dose (Table 5 and Figure 3). The mean cumulative flux of [¹⁴C]-Benzethonium Chloride through human skin was 0.03 µg equiv.cm⁻² at 24 h post dose (Table 6 and Figure 4).



4.2 Rat Skin

4.2.1 [¹⁴C]-Benzethonium Chloride in Water Formulation

A total of 13 samples of rat skin, taken from 7 different animals (4 male and 3 female), were dosed topically with [¹⁴C]-Benzethonium Chloride in water formulation (Tables 7-9, Figures 5-6).

Table 7 shows the distribution of radioactivity at 24 h post dose. Mean mass balance was 96.06% of the applied dose. The mean total unabsorbed material was 90.85% which was made up of skin wash + swabs (66.70%), cell wash (1.65%), cling wrap (1.30%), stratum corneum (10.69%) and dose site epidermis (11.20%). Mean total [¹⁴C]-Benzethonium Chloride absorbed was 5.21%, consisting of dose site dermis (4.51%), non dose site skin (0.53%), receptor fluid (0.15%) and receptor rinse (0.01%).

Dermal delivery was 5.05%. Total penetration (receptor fluid + receptor rinse) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was minimal (0.16% at 24 h). The mean cumulative recovery of radioactivity in the receptor fluid only at the end of the 24 h collection period was 0.15% of the applied dose (Table 8 and Figure 5). The mean cumulative flux of [¹⁴C]-Benzethonium Chloride through rat skin was 0.15 µg equiv.cm⁻² at 24 h post dose (Table 9 and Figure 6).

4.2.2 [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation

A total of 11 samples of rat skin, taken from 6 different animals (3 male and 3 female), were dosed topically with [¹⁴C]-Benzethonium Chloride in ethanol:water (95:5, v/v) formulation (Tables 10-12, Figures 7-8).

Table 10 shows the distribution of radioactivity at 24 h post dose. Mean mass balance was 95.36% of the applied dose. The mean total unabsorbed material was 90.31% which was made up of skin wash + swabs (68.93%), cell wash (1.68%), cling wrap (0.92%), stratum corneum (5.84%) and dose site epidermis (13.43%). Mean total [¹⁴C]-Benzethonium Chloride absorbed was 5.05%, consisting of dose site dermis (4.07%), non dose site skin (0.66%), receptor fluid (0.31%) and receptor rinse (0.02%).

Dermal delivery was 4.73%. Total penetration (receptor fluid + receptor rinse) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was minimal (0.32% at 24 h). The mean cumulative recovery of radioactivity in the receptor fluid only at the end of the 24 h collection period was 0.31% of the applied dose (Table 11 and Figure 7). The mean cumulative flux of [¹⁴C]-Benzethonium Chloride through rat skin was 0.31 µg equiv.cm⁻² at 24 h post dose (Table 12 and Figure 8).



5 Discussion

[¹⁴C]-Benzethonium Chloride was applied at a concentration of 1.0% (w/v) to human and rat dermatome skin membranes *in vitro* in 2 test formulations; water and ethanol:water (95:5, v/v).

A comparison of the distribution of [¹⁴C]-Benzethonium chloride at 24 h post dose can be found in Appendix 8.

For both formulations applied to human skin, mean penetration (Figure 9) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was 0.03% of the applied dose. When comparing total penetration (receptor fluid + receptor rinse), there was a 10-fold increase in penetration for the ethanol:water (95:5, v/v) formulation. This was due to a higher recovery of material in the receptor rinse of the ethanol:water (95:5, v/v) formulation. This was levelled by the lower dermal delivery seen for the ethanol:water (95:5, v/v) formulation. However, total absorbed was similar for both formulations (<1%).

For both formulations applied to rat skin, penetration (Figure 10) of [¹⁴C]-Benzethonium Chloride into the receptor fluid was low also (water formulation, 0.15%; ethanol:water (95:5, v/v) formulation, 0.31%). However dermal delivery increased to ca 5% of the applied dose.

The penetration (Figures 11 and 12) and dermal delivery of [¹⁴C]-Benzethonium chloride was higher in the rat than human for both formulations. Compared to the human, total absorption was 7.8-fold greater in the rat for the water formulation and 10.1-fold greater for the ethanol:water (95:5, v/v) formulation. Dermal delivery was 7.9-fold greater in the rat for the water formulation and 22-fold greater for the ethanol:water (95:5, v/v) formulation. Penetration into the receptor fluid was 5-fold greater in the rat for the water formulation and 10-fold greater for the ethanol:water (95:5, v/v) formulation than for the human skin (Figure 13). This was the same for penetration (receptor fluid + receptor rinse) for the water formulation but there was no difference in penetration (receptor fluid + receptor rinse) between human and rat in the ethanol:water (95:5, v/v) formulation.



6 Conclusions

In conclusion, [¹⁴C]-Benzethonium Chloride poorly penetrates human and rat skin in both test formulations; water and ethanol:water (95:5, v/v). Total absorption also was less than 1.0% in both test formulations with human skin. However, total absorption increased to approximately 5% of the applied dose in both test formulations with rat skin.

7 Tables

Table 1 *Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water Formulation to Human Dermatome Skin*

Recovery (%)	Cell Number and Donor										Mean	SD
	1 F/43Y	2 F/43Y	3 F/43Y	4 F/34Y	5 F/34Y	6 F/34Y	7 F/38Y	16 F/38Y	17 F/39Y	20 F/55Y		
Skin Wash + Swab	69.17	80.62	79.29	75.42	90.42	82.47	58.81	60.88	78.87	98.04	77.40	12.14
Cell Wash	2.32	1.89	2.63	6.55	2.29	6.22	5.16	1.57	1.81	1.38	3.18	1.99
Cling Wrap	0.15	0.27	0.12	0.01+	0.01+	0.03+	0.05	0.34	0.14	0.01+	0.11	0.12
Stratum Corneum	11.90	10.31	10.26	6.43	3.08	7.06	9.09	25.13	12.06	3.66	9.90	6.21
Epidermis (DS)	6.35	4.48	4.69	0.22	0.01	0.50	5.79	7.94	4.47	0.22	3.47	2.96
Dermis (DS)	2.17	1.27	0.54	0.21	0.11	0.21	0.50	0.54	0.36	0.04	0.60	0.65
Non Dose Site	0.07	0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.06	0.05	0.04	0.01
Receptor Fluid	0.02	0.04	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01
Receptor Rinse	0.00	0.00	0.00	0.00+	0.00	0.00	0.00+	0.00	0.00	0.00	0.00	0.00
Total Penetrated	0.02	0.04	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01
Dermal Delivery	2.23	1.30	0.58	0.24	0.14	0.25	0.53	0.57	0.43	0.09	0.64	0.66
Total Unabsorbed	89.88	97.57	96.99	88.62	95.81	96.27	78.90	95.86	97.35	103.31	94.06	6.71
Total Absorbed	2.26	1.34	0.61	0.26	0.18	0.29	0.55	0.61	0.45	0.11	0.67	0.66
Total Recovery	92.14	98.91	97.60	88.89	95.99	96.56	79.45	96.47	97.81	103.42	94.72	6.61

+ = Results obtained from data < 30 dpm above background

DS = Dose Site

SD = Standard Deviation





Table 2 Cumulative Penetration (% Applied Dose) of [¹⁴C]-Benzethonium Chloride in Water Formulation Through Human Dermotome Skin into Receptor Fluid

Time (h)	Cell Number and Donor										Mean	SD
	1 F/43Y	2 F/43Y	3 F/43Y	4 F/34Y	5 F/34Y	6 F/34Y	7 F/38Y	16 F/38Y	17 F/39Y	20 F/55Y		
0	0.00	0.00+	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00+	0.00	0.00
1	0.00	0.00	0.00	0.00	0.01	0.00	0.00+	0.01	0.00	0.00+	0.00	0.00
2	0.00	0.00+	0.00	0.01+	0.01+	0.00	0.00	0.01	0.01	0.00	0.01	0.00
3	0.01+	0.00+	0.01+	0.01+	0.01+	0.01	0.00+	0.01+	0.01+	0.00+	0.01	0.00
4	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.01+	0.01+	0.01+	0.00+	0.01	0.00
5	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.00
6	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.00
8	0.01	0.01	0.01	0.01+	0.01+	0.01	0.01	0.02+	0.01	0.01+	0.01	0.00
10	0.01	0.01	0.01	0.01	0.01+	0.02	0.01	0.03	0.01	0.01	0.01	0.01
12	0.01	0.02	0.02	0.01+	0.02	0.02	0.01	0.03+	0.01	0.01	0.02	0.01
14	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.02	0.01+	0.02	0.01
16	0.02	0.02	0.02	0.01+	0.02	0.02	0.01	0.03+	0.02	0.01	0.02	0.01
18	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.01
20	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.04	0.02	0.02	0.02	0.01
22	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01
24	0.02	0.04	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 3 Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [^{14}C]-Benzethonium Chloride in Water Formulation Through Human Dermotome Skin into Receptor Fluid

Time (h)	Cell Number and Donor										Mean	SD
	1 F/43Y	2 F/43Y	3 F/43Y	4 F/34Y	5 F/34Y	6 F/34Y	7 F/38Y	16 F/38Y	17 F/39Y	20 F/55Y		
0	0.00	0.00+	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00+	0.00	0.00
1	0.00	0.00	0.00	0.00	0.01	0.00	0.00+	0.01	0.00	0.00+	0.00	0.00
2	0.00	0.00+	0.01	0.01+	0.01+	0.00	0.00	0.01	0.01	0.00	0.01	0.00
3	0.01+	0.00+	0.01+	0.01+	0.01+	0.01	0.00+	0.01+	0.01+	0.00+	0.01	0.00
4	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.01+	0.01+	0.01+	0.00+	0.01	0.00
5	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.00
6	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01	0.00
8	0.01	0.01	0.01	0.01+	0.01+	0.01	0.01	0.02+	0.01	0.01+	0.01	0.00
10	0.01	0.01	0.01	0.01	0.01+	0.02	0.01	0.03	0.01	0.01	0.01	0.01
12	0.01	0.02	0.02	0.01+	0.02	0.02	0.01	0.03+	0.01	0.01	0.02	0.01
14	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.02	0.01+	0.02	0.01
16	0.02	0.02	0.02	0.01+	0.02	0.02	0.01	0.03+	0.02	0.01	0.02	0.01
18	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.01
20	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.04	0.02	0.02	0.02	0.01
22	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01
24	0.02	0.04	0.03	0.02	0.03	0.03	0.02	0.04	0.03	0.02	0.03	0.01

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 4 *Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Human Dermotome Skin*

Recovery (%)	Cell Number and Donor										Mean	SD
	8 F/43Y	9 F/43Y	10 F/43Y	11 F/34Y	12 F/34Y	13 F/34Y	14 F/38Y	24 F/39Y	27 F/55Y	28 F/55Y		
Skin Wash + Swab	89.29	87.31	81.82	89.74	94.05	95.12	87.54	97.72	93.36	92.28	90.82	4.63
Cell Wash	1.89	2.51	3.34	1.78	0.96	1.49	1.38	0.68	1.24	0.92	1.62	0.81
Cling Wrap	0.07	0.04+	0.07	0.00+	0.01+	0.01+	0.03+	0.03+	0.01+	0.00+	0.03	0.03
Stratum Corneum Epidermis (DS)	4.10	7.03	7.19	2.06	1.37	1.61	3.83	1.52	1.40	1.50	3.16	2.31
Dermis (DS)	2.95	3.11	4.39	0.53	0.12	0.29	1.25	1.78	0.19	0.05	1.47	1.54
Non Dose Site	0.51	0.25	0.65	0.05	0.05	0.03	0.16	0.13	0.02	0.01	0.19	0.22
Receptor Fluid	0.04	0.06	0.02	0.05	0.02	0.03	0.01	0.02	0.02	0.01	0.03	0.02
Receptor Rinse	0.02	0.03	0.04	0.02	0.04	0.02	0.02	0.03	0.03	0.02	0.03	0.01
Total Penetrated	1.35	0.39	0.00	0.17	0.21	0.25	0.26	0.00+	0.00+	0.00+	0.26	0.41
Dermal Delivery	1.37	0.42	0.04	0.19	0.25	0.27	0.27	0.03	0.03	0.02	0.29	0.40
Total Unabsorbed	0.55	0.31	0.67	0.10	0.07	0.07	0.16	0.15	0.04	0.03	0.22	0.23
Total Absorbed	98.30	100.00	96.81	94.12	96.51	98.53	94.03	101.72	96.19	94.75	97.09	2.55
Total Recovery	1.92	0.73	0.72	0.29	0.31	0.33	0.44	0.18	0.07	0.05	0.50	0.55
	100.22	100.73	97.52	94.41	96.82	98.86	94.47	101.90	96.25	94.79	97.60	2.73

+ = Results obtained from data < 30 dpm above background

DS = Dose Site

SD = Standard Deviation



Table 5 *Cumulative Penetration (% Applied Dose) of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Human Dermotome Skin into Receptor Fluid*

Time (h)	Cell Number and Donor										Mean	SD
	8 F/43Y	9 F/43Y	10 F/43Y	11 F/34Y	12 F/34Y	13 F/34Y	14 F/38Y	24 F/39Y	27 F/55Y	28 F/55Y		
0	0.00	0.00	0.00	0.01	0.00+	0.00+	0.00+	0.00	0.00	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.01	0.00+	0.00+	0.00+	0.00	0.01	0.00+	0.00	0.00
2	0.00+	0.00+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01	0.02	0.00+	0.01	0.00
3	0.01+	0.00+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01	0.02	0.01	0.01	0.00
4	0.01+	0.01+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01+	0.02+	0.01	0.01	0.01
5	0.01+	0.01+	0.01+	0.01+	0.00+	0.01+	0.01+	0.02+	0.02	0.01+	0.01	0.01
6	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.02+	0.02+	0.01+	0.01	0.01
8	0.01+	0.01	0.01	0.01	0.01	0.01+	0.01+	0.02+	0.02+	0.02+	0.01	0.01
10	0.01	0.01	0.01	0.01+	0.01	0.01	0.01+	0.02+	0.02+	0.02+	0.01	0.00
12	0.01	0.01	0.02	0.02+	0.01	0.01+	0.01+	0.02+	0.02+	0.02+	0.01	0.00
14	0.01	0.01	0.02	0.02	0.02	0.01+	0.01+	0.02+	0.02+	0.02+	0.02	0.00
16	0.02	0.02	0.02	0.02	0.02	0.01+	0.01+	0.02+	0.02+	0.02+	0.02	0.00
18	0.02	0.02	0.03	0.02	0.02	0.01+	0.01+	0.02+	0.02+	0.02+	0.02	0.01
20	0.02	0.02	0.03	0.02	0.03	0.01+	0.01+	0.02	0.03+	0.02+	0.02	0.01
22	0.02	0.02	0.04	0.02	0.04	0.01+	0.01+	0.02+	0.03+	0.02+	0.02	0.01
24	0.02	0.03	0.04	0.02	0.04	0.02	0.02+	0.03+	0.03+	0.02+	0.03	0.01

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 6 Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [^{14}C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Human Dermatome Skin into Receptor Fluid

Time (h)	Cell Number and Donor										Mean	SD
	8 F/43Y	9 F/43Y	10 F/43Y	11 F/34Y	12 F/34Y	13 F/34Y	14 F/38Y	24 F/39Y	27 F/55Y	28 F/55Y		
0	0.00	0.00	0.00	0.01	0.00+	0.00+	0.00+	0.00	0.00	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.01	0.00+	0.00+	0.00+	0.00	0.01	0.00+	0.00	0.00
2	0.00+	0.00+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01	0.02	0.00+	0.01	0.00
3	0.01+	0.00+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01	0.02	0.01	0.01	0.01
4	0.01+	0.01+	0.01+	0.01+	0.00+	0.00+	0.00+	0.01+	0.02+	0.01	0.01	0.01
5	0.01+	0.01+	0.01+	0.01+	0.00+	0.01+	0.01+	0.02+	0.02	0.01+	0.01	0.01
6	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.01+	0.02+	0.02+	0.01+	0.01	0.01
8	0.01+	0.01	0.01	0.01	0.01	0.01+	0.01+	0.02+	0.02+	0.02+	0.01	0.01
10	0.01	0.01	0.01	0.02+	0.01	0.01	0.01+	0.02+	0.02+	0.02+	0.01	0.00
12	0.01	0.01	0.02	0.02+	0.01	0.01+	0.01+	0.02+	0.02+	0.02+	0.01	0.00
14	0.01	0.01	0.02	0.02	0.02	0.01+	0.01+	0.02+	0.02+	0.02+	0.02	0.00
16	0.02	0.02	0.02	0.02	0.02	0.01+	0.01+	0.02+	0.03+	0.02+	0.02	0.00
18	0.02	0.02	0.03	0.02	0.02	0.01+	0.01+	0.02+	0.03+	0.02+	0.02	0.01
20	0.02	0.02	0.03	0.02	0.03	0.01+	0.01+	0.02	0.03+	0.02+	0.02	0.01
22	0.02	0.02	0.04	0.02	0.04	0.01+	0.01+	0.03+	0.03+	0.02+	0.02	0.01
24	0.02	0.03	0.04	0.03	0.04	0.02	0.02+	0.03+	0.03+	0.02+	0.03	0.01

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 7 **Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water Formulation to Rat Dermatome Skin**

Recovery (%)	Cell Number and Animal													Mean	SD
	29 001M	30 002M	31 002M	33 003F	34 004F	35 005F	43 002M	44 002M	45 003M	46 003M	47 003F	48 004F	49 004M		
Skin Wash + Swab	70.83	72.81	62.23	75.33	76.75	73.47	52.19	52.18	67.73	69.78	59.03	63.64	71.15	66.70	8.24
Cell Wash	2.23	1.28	0.95	1.30	1.43	1.76	1.84	2.19	2.93	1.75	1.84	1.41	0.57	1.65	0.60
Cling Wrap	3.33	1.47	0.71	0.48	1.33	0.50								1.30	1.08
Stratum Corneum	6.53	8.55	19.53	15.61	3.11	5.86	19.71	17.30	4.24	6.84	16.37	14.27	1.09	10.69	6.61
Epidermis (DS)	6.51	7.60	2.23	5.08	10.07	9.80	15.84	15.54	13.09	18.69	13.89	6.64	20.64	11.20	5.58
Dermis (DS)	6.08	3.17	4.02	2.21	1.14	2.62	6.01	7.05	6.30	5.18	2.34	3.06	9.49	4.51	2.39
Non Dose Site	0.18	0.32	0.26	0.34	2.29	0.27	0.40	0.34	1.03	0.07	0.23	0.26	0.95	0.53	0.60
Receptor Fluid	0.06	0.07	0.02	0.00	0.13	0.61	0.38	0.08	0.28	0.03	0.11	0.02	0.11	0.15	0.18
Receptor Rinse	0.01	0.01	0.00	0.01	0.01	0.03	0.02	0.02	0.02	0.00	0.02	0.01	0.02	0.01	0.01
Total Penetrated	0.07	0.08	0.02	0.01	0.14	0.64	0.40	0.10	0.30	0.04	0.13	0.02	0.14	0.16	0.18
Dermal Delivery	6.26	3.48	4.28	2.55	3.43	2.90	6.41	7.38	7.33	5.26	2.57	3.32	10.44	5.05	2.39
Total Unabsorbed	89.42	91.71	85.65	97.80	92.68	91.39	89.58	87.22	87.99	97.06	91.13	85.95	93.45	90.85	3.81
Total Absorbed	6.33	3.56	4.30	2.56	3.57	3.53	6.81	7.49	7.63	5.29	2.70	3.34	10.58	5.21	2.41
Total Recovery	95.75	95.27	89.95	100.36	96.25	94.92	96.39	94.71	95.62	102.36	93.83	89.29	104.03	96.06	4.22

DS = Dose Site

SD = Standard Deviation



Table 8 Cumulative Penetration (% Applied Dose) of [¹⁴C]-Benzethonium Chloride in Water Formulation Through Rat Dermatome Skin into Receptor Fluid

Time (h)	Cell Number and Animal													Mean	SD
	29 001M	30 002M	31 002M	33 003F	34 004F	35 005F	43 002M	44 002M	45 003M	46 003M	47 003F	48 004F	49 004M		
0	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.01	0.00	0.00	0.00+	0.00	0.00	0.00+	0.00	0.00
2	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.01	0.00	0.00+	0.00+	0.00+	0.00	0.00+	0.00	0.00
3	0.00+	0.00+	0.00+	0.00+	0.00+	0.01	0.01	0.00	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00
4	0.00+	0.00+	0.00+	0.00+	0.00+	0.02	0.01	0.01	0.01+	0.00+	0.00+	0.01+	0.00+	0.00	0.01
5	0.00+	0.00+	0.00+	0.00+	0.00+	0.04	0.01	0.01	0.01	0.00+	0.01+	0.01+	0.00+	0.01	0.01
6	0.00+	0.00+	0.00+	0.00+	0.00	0.05	0.02	0.01	0.01	0.00+	0.01	0.01+	0.00+	0.01	0.01
8	0.00+	0.00+	0.00+	0.00+	0.01	0.10	0.03	0.01	0.02	0.00	0.01+	0.01+	0.01+	0.02	0.03
10	0.00+	0.00+	0.00	0.00+	0.02	0.14	0.04	0.02	0.03	0.00+	0.01+	0.01+	0.01+	0.02	0.04
12	0.01+	0.00+	0.01	0.00+	0.03	0.19	0.07	0.03	0.05	0.01+	0.01	0.01	0.01+	0.03	0.05
14	0.01	0.01	0.01	0.00+	0.04	0.25	0.10	0.04	0.07	0.01+	0.01	0.01+	0.01	0.04	0.07
16	0.01	0.02	0.01	0.00+	0.06	0.30	0.13	0.05	0.10	0.01	0.02	0.01+	0.02	0.06	0.08
18	0.01	0.02	0.01	0.00+	0.07	0.35	0.18	0.06	0.13	0.01	0.03	0.01+	0.02	0.07	0.10
20	0.02	0.03	0.02	0.00+	0.10	0.41	0.24	0.07	0.17	0.02	0.05	0.01+	0.03	0.09	0.12
22	0.04	0.04	0.02+	0.00+	0.12	0.54	0.31	0.07	0.22	0.03	0.08	0.01	0.05	0.12	0.16
24	0.06	0.07	0.02	0.00+	0.13	0.61	0.38	0.08	0.28	0.03	0.11	0.02+	0.11	0.15	0.18

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 9 Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [^{14}C]-Benzethonium Chloride in Water Formulation Through Rat Dermotome Skin into Receptor Fluid

Time (h)	Cell Number and Animal													Mean	SD
	29 001M	30 002M	31 002M	33 003F	34 004F	35 005F	43 002M	44 002M	45 003M	46 003M	47 003F	48 004F	49 004M		
0	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.01	0.00	0.00	0.00+	0.00	0.00	0.00+	0.00	0.00
2	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.01	0.00	0.00+	0.00+	0.00+	0.00	0.00+	0.00	0.00
3	0.00+	0.00+	0.00+	0.00+	0.00+	0.01	0.01	0.01	0.00+	0.00+	0.00+	0.01+	0.00+	0.00	0.00
4	0.00+	0.00+	0.00+	0.00+	0.00+	0.02	0.01	0.01	0.01+	0.00+	0.00+	0.01+	0.00+	0.01	0.01
5	0.00+	0.00+	0.00+	0.00+	0.00+	0.04	0.02	0.01	0.01	0.00+	0.01+	0.01+	0.00+	0.01	0.01
6	0.00+	0.00+	0.00+	0.00+	0.00	0.06	0.02	0.01	0.01	0.00+	0.01	0.01+	0.01+	0.01	0.01
8	0.00+	0.00+	0.00+	0.00+	0.01	0.10	0.03	0.01	0.02	0.00	0.01+	0.01+	0.01+	0.02	0.03
10	0.00+	0.00+	0.00	0.00+	0.02	0.14	0.04	0.02	0.03	0.00+	0.01+	0.01+	0.01+	0.02	0.04
12	0.01+	0.00+	0.01	0.00+	0.03	0.20	0.07	0.03	0.05	0.01+	0.01	0.01	0.01+	0.03	0.05
14	0.01	0.01	0.01	0.00+	0.04	0.25	0.10	0.04	0.07	0.01+	0.01	0.01+	0.01	0.04	0.07
16	0.01	0.02	0.01	0.00+	0.06	0.31	0.14	0.05	0.10	0.01	0.02	0.01+	0.02	0.06	0.09
18	0.02	0.02	0.01	0.00+	0.07	0.36	0.18	0.06	0.13	0.02	0.03	0.01+	0.03	0.07	0.10
20	0.02	0.03	0.02	0.00+	0.10	0.42	0.25	0.07	0.18	0.02	0.05	0.01+	0.03	0.09	0.12
22	0.04	0.05	0.02	0.00+	0.12	0.56	0.32	0.08	0.23	0.03	0.08	0.02	0.05	0.12	0.16
24	0.06	0.08	0.02	0.00+	0.13	0.63	0.39	0.08	0.29	0.03	0.11	0.02+	0.12	0.15	0.18

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 10 *Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Rat Dermatome Skin*

Recovery (%)	Cell Number											Mean	SD
	37 002M	38 002M	40 003F	41 004F	42 005F	50 002M	51 002M	52 003M	53 003M	54 003F	56 004M		
Skin Wash + Swab	74.46	69.12	74.59	68.57	77.70	54.12	57.84	69.90	70.00	79.00	62.98	68.93	7.88
Cell Wash	1.59	0.90	1.14	1.01	2.13	1.35	3.25	1.77	2.02	1.59	1.75	1.68	0.65
Cling Wrap	2.18	2.41	0.00+	0.02+	0.00+							0.92	1.26
Stratum Corneum	7.86	12.55	6.52	3.85	5.94	9.82	5.66	1.36	3.79	4.07	2.81	5.84	3.25
Epidermis (DS)	6.53	5.28	10.54	19.16	8.94	13.58	5.26	19.63	11.87	16.09	30.88	13.43	7.71
Dermis (DS)	5.77	6.26	1.71	2.64	0.84	2.86	6.07	5.13	10.68	0.81	1.97	4.07	3.02
Non Dose Site	0.10	0.16	1.11	0.13	0.15	0.38	2.84	1.12	1.06	0.12	0.13	0.66	0.84
Receptor Fluid	0.11	0.03	0.24	0.74	0.44	0.11	0.21	0.05	0.29	0.46	0.68	0.31	0.25
Receptor Rinse	0.02	0.01	0.01	0.05	0.02	0.01	0.01	0.00	0.01	0.03	0.03	0.02	0.01
Total Penetrated	0.12	0.04	0.26	0.79	0.46	0.11	0.22	0.05	0.29	0.49	0.72	0.32	0.26
Dermal Delivery	5.87	6.42	2.82	2.77	0.98	3.24	8.91	6.25	11.74	0.93	2.10	4.73	3.44
Total Unabsorbed	92.63	90.26	92.79	92.61	94.71	78.87	72.01	92.67	87.67	100.76	98.42	90.31	8.30
Total Absorbed	5.99	6.46	3.07	3.56	1.44	3.36	9.13	6.30	12.04	1.42	2.82	5.05	3.32
Total Recovery	98.62	96.72	95.86	96.17	96.15	82.23	81.14	98.97	99.71	102.18	101.24	95.36	7.08

+ = Results obtained from data < 30 dpm above background

DS = Dose Site

SD = Standard Deviation



Table 11 Cumulative Penetration (% Applied Dose) of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Rat Dermatome Skin into Receptor Fluid

Time (h)	Cell Number										Mean	SD	
	37 002M	38 002M	40 003F	41 004F	42 005F	50 002M	51 002M	52 003M	53 003M	54 003F			56 004M
0	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00+	0.00+	0.00	0.00
2	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00+	0.00+	0.01	0.00+	0.00+	0.00	0.00
3	0.00+	0.00+	0.00	0.01	0.00	0.00+	0.00+	0.00+	0.01+	0.00+	0.00+	0.00	0.00
4	0.00+	0.00+	0.00	0.01	0.01	0.00+	0.01	0.00+	0.01+	0.00	0.00	0.00	0.00
5	0.00+	0.00+	0.01	0.02	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00
6	0.00+	0.00+	0.01	0.03	0.02	0.01+	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8	0.00+	0.00+	0.02	0.05	0.03	0.01	0.01	0.01	0.02	0.03	0.03	0.02	0.02
10	0.00+	0.00+	0.04	0.09	0.05	0.01	0.02	0.02	0.02	0.06	0.06	0.03	0.03
12	0.01	0.00+	0.05	0.17	0.07	0.01	0.03	0.02	0.04	0.09	0.10	0.05	0.05
14	0.02	0.00+	0.07	0.22	0.11	0.02	0.05	0.02	0.06	0.14	0.16	0.08	0.07
16	0.04	0.00+	0.10	0.29	0.17	0.03	0.08	0.02+	0.09	0.19	0.24	0.11	0.09
18	0.05	0.01	0.12	0.37	0.22	0.04	0.11	0.03+	0.13	0.25	0.34	0.15	0.13
20	0.09	0.02	0.17	0.47	0.28	0.06	0.15	0.04	0.17	0.31	0.44	0.20	0.16
22	0.10	0.03	0.20	0.59	0.37	0.08	0.19	0.04	0.23	0.38	0.55	0.25	0.20
24	0.11	0.03	0.24	0.74	0.44	0.11	0.21	0.05	0.29	0.46	0.68	0.31	0.25

+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



Table 12 Cumulative Flux ($\mu\text{g equiv.cm}^{-2}$) of [^{14}C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation Through Rat Dermotome Skin into Receptor Fluid

Time (h)	Cell Number											Mean	SD
	37 002M	38 002M	40 003F	41 004F	42 005F	50 002M	51 002M	52 003M	53 003M	54 003F	56 004M		
0	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00
1	0.00+	0.00+	0.00+	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00+	0.00+	0.00	0.00
2	0.00+	0.00+	0.00+	0.00	0.00+	0.00+	0.00	0.00+	0.01	0.00+	0.00+	0.00	0.00
3	0.00+	0.00+	0.00	0.01	0.00	0.00+	0.00+	0.00+	0.01+	0.00+	0.00+	0.00	0.00
4	0.00+	0.00+	0.00	0.01	0.01	0.00+	0.01	0.00+	0.01+	0.00	0.01	0.00	0.00
5	0.00+	0.00+	0.01	0.02	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00
6	0.00+	0.00+	0.01	0.03	0.02	0.01+	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8	0.00+	0.00+	0.02	0.06	0.03	0.01	0.01	0.01	0.02	0.03	0.03	0.02	0.02
10	0.00+	0.00+	0.04	0.09	0.05	0.01	0.02	0.02	0.03	0.06	0.06	0.03	0.03
12	0.01	0.00+	0.06	0.17	0.07	0.01	0.03	0.02	0.04	0.09	0.10	0.06	0.05
14	0.02	0.00+	0.08	0.23	0.11	0.02	0.05	0.03	0.06	0.14	0.16	0.08	0.07
16	0.04	0.00+	0.10	0.29	0.17	0.03	0.08	0.03	0.09	0.19	0.25	0.12	0.10
18	0.05	0.01	0.12	0.38	0.22	0.05	0.11	0.03	0.13	0.25	0.35	0.15	0.13
20	0.09	0.02	0.17	0.48	0.29	0.06	0.15	0.04	0.18	0.32	0.45	0.20	0.16
22	0.10	0.03	0.21	0.60	0.38	0.09	0.19	0.05	0.24	0.39	0.57	0.26	0.20
24	0.11	0.03	0.25	0.76	0.45	0.11	0.21	0.05	0.30	0.47	0.70	0.31	0.25

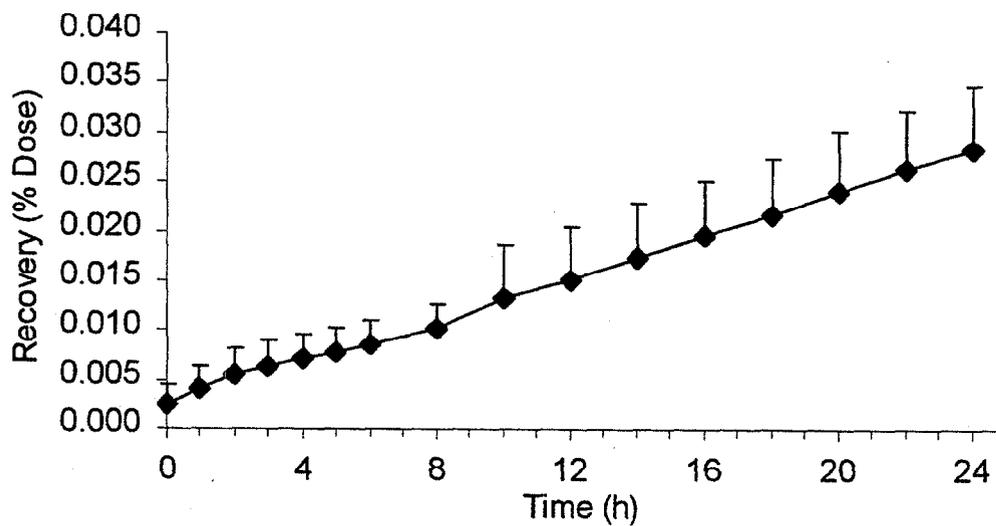
+ = Results obtained from data < 30 dpm above background

SD = Standard Deviation



8 Figures

Figure 1 Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water Formulation to Human Skin (Mean ± SD, n = 10)



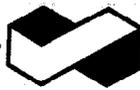


Figure 2 Cumulative Flux ($\mu\text{g equiv. cm}^{-2}$) of Benzethonium Chloride Through Human Skin Following Topical Application of [^{14}C]-Benzethonium Chloride in Water Formulation (Mean \pm SD, n = 10)

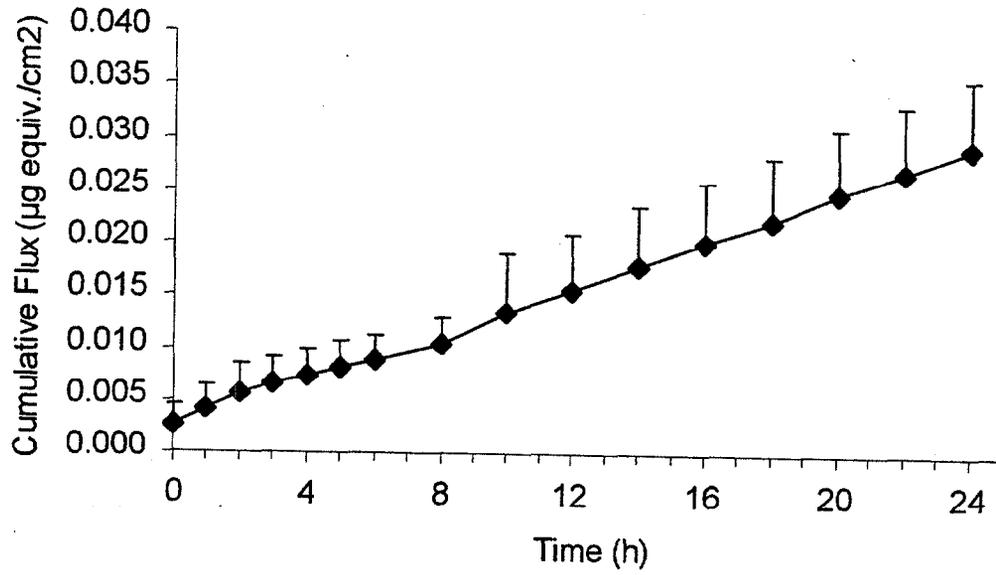




Figure 3 Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Human Skin (Mean ± SD, n = 10)

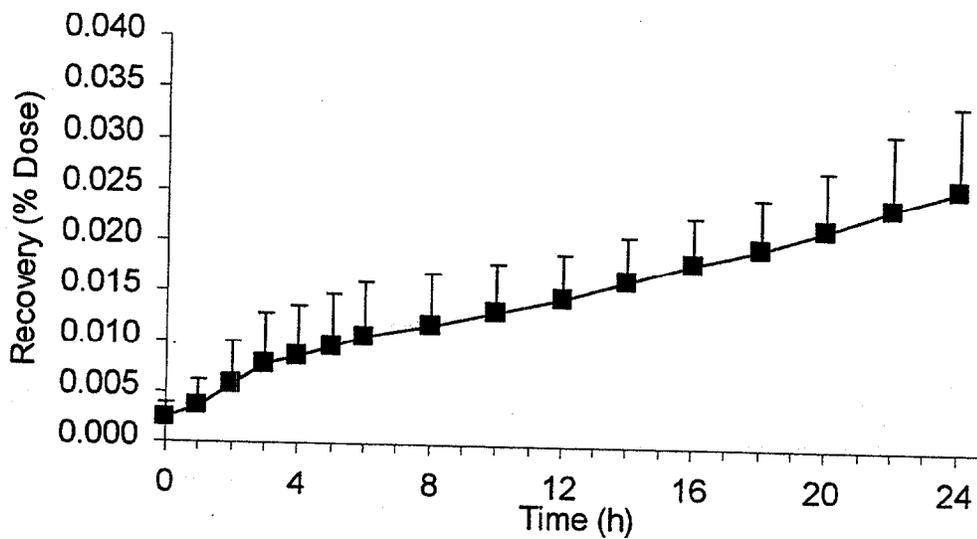




Figure 4 Cumulative Flux ($\mu\text{g equiv. cm}^{-2}$) of Benzethonium Chloride Through Human Skin Following Topical Application of [^{14}C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation (Mean \pm SD, n = 10)

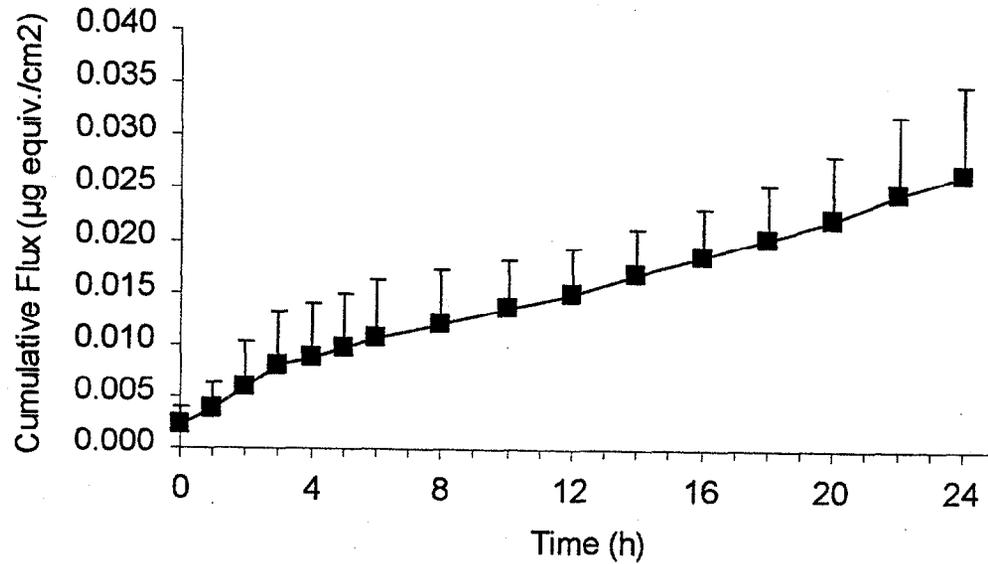




Figure 5 **Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water Formulation to Rat Skin (Mean ± SD, n = 13)**

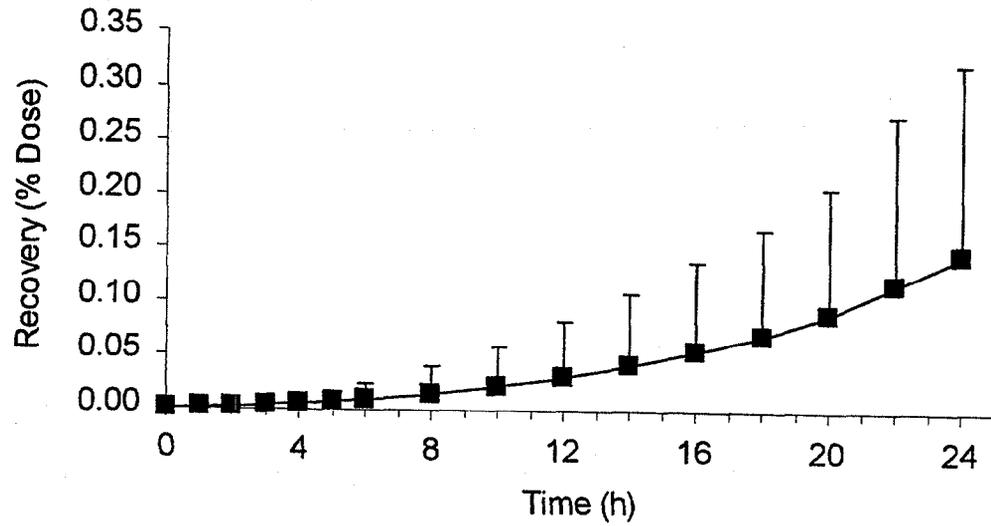




Figure 6 Cumulative Flux ($\mu\text{g equiv. cm}^{-2}$) of Benzethonium Chloride Through Rat Skin Following Topical Application of [^{14}C]-Benzethonium Chloride in Water Formulation (Mean \pm SD, $n = 13$)

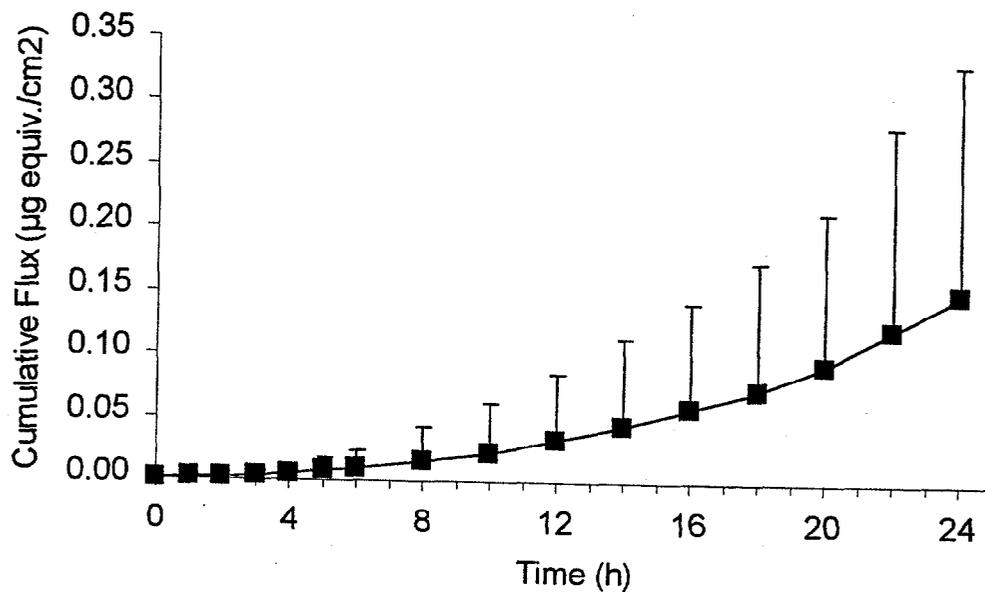




Figure 7 *Recovery of Radioactivity (% Applied Dose) in Receptor Fluid Following Topical Application of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation to Rat Skin (Mean ± SD, n = 11)*

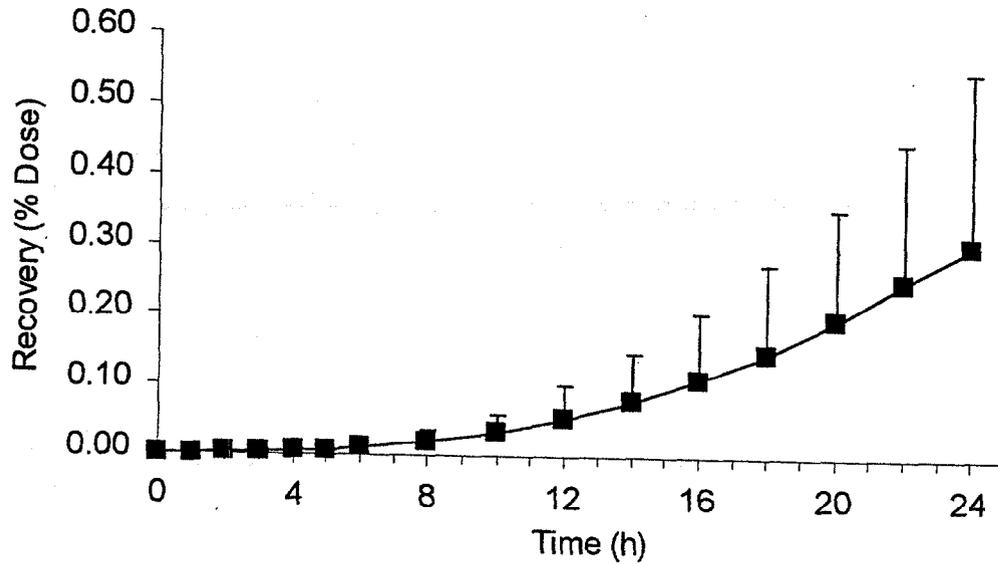




Figure 8 Cumulative Flux ($\mu\text{g equiv. cm}^{-2}$) of Benzethonium Chloride Through Rat Skin Following Topical Application of [^{14}C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation (Mean \pm SD, n = 11)

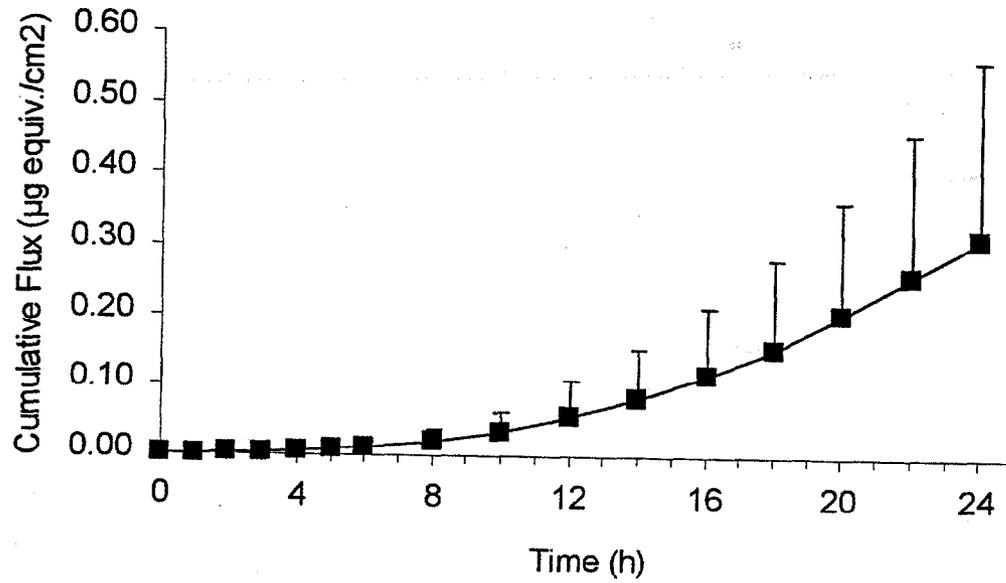




Figure 9 A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human Skin Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water (Mean ± SD, n = 10) and Ethanol:Water (95:5, v/v; Mean ± SD, n = 10) Formulations

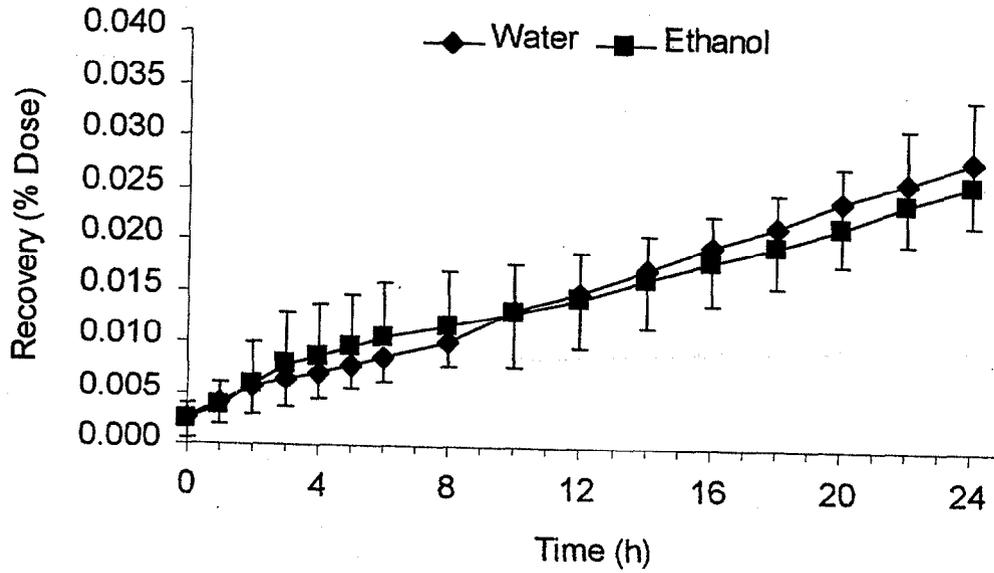




Figure 10 *A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Rat Skin Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water (Mean ± SD, n = 12) and Ethanol:Water (95:5, v/v; Mean ± SD, n = 11) Formulations*

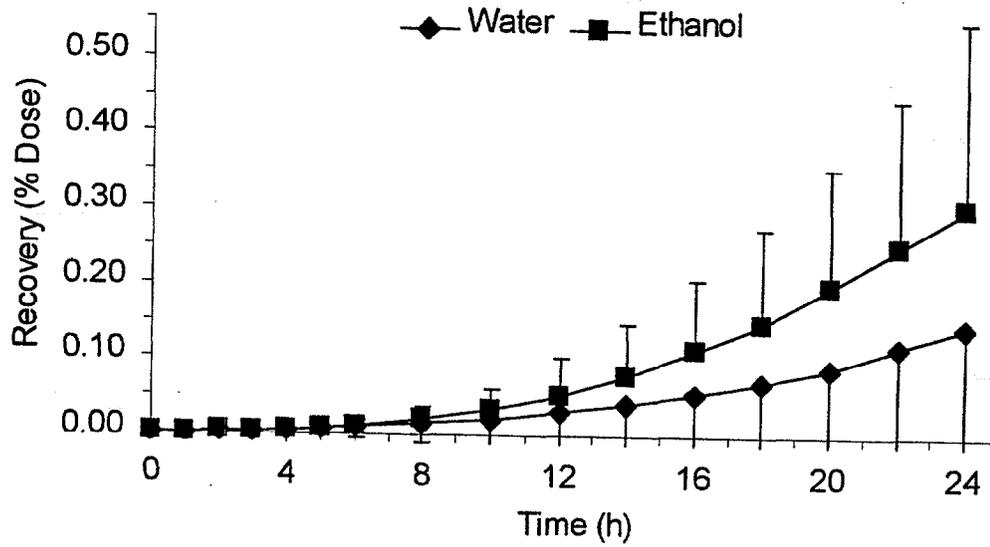




Figure 11 *A Comparison of the Recovery of Radioactivity (% Applied Dose) In Receptor Fluid of Human (Mean \pm SD, n = 10) and Rat (Mean \pm SD, n = 13) Skin Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water Formulation*

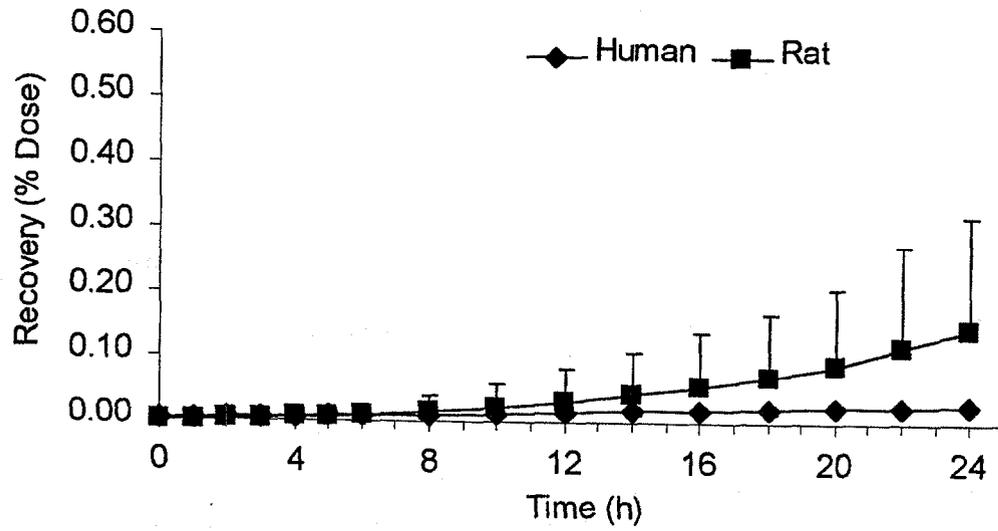




Figure 12 A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human (Mean \pm SD, n = 10) and Rat (Mean \pm SD, n = 11) Skin Following Topical Application of [¹⁴C]-Benzethonium Chloride in Ethanol:Water (95:5, v/v) Formulation

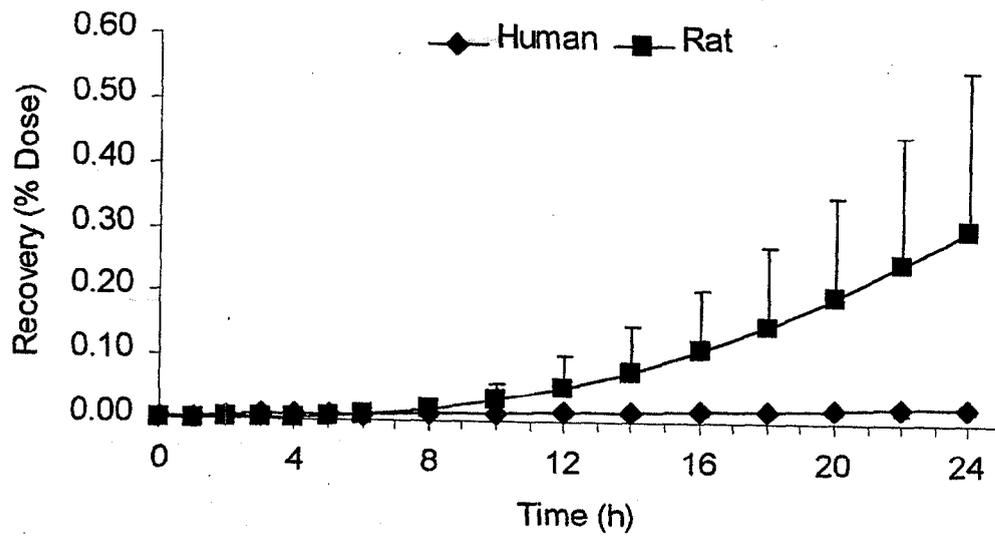
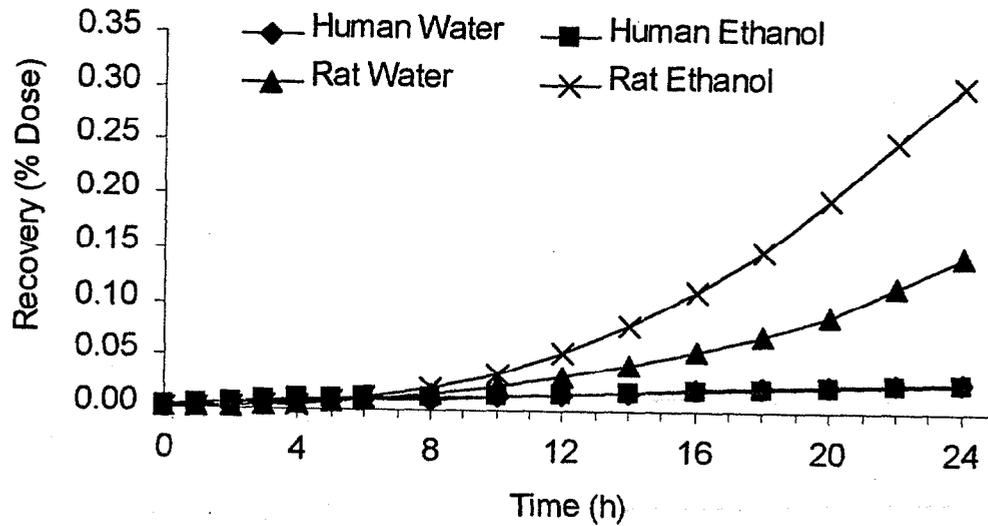




Figure 13 *A Comparison of the Recovery of Radioactivity (% Applied Dose) in Receptor Fluid of Human and Rat Skin Following Topical Application of [¹⁴C]-Benzethonium Chloride in Water and Ethanol:Water (95:5, v/v) Formulations (Mean)*





9 Appendices

Appendix 1 Product Information for [¹⁴C]-Benzethonium Chloride

Wizard Laboratories, Inc.



DATE: 4/7/00

LOT NO.: 000407

PRODUCT INFORMATION

COMPOUND..... Benzethonium chloride-N-CH₃-¹⁴C

ACTIVITY..... 5.0 mCi

SPECIFIC ACTIVITY..... 40.16 mCi/nmol

RADIOCHEMICAL PURITY... 97.54%

as determined by high-pressure liquid chromatography:

Column: Alltima C18, 4.6 x 250mm
Mobile Phase: 70:30 CH₃CN:H₂O (.5% TFA) isocratic, 1
ml/min, 254 nm

TLC, Silica gel, 8:1:0.1 CH₂Cl₂:MeOH:TFA

Packaging x 1.0 mCi in screw-cap vial under N₂

NOTE: The susceptibility of carbon-14 labeled compounds to radiolysis varies greatly. We urge you to use this product as soon as possible.

WARRANTY: Wizard Laboratories warrants this material to be as stated above. There is no warranty, expressed or implied, as to the fitness of this material for any particular purpose. The customer must notify the company, by registered mail, of any breach of warranty within 28 days of receipt.



Appendix 2 Certificate of Analysis for Benzethonium Chloride

Lonzagroup

Fine Chemicals and Specialties

CERTIFICATE OF ANALYSIS

Benzethonium Chloride
Trade Name: Hyamine 1622

Batch Number: 80102

<u>ANALYSIS</u>	<u>RESULTS</u>
Quaternary Ammonium Chloride (EW 448.1)	97.9%
Appearance	White, Fine Powder
Expiration Date:	March 30, 2001



Robert J. Sloan
Research Associate / Study Director, Technical Services
Specialty Chemicals Research & Development



Date

Reference: Product Registration Study SP-00030-A

This testing was performed according to the requirements of FIFRA Good Laboratory Practice Standards (40 CFR Part 160). Data supporting this document will be maintained in the archive at the Annandale, New Jersey, facility of Lonzagroup.

C:\Current Work\Documentation\Certificates Of Analysis\80102-3.Doc

Lonza Inc
Clinton Tel 908 730 1500
79 Route 22 East, PO Box 993 Fax 908 730 1546
Annandale, NJ 08801, USA www.lonzagroup.com



Appendix 3 Human Skin Donor Details

Hospital Number	Sex/Age	Site
571744	F/30Y	Breast
563011	F/34Y	Breast
559940	F/43Y	Breast
552133	F/38Y	Breast
266295	F/23Y	Breast
566827	F/28Y	Breast
541582	F/39Y	Abdomen
130735	F/55Y	Breast



Appendix 4 Thickness of Dermatome Skin Membranes

(a) Human

Donor (Age/Sex)	Membrane Thickness (μm)	
	Full Thickness Skin	Dermatome Skin
F/43Y	2250	450-500
F/34Y	1700	370-210
F/30Y	2500	NCS
F/38Y	2300	320-450
F/23Y	2600	330
F/39Y	2150	430
F/28Y	2140	360
F/55Y	1950	410-480

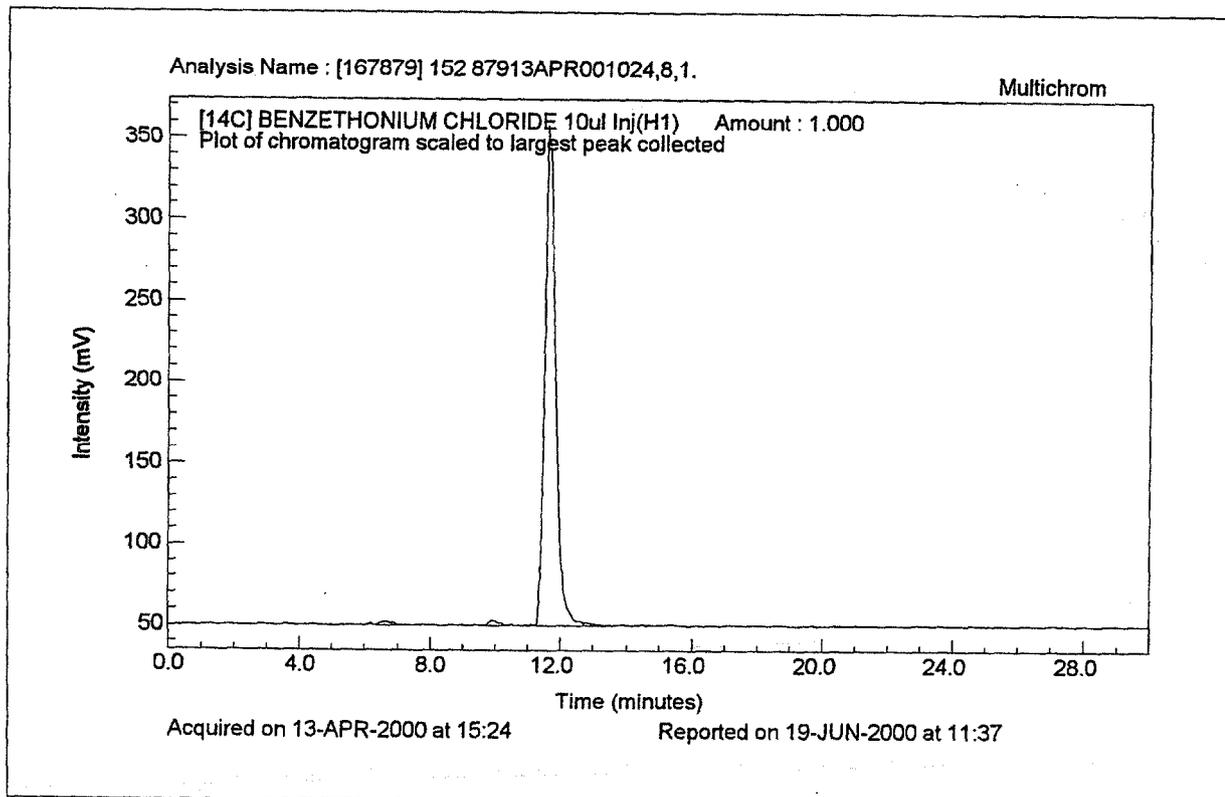
(b) Rat

Animal No.	Membrane Thickness (μm)	
	Full Thickness Skin	Dermatome Skin
001F	1520	370
002F	1380	NCS
003F	1080	430
004F	1180	350-480
005F	1810	430
001M	1800	380-480
002M	1350-1450	500
003M	1580	400-480
006F	1000	NCS
004M	1650	370-500
001F	1200	NCS

NCS = not cut successfully



Appendix 5 Radiochemical Purity of [¹⁴C]-Benzethonium Chloride by HPLC



Analyst Name : Gayle Holmes

Lims Id :

Comment : RADIOCHEMICAL PURITY CHECK OF [¹⁴C]-BENZETHONIUM CHLORIDE

Method Title : RADIOCHEMICAL PURITY CHECK OF [¹⁴C]-BENZETHONIUM CHLORIDE

Sample Name : [¹⁴C] BENZETHONIUM CHLORIDE 10ul Inj (H1)

Sample Id :

Sample Type : Sample Amount=1.00000

Bottle No : 8

PEAK INFORMATION

<u>RT mins</u>	<u>Hght uV</u>	<u>Area uVs</u>	<u>Area %</u>
6.699	2455	57157	0.79
9.904	3294	68033	0.94
11.600	307936	7105559	97.70
12.800	2040	42111	0.58
<u>Totals</u>			
Unknowns	315725	7272859	100.00
Named	0	0	0.00
	315725	7272859	100.00



Appendix 6 Temperature and Relative Humidity of the Laboratory

Experiment No.	Pre/ Post Dose	Temperature (°C)		Relative Humidity (%)	
		Mean	SD	Mean	SD
1	Pre	19.0	0.8	34.0	0.0
	Post	20.3	0.6	32.7	0.6
2	Pre	20.2	0.8	35.0	0.0
	Post	21.8	1.2	33.8	2.4
3	Pre	20.7	0.5	37.4	0.5
	Post	21.0	0.0	37.9	0.4
4	Pre	21.6	0.5	37.4	0.5
	Post	21.8	0.4	38.9	1.9



Appendix 7 Cross Reference of Skin Sample Number with Skin Donor and Tritiated Water Permeability Coefficient (Kp)

(a) Human Skin

Experiment No.	Skin Sample Number	Human Skin Donor	Kp ($\times 10^{-3}$ cm.h ⁻¹)
1	1	F/43Y	2.3
	2	F/43Y	1.8
	3	F/43Y	1.8
	4	F/34Y	1.2
	5	F/34Y	0.9
	6	F/34Y	1.6
	7	F/38Y	1.4
	8	F/43Y	1.9
	9	F/43Y	1.5
	10	F/43Y	1.6
	11	F/34Y	1.9
	12	F/34Y	1.6
	13	F/34Y	1.0
	14	F/38Y	1.3
2	15	F/38Y	8.4
	16	F/38Y	2.2
	17	F/39Y	1.9
	18	F/55Y	3.2
	19	F/55Y	2.9
	20	F/55Y	1.9
	21	F/55Y	2.7
	22	F/38Y	3.1
	23	F/38Y	2.6
	24	F/39Y	1.6
	25	F/55Y	3.4
	26	F/55Y	3.0
	27	F/55Y	1.4
	28	F/55Y	2.5

Rejection criterion, reject if Kp > 2.5×10^{-3} cm.h⁻¹



Appendix 7 **Cross Reference of Skin Sample Number with Skin Donor and Tritiated Water Permeability Coefficient (Kp)**
(continued)

(b) Rat Skin

Experiment No.	Skin Sample Number	Animal Number	Kp ($\times 10^{-3}$ cm.h ⁻¹)
3	29	001M	1.0
	30	002M	0.6
	31	002M	0.6
	32	003M	3.2
	33	003F	0.5
	34	004F	0.5
	35	005F	0.6
	36	001M	4.3
	37	002M	0.4
	38	002M	0.5
	39	003M	5.0
	40	003F	0.5
	41	004F	1.5
42	005F	0.5	
4	43	002M	1.3
	44	002M	1.3
	45	003M	1.3
	46	003M	0.8
	47	003F	0.8
	48	004F	1.8
	49	004M	1.3
	50	002M	0.7
	51	002M	1.9
	52	003M	0.7
	53	003M	0.9
	54	003F	0.3
	55	004F	3.8
	56	004M	1.2

Rejection criterion, reject if Kp > 2.5×10^{-3} cm.h⁻¹



**Appendix 8 A Comparison of the Distribution of Radioactivity
(% Applied Dose) at 24 h Post Dose Following Topical
Application of [¹⁴C]-Benzethonium Chloride in Water and
Ethanol:Water (95:5, v/v) Formulations to Human and Rat
Dermatome Skins**

Recovery (%)	Human Water	SD	Human Ethanol	SD	Rat Water	SD	Rat Ethanol	SD
Skin Wash + Swab	77.40	12.14	90.82	4.63	66.70	8.24	68.93	7.88
Cell Wash	3.18	1.99	1.62	0.81	1.65	0.60	1.68	0.65
Cling Wrap	0.11	0.12	0.03	0.03	1.30	1.08	0.92	1.26
Stratum Corneum Epidermis (DS)	9.90	6.21	3.16	2.31	10.69	6.61	5.84	3.25
	3.47	2.96	1.47	1.54	11.20	5.58	13.43	7.71
Dermis (DS)	0.60	0.65	0.19	0.22	4.51	2.39	4.07	3.02
Non Dose Site	0.04	0.01	0.03	0.02	0.53	0.60	0.66	0.84
Receptor Fluid	0.03	0.01	0.03	0.01	0.15	0.18	0.31	0.25
Receptor Rinse	0.00	0.00	0.26	0.41	0.01	0.01	0.02	0.01
Total Penetrated	0.03	0.01	0.29	0.40	0.16	0.18	0.32	0.26
Dermal Delivery	0.64	0.66	0.22	0.23	5.05	2.39	4.73	3.44
Total Unabsorbed	94.06	6.71	97.09	2.55	90.85	3.81	90.31	8.30
Total Absorbed	0.67	0.66	0.50	0.55	5.21	2.41	5.05	3.32
Total Recovery	94.72	6.61	97.60	2.73	96.06	4.22	95.36	7.08



10/10

B



Round Lake, Illinois 60073

REPORT

Study Number 10936	Class B	Type of Document Final Report
Title Preliminary Pharmacokinetics Study of Dermal Applied ¹⁴ C-Benzethonium Chloride in Rats		
Proposed Completion Date (protocol) or Period Covered (report) 5/31/00 - 10/04/00		Optional Tracking Number(s) A200009
Project Number CR302	Customer Lonzagroup	Customer's Department/Division
Additional Distribution (Original to the Customer) CRTS Archives, Toxicology, R. Eyre		

APPROVALS

Name	Department/Division	Signature	Date
Study Director			
Daniel M. Wilson, Ph.D., D.A.B.T	CASE/CRTS	<i>[Signature]</i>	Oct 4, 2000
Study Director's Management			
Randy D. White, Ph.D.	CASE/CRTS	<i>Randy D. White</i>	04 Oct 2000
Vice President, CASE/CRTS			
J. Gonder, D.V.M., Ph.D.	CASE/CRTS	<i>Jane C Gonder</i>	10/4/2000

For Reports: Authorized for appropriate use outside the company in compliance with Baxter policies regarding use of **BAXTER CONFIDENTIAL** information? Yes, JDW Initials of Study Director's Department Head or designee and approval signature required above.

Contributing Individuals (applicable for reports)

The date that the last person signs this document is to be used when referencing this document.

For Protocols: Study must not begin until all approval signatures are obtained
 For Reports: Report must not be issued until all approval signatures are obtained

REPORT

ABSTRACT

Purpose: The purpose of this study was to obtain preliminary information regarding the levels and time course of appearance and disappearance of radioactivity in the blood of rats following dermal application of an aqueous solution of ^{14}C -benzethonium chloride (^{14}C -BTC). This information was intended to be used to determine the feasibility of conducting a full GLP definitive toxicokinetic study and aid in the design of such a study.

Methods: Three male and three female rats received single 100 μl topical applications of ^{14}C -BTC at a concentration of 1% in an aqueous solution. The 1% concentration was determined to be the maximum (or close to the maximum) concentration of BTC that could be applied on a daily basis without producing more than slight dermal irritation (Baxter Study Number 10797). Blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 30, and 48 hours after the initial application of ^{14}C -BTC and urine and feces were collected over the 48 hours following the application. For each rat, duplicate blood samples from each time point and duplicate samples of urine and feces were assayed for radioactivity by liquid scintillation counting.

Results: Radioactivity was detected in the treated blood samples at either background or slightly above background (i.e., less than approximately 100 dpm above background levels). An average of approximately 0.37% and 7.3% of the administered radioactivity was recovered respectively, in the urine and feces collected over the 48 hour period following dosing.

Conclusion: Although quantifiable levels of radioactivity were detected in the urine and feces during the 48 hour period following dermal administration of a 1% aqueous solution of ^{14}C -BTC, the levels of radioactivity in the blood during this period were not sufficient to quantify. Therefore, it is not feasible to conduct the definitive toxicokinetic study that was scheduled to be conducted with this test article.

REPORT

TABLE OF CONTENTS

ABSTRACT.....	2
TABLE OF CONTENTS.....	3
PURPOSE.....	4
TEST ARTICLE AND DILUENT.....	4
TEST SYSTEM.....	4
ANIMAL HUSBANDRY.....	5
STUDY DESIGN.....	5
LABORATORY METHODS.....	5
Dosing.....	5
Blood Sampling.....	6
Urine and Feces Collection.....	6
Body Weights.....	6
Radioactivity Analysis.....	6
Necropsy.....	6
RESULTS.....	7
Body Weights.....	7
Radioactivity Analysis in Blood.....	7
Radioactivity Analysis in Urine.....	8
Radioactivity Analysis in Feces.....	9
DISCUSSION/CONCLUSION.....	9
RECORDS.....	10
PROTOCOL.....	10
REFERENCE.....	10
STANDARD PROCEDURES.....	10

TABLES

Table 1. Individual Initial Animal Body Weight Data.....	7
Table 2. Radioactivity in 50 µl Whole Blood Following Dermal Application of 38.1 µCi ¹⁴ C-BTC.....	7
Table 3. Radioactivity Recovered in Urine of Rats Following Dermal Application of 38.1 µCi ¹⁴ C-BTC.....	8
Table 4. Radioactivity Recovered in Feces of Rats Following Dermal Application of 38.1 µCi ¹⁴ C-BTC.....	9

FIGURE

Figure 1. Time Course of Radioactivity in Whole Blood Following Dermal Application of 38.1 µCi ¹⁴ C-BTC.....	8
--	---

APPENDIX

Appendix A. Study Protocol and Amendments	
---	--

PURPOSE

The purpose of this study was to obtain preliminary information regarding the levels and time course of appearance and disappearance of radioactivity in the blood of rats following dermal application of an aqueous solution of ^{14}C -benzethonium chloride (^{14}C -BTC).

TEST ARTICLE AND DILUENT

Test Articles: ^{14}C -benzethonium chloride (^{14}C -BTC)
Manufacturer: Wizard Laboratories, Inc.
Lot 000407
Position of ^{14}C -label: N-methyl position of the BTC molecule
Specific activity of ^{14}C -BTC: 40.16 mCi/mmol

Benzethonium Chloride (BTC)
Lonza Batch 80102, Lot 8K0909, Exp. 3/30/2001

Test Article Diluent: Sterile Water for Injection, USP, Baxter Lot C431171, Exp. JUL 00

Test Article Preparation: The test articles were prepared by diluting BTC and ^{14}C -BTC with Sterile Water for Injection to achieve a concentration of 1% BTC at an activity of 38.1 $\mu\text{Ci}/100\ \mu\text{l}$. Based on the results of a previous dermal irritation study (Ref 1), this concentration of BTC was expected to produce no more than slight dermal irritation if applied once per day for five consecutive days.

Handling and Storage: Test articles and diluent were stored at room temperature.

TEST SYSTEM

Species: Rat

Strain: F344

Source: Harlan Sprague Dawley, Inc. (Indianapolis, Indiana)

Age: Approximately 12 weeks (Date of Birth: Mar 3, 2000)

Weight: 202 to 222 g

Number and Sex: 6 rats (3 male and 3 female rats)

Acclimation/
Quarantine: 5 days

Identification: According to CV-036-001, rats were assigned a Baxter identification number. Rats were identified with the experimental number by ear punch according to CV-037-002.

REPORT

- Health Status:** Only rats showing no signs of clinical illness were used for this study. The rats were from a colony that was barrier raised and certified by the vendor to be specific pathogen free.
- Euthanasia:** Rats were euthanized by CO₂ induced hypoxia following the 48 hour blood sampling time point.
- Justification:** The rat was selected based upon established knowledge of its acceptability for use in this type of study. The dermal route of administration is a possible route of exposure in humans. A two year rat carcinogenicity study was conducted with BTC in the F344 strain of rat under the auspices of the National Toxicology Program.

ANIMAL HUSBANDRY

- Procurement:** Animals were ordered in accordance with CV-036-001, preconditioned in accordance with CV-036-013, and maintained in accordance with CV-036-010.
- Caging:** Rats were individually housed in plastic metabolism cages.
- Feed:** Rats were provided rodent feed *ad libitum*.
- Water:** Drinking water was provided *ad libitum*. According to CV-036-004, the water is periodically analyzed for microbial and chemical agents.
- Feed/Water Contaminants:** There were no known contaminants in the feed or water that would interfere with this study.

STUDY DESIGN

Three male and three female rats received a single 100 µl topical application of ¹⁴C-labeled test article at a concentration of 1% BTC in an aqueous solution. Blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 30 and 48 hours after application and urine and feces were collected over the 48 hours following application.

LABORATORY METHODS

- Dosing:** Within 24 hours prior to dermal application of the test article, the dorsum of each animal was clipped free of hair using an electric clipper. Animals received a single dermal 100 µl application of ¹⁴C-labeled BTC (38.1 µCi) in an aqueous solution spread over an approximately 10 cm² area of the dorsal skin. Then a protective, non-occlusive cover was secured over the application site to prevent the spread and loss of radioactivity. Following the 24 hour exposure period, the protective cover was removed and, in an effort to remove residual radioactivity, the application site was washed three times with distilled water and wiped with gauze pads after each wash. However, following

REPORT

washing the use of a survey meter demonstrated that radioactivity was still present on the backs of each animal.

Blood Sampling: Whole blood was collected by retro-orbital bleed (approximately 100 μ l to 150 μ l per draw) using EDTA-coated capillary pipettes into microcentrifuge tubes. Blood samples were collected at 0.5, 1, 2, 4, 8, 12, 24, 30, and 48 hours following initial application of 14 C-BTC.

Urine and Feces Collection: Immediately following application for the test article, each animal was placed in a plastic Nalgene metabolism cage. Except for Animal No. RA0427 (Exp. No. 6), urine and feces were collected in separate containers over the 48 hour period following application of the test article. The urine and feces for Animal No. RA0427 (Exp. No. 6) were inadvertently collected into a single container; therefore, radioactivity analyses for the urine and feces of this animal were not performed.

Body Weights: Individual animal body weights were measured immediately prior to treatment.

Radioactivity Analysis: Duplicate 50 μ l aliquots of whole blood were removed from the microcentrifuge tubes and solubilized with 100 μ l of Solvable™ Tissue and Gel Solubilizer. The samples were then decolorized by the addition of 10 μ l of 0.1 M EDTA and 50 μ l of 30% hydrogen peroxide, and heated in a 52°C waterbath for approximately one hour. After the samples had cooled to room temperature, 15 ml of Hionic-Fluor™ scintillation cocktail was added to each sample and the samples were assayed for radioactivity with a liquid scintillation counter.

Duplicate 1 ml aliquots of the urine collected over the 48 hour period following dosing were mixed with 15 ml of Hionic-Fluor™ scintillation cocktail and assayed for radioactivity with a liquid scintillation counter.

Duplicate dried and pulverized samples (approximately 20 mg to 25 mg) of the feces collected over the 48 hour period following dosing were rehydrated overnight with 1 ml of water. The samples were then solubilized by the addition of 1 ml of Solvable™ Tissue and Gel Solubilizer and heated in a 50°C waterbath for approximately two hours followed by the addition of 1 ml of isopropyl alcohol and heating at 50°C for approximately 2 hours. The samples were then decolorized by the addition of 200 μ l of 30% hydrogen peroxide, and heated in a 50°C waterbath for approximately two hours. After the samples had cooled to room temperature, 15 ml of Hionic-Fluor™ scintillation cocktail was added to each sample and the samples were assayed for radioactivity with a liquid scintillation counter.

Necropsy: Necropsies were not performed.

RESULTS

Body Weights: Individual initial animal body weights are presented in Table 1.

Table 1. Individual Initial Animal Body Weight Data

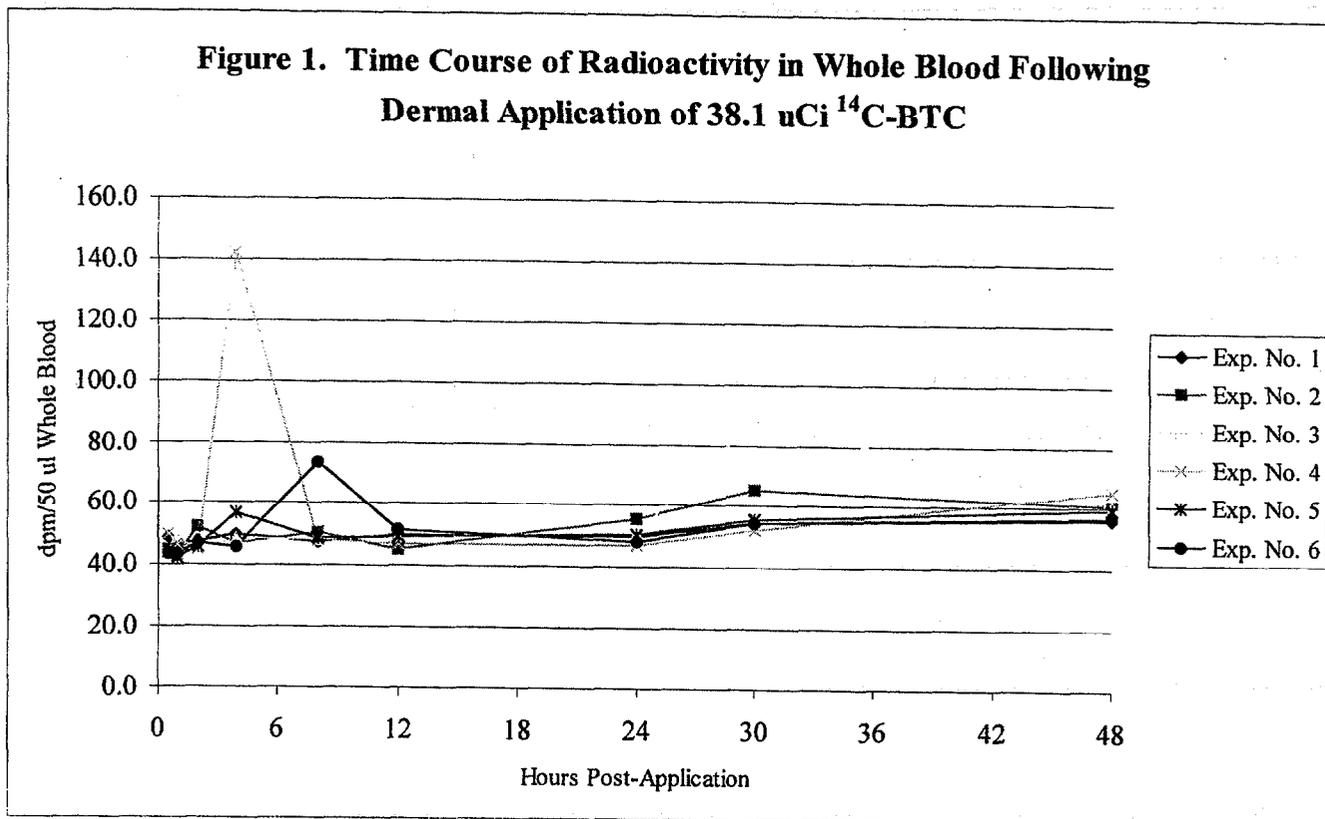
Rat ID No	EXP. No.	Sex	Body Weight (g)
RA0420	1	Male	216
RA0421	2	Male	206
RA0422	3	Male	222
RA0425	4	Female	206
RA0426	5	Female	209
RA0427	6	Female	202

Radioactivity

Analysis in Blood: The mean radioactivity (dpm) detected in 50 μ l of whole blood from each animal at each time point is presented in Table 2 and is graphically represented in Figure 1. Background radioactivity of an untreated 50 μ l whole blood sample ranged between approximately 48.1 dpm and 40.6 dpm. Radioactivity measured in the treated blood samples was either at background or slightly above background (i.e., less than approximately 100 dpm above background). The greatest increase in radioactivity in the blood samples was approximately 100 dpm above background and was seen at the four hour sample collection time point for Animal No. RA0425 (Exp. No. 4).

Table 2. Radioactivity in 50 μ l Whole Blood Following Dermal Application of 38.1 μ Ci 14 C-BTC

Rat ID No	EXP. No.	Sex	Mean DPM/50 μ l of whole blood/time point									
			0.5 hr	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr	30 hr	48 hr	
RA0420	1	Male	48.5	46.3	47.7	50.0	47.4	49.9	50.0	54.3	56.3	
RA0421	2	Male	44.8	43.0	51.9	46.8	50.6	45.4	55.9	65.1	61.0	
RA0422	3	Male	44.3	48.9	44.1	48.7	48.1	49.2	50.8	88.5	60.9	
RA0425	4	Female	50.1	46.6	45.5	142.3	48.2	46.8	46.9	52.1	65.6	
RA0426	5	Female	44.2	42.2	46.1	56.9	49.0	49.5	50.7	55.9	59.7	
RA0427	6	Female	43.3	43.7	46.9	46.0	73.3	51.8	48.1	54.5	56.8	



Radioactivity

Analysis in Urine: The amount of radioactivity recovered in the urine over the 48 hour period following dosing is reported in Table 3. An average of approximately 0.37% of the administered radioactivity was recovered in the urine collected over the 48 hour period following dosing.

Table 3. Radioactivity Recovered in Urine of Rats Following Dermal Application of 38.1 μCi ^{14}C -BTC

Rat ID No	EXP No.	Sex	Mean DPM/ml Urine	48 Hour Urine Volume (ml)	Total DPM	
					Recovered in Urine	Percent of Administered Dose Recovered in Urine
RA0420	1	Male	43171	8.4	362632	0.43
RA0421	2	Male	43028	7.5	322712	0.38
RA0422	3	Male	56512	6.5	367329	0.43
RA0425	4	Female	42590	6.7	285350	0.34
RA0426	5	Female	27666	7.9	218559	0.26
RA0427	6	Female	a	a	a	a

^aSample was contaminated with feces and, therefore, not counted.

REPORT

Radioactivity

Analysis in Feces: The amount of radioactivity recovered in the feces over the 48 hour period following dosing is reported in Table 4. An average of approximately 7.3% of the administered radioactivity was recovered in the feces over the 48 hour period following the initial application.

Table 4. Radioactivity Recovered in Feces of Rats Following Dermal Application of 38.1 μ Ci 14 C-BTC

Rat ID No	EXP No.	Sex	Mean DPM/mg Feces	48 Hour Fecal Weight (g)	Total DPM Recovered in Feces	Percent of Administered Dose Recovered in Feces
RA0420	1	Male	1719.9	3.345	5752991	6.8
RA0421	2	Male	2369.7	2.724	6455149	7.6
RA0422	3	Male	1738.3	2.096	3643471	4.3
RA0425	4	Female	189.1	2.939	555687	0.7
RA0426	5	Female	3250.8	3.931	12779076	15.1
RA0427	6	Female	a	a	a	a

^aSample was contaminated with urine and, therefore, not counted.

DISCUSSION/CONCLUSION

The conditions of this study represented the maximum (or close to the maximum) concentration of BTC that can be applied to the back of a rat on a daily basis without producing more than slight skin irritation (Baxter Report Number 10797). The volume of dosing solution represented the maximum (or close to the maximum) amount of solution (100 microliters) that could be applied in a contained manner to the back of a rat of the F344 strain. The F344 strain rat was used in this study because it was the strain of rat used in the carcinogenicity study conducted on BTC under the auspices of the National Toxicology Program (NTP). The same dose volume was employed in the NTP studies (personal communication with Sponsor's Representative). The total amount of radioactivity applied to each animal (38 microCurie/animal) also represents a high level of total radioactivity. The total volume of blood sampled from each animal (0.1 ml) represented the maximum amount of blood that could be sampled over the 48-hr test period. Under these conditions, quantifiable levels of radioactivity were not observed in the blood of the test animals. Since it is very unlikely that modifications to this study design could be made such that quantifiable levels of radioactivity could be obtained in a study of this type, a decision was made not to go forward with the definitive toxicokinetic study that was scheduled to be conducted on BTC.

While quantifiable levels of radioactivity were not observed in the blood, approximately 0.4% and 7% of the applied radioactivity was recovered in the urine and feces, respectively, over the 48-hr collection period. These data demonstrate that BTC can penetrate through rat skin and that the major route of excretion for dermally applied BTC appears to be through the enterohepatic pathway.

REPORT

RECORDS

The original data listed below will be sent to the Sponsor for retention after finalization of the report:

Protocol and protocol amendment

Test article usage/inventory logs

Dose preparation records

In-life animal records:

Body weights

Dose administration

Sample Collection

Observations

Euthanasia

Scintillation counter printouts

The following supporting records shall be retained in the Baxter Healthcare Corporate Research and Technical Services Archives:

Animal receipt/acclimation records

Water and feed analysis records

Animal room temperature and humidity records

Test article storage area temperature records

Instrument calibration and maintenance records

PROTOCOL

Preliminary Pharmacokinetics Study of Dermally Applied ¹⁴C-Benzethonium Chloride in Rats. Corporate Research and Technical Services Study No. 10936. Project No. CR302. 31 May, 2000. Amendment No. 1, Jun 28, 2000. Amendment No. 2, Oct 4, 2000. Study Director: Daniel Wilson.

REFERENCE

1. Dermal Irritation of Benzethonium Chloride in Rats. Corporate Research and Technical Services Study No. 10797. Project No. CR302. May 6, 2000. Study Director: Russell Eyre.

STANDARD PROCEDURESGeneral

Corporate Research and Technical Services Standard Procedure YY-016-001. Corporate Research and Technical Services Archives Procedure. Nov 15, 1999.

Corporate Research and Technical Services Standard Procedure YY-005-003. Writing Protocols. Nov 15, 1999.

Corporate Research and Technical Services Standard Procedure YY-005-004. Recording Original Data. Nov 15, 1999.

Corporate Research and Technical Services Standard Procedure YY-005-005. Writing Laboratory Reports. Nov 15, 1999.

REPORT

Applied Sciences Standard Procedure 06GH13. Handling and Disposal of Hazardous Waste. Jun 12, 1995.

IV Systems Authorizing Document P1S002. Chemical Hygiene/Lab Safety Procedure. Feb 4, 1999.

Corporate Research and Technical Services Standard Procedure YY-035-002. General Handling of Blood, Fluid, and Tissue Samples. Jul 22, 1996.

Corporate Research and Technical Services Standard Procedure CY-037-002. Blood Collection in Laboratory Animals. Apr 10, 2000.

Corporate Research and Technical Services Standard Procedure YY-035-003. Use of Radioactive Materials under Broadscope License. Mar 9, 1998.

Veterinary Resources

Corporate Research and Technical Services Standard Procedure CV-036-010. Care and Use of Laboratory Animals. Apr 27, 1998.

Corporate Research and Technical Services Standard Procedure CV-036-013. Quarantine and Conditioning of Laboratory Animals for Study Assignment. Mar 22, 1999.

Corporate Research and Technical Services Standard Procedure CV-037-002. Identification of Animals. May 8, 2000.

Corporate Research and Technical Services Standard Procedure CV-037-003. Euthanasia of Laboratory Animals. Mar 22, 1999.

Corporate Research and Technical Services Standard Procedure CV-036-001. Ordering and Receiving Laboratory Animals. Oct 26, 1998.

Corporate Research and Technical Services Standard Procedure CV-036-004. Microbial and Chemical Monitoring - Veterinary Resources. Mar 27, 2000.

Appendix A

Study Protocol and Amendments

Study Number: 10936

PROTOCOL

Page 2 of 6

TITLEPreliminary Pharmacokinetics Study of Dermally Applied ^{14}C -Benzethonium Chloride in Rats**PURPOSE**

The purpose of this study is to obtain preliminary information regarding the levels and time course of appearance and disappearance of radioactivity in the blood of rats following dermal application of an aqueous solution of ^{14}C -benzethonium chloride (^{14}C -BTC).

TEST ARTICLE AND DILUENT

Test Articles: ^{14}C -benzethonium chloride (^{14}C -BTC) (Supplied by the Sponsor)
Position of ^{14}C -label: N-methyl position of the BTC molecule
Specific activity of ^{14}C -BTC: Approximately 40 mCi/mmol
Unlabeled benzethonium chloride (BTC) (Supplied by the Sponsor)

Diluent: Sterile Water for Injection, USP

Handling and Storage: Test articles and diluent will be stored at room temperature.

TEST ARTICLE PREPARATION

Dosing solutions will be prepared by diluting BTC and ^{14}C -BTC with Sterile Water for Injection to achieve a concentration of 1% BTC. The initial high concentration (1% BTC) will be prepared at an activity of 40 $\mu\text{Ci}/100\ \mu\text{l}$. This concentration of BTC is expected to produce no more than slight dermal irritation if applied once per day for five consecutive days.

TEST SYSTEM

Species: Rat

Strain: F344

Source: Charles River

Age: 12 to 14 weeks

Weight: Approximately 150 to 200 g

Number and Sex: Two groups of 6 rats (3 male and 3 female rats/group)

Additional animals may be used if needed to achieve the purposes of the study.

Acclimation/
Quarantine: At least 3 days

BAXTER CONFIDENTIAL

Study Number: 10936

Page 3 of 6

PROTOCOL

- Identification:** According to CV-036-001, rats will be assigned a Baxter identification number. Rats will be identified with the experimental number by ear punch according to CV-037-002.
- Health Status:** Only rats showing no signs of clinical illness will be used for this study. The rats will be from a colony that is barrier raised and certified by the vendor to be specific pathogen free.
- Euthanasia:** Rats will be euthanized by CO₂ induced hypoxia.
- Justification:** The rat was selected based upon established knowledge of its acceptability for use in this type of study. The dermal route of administration is a possible route of exposure in humans.

ANIMAL HUSBANDRY

- Procurement:** Animals will be ordered in accordance with CV-036-001, preconditioned in accordance with CV-036-013, and maintained in accordance with CV-036-010.
- Caging:** Rats will be individually housed in plastic metabolism cages.
- Feed:** Rats shall be provided rodent feed ad libitum.
- Water:** Drinking water will be provided *ad libitum*. According to CV-036-004, the water is periodically analyzed for microbial and chemical agents.
- Feed/Water Contaminants:** There are no known contaminants in the feed or water that will interfere with this study.

STUDY DESIGN

Two groups of three male and three female rats per group will receive a single topical application of ¹⁴C-labeled test article to ascertain the optimal time points for blood collection to be used in the subsequent GLP pharmacokinetic study and to determine doses needed in order to administer enough radioactivity for analytical purposes. Additional animals may be used if additional groups of animals are required to achieve this purpose.

Initially, three male and three female animals will receive single 100 µl (40 µCi) topical applications of ¹⁴C-labeled test article at a concentration of 1% BTC in an aqueous solution. If a suitable level of radioactivity is observed in the blood of the rats in the initial experiment, an additional three male and three female animals may receive an equal volume of the ¹⁴C-labeled test article at a lower concentration. It is anticipated that this lower concentration will correspond to 1/5th to 1/10th of the concentration used in the initial experiment. Blood samples will be collected at specified time points after application.

BAXTER CONFIDENTIAL

Study Number: 10936

Page 4 of 6

PROTOCOL

LABORATORY METHODS

- Dosing:** Within 24 hours prior to dermal application of the test article, the dorsum of each animal will be clipped free of hair using an electric clipper. Animals will receive a single dermal 100 μ l application of 14 C-labeled BTC in an aqueous solution spread over an approximately 10 cm^2 area of the dorsal skin. A protective, non-occlusive cover will be secured over the application site to prevent the spread and loss of radioactivity. Following the 24 hour exposure period, the protective cover will be removed and the application site will be washed to remove residual radioactivity.
- Blood Sampling:** Whole blood will be collected by retro-orbital bleed (approximately 100 μ l to 150 μ l per draw) using EDTA-coated capillary pipettes into microcentrifuge tubes. Initially, blood samples will be collected at 0.5, 1, 2, 4, 8, 12, 24, 30, and 48 hours following initial application of 14 C-BTC. These sample collection time points may be changed for subsequent test groups if deemed necessary to achieve the proposed objectives of this study.
- Body Weights:** Immediately prior to treatment.
- Radioactivity Analysis:** Duplicate aliquots of whole blood will be removed from the micro-centrifuge tubes, solubilized with a tissue solubilizer and then decolorized. Scintillation cocktail will be added to the samples and they will be assayed for radioactivity with a liquid scintillation counter.
- Necropsy:** Animals will be discarded without a necropsy.

EVALUATION OF RESULTS

Data from the initial two groups of animals will be assessed to determine if enough absorption occurred to adequately evaluate the levels of radioactivity in the blood at the doses given. If adequate absorption occurred, the data will be evaluated to assess if the appropriate time points were selected for determination of C_{max} , T_{max} , and $t_{1/2}$ in a subsequent pharmacokinetic study. If it appears that the time points selected were not optimal or systemic absorption was not sufficient for analytical purposes, the study may be repeated in additional animals. If dosing of additional animals is necessary, either the specific activity, the BTC concentration, or both specific activity and BTC concentration may be altered as needed.

REPORT

A non-GLP report will be written to include test system identification (species, sex, and age), individual animal identification, body weight, treatment, description of sample collection methods, results of sample evaluation (dpm/blood sample for each time point collected). Additionally, recommendations for dose levels and blood sample time points to be used for a possible subsequent GLP pharmacokinetics study will be included.

BAXTER CONFIDENTIAL

Study Number: 10936

Page 5 of 6

PROTOCOL

RECORDS

Numbered laboratory notebooks, data sheets, and instrument readouts will be used to record original data. The original data, or copies thereof, shall be available at Baxter Healthcare Corporate Research and Technical Services (Round Lake, Illinois facility) to facilitate auditing of the study during its progress and before acceptance of the final report. After the final report is signed, all original data listed below will be sent to the Sponsor for retention:

Protocol and protocol amendments (if applicable)

Test article usage/inventory logs

Dose preparation records

In-life animal records:

Body weights

Dose administration

Sample Collection

Observations

Euthanasia

Scintillation counter printouts

The following supporting records shall be retained in the Baxter Healthcare Corporate Research and Technical Services Archives:

Animal receipt/acclimation records

Water and feed analysis records

Animal room temperature and humidity records

Test article storage area temperature records

Instrument calibration and maintenance records

STANDARD PROCEDURESGeneral

Corporate Research and Technical Services Standard Procedure YY-016-001. Corporate Research and Technical Services Archives Procedure. Current Issue.

Corporate Research and Technical Services Standard Procedure YY-005-003. Writing Protocols. Current Issue.

Corporate Research and Technical Services Standard Procedure YY-005-004. Recording Original Data. Current Issue.

Corporate Research and Technical Services Standard Procedure YY-005-005. Writing Laboratory Reports. Current Issue.

Applied Sciences Standard Procedure 06GH13. Handling and Disposal of Hazardous Waste. Current Issue.

BAXTER CONFIDENTIAL

Study Number: 10936

Page 6 of 6

PROTOCOL

IV Systems Authorizing Document P1S002. Chemical Hygiene/Lab Safety Procedure. Current Issue.

Corporate Research and Technical Services Standard Procedure YY-035-002. General Handling of Blood, Fluid, and Tissue Samples. Current Issue.

Corporate Research and Technical Services Standard Procedure CY-037-002. Blood Collection in Laboratory Animals. Current Issue.

Corporate Research and Technical Services Standard Procedure YY-035-003. Use of Radioactive Materials under Broadscope License. Current Issue.

Veterinary Resources

Corporate Research and Technical Services Standard Procedure CV-036-010. Care and Use of Laboratory Animals. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-036-013. Quarantine and Conditioning of Laboratory Animals for Study Assignment. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-037-002. Identification of Animals. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-037-003. Euthanasia of Laboratory Animals. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-036-012. Disposition of Laboratory Animals That Die on Test. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-036-001. Ordering and Receiving Laboratory Animals. Current Issue.

Corporate Research and Technical Services Standard Procedure CV-036-004. Microbial and Chemical Monitoring - Veterinary Resources. Current Issue.

BAXTER CONFIDENTIAL

** TOTAL PAGE.07 **

Study Number 10936	Class B	Type of Document Protocol Amendment No. 1
Title Preliminary Pharmacokinetics Study of Dermal Applied ¹⁴ C-Benzethonium Chloride in Rats		
Proposed Completion Date (protocol) or Period Covered (report) 30 Sep. 2000		Optional Tracking Number(s) A200009
Project Number CR302	Customer Lonzagroup	Customer's Department/Division
Additional Distribution (Original to Archives) See attached distribution list		

APPROVALS

Name	Department/Division	Signature	Date
Study Director			
Russell Eyre, Ph.D., D.A.B.T	CASE/CRTS	<i>Russell Eyre</i>	6/28/00
Study Director's Management			
Dan M. Wilson, Ph.D., D.A.B.T	CASE/CRTS	<i>Dan M. Wilson</i>	7/6/00
Director, Toxicology			
Randy D. White, Ph.D.	CASE/CRTS	<i>Randy D. White</i>	7/6/00
Sponsors Representative			
Gerald P. Schoenig, Ph.D	Toxicology/Regulatory Services, Inc.	<i>Gerald P. Schoenig</i>	9/18/00

For Reports: Authorized for appropriate use outside the company in compliance with Baxter policies regarding use of **BAXTER CONFIDENTIAL** information? Yes, _____ Initials of Study Director's Department Head or designee and approval signature required above.

Contributing Individuals (applicable for reports)

The date that the last person signs this document is to be used when referencing this document.

For Protocols: Study must not begin until all approval signatures are obtained

For Reports: Report must not be issued until all approval signatures are obtained

Based on the low levels of radioactivity in the blood and in consultation with the Sponsor Representative, it was decided to determine the levels of radioactivity in the urine and feces. Therefore, add the following paragraph to the Radioactivity Analysis section:

“Duplicate 1 ml aliquots of the urine collected over the 48 hour period following dosing will be mixed with 15 ml of scintillation cocktail and assayed for radioactivity with a liquid scintillation counter. Duplicate 20 mg dried and pulverized samples of the feces collected over the 48 hour period following dosing will be solubilized and decolorized then mixed with an appropriate volume of scintillation cocktail and assayed for radioactivity with a liquid scintillation counter.”

Also, add the following sentence after the first sentence of the REPORT section:

“For the urine and feces, the percent total dosed radioactivity in the urine and feces will be reported.”



Round Lake, Illinois 60073

AMENDMENT

Study Number 10936	Class B	Type of Document Protocol Amendment No. 2
Title Preliminary Pharmacokinetics Study of Dermally Applied ¹⁴ C-Benzethonium Chloride in Rats		
Proposed Completion Date (protocol) or Period Covered (report) Oct 4, 2000		Optional Tracking Number(s) A200009
Project Number CR302	Customer Lonzagroup	Customer's Department/Division
Additional Distribution (Original to Archives) See attached distribution list		

APPROVALS

Name	Department/Division	Signature	Date
Study Director Daniel M. Wilson, Ph.D., D.A.B.T CASE/CRTS			Oct 4, 2000
Director, Toxicology Randy D. White, Ph.D.	CASE/CRTS		04 Oct 2000

For Reports: Authorized for appropriate use outside the company in compliance with Baxter policies regarding use of **BAXTER CONFIDENTIAL** information? Yes, _____ Initials of Study Director's Department Head or designee and approval signature required above.

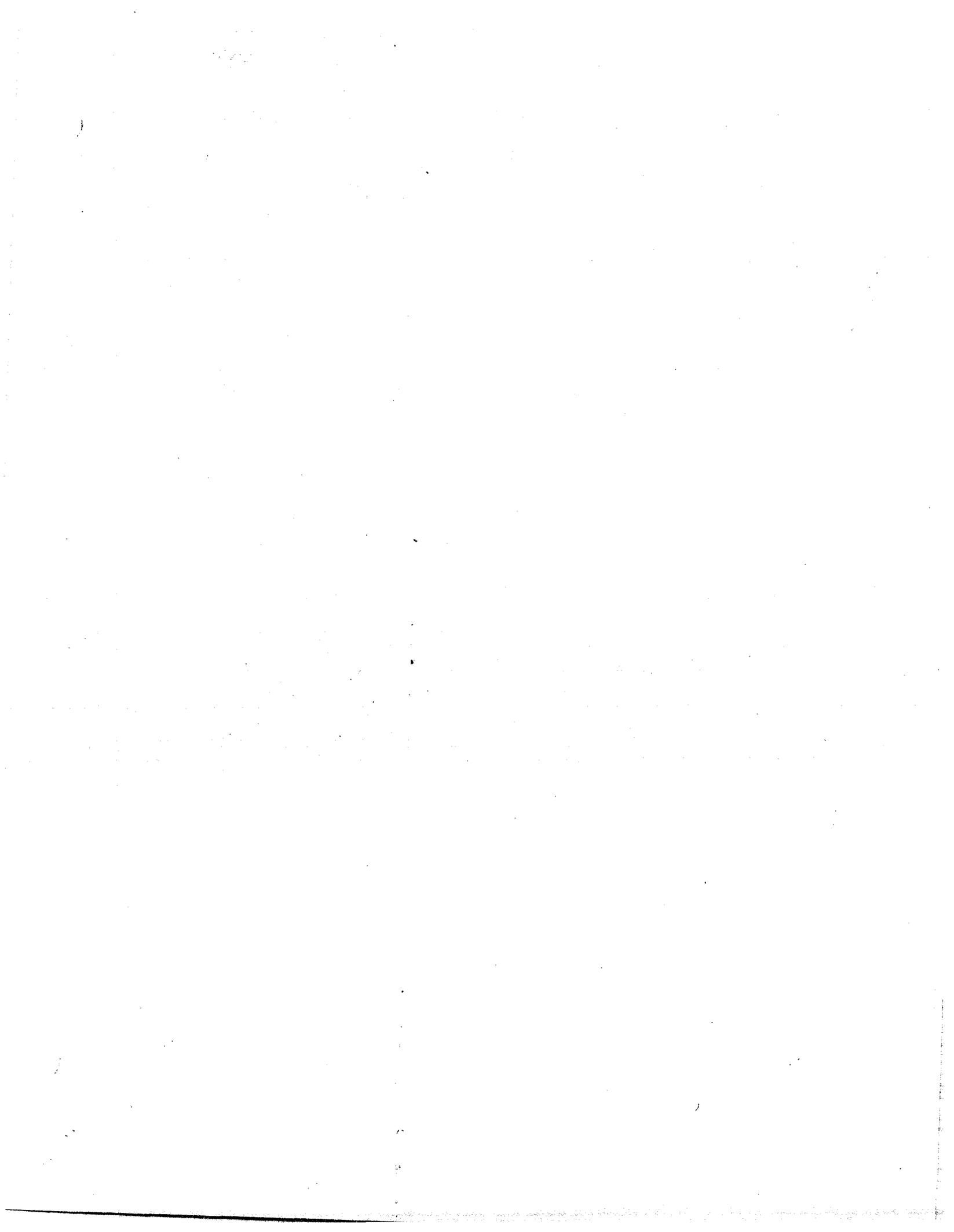
Contributing Individuals (applicable for reports)

The date that the last person signs this document is to be used when referencing this document.

For Protocols: Study must not begin until all approval signatures are obtained

For Reports: Report must not be issued until all approval signatures are obtained

Due to the previous Study Director leaving Baxter, change the Study Director for this study to Daniel M. Wilson.



C



Round Lake, Illinois 60073

REPORT

Study Number 10797	Class C	Type of Document Final Report
Title Dermal Irritation of Benzethonium Chloride in Rats		
Proposed Completion Date (protocol) or Period Covered (report) 5/5/00 – 10/3/00		Optional Tracking Number(s) A200009
Project Number CR302	Customer Lonzagroup	Customer's Department/Division
Additional Distribution (Original to the Customer) CRTS Archives, Toxicology, R. Eyre		

APPROVALS

Name	Department/Division	Signature	Date
Study Director			
Daniel M. Wilson, Ph.D., D.A.B.T	CASE/CRTS	<i>Daniel M. Wilson</i>	Oct 4, 2000
Study Director's Management			
Randy D. White, Ph.D.	CASE/CRTS	<i>Randy D. White</i>	04 Oct 2000
Vice President, CASE/CRTS			
J. Gonder, D.V.M., Ph.D.	CASE/CRTS	<i>Janet C. Gonder</i>	10/4/2000
For Reports: Authorized for appropriate use outside the company in compliance with Baxter policies regarding use of BAXTER CONFIDENTIAL information? <input checked="" type="checkbox"/> Yes, <i>DMW</i> Initials of Study Director's Department Head or designee and approval signature required above.			
Contributing Individuals (applicable for reports)			
The date that the last person signs this document is to be used when referencing this document.			

For Protocols: Study must not begin until all approval signatures are obtained

For Reports: Report must not be issued until all approval signatures are obtained

TABLE OF CONTENTS

TABLE OF CONTENTS.....2
PURPOSE.....3
TEST ARTICLE AND DILUENT3
TEST ARTICLE PREPARATION.....3
TEST SYSTEM3
ANIMAL HUSBANDRY.....4
STUDY DESIGN.....4
LABORATORY METHODS.....4
 Dosing.....4
 Observations4
 Body Weights.....4
 Necropsy4
RESULTS5
 Body Weights.....5
 Observations5
CONCLUSION.....6
RECORDS6
REFERENCE.....6
STANDARD PROCEDURES.....7

TABLES

Table 1. Draize Classification System for Skin Reactions5
Table 2. Individual Animal Body Weight Data5
Table 3. Individual Dermal Irritation Scores6

PURPOSE

The purpose of this study was to determine if a 1% (w/v) aqueous solution of benzethonium chloride can be applied dermally once per day for five consecutive days without producing more than slight dermal irritation.

TEST ARTICLE AND DILUENT

Test Article: Benzethonium Chloride (BTC)
Lonza Batch 80102, Lot 8K0909, Exp. 3/30/2001

Test Article Diluent: Sterile Water for Injection, USP, Baxter Lot 431171, Exp. JUL 00

Handling and Storage: Test article and diluent were stored at room temperature.

TEST ARTICLE PREPARATION

The dosing solution was prepared by dissolving BTC in Sterile Water for Injection to achieve a concentration of 1% BTC (w/v).

TEST SYSTEM

Species: Rat

Strain: F344

Source: Harlan Sprague Dawley, Inc. (Indianapolis, Indiana)

Age: 11 to 12 weeks (Date of Birth: males, Feb 18, 2000; females, Feb 11, 2000)

Weight: 144 to 183 g

Number and Sex: 6 rats (3 male and 3 female rats)

Acclimation/
Quarantine: 3 days

Identification: Upon receipt, rats were assigned a Baxter identification number.

Health Status: Only rats showing no signs of clinical illness were used for this study. The rats were from a colony that was barrier raised and certified by the vendor to be specific pathogen free.

Euthanasia: Rats were euthanized by CO₂ induced hypoxia following the five day observation period.

Justification: The rat was selected based upon established knowledge of its acceptability for use in this type of study. The dermal route of administration is a possible route of exposure in humans.

ANIMAL HUSBANDRY

- Caging:** Rats were individually housed in suspended stainless steel cages.
- Feed:** Rats were provided rodent feed *ad libitum*.
- Water:** Drinking water was provided *ad libitum*. The water is periodically analyzed for microbial and chemical agents.
- Feed/Water Contaminants:** There were no known contaminants in the feed or water that would interfere with this study.

STUDY DESIGN

Three male and three female rats received five consecutive daily topical applications of the test article at an aqueous concentration of 1% (w/v). Approximately 24 hours after each application, the treatment site was observed for signs of erythema and edema and washed with water prior to receiving the next application.

LABORATORY METHODS

- Dosing:** On Day 0 approximately 30 minutes prior to dermal application of the test article, the dorsum of each animal was clipped free of hair using an electric clipper. Beginning on Day 0, three male and three female rats received five consecutive daily topical applications (100 μ l per application) of the test article at an aqueous concentration of 1% (w/v). The 100 μ l application was applied to the back of rats over a surface area of approximately 10 cm². Approximately 24 hours following each of the first four applications, the treatment site was washed with water (i.e., after treatment site observation and prior to next application).
- Observations:** Approximately 24 hours following each application, the treatment site was evaluated for erythema and edema according to Draize (Ref 1) as shown in Table 1.
- Body Weights:** Individual animal body weights were measured immediately prior to the first treatment.
- Necropsy:** Necropsies were not performed.

Table 1. Draize Classification System for Skin Reactions

Reaction	Numerical Grading
<u>Erythema and Eschar Formation</u>	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation	4
<u>Edema Formation</u>	
No edema	0
Very slight edema (barely perceptible)	1
Well defined edema (edges of area are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond exposure area)	4

RESULTS

Body Weights: Individual initial animal body weights are presented in Table 2.

Table 2. Individual Animal Body Weight Data

Rat ID No	Sex	Body Weight (g)
RA0371	Male	179
RA0372	Male	183
RA0373	Male	183
RA0375	Female	144
RA0376	Female	146
RA0377	Female	149

Observations: Dermal irritation scores determined approximately 24 hours following each application are presented in Table 3. There was no evidence of irritation in one male rat. One female rat exhibited barely perceptible erythema after the first treatment with no evidence of irritation after the second through the fifth treatment. Two male rats exhibited barely perceptible erythema after each treatment. One female rat developed barely perceptible erythema after the third treatment and non-confluent well defined erythema over approximately 10% of the treatment sight with barely perceptible erythema over the remaining treatment site area after the fourth and fifth treatment. One female exhibited barely perceptible erythema after the first and second treatment, non-confluent well defined erythema and barely perceptible edema after the third and fourth treatment, and non-confluent well defined erythema with no evidence of edema after the fifth treatment.

REPORT

Table 3. Individual Dermal Irritation Scores

Rat ID No	Sex	Day 1		Day 2		Day 3		Day 4		Day 5	
		Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
RA0371	Male	1	0	1	0	1	0	1	0	1	0
RA0372	Male	1	0	1	0	1	0	1	0	1	0
RA0373	Male	0	0	0	0	0	0	0	0	0	0
RA0375	Female	1	0	1	0	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a	0 ^a
RA0376	Female	1	0	0	0	0	0	0	0	0	0
RA0377	Female	0	0	0	0	1	0	2 ^b	0	2 ^b	0

^aIrritation scores were not confluent. Areas of irritation consisted of approximately 20% to 25% of the treatment site area.

^bScore of 2 erythema was not confluent. Area of 2 erythema consisted of approximately 10% of the treatment site area. The remaining treatment site area exhibited an erythema score of 1.

CONCLUSION

A 1% (w/v) aqueous solution of benzethonium chloride can be applied dermally once per day for five consecutive days without producing more than slight dermal irritation.

RECORDS

The original data listed below will be sent to the Sponsor for retention after finalization of the report:

Protocol and protocol amendments (if applicable)

Test article usage/inventory logs

Dose preparation records

In-life animal records:

Body weights

Dose administration

Observations

Euthanasia

The following supporting records shall be retained in the Baxter Healthcare Corporate Research and Technical Services Archives:

Animal receipt/acclimation records

Water and feed analysis records

Animal room temperature and humidity records

Test article storage area temperature records

Instrument calibration and maintenance records

REFERENCE

1. Draize, J. H. Dermal Toxicity. U.S. Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, p. 46 - 59. Austin 1, Texas: The Association of Food and Drug Officials of the United States Business Office, Bureau of Food and Drugs, Texas State Department of Health, 1959.

STANDARD PROCEDURESGeneral

Corporate Research and Technical Services Standard Procedure YY-016-001. Corporate Research and Technical Services Archives Procedure. Nov 15, 1999.

Corporate Research and Technical Services Standard Procedure YY-005-004. Recording Original Data. Nov 15, 1999.

Corporate Research and Technical Services Standard Procedure YY-005-005. Writing Laboratory Reports. Nov 15, 1999.

IV Systems Authorizing Document P1S002. Chemical Hygiene/Lab Safety Procedure. Feb 4, 1999.

Corporate Research and Technical Services Standard Procedure YY-035-002. General Handling of Blood, Fluid, and Tissue Samples. Jul 22, 1996.

Corporate Research and Technical Services Standard Procedure CY-037-002. Blood Collection in Laboratory Animals. Apr 10, 2000.

Veterinary Resources

Corporate Research and Technical Services Standard Procedure CV-036-010. Care and Use of Laboratory Animals. Apr 27, 1998.

Corporate Research and Technical Services Standard Procedure CV-036-013. Quarantine and Conditioning of Laboratory Animals for Study Assignment. Mar 22, 1999.

Corporate Research and Technical Services Standard Procedure CV-037-002. Identification of Animals. May 8, 2000.

Corporate Research and Technical Services Standard Procedure CV-037-003. Euthanasia of Laboratory Animals. Mar 22, 1999.

Corporate Research and Technical Services Standard Procedure CV-036-001. Ordering and Receiving Laboratory Animals. Oct 26, 1998.

Corporate Research and Technical Services Standard Procedure CV-036-004. Microbial and Chemical Monitoring - Veterinary Resources. Mar 27, 2000.