October 11, 2004

FINAL REPORT #040706-201

1.0 TITLE: AN EVALUATION OF ONE TEST PRODUCT FOR ITS ANTIMICROBIAL PROPERTIES WHEN CHALLENGED WITH VARIOUS MICROORGANISM STRAINS USING AN IN-VITRO TIME-KILL METHOD

2.0 SPONSOR: B4 BRANDS
313 3rd Avenue South
Mount Vernon, Iowa 52314

3.0 COMPANY: BIOSCIENCE LABORATORIES, INC.
300 N. Wilson Avenue
Bozeman, Montana 59715

4.0 STUDY DIRECTORS:

Terri Eastman- Principal Study Director
Lisa Lehman- Associate Study Director

5.0 PURPOSE:

This evaluation used an In-Vitro Time-Kill Method to assess the broad-spectrum antimicrobial efficacy of one (1) test product, an alcohol-based hand sanitizer, when challenged with fifty-three (53) microorganism strains. The test product was evaluated at a concentration of 99% (v/v). All testing was performed in accordance with Good Laboratory Practices, as specified in 21 CFR Part 58.

6.0 SCOPE:

An In-Vitro Time-Kill evaluation was performed for one (1) test product using challenge suspensions of fifty-three (53) different microorganism strains. The microorganism strains evaluated included twenty-five (25) American Type Culture Collection (ATCC) strains and twenty-five (25) Clinical Isolates of those same species, as described in the Tentative Final Monograph, *Federal Register*, 17 June 1994, vol. 59:116, p. 31444, as well as *Clostridium difficile* (ATCC #9689), *Salmonella choleraesuis,* serotype Typhi (ATCC #6539), and *Trichophyton mentagrophytes* (ATCC #9533). Each of the challenge species was exposed to the test product for a single contact time- fifteen (15) seconds, thirty (30) seconds, or one (1) minute – depending upon the challenge strain (reference Table II). The Percent and Log_{10} Reductions from the initial populations were determined for each challenge microorganisms following the appropriate timed exposure to the test product. All agar-plating was performed in duplicate. The Study Protocol, included in Addendum I of this Final Report, present the study methodology in detail, as do the General Data Gathering Forms (Form No. 91-L-002) in Addendum VI of this Final Report. One (1) deviation from the methodology presented in the Protocol occurred (see Section 11.0 of this Final Report), which had no adverse effect upon the Study outcome. The Protocol and/or SOP Deviation Recording Form (Form No. 99-QA-004) which details the deviation, is included in Addendum I of this Final Report.

7.0 TEST MATERIAL:

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

Test Product #1:	Avant Original™ Instant Hand Sanitizer
Lot Number:	9664
Manufacture Date:	Not Provided
Expiration Date:	9/13/06

8.0 EQUIPMENT AND SUPPLIES:

The equipment and supplies used in this study are as described in the Study Protocol in Addendum 1 of this Final Report. Additional details are recorded on Equipment Tracking Forms (Form No. 98-L-007) in Addendum VIII of this Final Report.

9.0 GROWTH MEDIA AND DILUTING FLUIDS:

The growth media and diluting fluids used in this study are as described in the Study Protocol in Addendum I of this Final Report. Additional details are recorded on Media/Diluent Tracking Forms (Form No. 97-L-007) in Addendum V of this Final Report.

10.0 NEUTRALIZATION STUDY:

Neutralization studies (SOP L-2007) were performed for the test product versus *Clostridium difficile* (ATCC #9689), *Escherichia coli* (ATCC #11229), and *Streptococcus pneumoniae* (ATCC #49619) to ensure that the neutralizing solution employed (BBP++) was effective in neutralizing the antimicrobial properties of the product. This neutralization procedure followed guidelines set forth in ASTM E-1054-02, *Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*, and demonstrated the effective neutralization of the antimicrobial activity of the test product. All data resulting from the Neutralization Assay are included in Addendum IV of this Final Report.

11.0 PROTOCOL DEVIATION:

Staphylococcus aureus (Clinical Isolate; BSLI #032301MMRSal3) was replaced with *Staphylococcus aureus* (Clinical Isolate; BSLI #032301MMRSa3) due to the non-availability of the Protocol-specified strain. The intent of this evaluation was to assess the broad-spectrum antimicrobial efficacy of the test product when challenged with one (1) ATCC strain and one (1) Clinical Isolate of each of the species listed in the Tentative Final Monograph, *Federal Register*, 17 June 1994, vol. 59:116, p. 31444. The 1994 TFM does not specify specific Clinical Isolate strains to be used, only the species. Substituting one (1) Clinical Isolate strain for another of the same species had no adverse effect upon the outcome of the study.

12.0 CLINICAL ISOLATES:

The origin of each of the Clinical Isolates evaluated is provided in Table I.

TABLE 1: ORIGIN OF CLINICAL ISOLATES

Organism	Date Isolated	Specimen	Patient Age/Sex	Source	BSLI ID No.
Acinetobacter baumannii	Unknown	Urine	Unknown	MRL	061700Ab16
Bacteroides fragilis	Unknown	Peritoneal fluid	23/M	ARUP	090800Bf
Candida albicans	02/01/00	Bronchial lavage	81/M	CSMH	040400Ca16
Candida tropicalis	10/21/99	subhepatic fluid	47/M	UW/HMC	121799Ct
Enterobacter cloacae	06/23/00	Left hip	47/F	U of U	070700Ec11
Enterococcus faecalis	Unknown	Rectal swab	Unknown	MRL	061700Efs12
Enterococcus faecium	Unknown	Rectal swab	Unknown	MRL	061700Efm15
Escherichia coli	07/03/99	Urine	Unknown	WCMC	070399Ec
Escherichia coli	06/01/99	Blood	34/F	ARUP	060199Ec
Haemophilus influenzae	12/11/99	Sputum	35/M	UW/HMC	121699Hi3
Klebsiella oxytoca	06/01/99	Urine	71/F	UW/HMC	060199Ko

Klebsiella pneumoniae	Unknown	Urine	Unknown	U of U	040400Kpn2
Micrococcus luteus	Unknown	Blood	64/M	JG	061901M13
Proteus mirabilis	11/30/99	Urine	34/F	UW/HMC	121699Pm2
Pseudomonas aeruginosa	07/01/99	Wound	Unknown	WCMC	070199Pa
Pseudomonas aeruginosa	Unknown	Urine	Unknown	U of U	040400Pa5
Serratia marcescens	Unknown	Unknown	Unknown	WMC	081499Sm
Staphylococcus aureus	Unknown	Sputum	79/M	JG	032301MMRSa3
Staphylococcus aureus	02/21/00	Urine	35/M	U of U	040400Sa8
Staphylococcus epidermidis	Unknown	Eye	Unknown	MRL	061700Se2
Staphylococcus haemolyticus	06/05/00	Wound	72/M	WMC	062900Sha
Staphylococcus hominis	Unknown	Unknown	Unknown	MRL	060700Sho4
Staphylococcus saprophyticus	Unknown	Urine	62/M	WMC	062900Ss
Streptococcus pneumoniae	Unknown	Pulmonary	22/M	JG	061901Spn1
Streptococcus pyogenes	Unknown	Throat	Unknown	U of U	040400Spy4

ARUP = Associated Regional and University Pathologist Laboratories in Salt Lake City, UT
CSMH = Christus St. Mary Hospital in Port Arthur, Texas
JG = Jones Group
MRL = MRL Research Laboratory in Cypress, California
U of U = University of Utah Hospital and Clinics in Salt Lake City, Utah
UW/HMC = University of Washington, Washington/Harborview Medical Center
WCMC = Westchester County Medical Center in Valhalla, New York
WMC = Western Montana Clinic in Missoula, Montana

13.0 RESULTS: TABLE II:

Table II presents the Log_{10} and percent reductions observed for Test Product #1 (Avant Original™ Instant Hand Sanitizer [Lot Number 9664] versus each of the fifty-three (53) microorganisms tested.

No.	Microoganism Species	(ATCC or Clinical Isolate*)	Exposure Time	Log_{10} Reduction	Percent Reduction
1	*Acinetobacter baumannii*	ATCC #19003	15 Seconds	5.9777	99.9999%
2*	*Acinetobacter baumannii*	BSLI #061700Ab16	15 Seconds	5.8692	99.9999%
3	*Bacteroides fragilis*	ATCC #43858	15 Seconds	7.6284	99.9999%
4*	*Bacteroides fragilis*	BSLI #090800Bf	15 Seconds	7.6175	99.9999%
5	*Candida albicans*	ATCC #10231	30 Seconds	6.2227	99.9999%
6*	*Candida albicans*	BSLI #040400Ca16	30 Seconds	6.3010	99.9999%
7	*Candida tropicalis*	ATCC #750	30 Seconds	6.2867	99.9999%
8*	*Candida tropicalis*	BSLI #121799Ct	30 Seconds	6.3054	99.9999%
9	*Clostridium difficile*	ATCC #9689	15 Seconds	5.4749	99.9997%
10	*Enterobacter aerogenes*	ATCC #29007	15 Seconds	6.1717	99.9999%
11*	*Enterobacter cloacae*	BSLI #070700Ec11	15 Seconds	6.3579	99.9999%
12	*Enterococcus faecalis*	ATCC #29212	15 Seconds	6.4793	99.9999%

13*	*Enterococcus faecalis;* VRE	BSLI #061700Efs12	15 Seconds	6.4346	99.9999%
14	*Enterococcus faecium;* MDR	ATCC # 51559	15 Seconds	6.2355	99.9999%
15*	*Enterococcus faecium;* VRE	BSLI #061700Efm15	15 Seconds	6.0354	99.9999%
16	*Escherichia coli*	ATCC #11229	15 Seconds	6.1945	99.9999%
17*	*Escherichia coli*	BSLI #060199Ec	15 Seconds	6.1367	99.9999%
18	*Escherichia coli*	ATCC #25922	15 Seconds	6.1492	99.9999%
19*	*Escherichia coli*	BSLI #070399Ec	15 Seconds	6.4074	99.9999%
20	*Haemophilus influenzae*	ATCC #8149	15 Seconds	6.5911	99.9999%
21*	*Haemophilus influenzae*	BSLI #121699Hi3	15 Seconds	5.9614	99.9999%
22	*Klebsiella oxytoca*	ATCC #15764	15 Seconds	6.1399	99.9999%
23*	*Klebsiella oxytoca*	BSLI #060199Ko	15 Seconds	6.2707	99.9999%
24	*Klebsiella pneumoniae*	ATCC #29019	15 Seconds	6.1351	99.9999%
25*	*Klebsiella pneumoniae*	BSLI #040400Kpn2	15 Seconds	6.3128	99.9999%
26	*Micrococcus luteus*	ATCC #7468	15 Seconds	5.9708	99.9999%
27*	*Micrococcus luteus*	BSLI #061901M13	15 Seconds	6.4556	99.9999%
28	*Proteus mirabilis*	ATCC #7002	15 Seconds	5.9138	99.9999%
29*	*Proteus mirabilis*	BSLI #121699Pm2	15 Seconds	6.0531	99.9999%
30	*Pseudomonas aeruginosa*	ATCC #15442	15 Seconds	6.1717	99.9999%
31*	*Pseudomonas aeruginosa*	BSLI #070199Pa	15 Seconds	6.5563	99.9999%
32	*Pseudomonas aeruginosa*	ATCC #27853	15 Seconds	6.1255	99.9999%
33*	*Pseudomonas aeruginosa*	BSLI #040400Pa5	15 Seconds	6.5185	99.9999%
34	*Salmonella typhi*	ATCC #6539	15 Seconds	6.4764	99.9999%
35	*Serratia marcescens*	ATCC #14756	15 Seconds	6.5119	99.9999%
36*	*Serratia marcescens*	BSLI #081499Sm	15 Seconds	6.3729	99.9999%
37	*Staphylococcus aureus*	ATCC #6538	15 Seconds	6.4814	99.9999%
38*	*Staphylococcus aureus* MMRSA	BSLI #032301MMRSa3	15 Seconds	6.6180	99.9999%
39	*Staphylococcus aureus*	ATCC #29213	15 Seconds	6.6857	99.9999%
40*	*Staphylococcus aureus* MRSA	BSLI #040400Sa8	15 Seconds	6.3636	99.9999%
41	*Staphylococcus epidermidis*	ATCC #12228	15 Seconds	6.6580	99.9999%
42*	*Staphylococcus epidermidis*	BSLI #061700Se2	15 Seconds	6.1931	99.9999%
43	*Staphylococcus haemolyticus*	ATCC #29970	15 Seconds	6.0663	99.9999%
44*	*Staphylococcus haemolyticus*	BSLI #062900Sha	15 Seconds	6.4362	99.9999%
45	*Staphylococcus hominis*	ATCC #27844	15 Seconds	5.9754	99.9999%
46*	*Staphylococcus hominis*	BSLI #060700Sho4	15 Seconds	6.5966	99.9999%
47	*Staphylococcus saprophyticus*	ATCC #35552	15 Seconds	6.6812	99.9999%
48*	*Staphylococcus saprophyticus*	BSLI #062900Ss	15 Seconds	6.3953	99.9999%
49	*Streptococcus pneumoniae*	ATCC #49619	15 Seconds	5.9165	99.9999%
50*	*Streptococcus pneumoniae*	BSLI #061901Spn1	15 Seconds	7.2589	99.9999%
51	*Streptococcus pyogenes*	ATCC #19615	15 Seconds	5.8195	99.9998%

52*	Streptococcus pyogenes	BSLI #040400Spy4	15 Seconds	6.9542	99.9998%
53	Trichophyton mentagrophytes	ATCC #9533	1 Minute	5.3284	99.9995%

* = Clinical Isolate
MDR = Multi-Drug Resistent
VRE = Vancomycin-Resistent *Enterococcus*
MRSA = Methicillin-Resistent *Staphylococcus aureus*
MMRSA = Mupirocin-Resistent, Methicillin-Resistent *Staphylococcus aureus*

14.0 ACCEPTANCE:

BIOSCIENCE LABORATORIES, INC.
300 N. Wilson Avenue
Bozeman, Montana 59715

President
and CEO: _____ _____
Daryl S. Paulson, Ph.D. Date

Principal
Study Director: _____ _____
Terri Eastman Date of Study Completion

Associate
Study Director: _____ _____
Lisa Lehman 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:

Phase	Date
Neutralization Assay	09/21/04
Product Testing	09/21/04
Data Audit	10/04/04
Final Report Review	10/11/04
Reports to Study Director And Management	09/21/04 & 10/11/04

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

Director of
Quality
Assurance: _____ _____
John A. Michell, Ph.D. Date