Hill Top RESEARCH

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REPORT FOR

ASSESSMENT OF RAPID GERMICIDAL (TIME KILL) ACTIVITY FOR UltraBlu™ TOOTHBRUSH

HTR Study No.: 08-129295-106

November 26, 2008

FOR

TECHLIGHT SYSTEMS, INC.
P.O. Box 190
Dunnellon, FL 34430

BY
HILL TOP RESEARCH CORPORATION
6088 Main & Mill Streets
Miamiville, Ohio 45147

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APPENDICES

1.0 SUMMARY

- The test article identified as UltraBlu™ Toothbrush was received from TechLight Systems, Inc. for use in this test.

- The test article was tested in triplicate against the test organisms contained in whole saliva collected from healthy human volunteers (unrestricted), *Candida albicans*, ATCC 18804, *Fusobacterium nucleatum*, ATCC 10953 and *Porphyromonas gingivalis*, ATCC 33277 with a 2 minute exposure to the UltraBlu™ Toothbrush "blue light."

- After an exposure period of two minutes, no reduction in numbers of *F. nucleatum* or organisms contained in human saliva was seen. Under the same test conditions, The test organism *C. albicans* showed a 7% reduction in yeast organisms and the test organism *P. gingivalis* showed a 17% reduction in the number of bacteria.

- An additional test article, identified as UltraBlu™ Toothbrush (engineering prototype) was received from TechLight Systems, Inc. on October 29, 2008 for use in this test as directed in Protocol Amendment #1. This test exposed the test organism *P. gingivalis* to ten, 2 minute exposures over a 29 minute period (2 minutes on, 1 minute off; 10 times).

- When compared to an untreated control, the test article, UltraBlu™ Toothbrush (engineering prototype) showed a 99.39% reduction (2.22 $\log_{10}$) in numbers of the test organism *P. gingivalis* after 10 - 2 minute exposures over 29 minutes.

2.0 PURPOSE

The purpose of this study was to evaluate the antibacterial effect of the UltraBlu™ Toothbrush "blue light" in reducing bacteria found in human saliva and other selected microorganisms known to be present in the mouth when artificially deposited on a glass surface and exposed to the "blue light" under simulated use conditions without brushing.

3.0 STUDY SPONSOR

TechLight Systems, Inc.
P.O. Box 190
Dunnellon, FL 34430

SPONSOR REPRESENTATIVE: Michael Barnes

4.0 INVESTIGATIVE PERSONNEL

Study Director:	Kathleen A. Baxter, B.S.
Study Manager:	Jane M. Young, B.S.
Study Coordinator:	Margaret K. Haines, B.S.

5.0 RESEARCH STANDARDS

This study was conducted according to standardized microbiological laboratory practices as outlined in the Standard Operating Procedures of Hill Top Research Corporation.

6.0 PROTOCOL

The study protocol and Protocol Amendment #1 as described in Appendix III were followed.

7.0 STUDY SCHEDULE

Date Study Initiated: October 9, 2008
Date Study Completed: November 26, 2008

8.0 TEST ARTICLES

The test article identified as UltraBlu™ Toothbrush was received from TechLight Systems, Inc. on September 23, 2008, for use in this test.

An additional test article, identified as UltraBlu™ Toothbrush (engineering prototype) was received from TechLight Systems, Inc. on October 29, 2008 for use in this test as directed in Protocol Amendment #1.

The test article received October 29, 2008 will be returned to the sponsor upon completion of testing.

9.0 PROTOCOL

The study protocol and Protocol Amendment #1 were followed.

10.0 RESULTS

The Summary Table and Tables of Results are presented in Appendix I for the 2 minute exposure period and the Summary Table and Table of Results-Protocol Amendment #1 (10, 2 minute exposure periods) is presented in Appendix II.

11.0 CONCLUSION

Conclusions, as a result of testing, are as follows:

- The test article, identified as UltraBlu™ Toothbrush, was tested in triplicate against the test organisms contained in whole saliva collected from healthy human volunteers (unrestricted), *Candida albicans*, ATCC 18804, *Fusobacterium nucleatum*, ATCC 10953 and *Porphyromonas gingivalis*, ATCC 33277 with a 2 minute exposure to the UltraBlu™ Toothbrush "blue light."

11.0 CONCLUSION CON'T.

- After an exposure period of two minutes, no reduction in numbers of *F. nucleatum* or organisms contained in human saliva was seen. Under the same test conditions, the test organism *C. albicans* showed a 7% reduction in yeast organisms and the test organism *P. gingivalis* showed a 17% reduction in the number of bacteria.

- An additional test article, identified as UltraBlu™ Toothbrush (engineering prototype) was received from TechLight Systems, Inc. on October 29, 2008 for use in this test as directed in Protocol Amendment #1. This test exposed the test organism *P. gingivalis* to ten, 2 minute exposures over a 29 minute period (2 minutes on, 1 minute off; 10 times).

- When compared to an untreated control, the test article, UltraBlu™ Toothbrush (engineering prototype) showed a 99.39% reduction (2.22 $\log_{10}$) in numbers of the test organism *P. gingivalis* after 10 - 2 minute exposures over 29 minutes.

12.0 SIGNATURE

HILL TOP RESEARCH CORPORATION

Kathleen A. Baxter 11/26/08

Kathleen A. Baxter, B.S. Date
Study Director
Microbiology Business Unit

APPENDIX I

HILL TOP RESEARCH CORPORATION

APPENDIX I

SUMMARY TABLE AND TABLES OF RESULTS

SUMMARY TABLE OF RESULTS
LOG_{10} REDUCTION with UltraBlu™ Toothbrush after Two Minutes Exposure

Test Organism		Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Candida albicans, ATCC 18804	Numbers Control at 2 minutes	1.3×10^6	6.11	NA	NA
	Treatment at Exposure Time of 2 minutes	1.2×10^6	6.08	7.69	0.03
Fusobacterium nucleatum, ATCC 10953	Numbers Control at 2 minutes	4.4×10^5	5.64	NA	NA
	Treatment at Exposure Time of 2 minutes	4.7×10^5	5.67	NR	NR
Porphyromonas gingivalis, ATCC 33277	Numbers Control at 2 minutes	2.9×10^5	5.46	NA	NA
	Treatment at Exposure Time of 2 minutes	2.4×10^5	5.38	17.24	0.08
Saliva	Numbers Control at 2 minutes	2.1×10^7	7.32	NA	NA
	Treatment at Exposure Time of 2 minutes	2.2×10^7	7.41	NR	NR

NA = Not Applicable
NR = No Reduction

TABLE I OF RESULTS
REDUCTION IN NUMBERS OF *Candida albicans*, ATCC 18804
With UltraBlu™ Toothbrush

Test Period: October 13 – 15, 2008
Test Organism: *C. albicans*, ATCC 18804
Incubation: 48 ± 4 hours at 25 ± 2°C
Exposure Period: 2 minutes at 37 ± 2°C

	Replicate	Bottle*			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Count (CFU/mL)	Count (CFU/surface)	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Numbers Control at 2 minutes	A	TNTC[a]	TNTC	TNTC	TNTC	TNTC	37[b]	8	0	3.1×10^4	6.2×10^5	1.3×10^6	6.11	NA[c]	NA
		TNTC	TNTC	TNTC	TNTC	TNTC	25	4	0						
	B	TNTC	TNTC	TNTC	TNTC	TNTC	52	8	1	7.4×10^4	1.5×10^6				
		TNTC	TNTC	TNTC	TNTC	TNTC	95	12	0						
	C	TNTC	TNTC	TNTC	TNTC	TNTC	81	8	0	8.8×10^4	1.8×10^6				
		TNTC	TNTC	TNTC	TNTC	TNTC	94	13	1						
Treatment at Exposure Time of 2 minutes	A	TNTC	TNTC	TNTC	TNTC	TNTC	59	11	0	7.0×10^4	1.4×10^6	1.2×10^6	6.08	7.69	0.03
		TNTC	TNTC	TNTC	TNTC	TNTC	82	7	0						
	B	TNTC	TNTC	TNTC	TNTC	TNTC	64	4	0	7.7×10^4	1.5×10^6				
		TNTC	TNTC	TNTC	TNTC	TNTC	90	7	0						
	C	TNTC	TNTC	TNTC	TNTC	TNTC	30	3	0	3.6×10^4	7.2×10^5				
		TNTC	TNTC	TNTC	TNTC	TNTC	41	3	0						

Inoculum Count

10^{-5}		10^{-6}		10^{-7}		Count (CFU/mL)	Theoretical CFU/surface (CFU/mL X 30 µl X 0.001)
TNTC	TNTC	57	54	4	1	5.6×10^7	1.7×10^6

*1.0 mL plated across 3 plates in duplicate
[a] TNTC = Too Numerous To Count
[b] Underlined values used in calculations
[c] NA = Not Applicable
[d] LA = Lab Accident; gross plate contamination

TABLE II OF RESULTS
REDUCTION IN NUMBERS OF *Fusobacterium nucleatum*, ATCC 10953
With UltraBlu™ Toothbrush

Test Period: October 13 – 15, 2008
Test Organism: *F. nucleatum*, ATCC 10953
Incubation: 48 ± 4 hours at 25 ± 2°C
Exposure Period: 2 minutes at 37 ± 2°C

	Replicate	Bottle*			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Count (CFU/mL)	Count (CFU/surface)	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Numbers Control at 2 minutes	A	TNTC[a]	TNTC	TNTC	202[b]	19	0	0	0	1.8×10^3	3.6×10^4	4.4×10^5	5.64	NA[c]	NA
		TNTC	TNTC	TNTC	151	19	0	0	0						
	B	TNTC	TNTC	TNTC	TNTC	TNTC	49	2	0	4.4×10^4	8.8×10^5				
		TNTC	TNTC	TNTC	TNTC	TNTC	38	3	1						
	C	TNTC	TNTC	TNTC	TNTC	235	22	2	0	2.1×10^4	4.2×10^5				
		TNTC	TNTC	TNTC	TNTC	194	20	0	1						
Treatment at Exposure Time of 2 minutes	A	TNTC	TNTC	TNTC	TNTC	TNTC	33	5	0	2.9×10^4	5.8×10^5	4.7×10^5	5.67	NR[d]	NR
		TNTC	TNTC	TNTC	TNTC	TNTC	25	2	0						
	B	TNTC	TNTC	TNTC	TNTC	292	23	1	0	3.0×10^4	6.0×10^5				
		TNTC	TNTC	TNTC	TNTC	318	38	6	0						
	C	TNTC	TNTC	TNTC	TNTC	126	12	0	0	1.2×10^4	2.4×10^5				
		TNTC	TNTC	TNTC	TNTC	124	6	2	0						

Inoculum Count

10^{-5}		10^{-6}		10^{-7}		Count (CFU/mL)	Theoretical CFU/surface (CFU/mL X 30 µl X 0.001)
TNTC	TNTC	27	34	1	3	3.0×10^7	9.0×10^5

*1.0 mL plated across 3 plates in duplicate
[a] TNTC = Too Numerous To Count
[b] Underlined values used in calculations
[c] NA = Not Applicable
[d] NR = No Reduction

TABLE III OF RESULTS
REDUCTION IN NUMBERS OF *Porphyromonas gingivalis*, ATCC 33277
With UltraBlu™ Toothbrush

Test Period: October 13 – 15, 2008
Test Organism: *P. gingivalis*, ATCC 33277
Incubation: 48 ± 4 hours at 25 ± 2°C
Exposure Period: 2 minutes at 37 ± 2°C

	Replicate	Bottle*			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Count (CFU/mL)	Count (CFU/surface)	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Numbers Control at 2 minutes	A	TNTC[a]	TNTC	TNTC	TNTC	98[b]	6	0	0	9.7×10^3	1.9×10^5	2.9×10^5	5.46	NA[c]	NA
		TNTC	TNTC	TNTC	TNTC	96	7	1	0						
	B	TNTC	TNTC	TNTC	TNTC	166	26	1	0	1.8×10^4	3.6×10^5				
		TNTC	TNTC	TNTC	TNTC	152	13	0	0						
	C	TNTC	TNTC	TNTC	TNTC	154	11	3	0	1.6×10^4	3.2×10^5				
		TNTC	TNTC	TNTC	TNTC	160	18	3	0						
Treatment at Exposure Time of 2 minutes	A	TNTC	TNTC	TNTC	TNTC	96	LA[d]	0	0	1.0×10^4	2.0×10^5	2.4×10^5	5.38	17.24	0.08
		TNTC	TNTC	TNTC	TNTC	115	11	0	0						
	B	TNTC	TNTC	TNTC	TNTC	164	10	2	0	1.7×10^4	3.4×10^5				
		TNTC	TNTC	TNTC	TNTC	176	15	1	0						
	C	TNTC	TNTC	TNTC	TNTC	96	8	1	0	8.6×10^3	1.7×10^5				
		TNTC	TNTC	TNTC	TNTC	76	14	0	0						

Inoculum Count

10^{-4}		10^{-5}		10^{-6}		Count (CFU/mL)	Theoretical CFU/surface (CFU/mL X 30 µl X 0.001)
TNTC	TNTC	143	96	14	6	1.2×10^7	3.6×10^5

*1.0 mL plated across 3 plates in duplicate
[a] TNTC = Too Numerous To Count
[b] Underlined values used in calculations
[c] NA = Not Applicable

TABLE IV OF RESULTS
REDUCTION IN NUMBERS OF Saliva (unrestricted)
With UltraBlu™ Toothbrush

Test Period: October 13 – 15, 2008
Test Organism: Saliva (unrestricted)
Incubation: 48 ± 4 hours at 25 ± 2°C
Exposure Period: 2 minutes at 37 ± 2°C

	Replicate	Bottle*			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Count (CFU/mL)	Count (CFU/surface)	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Numbers Control at 2 minutes	A	TNTC[a]	TNTC	TNTC	TNTC	TNTC	TNTC	67[b]	6	6.8×10^5	1.4×10^7	2.1×10^7	7.32	NA[d]	NA
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	70	12						
	B	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	70	8	7.8×10^5	1.6×10^7				
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	86	LA[c]						
	C	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	119	3	1.6×10^6	3.2×10^7				
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	207	5						
Treatment at Exposure Time of 2 minutes	A	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	117	9	9.0×10^5	1.8×10^7	2.6×10^7	7.41	NR[e]	NR
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	62	6						
	B	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	155	35	1.9×10^6	3.8×10^7				
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	218	4						
	C	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	149	2	1.1×10^6	2.2×10^7				
		TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	77	3						

Inoculum Count

10^{-5}		10^{-6}		10^{-7}		Count (CFU/mL)	Theoretical CFU/surface (CFU/mL X 20 µl X 0.001)
TNTC	TNTC	TNTC	TNTC	80	116	9.8×10^8	2.0×10^7

*1.0 mL plated across 3 plates in duplicate
[a] TNTC = Too Numerous To Count
[b] Underlined values used in calculations
[c] NA = Not Applicable
[d] LA = Lab Accident; gross plate contamination
[e] NR = No Reduction

APPENDIX II

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APPENDIX II

SUMMARY TABLE AND TABLE OF RESULTS
Protocol Amendment #1

SUMMARY TABLE OF RESULTS
LOG_{10} REDUCTION With UltraBlu™ Toothbrush (engineering prototype)

Test Organism	Exposure Period	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Porphyromonas gingivalis, ATCC 33277	Numbers Control at 29 minute exposure period	1.8×10^5	5.26	NA	NA
	Treatment at Exposure Time of 10 - 2 minute exposures over 29 minutes (2 min. on; 1 min. off; repeat cycles)	1.1×10^3	3.04	99.39	2.22

NA = Not Applicable
NR = No Reduction

TABLE I OF RESULTS
REDUCTION IN NUMBERS OF *Porphyromonas gingivalis*, ATCC 33277
With UltraBlu™ Toothbrush

Test Period: October 31 – November 3, 2008 **Incubation:** 48 ± 4 hours at 25 ± 2°C
Test Organism: *P. gingivalis*, ATCC 33277 **Exposure Period:** 10, 2 minute exposures at 37 ± 2°C

	Replicate	Bottle*			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Count (CFU/mL)	Count (CFU/surface)	Average Count (CFU/surface)	Average Log_{10} CFU/surface	Percent Reduction	Log_{10} Reduction
Numbers Control at 10 - 2 minute exposures over 29 minutes	A	TNTC[a]	TNTC	TNTC	TNTC	151[b]	14	0	0	1.5×10^4	3.0×10^5	1.8×10^5	5.26	NA[c]	NA
		TNTC	TNTC	TNTC	TNTC	150	19	2	0						
	B	TNTC	TNTC	TNTC	TNTC	119	12	1	0	1.1×10^4	2.2×10^5				
		TNTC	TNTC	TNTC	TNTC	105	3	1	0						
	C	TNTC	TNTC	TNTC	162	11	6	0	0	1.4×10^3	2.8×10^4				
		TNTC	TNTC	TNTC	109	11	1	0	0						
Treatment at Exposure Time of 10 - 2 minute exposures over 29 minutes	A	12	11	14	55	2	1	0	0	3.6×10^1	7.2×10^2	1.1×10^3	3.04	99.39	2.22
		13	13	9	3	1	2	0	0						
	B	32	24	25	10	2	0	0	0	8.6×10^1	1.7×10^3				
		26	30	34	9	0	0	0	0						
	C	15	18	24	8	2	0	0	0	5.2×10^1	1.0×10^3				
		16	16	15	5	1	0	0	0						

Inoculum Count

10^{-5}		10^{-6}		10^{-7}		Count (CFU/mL)	Theoretical CFU/surface (CFU/mL X 30 µl X 0.001)
190	175	22	11	2	2	1.8×10^7	1.8×10^5

*1.0 mL plated across 3 plates in duplicate
[a] TNTC = Too Numerous To Count
[b] Underlined values used in calculations
[c] NA = Not Applicable

APPENDIX III

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APPENDIX III

COPY OF PROTOCOL

Hill Top RESEARCH

Hill Top Research Confidential

PROTOCOL FOR

ASSESSMENT OF RAPID GERMICIDAL (TIME KILL) ACTIVITY FOR UltraBlu™ TOOTHBRUSH

For:
TechLight Systems, Inc.

HTR Ref.: 08-129295-106

HTR Ref.: 08-129295-106; *Hill Top Research Confidential*
Germicidal (Time Kill)
Activity Protocol

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HTR Ref.: 08-129295-106; *Hill Top Research Confidential*
Germicidal (Time Kill)
Activity Protocol

1.0 INTRODUCTION

The UltraBlu™ Toothbrush was developed with blue light technology that has been proposed to be of benefit to people with gum disease. The toothbrush was selected as the vehicle for incorporation of the blue light since it is used daily by everyone and would therefore be most beneficial to the greatest number of people. Since the growth of microorganisms in the mouth has been associated with gum disease, the effect of the blue light on microorganisms occurring in the mouth is being investigated in an *in vitro* test procedure.

2.0 PURPOSE

To evaluate the antibacterial effect of the UltraBlu™ Toothbrush "blue light" in reducing bacteria found in human saliva and other selected microorganisms known to be present in the mouth when artificially deposited on a glass surface and exposed to the "blue light" under simulated use conditions without brushing.

3.0 STUDY SPONSOR AND SPONSOR REPRESENTATIVES

TechLight Systems, Inc.
P.O. Box 190
Dunnellon, FL 34430

REPRESENTATIVE: Michael Barnes
Telephone No.: (352) 465-4101
Fax No.: (352) 465-8299

4.0 TEST FACILITY AND INVESTIGATIVE PERSONNEL

Hill Top Research Corporation
6088 Main and Mill Streets
Miamiville, Ohio 45147
Telephone No: (513) 831-3114
Fax No.: (513) 831-1217

Study Director: Kathleen A. Baxter, B.S.
Study Manager: Jane M. Young, B.S.
Study Coordinator: Margaret K. Haines, B.S.

5.0 RESEARCH STANDARDS

This study will be run according to the good laboratory practices and Standard Operating Procedures of Hill Top Research Corporation.

6.0 EXPERIMENTAL DESIGN

The test article is brought into contact with the glass surface previously inoculated with pooled human saliva or other microorganisms for a specified period of time. At the end of a specified exposure period, the microorganisms are eluted from the glass surface and are then plated to enumerate the survivors. The percent reduction and the $\log_{10}$ reduction from the original population are calculated.

7.0 PROPOSED EXPERIMENTAL STARTING AND COMPLETION DATES

Proposed Experimental Starting Date:	October 13, 2008
Proposed Experimental Termination Date:	October 20, 2008
Proposed Completion Date:	November 3, 2008

8.0 TEST ARTICLE IDENTIFICATION

The test article, received on September 23, 2008 and identified by the sponsor as UltraBlu™ Toothbrush will be used for testing. The test article will be assigned a Hill Top Research code for generation of the test data.

9.0 TEST ARTICLE CHARACTERIZATION

Not applicable.

10.0 TEST SYSTEM JUSTIFICATION

The test system has been used historically for this type of study.

11.0 TEST SYSTEM IDENTIFICATION

The test organisms to be used in this study will be those contained in whole saliva collected from healthy human volunteers (unrestricted) and those identified in Appendix II. The saliva will be collected from a

number of individuals, pooled, and maintained at 4-8°C prior to use. The test cultures will be propagated and recovered as outlined in Appendix II.

12.0 TEST PROCEDURE

12.1 A 10-30 µL aliquot of the test culture or human saliva will be inoculated on the interior surface (1" X 1") of a sterile milk dilution bottle and allowed to dry for 30 minutes at ~35°C. The expected numbers of organisms on the surface of the bottle will be ~1 X 10^6 CFU/1" X 1" surface area. The exterior of the bottle will be covered with foil to simulate the mouth (darkness). The brush head will be removed from the toothbrush and the brush will then be inserted into the opening of the bottle so that the "blue light" aligns with the inoculated surface. The brush will then be turned on to activate the "blue light" for an exposure period of 2 minutes. The brush will then be removed from the bottle, 20 mL of PBS and glass beads added to the bottle, and the bottle will be agitated for 30 seconds to remove the surviving organisms. [The brush head will be sanitized with an alcohol wipe between tests to limit any cross-contamination between tests.] The untreated control will have the same exposure time as the "blue light" brush head. After elution with the 20 mL of PBS, all samples will be serially diluted in PBS (9.0 mL) and plated in duplicate by the Spread Plate Technique according to the scheme outlined in Appendix II. Testing will be conducted in triplicate.

12.2 Plates and diluent used for anaerobic incubation will be pre-reduced in an anaerobic chamber for at least 4 hours prior to use. [Note: Testing with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* will be conducted in an anaerobic environment.] Following incubation, colony forming units (CFU) per surface (1' X 1") will be counted and numbers of surviving organisms will be calculated.

12.3 Both the percent reduction in numbers and the $\log_{10}$ reductions will be reported for both the human saliva and the test organisms for the "blue light" treatment. A percent reduction, as compared to the numbers control, will be determined against the average result of the three replicates for the exposure period. It will be determined as illustrated below.

$$\% \text{ Reduction} = \frac{[\text{NC(CFU/surface)} - \text{TA(CFU/surface)}]}{\text{NC(CFU/surface)}} \times 100$$

NC = Average Numbers Control population
TA = Average Test Article population

13.0 STATISTICAL METHOD

Not applicable.

14.0 REPORT

The report will include (but not be limited to) identification of the test organism, test procedure, protocol modification (if any), identification of the test material, solvent (if any), test concentration, subculture media, results, statistical analysis, and summary.

15.0 DATA RETENTION

The final report will be sent to the sponsor following completion of the study. All records that would be required to reconstruct the study and demonstrate adherence to the Protocol will be maintained. The raw data and the original of the final report will be on file at the testing facility for a period of not less than two years. Permanent records will be in the form of microfilm or other readily retrievable forms.

Upon completion of testing, the test articles will be retained for a period of thirty (30) days, and then destroyed. The test articles will be returned to the sponsor only if the sponsor so requests and agrees to pay the cost of shipping.

16.0 NOTICE

If it becomes necessary to make changes in the approved protocol, the revisions and reasons for change will be documented, reported to the sponsor and will become part of the permanent file for that study.

Similarly, the sponsor will be notified as soon as is practical whenever an event occurs that is unexpected and may have an effect on the validity of the study.

17.0 **PROTOCOL APPROVAL FORM**

HILL TOP RESEARCH

PROTOCOL TITLE	REFERENCE CODE
Assessment of Rapid Germicidal (Time Kill) Activity	DISF\PRO\TK\TechLight

PROTOCOL APPROVED FOR: HILL TOP RESEARCH CORPORATION

(11) See page 12

BY: _____ _____
Kathleen A. Baxter, B.S. Date
Study Director
Microbiological Services Division

Protocol Approved By (Sponsor):

_____ 10/9/08 _____
Signed Date

_____ _____
Signed Date

PO Box 190, Dunnellon, FL 34430
Address

Draft Protocol approved 10/9/08. received us mail 1c
JS 10/20/(

17.0 **PROTOCOL APPROVAL FORM**

HILL TOP RESEARCH

PROTOCOL TITLE
Assessment of Rapid Germicidal (Time Kill) Activity

REFERENCE CODE
DISF\PRO\TK\TechLight

PROTOCOL APPROVED FOR: HILL TOP RESEARCH CORPORATION

BY: Kathleen A. Baxter 10-9-08
Kathleen A. Baxter, B.S. Date
Study Director
Microbiological Services Division

Protocol Approved By (Sponsor):

Signed 10/16/08
Date

Signed Date

PO Box 190, Dunnellon, FL 34430
Address

HTR Ref.: 08-129295-106; *Hill Top Research Confidential*
Germicidal (Time Kill)
Activity Protocol

Appendix I
Materials and Reagents

Appendix I

1.0 MATERIALS AND REAGENTS

1.1 Phosphate Buffered Saline (PBS); 20 mL/tube; 9.0 ml/tube (HTR I-142)
1.2 Schaedler Anaerobic Blood Agar (HTR I-164); 22-25 mL/dish
1.3 Schaedler Anaerobic Broth (Difco or equivalent) with additives; 10 mL/tube
1.3 OOP's III Agar (HTR I-161); 22-25 mL/dish
1.4 Sabouraud Dextrose Agar (HTR I-086); 22-25 mL/dish
1.5 Sabouraud Dextrose Broth (HTR I-083); 10 mL/tube
1.5 Sterile, wide-mouth milk dilution bottles, Corning #1368
1.6 Sterile Petri dishes
1.7 Sterile Pipets, 1.0 mL
1.8 ColiRollers™ Plating Beads (Novagen)
1.8 Incubators, 33-38°C; anaerobic, 35±2°C, 25±2°C; aerobic
1.9 Laminar flow hood
1.10 Sterile 10µL inoculating loops
1.11 Miscellaneous laboratory equipment

2.0 REFERENCES

2.1 Official Methods of Analysis of AOAC International, 18th Edition, Revision 1, 2006, Chapter 6, Section 6.3.03 A.
2.2 Hill Top Research Media Standard Operating Procedures Manual
2.3 Hill Top Research Standard Operating Procedures Manual

HTR Ref.: 08-129295-106; *Hill Top Research Confidential*
Germicidal (Time Kill)
Activity Protocol

Appendix II
Test Organisms

Test Organisms

Test Organism	ATCC #	Incubation for Growth		Growth Medium	Incubation for Recovery		Recovery Medium
		Time (± 4hrs)	Temp (± 2°C)		Time (± 4hrs)	Temp (± 2°C)	
Candida albicans	18804	72	25***	SDB	72	25***	SDA
Fusobacterium nucleatum	10953	48-72	33-38**	MSB	48-72	33-38**	MSA
Porphyromonas gingivalis	33277	48-72	33-38**	MSB	48-72	33-38**	MSA
Human Saliva	NA	NA	NA	NA	5 days	33-38**	OOP's III

** Anaerobic ***Aerobic NA = Not applicable

MSB/MSA = Modified Schaedler Broth/Agar
SDB/SDA = Sabouraud Dextrose Broth/Agar
OOP's III = B.F. Turng, G. E. Minah and W.A Falkler. Development of an Agar Medium for Detection of Oral H_2S Producing Organisms. J. Dent. Res. 76: 226, 1997

Hill Top

HILL TOP RESEARCH
6088 MAIN AND MILL STREETS
MIAMIVILLE, OHIO 45147

PROTOCOL AMENDMENT #1	ASSESSMENT OF RAPID GERMICIDAL (TIME KILL) ACTIVITY FOR UltraBlu™ TOOTHBRUSH
HTR STUDY NO.:	09-129295-106
SPONSOR & ADDRESS:	TechLight Systems, Inc. P.O. Box 190 Dunnellon, FL 34430
SPONSOR'S REPRESENTATIVES:	Michael Barnes

PROTOCOL MODIFICATIONS:

The following sections of the protocol will be amended to read:

Section 8.0 **TEST ARTICLE IDENTIFICATION**

An additional test article, received on October 29, 2008 and identified by the sponsor as UltraBlu™ Toothbrush (engineering prototype) will be used for testing. The test article will be assigned a Hill Top Research code for generation of the test data.

Section 11.0 **TEST SYSTEM IDENTIFICATION**

The test organism to be used for testing, with the engineering prototype brush received on October 29, will only be *Porphyromonas gingivalis* as outlined in Appendix II.

Section 12.0 **TEST PROCEDURE**

12.1 A 10 µL aliquot of the test culture will be inoculated on the interior surface (~5 mm X 5 mm) of a sterile milk dilution bottle and allowed to dry for 30 minutes at ~35°C. The expected numbers of organisms on the surface of the bottle will be ~1 X 10^5 CFU/5 mm X 5 mm surface area. The exterior of the bottle will be covered with foil to simulate the mouth (darkness). The brush (no bristles) will then be inserted into the opening of the bottle so that the "blue light" aligns with the inoculated surface. The brush will then be turned on to activate the "blue light" for 10, 2 minute exposure periods over 29 minutes of time (2 minutes on, 1 minute off, etc) for a total of 20 minutes of exposure to the "blue light." The brush will then be removed from the bottle, 20 mL of PBS and glass beads added to the bottle, and the bottle will be agitated for 30 seconds to remove the surviving organisms. [The brush head will be sanitized with an alcohol wipe and allowed to air dry between tests to limit any cross-contamination between tests.] The

untreated control will have the same exposure time as the "blue light" brush head. After elution with the 20 mL of PBS, all samples will be serially diluted in PBS (9.0 mL) and plated in duplicate by the Spread Plate Technique according to the scheme outlined in Appendix II. Testing will be conducted in triplicate.

APPROVED FOR: HILL TOP RESEARCH CORPORATION

BY: Kathleen A. Baxter 10/29/08
Kathleen A. Baxter, B.S. Date
Study Director

ACCEPTED BY: TechLight Systems, Inc.

BY: 10-31-08
Michael Barnes Date

RECORD RETENTION AND PUBLICATION NOTICE

Hill Top Research Corporation submits this report with the understanding that the Sponsor may use the Study report for its own purposes. The Sponsor agrees, however, not to use the name Hill Top Research Corporation, or any derivation thereof, in advertising or marketing without the prior written consent of Hill Top.

All study documents and a copy of the final report will be on file in the Hill Top Research Records Center at Main and Mill Streets, Miamiville, Ohio 45147, for a period of not less than two years, unless indicated otherwise on the study protocol or financial contract. A permanent record will be retained in the form of microfilm or in another readily retrievable form.