CONFIDENTIAL	Report # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

An Evaluation of the Antimicrobial Efficacy of HEV Light

Final Report

January 27, 2021

Proposal Number

QL20343-2A

Study Director

Benjamin J. Bastin

Study Sponsor

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Testing Facility

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Cincinnati, OH 45204

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Approval Page

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Table of Contents

List of Tables

List of Attached Documents

1.0 PURPOSE

1.1 The evaluation of the microbicidal properties was analyzed by measuring the changes of a population of aerobic microorganisms within 4 exposure times and 3 distances when tested against antimicrobial test device provided by study sponsor.

2.0 SCOPE

2.1 The design of this evaluation was based on the guidance provided in the on ASTM Committee E1153-03 "*Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces*" [Section 10.1] and E3515-18 "*Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil*." [Section 10.2]

3.0 SUMMARY

3.1 **Sponsor:** Buddy Technologies USA 300 Lenora Street, #1591, Seattle, WA 98121

3.2 Test Device:

- 3.2.1 The test devices evaluated were provided to the testing facility by the study sponsor, complete with appropriate documentation.
- 3.2.2 Test Device: Buddy Technologies HEV light
- 3.2.3 The study parameters are presented in Table 1.

Table 1: Efficacy Study Parameters

Test Device	Organisms	Distance (cm)	Exposure Times (hours)	Test Replicates	Negative Controls	Positive Controls
Buddy Technologies HEV light	*Escherichia coli* ATCC 11229	40	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		80	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		121.92	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
	Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	40	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		80	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		121.92	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1

4.0 EQUIPMENT/APPARATUS

4.1 Incubator, temperature range 35 ± 1 °C
4.2 Incubator thermometer, NIST traceable
4.3 Steam autoclave
4.4 Vortex mixer
4.5 Calibrated, traceable minute/second timer
4.6 Refrigerator 2 - 8 °C
4.7 Refrigerator thermometer, NIST traceable
4.8 Traceable thermometer/clock/humidity monitor
4.9 Adjustable pipettor, 10 - 100 µL, 20 - 200 µL, and 100 µL - 1000 µL capacity
4.10 Reichert Quebec® colony counter
4.11 Hand tally
4.12 Centrifuge, capable of up to 4,500 rpm
4.13 Ultralow freezer, capable of maintaining - 70 °C
4.14 TorchStar Desk Lamp (used to fix the HEV bulb)
4.15 Smartphone

5.0 MATERIALS

5.1 Micropipette tips, 10 - 100 µL, 20 - 200 µL, and 100 - 1000 µL
5.2 Serological pipettes, 1, 2, 5, 10, and 25 mL
5.3 Test tubes
5.4 Disposable Petri dishes, 100 x 15 mm
5.5 Flasks or Containers
  5.5.1 Appropriate sizes with closures for preparation of culture medium and sterile deionized water.
  5.5.2 Volumetric, 100 and 1000 mL
5.6 Disposable loops
5.7 50 mL conical vials
5.8 Glass slide carriers
Note: As appropriate, materials were purchased sterile or sterilized via autoclaving.

6.0 MEDIA/CHEMICALS/REAGENTS

6.1 Tryptic Soy Agar with 5% Sheep Blood (SBA) Commercially available from BD, PN 221261, or equivalent
6.2 Microbial Content Test (MCT) agar MP107*
6.3 Tryptic Soy Broth (TSB) MP058
6.4 Phosphate Buffered Saline (PBS) MP416

*Note: As appropriate, media/chemicals/reagents were purchased sterile or sterilized via autoclaving. *The MP number refers to Q Laboratories media preparation number.*

7.0 METHOD DESCRIPTION

7.1 Test Microorganism Preparation:

7.1.1 Seed-lot culture maintenance techniques were used so that the viable microorganisms used for inoculation were not more than five passages removed from the original master seed lot.

7.1.2 The Test Microorganism cultures were prepared as follows:

7.1.2.1 Cultures were propagated on SBA for 18-24 hours at 35 ± 1 ˚C from a Q Laboratories frozen stock culture stored at -70 ˚C.

7.1.2.2 Cultures were transferred using TSB.

7.1.2.3 Each daily transfer was incubated at the appropriate temperature for growth for 24 ± 2 hours.

7.1.2.4 Daily transfers were performed for each culture by aseptically transferring growth.

7.1.2.5 The test culture was centrifuged at 4,500 rpm for 15 minutes.

7.1.2.6 The supernatant was removed and the pellet was rehydrated with PBS.

7.1.2.7 Each test culture was adjusted to an approximate concentration of 10^6.

7.2 Preparation of Carriers:

7.2.1 Prior to conducting the analysis, glass slide carriers were sterilized via autoclave.

7.2.2 With gloved hands, the glass slide carriers were placed on a disinfected flat surface. A 25 µL aliquot of each test culture was applied to the carrier. The inoculum was immediately spread to contact uniformly using the micropipette tip. The inoculum did not contact the edge of the glass slide carriers.

7.2.3 After inoculation, carriers were allowed to dry for 30 to 60 minutes at room temperature (20-25 °C). Carriers were visually inspected to ensure the culture was uniformly dried before initiating the test procedure.

7.3 Test Procedure:

7.3.1 The HEV light device was placed at the appropriate distance perpendicular from the carrier. Refer to Table 1 for the distance the carrier was placed from the device. Figure 1 below is an image of the HEV light exposure to the carriers. Care was taken not to contact the HEV bulb.

Figure 1: Buddy Technologies HEV light during exposure to the Carriers.

7.3.2 The device was turned on and the group was expanded that the device was saved in.

7.4 The group was expanded so that you can see the individual bulb.

7.5 The bulb was turned OFF and placed into Clean Mode.
7.6 The brightness setting was at 100%.
7.7 The bulb was a blue-ish/violet hue.
7.8 The device was turned toward the carrier.
7.9 The device was held appropriate contact time. See Table 1.
  7.9.1 Note that the acceptable deviation of the contact time was 5 minutes.
7.10 During the contact time, a photo was taken of the test device's activity on the carriers.
7.11 Recovery and Analysis:
  7.11.1 The carrier was added to 20.0 mL of PBS and thoroughly vortexed.
  7.11.2 Ten-fold serial dilutions were prepared of the sample extract by transferring 1.0 mL from the initial dilution into 9.0 mL of PBS.
  7.11.3 Each dilution was plated in duplicate into sterile Petri dishes and 12-15 mL of tempered MCT was added. After thoroughly mixing, the plates were allowed to solidify, inverted and incubated at 35 ± 1 °C for 48 ± 2 h.
  7.11.4 After incubation, typical colonies were enumerated, raw data was recorded as CFU/plate, and photos were taken of the control and test carrier plates. Figures 2 and 3 are the photos of the control and test carrier plates. Duplicate plates were averaged and multiplied by the dilution factor to arrive at CFU/test device. Raw values were recorded and used for the calculations in Section 11.0.

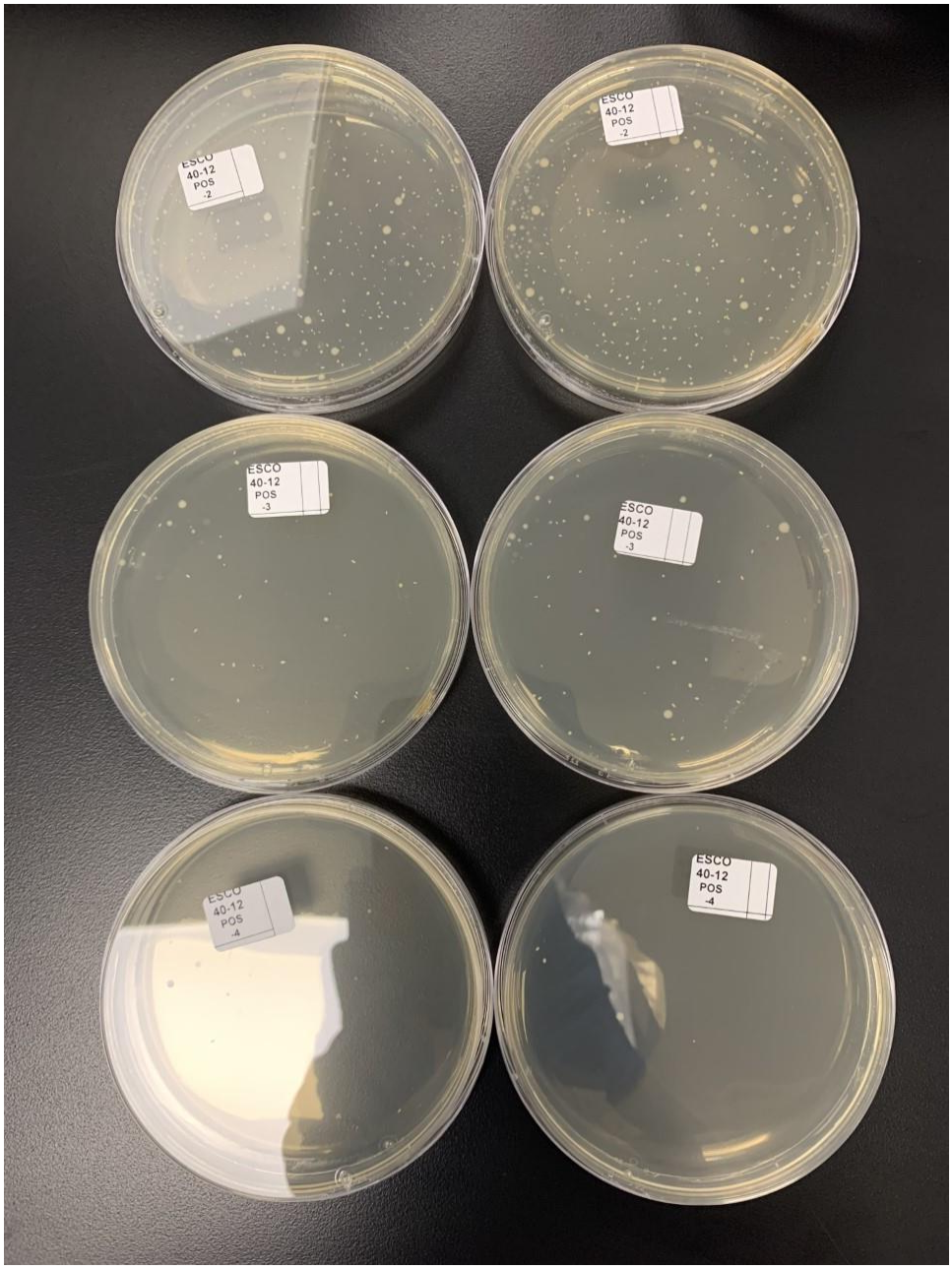

Figure 2: Buddy Technologies HEV light Control Carriers Plates.

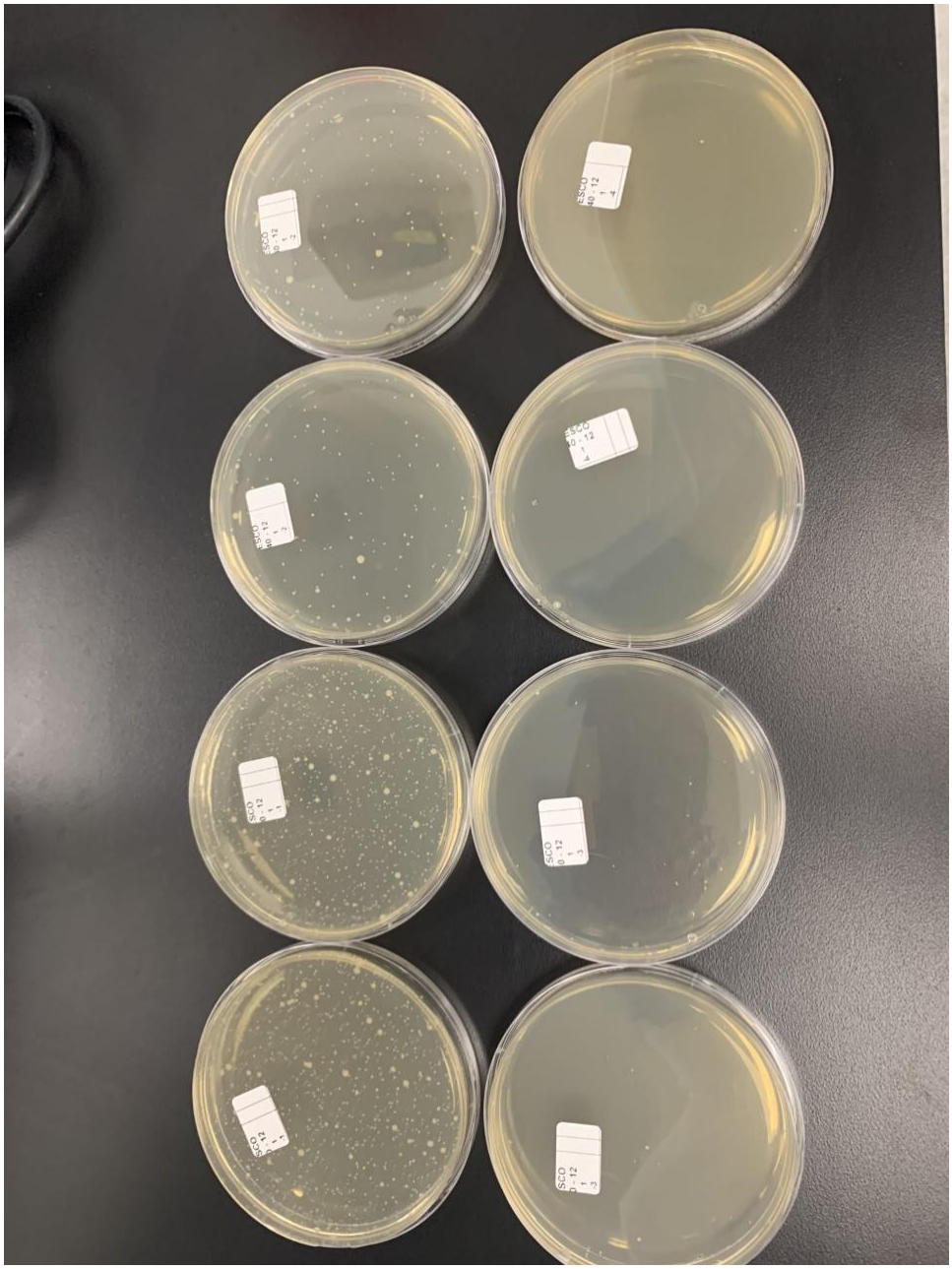

Figure 3: Buddy Technologies HEV light Test Carriers Plates.

7.12 Study Controls:

7.12.1 Negative Control

7.12.1.1 One uninoculated carrier was evaluated for total viable organisms at each contact time following procedures outlined in Sections 7.4 - 7.12.

7.12.2 Positive Control

7.12.2.1 One inoculated carrier was evaluated to determine the total viable organisms remaining on the carrier at each contact time. This control served as the basis for determining the percent and Log reduction for the test device replicates. The positive carrier was inoculated according to the procedure outlined in Section 7.3. The positive carrier was not exposed to the light of the test device. The remaining microorganisms were determined using the procedure in Section 7.12.

7.13 Test Culture Viability:

7.13.1 The inoculum was enumerated to verify the viability at the start of the testing phase. Inoculum populations were determined by preparing ten-fold serial dilutions of each challenge organism suspension in duplicate by standard microbiological procedures and incubating at 35 ± 1 °C for 48 ± 2 h.

7.13.2 Colonies were enumerated and recorded as CFU/plate. Duplicate plates were averaged and multiplied by the dilution factor to calculate the microbial population (CFU/mL) of the control suspension.

7.14 Media Quality Controls:

7.14.1 For MCT:

7.14.1.1 Duplicate plates were inoculated with 1 - 100 CFU of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 and incubated at 30-35 °C for 3 days or less.

7.14.1.2 Duplicate plates of an equivalent medium were inoculated with 1 - 100 CFU of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 and incubated at 30-35 °C for 3 days or less.

7.14.1.3 One (1) plate was incubated at 30-35 °C for 3 days or more that served as the sterility control.

7.14.1.4 Comparable growth acceptance was within 50 - 200 % between the media. Sterility acceptance was no growth.

7.14.2 Sterility assessment for PBS and TSB:

7.14.2.1 One (1) tube of media was incubated at 30-35 °C for 1 day or more and served as the sterility control.
7.14.2.2 After incubation, the tube was streaked to a general growth agar. The plate was incubated for 1 day or less at 30-35 °C.
7.14.2.3 The acceptance criterion was no growth from the sterility controls.

8.0 RECORDS TO BE MAINTAINED

8.1 All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between Q Laboratories and the sponsor will be stored in the archives at Q Laboratories, 1930 Radcliff Drive, Cincinnati, Ohio 45204, according to Q Laboratories SOP 20-ADMN-ISO-008, for a period of at least seven (7) years.

9.0 STUDY DATES AND FACILITIES

9.1 The analysis phase of this test was conducted at Q Laboratories in the Microbiology Research and Development Laboratory, 1930 Radcliff Drive, Cincinnati, Ohio 45204 on 12-25-2020 through 01-18-2021. The final report was released 01-26-2021.

10.0 REFERENCES

10.1 ASTM Committee E1153-03 "*Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces*"
10.2 ASTM Committee E3515-18 "*Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil*."

11.0 CALCULATIONS

11.1 A logarithmic transformation measuring surviving microbial populations of the positive control device and test replicates for each microorganism was performed.

11.1.1 The Log reduction was calculated as follows:

$$PC = Positive\ Control$$
$$TC = Test\ Carrier$$
$$LR = Log\ Reduction$$

$$\mathrm{LR} = Log_{10}PC - Log_{10}\mathrm{TC}$$

11.1.2 Percent reduction was calculated as follows:

$$PR = Percent\ Reduction$$

$$\mathrm{PR} = \left\{\frac{PC\ CFU - \mathrm{TC}\ CFU}{[PC\ CFU]}\right\} \times 100$$

Note: Calculated <2.0 x 10^1 as "20" to obtain the percent and Log reduction.

12.0 PERFORMANCE

12.1 In order to demonstrate disinfection against the culture suspension containing *E. coli* or *S. aureus* (MRSA), the Sponsor's disinfection procedure must have achieved a Log reduction of the test organisms as compared to the positive control.

13.0 ACCEPTANCE CRITERIA:

13.1 The study controls below must have performed according to the criteria detailed for the data to be considered acceptable.

13.1.1 All negative controls must be negative for growth.

13.1.2 Growth Promotion of media must meet specifications in Section 7.15.

14.0 SUMMARY OF RESULTS

14.1 The results for the inoculum viability are presented in Table 2.

14.2 The results for the percent and Log reduction efficiency for the 40 cm distance are presented in Tables 3-6.

14.3 The results for the percent and Log reduction efficiency for the 80 cm distance are presented in Tables 7-10.

14.4 The results for the percent and Log reduction efficiency for the 121.92 cm distance are presented in Tables 11-14.

15.0 RESULTS

Table 2: Results of the Inoculum Viability.

Distance (cm)	Challenge Organism	Initial Count (CFU/mL)	Log Initial Counts
40	*Escherichia coli* ATCC 11229	5.2E+06	6.7160
	Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	3.6E+06	6.5563
80	*Escherichia coli* ATCC 11229	2.1E+06	6.3222
	Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	4.4E+06	6.6435
121.92	*Escherichia coli* ATCC 11229	5.2E+06	6.7160
	Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	4.2E+06	6.6232

Table 3: Results at a 40 cm Distance and 2 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	3.1E+03	1.0E+02	2.0E+02	6.0E+02
	Log CFU/mL	3.4914	2.0000	2.3010	2.7782
	% reduction	N/A	96.77419%	93.54839%	80.64516%
	Log reduction	N/A	1.4914	1.1904	0.7132
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	9.4E+04	5.7E+04	8.3E+04	8.4E+04
	Log CFU/mL	4.9731	4.7559	4.9191	4.9243
	% reduction	N/A	39.36170%	11.70213%	10.63830%
	Log reduction	N/A	0.2172	0.0540	0.0488

Table 4: Results of 40 cm Distance and 4 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	2.5E+03	8.5E+01	1.1E+02	1.0E+02
	Log CFU/mL	3.3979	1.9294	2.0414	2.0000
	% reduction	N/A	96.60000%	95.60000%	96.00000%
	Log reduction	N/A	1.4685	1.3565	1.3979
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	9.7E+04	2.5E+04	3.1E+04	3.8E+04
	Log CFU/mL	4.9868	4.3979	4.4914	4.5798
	% reduction	N/A	74.22680%	68.04124%	60.82474%
	Log reduction	N/A	0.5889	0.4954	0.4070

Table 5: Results of 40 cm Distance and 8 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	2.2E+03	3.5E+01	2.0E+01	6.5E+01
	Log CFU/mL	3.3424	1.5441	1.3010	1.8129
	% reduction	N/A	98.40909%	99.09091%	97.04545%
	Log reduction	N/A	1.7983	2.0414	1.5295
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	3.4E+04	3.9E+03	5.0E+03	3.8E+03
	Log CFU/mL	4.5315	3.5911	3.6990	3.5798
	% reduction	N/A	88.52941%	85.29412%	88.82353%
	Log reduction	N/A	0.9404	0.8325	0.9517

Table 6: Results of 40 cm Distance and 12 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	2.9E+03	2.0E+01	1.0E+01	1.0E+01
	Log CFU/mL	3.4624	1.3010	1.0000	1.0000
	% reduction	N/A	99.31034%	99.65517%	99.65517%
	Log reduction	N/A	2.1614	2.4624	2.4624
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	1.8E+04	2.4E+02	2.6E+02	2.2E+02
	Log CFU/mL	4.2553	2.3802	2.4150	2.3424
	% reduction	N/A	98.66667%	98.55556%	98.77778%
	Log reduction	N/A	1.8751	1.8403	1.9129

Table 7: Results at a 80 cm Distance and 2 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	1.9E+03	7.0E+02	2.5E+02	7.5E+02
	Log CFU/mL	3.2788	2.8451	2.3979	2.8751
	% reduction	N/A	63.15789%	86.84211%	60.52632%
	Log reduction	N/A	0.4337	0.8809	0.4037
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	3.4E+03	9.4E+02	9.6E+02	1.2E+03
	Log CFU/mL	3.5315	2.9731	2.9823	3.0792
	% reduction	N/A	72.35294%	71.76471%	64.70588%
	Log reduction	N/A	0.5584	0.5492	0.4523

Table 8: Results of 80 cm Distance and 4 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	1.2E+03	2.4E+02	3.5E+02	1.7E+02
	Log CFU/mL	3.0792	2.3802	2.5441	2.2304
	% reduction	N/A	80.00000%	70.83333%	85.83333%
	Log reduction	N/A	0.6990	0.5351	0.8488
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	1.3E+03	2.7E+02	3.1E+02	2.0E+02
	Log CFU/mL	3.1139	2.4314	2.4914	2.3010
	% reduction	N/A	79.23077%	76.15385%	84.61538%
	Log reduction	N/A	0.6825	0.6225	0.8129

Table 9: Results of 80 cm Distance and 8 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	4.5E+03	2.5E+02	3.9E+02	3.2E+02
	Log CFU/mL	3.6532	2.3979	2.5911	2.5051
	% reduction	N/A	94.44444%	91.33333%	92.88889%
	Log reduction	N/A	1.2553	1.0621	1.1481
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	5.6E+03	6.7E+02	9.1E+02	2.5E+02
	Log CFU/mL	3.7482	2.8261	2.9590	2.3979
	% reduction	N/A	88.03571%	83.75000%	95.53571%
	Log reduction	N/A	0.9221	0.7892	1.3503

Table 10: Results of 80 cm Distance and 12 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	4.0E+03	1.2E+02	1.1E+02	3.3E+02
	Log CFU/mL	3.6021	2.0792	2.0414	2.5185
	% reduction	N/A	97.00000%	97.25000%	91.75000%
	Log reduction	N/A	1.5229	1.5607	1.0836
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	6.3E+03	1.0E+02	1.4E+02	1.2E+02
	Log CFU/mL	3.7993	2.0000	2.1461	2.0792
	% reduction	N/A	98.41270%	97.77778%	98.09524%
	Log reduction	N/A	1.7993	1.6532	1.7201

Table 11: Results at a 121.92 cm Distance and 2 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	3.2E+04	1.3E+04	1.8E+04	2.0E+04
	Log CFU/mL	4.5051	4.1139	4.2553	4.3010
	% reduction	N/A	59.37500%	43.75000%	37.50000%
	Log reduction	N/A	0.3912	0.2498	0.2041
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	6.7E+03	3.9E+03	5.0E+03	4.8E+03
	Log CFU/mL	3.8261	3.5911	3.6990	3.6812
	% reduction	N/A	41.79104%	25.37313%	28.35821%
	Log reduction	N/A	0.2350	0.1271	0.1449

Table 12: Results of 121.92 cm Distance and 4 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	9.3E+03	4.5E+03	3.9E+03	3.3E+03
	Log CFU/mL	3.9685	3.6532	3.5911	3.5185
	% reduction	N/A	51.61290%	58.06452%	64.51613%
	Log reduction	N/A	0.3153	0.3774	0.4500
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	1.7E+04	9.0E+03	7.5E+03	1.1E+04
	Log CFU/mL	4.2304	3.9542	3.8751	4.0414
	% reduction	N/A	47.05882%	55.88235%	35.29412%
	Log reduction	N/A	0.2762	0.3553	0.1890

Table 13: Results of 121.92 cm Distance and 8 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	5.1E+03	1.6E+03	2.2E+03	1.1E+03
	Log CFU/mL	3.7076	3.2041	3.3424	3.0414
	% reduction	N/A	68.62745%	56.86275%	78.43137%
	Log reduction	N/A	0.5035	0.3652	0.6662
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	5.0E+03	2.8E+03	1.8E+03	2.6E+03
	Log CFU/mL	3.6990	3.4472	3.2553	3.4150
	% reduction	N/A	44.00000%	64.00000%	48.00000%
	Log reduction	N/A	0.2518	0.4437	0.2840

Table 14: Results of 121.92 cm Distance and 12 Hour Exposure Time. Reported in CFU/mL recovered, Percent and Log_{10} Reduction.

Challenge Organism	Units	Positive Control (CFU/mL)	Replicate A	Replicate B	Replicate C
Escherichia coli ATCC 11229	CFU/mL	2.6E+03	3.5E+02	5.0E+02	3.5E+02
	Log CFU/mL	3.4150	2.5441	2.6990	2.5441
	% reduction	N/A	86.53846%	80.76923%	86.53846%
	Log reduction	N/A	0.8709	0.7160	0.8709
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	CFU/mL	1.0E+04	3.1E+03	2.8E+03	2.2E+03
	Log CFU/mL	4.0000	3.4914	3.4472	3.3424
	% reduction	N/A	69.00000%	72.00000%	78.00000%
	Log reduction	N/A	0.5086	0.5528	0.6576

16.0 CONCLUSION

16.1 Based on the results presented in this study report:

16.1.1 The Buddy Technologies HEV light tested at a distance of 40, 80, and 121.92 cm and an exposure time of 2, 4, 8, 12 hours showed a Log reduction of *Escherichia coli* ATCC 11229 and Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592.

16.2 All negative controls were negative for growth.

16.3 Growth Promotion of media passed specification.

Appendix - Signed Protocol

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

Study Title
An Evaluation of the Antimicrobial Efficacy of HEV Light

Test Device
HEV light

Testing Facility
Q Laboratories
1930 Radcliff Drive
Cincinnati, OH 45204
(513) 471-1300

Prepared For
Buddy Technologies USA (Study Sponsor)
300 Lenora Street, #1591
Seattle, WA 98121

Proposal Number and Laboratory Project Number (Study File)
QL20343-2A

Preparation Date
December 15, 2020

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

Table of Contents

List of Tables

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

1.0 PURPOSE

1.1 The evaluation of the microbicidal properties will be analyzed by measuring the changes of a population of aerobic microorganisms within 4 exposure times and 3 distances when tested against antimicrobial test device provided by study sponsor.

2.0 SCOPE

2.1 The design of this evaluation is based on the guidance provided in the on ASTM Committee E1153-03 "*Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces*" [Section 14.1] and E3515-18 "*Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil.*" [Section 14.2]

3.0 SUMMARY

3.1 **Sponsor:** Buddy Technologies USA 300 Lenora Street, #1591, Seattle, WA 98121

3.2 Test Device:

- 3.2.1 The test device to be evaluated will be provided to the testing facility by the study sponsor, complete with appropriate documentation.
- 3.2.2 Test Device: Buddy Technologies HEV light
- 3.2.3 The study parameters are presented in Table 1.

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

Table 1: Efficacy Study Parameters

Test Device	Organisms	Distance (cm)	Exposure Times (hours)	Test Replicates	Negative Controls	Positive Controls
Buddy Technologies HEV light	*Escherichia coli* ATCC 11229	40	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		80	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		121.92	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
	Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	40	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		80	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1
		121.92	2	3	1	1
			4	3	1	1
			8	3	1	1
			12	3	1	1

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

4.0 EQUIPMENT/APPARATUS

4.1 Incubator, temperature range 35 ± 1 °C
4.2 Incubator thermometer, NIST traceable
4.3 Steam autoclave
4.4 Vortex mixer
4.5 Calibrated, traceable minute/second timer
4.6 Refrigerator 2 - 8 °C
4.7 Refrigerator thermometer, NIST traceable
4.8 Traceable thermometer/clock/humidity monitor
4.9 Adjustable pipettor, 10 - 100 µL, 20 - 200 µL, and 100 µL - 1000 µL capacity
4.10 Reichert Quebec® colony counter
4.11 Hand tally
4.12 Centrifuge, capable of up to 4,500 rpm
4.13 Ultralow freezer, capable of maintaining - 70 °C
4.14 TorchStar Desk Lamp (used to fix the HEV bulb)
4.15 Smartphone
4.16 *Note: Substitutions of equivalent equipment made be made without sponsor approval. As appropriate, equipment/apparatus are purchased sterile or sterilized via autoclaving.*

5.0 MATERIALS

5.1 Micropipette tips, 10 - 100 µL, 20 - 200 µL, and 100 - 1000 µL
5.2 Serological pipettes, 1, 2, 5, 10, and 25 mL
5.3 Test tubes
5.4 Disposable Petri dishes, 100 x 15 mm
5.5 Flasks or Containers
  5.5.1 Appropriate sizes with closures for preparation of culture medium and sterile deionized water.
  5.5.2 Volumetric, 100 and 1000 mL
5.6 Disposable loops
5.7 50 mL conical vials
5.8 Glass slide carriers
5.9 *Note: Substitutions of equivalent materials made be made without sponsor approval. As appropriate, equipment/apparatus are purchased sterile or sterilized via autoclaving.*

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

6.0 MEDIA/CHEMICALS/REAGENTS

6.1 Tryptic Soy Agar with 5% Sheep Blood (SBA) Commercially available from BD, PN 221261, or equivalent
6.2 Microbial Content Test (MCT) agar MP107*
6.3 Tryptic Soy Broth (TSB) MP058
6.4 Phosphate Buffered Saline (PBS) MP416
6.5 *Note: Substitutions of equivalent materials or reagents made be made without sponsor approval. As appropriate, media/chemicals/reagents are purchased sterile or sterilized via autoclaving. *The MP number refers to Q Laboratories master preparation number.*

7.0 METHOD DESCRIPTION

7.1 Test Microorganism Preparation:
- 7.1.1 Seed-lot culture maintenance techniques are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed lot.
- 7.1.2 The Test Microorganism cultures will be prepared as follows:
  - 7.1.2.1 Propagate on SBA for 18-24 hours at 35 ± 1 °C from a Q Laboratories frozen stock culture stored at -70 °C.
  - 7.1.2.2 Transfer cultures using TSB.
  - 7.1.2.3 Incubate each daily transfer at the appropriate temperature for growth for 24 ± 2 hours.
  - 7.1.2.4 Perform daily transfers of each culture by aseptically transferring growth.
  - 7.1.2.5 Centrifuge the test culture at 4,500 rpm for 15 minutes.
  - 7.1.2.6 Remove the supernatant and rehydrate the pellet with PBS.
  - 7.1.2.7 Adjust each test culture to an approximate concentration of 10^6.

7.2 Preparation of Carriers:
- 7.2.1 Prior to conducting the analysis, glass slide carriers will be sterilized via autoclave.
- 7.2.2 With gloved hands, place the glass slide carriers on a disinfected flat surface. Apply 25 µL of each test culture to the carrier. Immediately spread the inoculum to contact uniformly using the micropipette tip. Do not allow the inoculum to contact the edge of the glass slide carriers.
- 7.2.3 After inoculation, allow carriers to dry for 30 to 60 minutes at room temperature (20-25 °C). Visually inspect carriers to ensure the culture is uniformly dried before initiating the test procedure.

7.3 Test Procedure:

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

7.3.1 Verify the HEV light device is placed at the appropriate distance perpendicular from the carrier. Refer to Table 1 for the distance the carrier should be placed from the device. Care should be taken not to contact the UV bulb.

7.3.2 Turn on the device and expand the group the device is saved in.

7.3.2.1 See manufacturer's instructions for connecting the device to a smartphone and operation of the device with a smartphone.

7.3.3 Expand the group so that you can see the individual bulb.

7.3.4 Turn the bulb OFF and this should place it into Clean Mode.

7.3.5 Double check that the brightness settings are at 100%

7.3.6 Ensure the bulb turns to a blue-ish/violet hue.

7.3.7 Direct the device toward the carrier.

7.3.8 Hold for the appropriate contact time. See Table 1.

7.3.8.1 Note that the acceptable deviation of the contact time is 5 minutes.

7.3.9 During the contact time, take a photo of the test device's activity on the carriers.

7.4 Recovery and Analysis:

7.4.1 Add the carrier to 20.0 mL of PBS, listed in Table 2, and thoroughly vortex.

7.4.2 Prepare ten-fold serial dilutions of the sample extract by transferring 1.0 mL from the initial dilution into 9.0 mL of appropriate PBS. Continue diluting as necessary.

7.4.3 Plate each dilution in duplicate into sterile Petri dishes and add 12 - 15 mL of tempered MCT. Mix thoroughly and allow the plates to solidify, invert and incubate at 35 ± 1 °C for 48 ± 2 h.

7.4.4 After incubation, enumerate typical colonies, recorded raw data record as CFU/plate, and take photos of the control and test carrier plates. Average duplicate plates multiply by the dilution factor to arrive at CFU/test device. Record raw values and use for the calculations in Section 9.0.

7.5 Study Controls:

7.5.1 Negative Control

7.5.1.1 Evaluate one uninoculated carrier for total viable organisms at each contact time following procedures outlined in Sections 7.3 – 7.4.

7.5.2 Positive Control

7.5.2.1 Evaluate one inoculated carrier to determine the total viable organisms remaining on the carrier at each contact time. This control will serve as the basis for determining the percent and Log reduction for the test device replicates. Inoculate positive carrier according to the procedure outlined in Section 7.2. Do

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

not expose the positive carrier to the light of the test device. Determine the remaining microorganisms using the procedure in Section 7.4.

8.0 TEST CULTURE VIABILITY

8.1 To verify the viability of the inoculum, enumerate at the start of the testing phase. Determine inoculum populations by preparing ten-fold serial dilutions of each challenge organism suspension in duplicate by standard microbiological procedures and incubating at 35 ± 1 °C for 48 ± 2 h.

8.2 Enumerate colonies and record as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate the microbial population (CFU/mL) of the control suspension.

9.0 STATISTICAL ANALYSIS

9.1 Perform a logarithmic transformation measuring surviving microbial populations of the positive control and test device replicates for each microorganism.

9.1.1 Calculate the Log reduction as follows:

$$PC = Positive\ Control$$
$$TD = Test\ Device$$
$$LR = Log\ Reduction$$
$$\mathrm{LR} = Log_{10}PC - Log_{10}\mathrm{TD}$$

9.1.2 Calculate percent reduction as follows:

$$PR = Percent\ Reduction$$
$$\mathrm{PR} = \left\{\frac{PC\ CFU - \mathrm{TD}\ CFU}{PC\ CFU}\right\} \times 100$$

Note: Calculated <2.0 x 10^1 as "20" to obtain the percent and Log reduction.

10.0 MEDIA QUALITY CONTROLS

10.1 For MCT:

10.1.1 Inoculate plates with 1 - 100 CFU of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 and incubate at 30-35 °C for 3 days or less.

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

10.1.2 Inoculate plates of an equivalent medium with 1 - 100 CFU of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231 and incubate at 30-35 °C for 3 days or less.

10.1.3 Incubate 1 plate at 30-35 °C for 3 days or more to serve as the sterility controls.

10.1.4 Comparable growth acceptance will be within 50 - 200 % between the media. Sterility acceptance is no growth.

10.2 For PBS and TSB:

10.2.1 Incubate 1 tube at 30-35 °C for 1 day or more to serve as the sterility controls.

10.2.2 After incubation, streak to a general growth agar. Incubate for 1 day or less at 30-35 °C.

10.2.3 The acceptance criterion is no growth from the sterility controls.

11.0 PERFORMANCE CRITERIA

11.1 In order to demonstrate disinfection against the mixed culture suspension containing *E. coli*, and *S. aureus* (MRSA), the Sponsor's disinfection procedure must have achieved a Log reduction of the test organisms as compared to the positive control.

12.0 ACCEPTANCE CRITERIA

12.1 The study controls below must perform according to the criteria detailed for the data to be considered acceptable.

12.1.1 All negative device controls must be negative for growth.

12.1.2 For media quality controls, comparable growth acceptance will be within 50 - 200 %. Sterility acceptance is no growth.

13.0 REFERENCES

13.1 ASTM Committee E1153-03 "*Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces*"

13.2 ASTM Committee E3515-18 "*Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil.*"

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

14.0 FINAL REPORT

14.1 A final validation report will be prepared upon completion of the study, including a tabularized summary of data and a description of results of the study.

15.0 RECORDS TO BE MAINTAINED

15.1 All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between Q Laboratories and the sponsor will be stored in the archives at Q Laboratories, 1930 Radcliff Drive, Cincinnati, Ohio 45204, according to Q Laboratories SOP 20-ADMN-ISO-008, for a period of at least even (7) years.

16.0 QUALITY COMPLIANCE

16.1 Q Laboratories has developed and implemented a quality management system that enhances our ability to provide testing services that consistently meet client expectations and regulatory requirements. Q Laboratories quality documentation requirements are defined by ISO 17025, FDA Quality System Regulations (QSR), FDA Current Good Manufacturing Practices (cGMPs), FDA Good Laboratory Practices (GLP), and EPA Good Laboratory Practices standards (GLPs).

Q Laboratories applies the following standards as applicable:

- ISO 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories
- FDA 21 CFR Part 820 Quality System Regulation
- FDA 21 CFR Part 58 Good Laboratory Practice for Non Clinical Laboratory Studies
- FDA 21 CFR Part 211 Current Good Manufacturing Practice for Finished Pharmaceuticals
- FDA 21 CFR Part 210 Current Good Manufacturing Practice in Manufacturing Processing, Packing or Holding of Drugs; General
- EPA 40 CFR Part 160 FIFRA Good Laboratory Practice Standards

17.0 PROTOCOL MODIFICATIONS

17.1 During the testing phase, changes to the protocol may be required. The study sponsor will be notified immediately of any modifications to the protocol. Approval of the modifications is required before any additional analysis is

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

conducted. The modifications will be added to the protocol as an amendment and approved by both the study director and study sponsor.

18.0 DEVICE DISPOSAL

18.1 All unused test devices will be offered for return to the Study Sponsor at the expense of Study Sponsor. If not desired by Study Sponsor, all unused test material to be disposed of within 90 days following the study completion.

CONFIDENTIAL	Protocol # QL20343-2A
Q Laboratories	Title: **An Evaluation of the Antimicrobial Efficacy of HEV Light**

19.0 **ACCEPTANCE OF STUDY PROTOCOL**

An Evaluation of the Antimicrobial Efficacy of HEV Light

Q Laboratories (Testing Facility)
1930 Radcliff Drive
Cincinnati, OH 45204

Study Director: _____ / BB — 12-30-2020
Benjamin J. Bastin — Date
Microbiology R&D Laboratory Supervisor

Buddy Technologies USA (Study Sponsor)
300 Lenora Street, #1591
Seattle, WA 98121

April Wright — 12/30/2020
Representative — Date

Senior Product Manager
Title