Efficacy of Tadiran Air Care O_2 System against Aerosolized *Methicillin Resistant Staphylococcus Epidermidis (MRSE)*

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Background: This in-vitro study characterized the decontamination efficacy of the Tadiran Air Care O2 system against aerosolized methicillin resistant *Staphylococcus epidermidis* (MRSE, Gram +). The Tadiran Air Care O2 system is a device based on PeroxMaker™ technology designed to reduce airborne pathogens. The effectiveness of the system was assessed in a 1m^3 bioaerosol chamber for a single (1) vegetative bacterium: methicillin resistant *Staphylococcus epidermidis* (Gram +), tested in triplicate, in addition to a single control trial.

Methods: Methicillin resistant *Staphylococcus epidermidis* was aerosolized into a sealed 1m^3 environmental chamber containing the Tadiran Air Care O2 system. Midget impingers and viable cascade impactors were used to determine chamber bioaerosol concentrations at pre-determined sampling times. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Chamber control trial data was subtracted from Tadiran Air Care O2 trial data to yield net LOG reduction in the chamber for viable bioaerosol concentration.

Results: Three trials were conducted to evaluate the Tadiran Air Care O2 system efficacy at removing viable *Staphylococcus epidermis* from the air. The Tadiran Air Care O2 device yielded over a 4.0 net LOG reduction in a 120-minute time period and on average showed a 4.58 +/- 0.12 net LOG reduction in a 150 minute period in a 1m^3 test chamber. This corresponds to a 99.9973% +/- 0.0007%% net reduction in viable bioaerosol on average compared to the control in 150 minutes.

Summary: Overall, the Tadiran Air Care O2 system performed very well with a 99.9994% net reduction in viable bioaerosol concentration within a 150 minute period. Testing was conducted using aerosolized methicillin resistant *Staphylococcus epidermidis.* This testing confirms that, in theory, the Tadiran Air Care O2 system should show efficacy at reducing the risk of pulmonary MRSE infections.

This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Overview

This study was conducted to evaluate the efficacy of the Tadiran Air Care O2 (Afula, Israel) based on PeroxMaker™ technology at removing viable bioaerosols from the air. A picture of the device can be found in **Figure 1**.

Testing was conducted in a 1m^3 custom bioaerosol exposure chamber. The Tadiran Air Care O2 device effectiveness was tested against the vegetative bacterium methicillin resistant *Staphylococcus epidermidis* in order to evaluate the system's net LOG reduction of viable bioaerosol within the chamber.

The effectiveness of the Air Care system was evaluated against a single vegetative bacterium. Testing was conducted in triplicate trials plus a control trial to demonstrate the capability of reducing viable bioaerosol concentrations. There were a total of four (4) independent trials in this study.

During the control trial, the Air Care O2 system remained inside the test chamber but was never turned on. During test trials, the system was turned on after initial chamber concentration sampling and remained running until the completion of the trial. Methicillin resistant *Staphylococcus epidermidis* was aerosolized into the test chamber and impinger samples were collected at set time points throughout each trial. Trials with the Air Care O2 device turned on were compared to control trials in determine net LOG reduction of viable bioaerosols within the chamber.

Figure 1*: Tadiran Air Care O2 Device*

Test Location and Conditions

Testing was conducted at Aerosol Research and Engineering labs located at 15320 S. Cornice Street in Olathe, Kansas 66062. Laboratory conditions were approximately 76°F with 41% relative humidity.

Testing Chamber

The primary aerosol exposure chamber containing the Tadiran Air Care O2 system is a sealed 1m3 environmental chamber constructed of 3/8" Lexan and outfitted with all necessary pass-through and sub-systems sampling ports. The chamber is equipped with HEPA filtered house air in order to maintain a clean background environment prior to all testing and to allow rapid air flushing through the chamber after completion of each exposure to ensure a clean background at before conducting subsequent trials.

During the aerosolization of the bioaerosols, the chamber was operated in a balanced push/pull aerosol inlet and vacuum to eliminate over or under pressure in the chamber. The chamber was operated at a slightly negative pressure, -0.3 inH2O, for technician safety. Once aerosolization of the challenge organism at the beginning of each trial was complete, the inlet and vacuum balance were cut off and the chamber sat idly until air sample collections.

The chamber is outfitted with a impinger sample ports located at each of the four corners on the top of the box. The viable cascade impacter sampling was done from the back right corner sample port

The chamber was equipped with four (4) mixing fans to ensure spatial homogeneity of bioaerosols during their aerosolization and sampling. These fans were switched on during the aerosolization of the bioaerosol into the chamber and remained on for the duration of the trials to ensure spatial homogeneity. **Figure 2** shows a picture of the full chamber setup containing the device.

Figure 2: *Exposure Test Chamber*

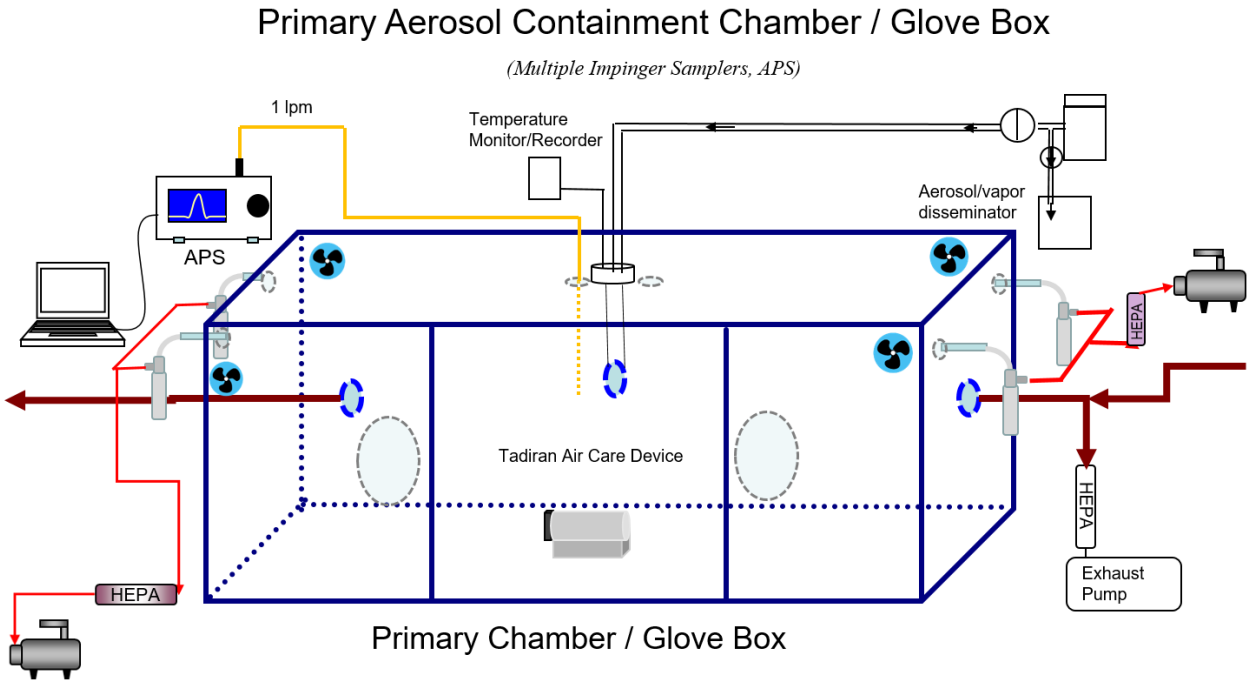


Figure 3: *Test Chamber Flow diagram for testing*

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 62-jet nebulizer (BGI Inc., Waltham MA) driven by purified filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the Collison nebulizer.

Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 35 psi, which obtained an output volumetric flow rate of approximately 35 lpm with a fluid dissemination rate of approximately 1 ml/min. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN).

Bioaerosol Sampling and Monitoring System

A midget impinger (Chem Glass Inc., Vineland NJ) was used for bioaerosol collection of biological aerosols to determine the chamber concentration. This impinger was connected to the bioaerosol chamber via a sample port located near the center of the exposure box.

The midget impinger's vacuum source was maintained at a negative pressure of 18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The midget sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

The impingers were filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms for proper plate counts. Impingers were taken in quadruplicate and pooled for an overall average of chamber concentration.

Starting at the 120 minute time point, sample collections were obtained using a viable cascade impactor. A viable cascade impactor (SKC Inc., Valley View PA) is comprised of an inlet cone, precision-drilled 400-hole impactor stage, and a base that holds a standard-size agar plate. A high flow pump pulls microorganisms in air through the holes (jets) where they are impacted on the agar surface. Viable cascade impactors were operated at a flow rate of 30 lpm for all samples. **Figure 4** shows a picture of a viable cascade impactor.

This method of bioaerosol collection was chosen due to greater sensitivity and more accurate sampling process for quantification. With viable collection enumeration detection at one colony forming unit (cfu), direct collection onto agar plates and immediate incubation after sample collection provides the highest sensitivity for low concentration viable bioaerosol

collection and measurement. However, this method of collection was not a feasible option for control testing due to high bioaerosol concentrations throughout the trials.

Figure 4*: SKC BioStage Viable Cascade Impactor.*

Tadiran Air Care Testing Matrix

Trial	Run	Challenge Organism	Chamber Size	Trial Time (minutes)	Sampling	Equipment
1 2 3 4	Challenge Challenge Challenge Control	*Staphylococcus epidermidis* (ATCC 14190)	$1m^3$	150	*Glass Midget Impingers*	*6-Jet Collison Nebulizer, TSI Aerodynamic Particle Sizer (APS)*

Figure 5: *Testing matrix for the chamber test*

Test Matrix

To accurately test the Tadiran Air Care O2 device, triplicate challenge trials were performed in the test chamber. In order to characterize the device's performance while taking into account the natural reduction of the bioaerosol in the chamber, a control trial was ran. A testing matrix for the device can be found in **Figure 5.**

Species Selection

This testing utilized methicillin resistant *Staphylococcus epidermidis* which is a bio-safety level 1 (BSL1) pathogen. This organism is a common pathogen with a history of being prevalent in hospitals. It is known to cause pulmonary infections.

Staph Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). Working stock cultures were prepared using sterile techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 100 ml of methicillin resistant *Staphylococcus epidermidis* stock was prepared in tryptic soy broth media, and incubated for 24 hours with oxygen infusion (1 cc/min) at 37°C. Biological stock concentrations were greater than 1 x 10^9 cfu/ml using this method.

Stock cultures were centrifuged for 10 minutes at 4000 rpm in sterile 50 mL conical tubes, growth media was decanted, and the cells re-suspended in sterile PBS buffer for aerosolization. Fresh cultures were prepared for each day of testing. On days where multiple tests were conducted, the same culture was used for all trials.

Bioaerosol Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates in a class 2 biosafety cabinet. The plated cultures were incubated for 24 hours, enumerated and recorded for data analysis.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS. The APS has a dynamic measurement range of 0.5 to 20 µm and was programmed to take consecutive real time one minute aerosol samples throughout the duration of each aerosol trial. Data was logged in real time to an Acer laptop computer, regressed, and plotted. Aerosol particle size distribution for *Staph epidermidis* is shown in **Figure 6**

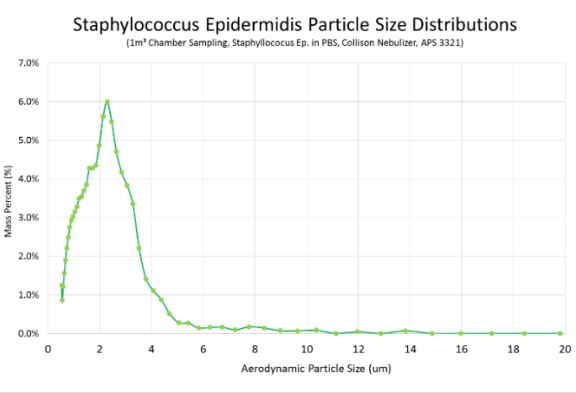


Figure 6: Staph epidermidis *Particle Size Distribution*

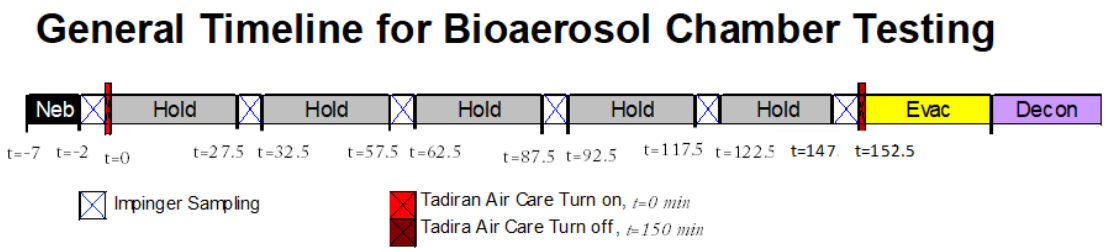


Figure 7: *General trial timeline for bioaerosol testing.*

Control Testing Method

To accurately assess the Tadiran Air Care O2 unit, test chamber pilot control trials were performed with methicillin resistant *Staphylococcus epidermidis* for 150 minute periods without the system in operation to characterize the biological challenge aerosol for aerosol delivery/collection efficiency, decay rate and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual viable bioaerosol reduction from the Air Care O2 challenge testing and verify that contaminant concentrations persisted above the required concentrations over the entire pilot control test period. **Figure 7** shows a test matrix for all conducted testing.

Tadiran Air Care O2 Testing Method

For each control and challenge test, the Collison nebulizer was filled with approximately 50 mL of biological stock and operated at 35 psi for a period of 5 minutes. For control and system trials, the impinger was filled with 20 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 has been shown to increase the impinger collection efficiency and de-agglomeration of micro-organisms.

The chamber mixing fans were turned on during bioaerosol generation to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample. For the remainder of both control and test trials, mixing fans remained on to ensure bioaerosol homogeneity.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and challenge test by sampling with a midget impinger located near the center of the chamber. Midget samples were collected for 2 or 5 minutes depending on which time point the sample was taken. Longer samples were taken towards the end of each test in order to collect enough viable bioaerosol for plating and enumeration.

Aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval, and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

For device testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the trial length of 150 minutes. Subsequent impinger samples were taken at intervals of 30 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the system over time. **Figure 7** outlines the general timeline for the testing procedure with the Tadiran Air Care O2 system. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 2 log dilution range.

Plates were incubated and enumerated for viable colony forming unit (cfu) counts to calculate bioaerosol challenge concentrations in the chamber and reduction of viable microorganisms.

This testing method was designed to assess the viable bioaerosol reduction in the test chamber, it did not directly assess the killing of the microorganism.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of thirty minutes between tests and analyzed with a TSI Aerodynamic Particle Sizer (APS) for particle concentration decrease to baseline levels between each test. At the conclusion of testing, the chamber was decontaminated using 35% vaporous, food grade hydrogen peroxide. The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

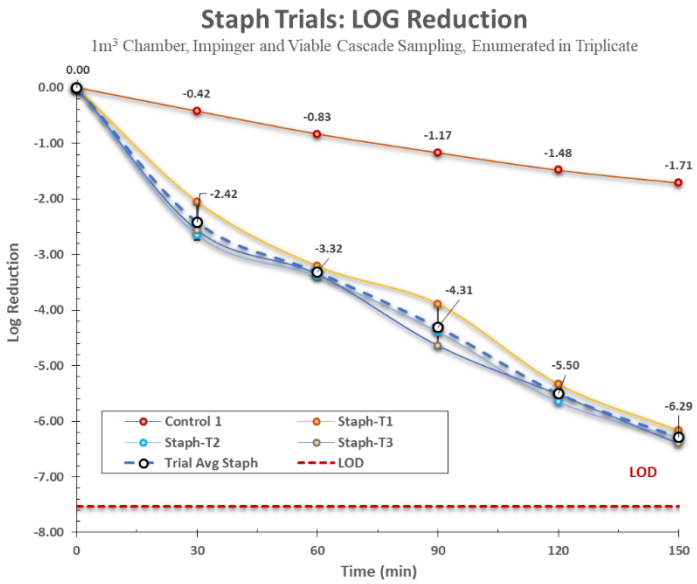


Figure 8: *LOG reduction of* S. epidermidis *in control and device challenge trials.*

Data Analysis

The data analysis shows the results of the triplicate trials conducted for this study, as well as an average at each time point for the group. All trials show individual and group average +/- standard deviations for Net LOG reduction on a per trial basis.

Tadiran Air Care O2 Results

Staphylococcus epidermidis cultures were initiated the day prior to testing and grew to a concentration greater than $1e^{10}$ cfu/ml. The control trial experienced a 1.71 LOG reduction of *Staph* after 150 minutes of sample collections using the midget impingers.

The device showed a consistent reduction throughout all of the trials. At the 30 minute time point, the device showed an average 2.00 net LOG reduction. At the 60 minute time point, the device had an average 2.49 net LOG reduction. At the final time point of 150 minutes, the device had an average net LOG reduction of 4.58 +/- 0.12 which is equivalent to a 99.9973% +/- 0.0007% reduction above the natural reduction rate. The reduction rate of the device as well as an average for all the trials can be found in LOG reduction in **Figure 8** and net LOG reduction in **Figure 9**.

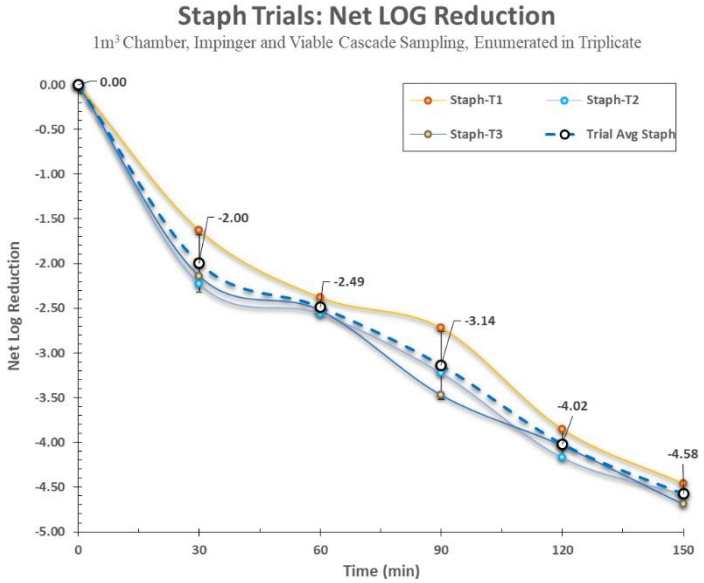


Figure 9: *Net LOG reduction of* S. epidermidis *in challenge trials*

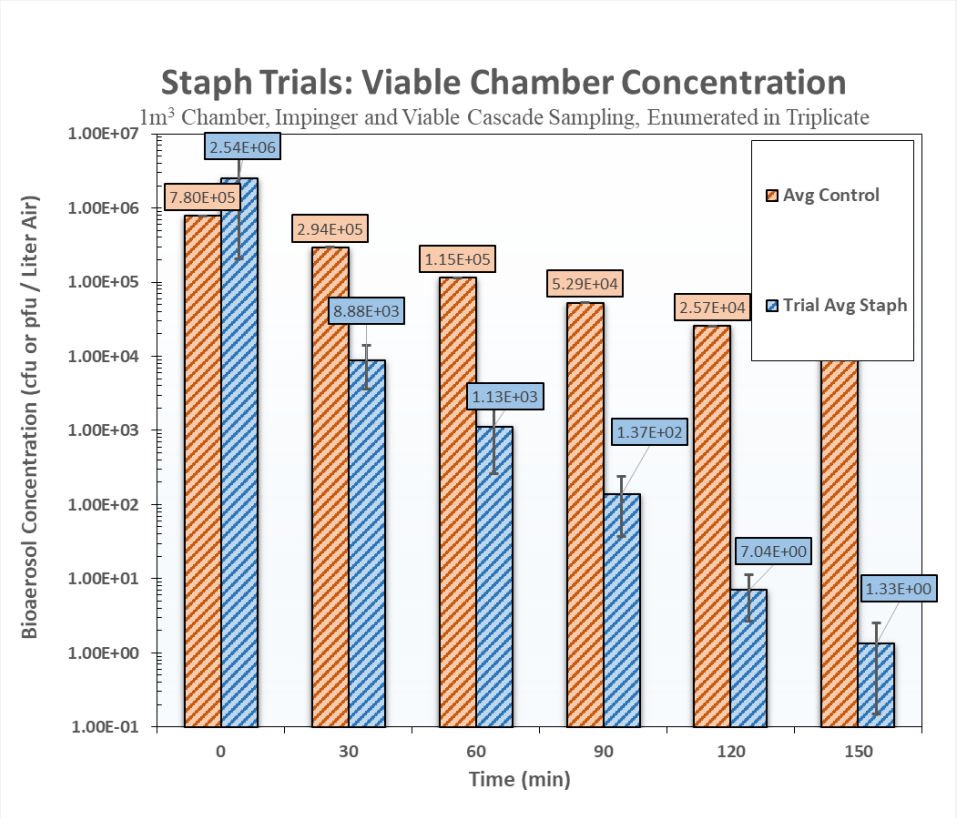


Figure 10: *Total net LOG reduction for the Tadiran Air Care O2 trials, as well as the average*

Summary

Overall the Tadiran Air Care O2 system performed extremely well with a reduction rate of over 4.0 net LOG in viable airborne bioaerosol concentration within a 120 minute period and yielding 4.58 net LOG reduction in the 150-minute period, when placed in a 1m³ enclosure. A comparative graph showing the chamber concentration over time for the Staph trial average and the control can be found in **Figure 10**. The results for the trials including group averages and standard deviation can be found in a summary table in **Figure 11**.

The results from the Tadiran Air Care O_2 device trials showed a steady reduction of bioaerosol throughout all of the trials. The results indicate the efficacy of the Tadiran Air Care O2 device in the 1m³ chamber and demonstrate that, in theory, this device could reduce the risk of MRSE pulmonary infections.

Staphylococcus epidermidis Trial Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)				
				30	60	90	120	150
Virus	*epidermidis* (RNA E. coli phage)	*Staph-T1*	*Net Log Reduction*	-1.63	-2.38	-2.72	-3.85	-4.46
			Net % Reduction	97.6465%	99.5831%	99.8099%	99.9859%	99.9965%
Virus	*epidermidis* (RNA E. coli phage)	*Staph-T2*	*Net Log Reduction*	-2.23	-2.56	-3.22	-4.17	-4.58
			Net % Reduction	99.4073%	99.7250%	99.9404%	99.9932%	99.9974%
Virus	*epidermidis* (RNA E. coli phage)	*Staph-T3*	*Net Log Reduction*	-2.14	-2.52	-3.47	-4.04	-4.69
			Net % Reduction	99.2770%	99.7010%	99.9663%	99.9908%	99.9980%
All Trial Averages			***Net Log Reduction***	**-2 +/- 0.32**	**-2.49 +/- 0.1**	**-3.14 +/- 0.38**	**-4.02 +/- 0.16**	**-4.58 +/- 0.12**
			Net % Reduction	***98.7769% +/- 0.9812%***	***99.6697% +/- 0.076%***	***99.9055% +/- 0.0839%***	***99.99% +/- 0.0037%***	***99.9973% +/- 0.0007%***

Figure 11: *Summary Data Table for Net LOG Reduction of the Tadiran Air Care O2*

References

T. Reponen, K. Willeke, V. Ulevicius et al. *Techniques of Dispersion of Microorganisms in Air.* Aerosol Science and Technology. 27: 1997. pp. 405-421.

Ding and Wing. *Effects of Sampling Time on the Total Recovery rate of AGI-30 Impingers for E. coli*. Aerosol and Air Quality Research, Vol. 1, No. 1, 2001, pp. 31-36.

Analytical GLP Certificate

Aerosol Research and Engineering Labs, Inc.
15320 S. Cornice Street
Olathe, KS 66062

Project

10867.70

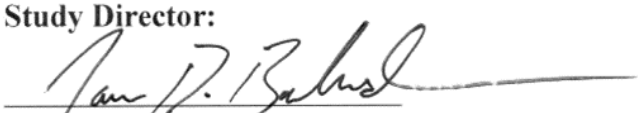
Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Study Director:



Jamie D. Balarashti
Study Director
ARE Labs, Inc.

3/21/2021
Date

Principal Investigator:

Weston Schaper, M.S.
Principal Investigator
ARE Labs, Inc.

3/21/2021
Date

Appendix A: Raw Data

Trial Information

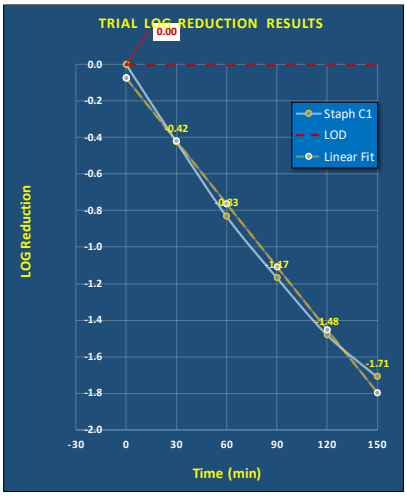
TEST DATE:	Thursday, March 4, 2021
TRIAL PERFORMED BY:	JCT
TRIAL NUMBER:	C1
TEST ORGANSIM:	Staphylococcus epidermidis
TRIAL NAME ID (GRAPHS/TABLES):	Staph C1

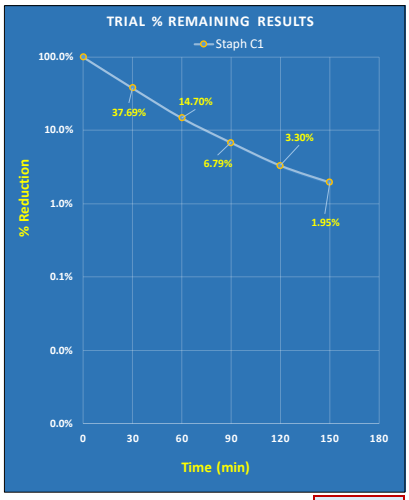
Device Information

MANUFACTURER:	
UNIT MODEL:	
FAN SPEED (CFM):	
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	16
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	30	60	90	120	150			LOD
IMPINGER USED (y / n)	y	y	y	y	y	y			n
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n			y
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.800E+05	2.940E+05	1.147E+05	5.293E+04	2.573E+04	1.520E+04			
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	14.29%	9.09%		1.50%	7.00%	10.00%			
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**7.800E+05**	**2.940E+05**	**1.147E+05**	**5.293E+04**	**2.573E+04**	**1.520E+04**			
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**37.6923%**	**14.7009%**	**6.7863%**	**3.2991%**	**1.9487%**			
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**62.3077%**	**85.2991%**	**93.2137%**	**96.7009%**	**98.0513%**			
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.42**	**-0.83**	**-1.17**	**-1.48**	**-1.71**			

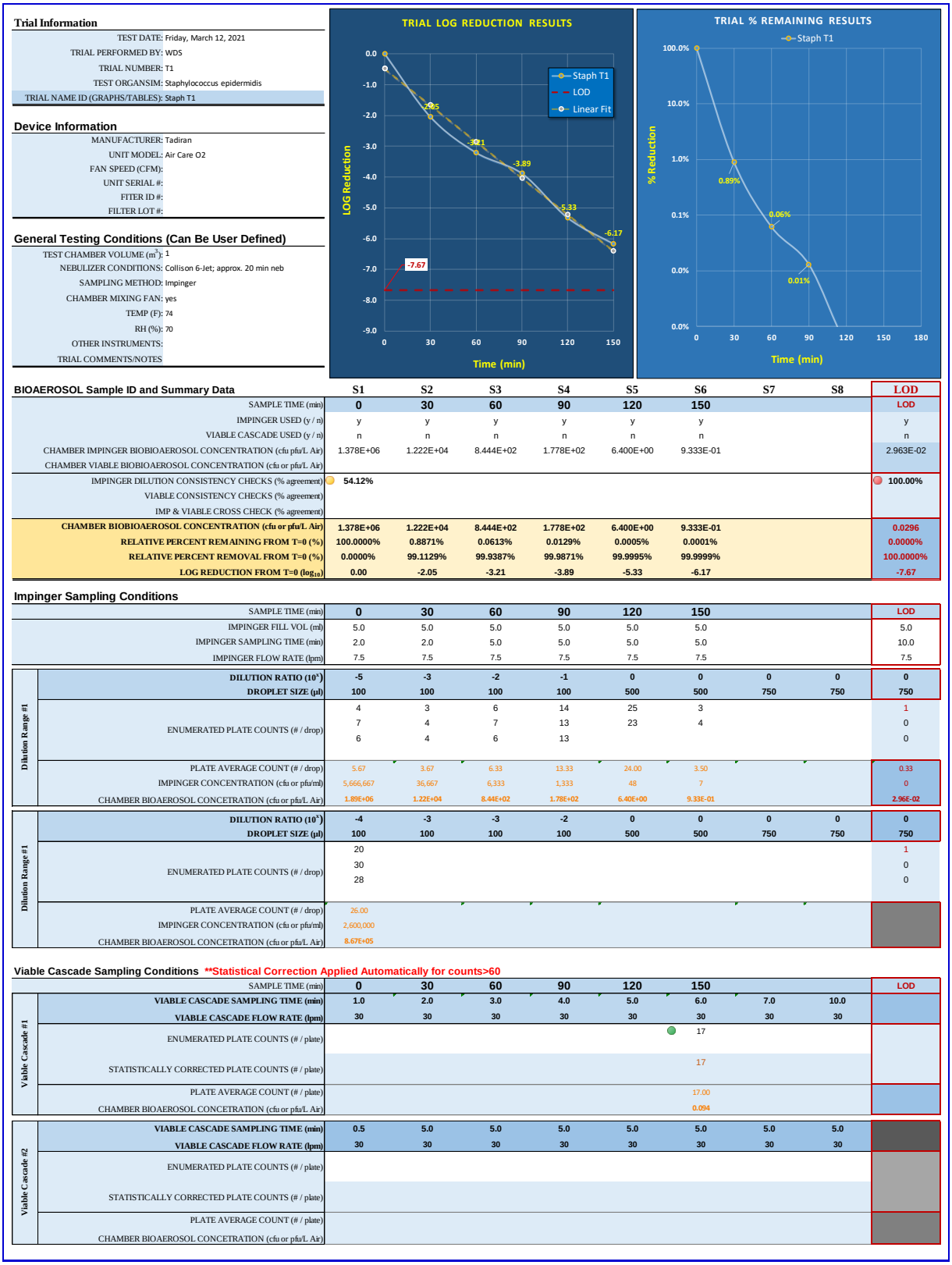
Impinger Sampling Conditions

SAMPLE TIME (min)	0	30	60	90	120	150			LOD
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0			20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0			5.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5			12.5
Dilution Range #1 — **DILUTION RATIO (10^x)**	-3	-3	-3	-3	-2	-2	-4	-4	0
DROPLET SIZE (µl)	100	100	100	100	100	100	100	100	750
ENUMERATED PLATE COUNTS (# / drop)	114		19	7	35				1
	106	42	15	8	31	17			0
	95	35	9	5	27	19			0
PLATE AVERAGE COUNT (# / drop)	105.00	38.50	14.33	6.67	31.00	18.00			0.33
IMPINGER CONCENTRATION (cfu or pfu/ml)	1,050,000	385,000	143,333	66,667	31,000	18,000			0
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.40E+05	3.08E+05	1.15E+05	5.33E+04	2.48E+04	1.44E+04			1.42E-01
Dilution Range #1 — **DILUTION RATIO (10^x)**	-4	-4	-4	-2	-3	-3	-1	-1	-1
DROPLET SIZE (µl)	100	100	100	100	100	100	750	750	750
ENUMERATED PLATE COUNTS (# / drop)	7	4		78	1	4			1
	8	3		62	4	2			0
	12			57	5	0			0
PLATE AVERAGE COUNT (# / drop)	9.00	3.50		65.67	3.33	2.00			
IMPINGER CONCENTRATION (cfu or pfu/ml)	900,000	350,000		65,667	33,333	20,000			
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.20E+05	2.80E+05		5.25E+04	2.67E+04	1.60E+04			

Viable Cascade Sampling Conditions ***Statistical Correction Applied Automatically for counts>60**

SAMPLE TIME (min)	0	30	60	90	120	150			LOD
Viable Cascade #1 — **VIABLE CASCADE SAMPLING TIME (min)**	1.0	2.0	3.0	4.0	5.0	6.0	7.0	10.0	
VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
ENUMERATED PLATE COUNTS (# / plate)									
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)									
PLATE AVERAGE COUNT (# / plate)									
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)									
Viable Cascade #2 — **VIABLE CASCADE SAMPLING TIME (min)**	0.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
ENUMERATED PLATE COUNTS (# / plate)									
STATISTICALLY CORRECTED PLATE COUNTS (# / plate)									
PLATE AVERAGE COUNT (# / plate)									
CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)									

Figure 1a: *Staph Control*



Trial Information

TEST DATE:	Friday, March 12, 2021
TRIAL PERFORMED BY:	WDS
TRIAL NUMBER:	T1
TEST ORGANSIM:	Staphylococcus epidermidis
TRIAL NAME ID (GRAPHS/TABLES):	Staph T1

Device Information

MANUFACTURER:	Tadiran
UNIT MODEL:	Air Care O2
FAN SPEED (CFM):	
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	
TRIAL COMMENTS/NOTES	

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SAMPLE TIME (min)	0	30	60	90	120	150			LOD
IMPINGER USED (y / n)	y	y	y	y	y	y			y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n			n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.378E+06	1.222E+04	8.444E+02	1.778E+02	6.400E+00	9.333E-01			2.963E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	54.12%								100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.378E+06	1.222E+04	8.444E+02	1.778E+02	6.400E+00	9.333E-01			0.0296
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.8871%	0.0613%	0.0129%	0.0005%	0.0001%			0.0000%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.1129%	99.9387%	99.9871%	99.9995%	99.9999%			100.0000%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-2.05	-3.21	-3.89	-5.33	-6.17			-7.67

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
	IMPINGER FILL VOL (ml)	5.0	5.0	5.0	5.0	5.0	5.0			5.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	5.0	5.0	5.0	5.0			10.0
	IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5	7.5			7.5
Dilution Range #1	**DILUTION RATIO (10^x)**	-5	-3	-2	-1	0	0	0	0	0
	DROPLET SIZE (µl)	100	100	100	100	500	500	750	750	750
	ENUMERATED PLATE COUNTS (# / drop)	4	3	6	14	25	3			1
		7	4	7	13	23	4			0
		6	4	6	13					0
	PLATE AVERAGE COUNT (# / drop)	5.67	3.67	6.33	13.33	24.00	3.50			0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	5,666,667	36,667	6,333	1,333	48	7			0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.89E+06	1.22E+04	8.44E+02	1.78E+02	6.40E+00	9.33E-01			2.96E-02
Dilution Range #1	**DILUTION RATIO (10^x)**	-4	-3	-3	-2	0	0	0	0	0
	DROPLET SIZE (µl)	100	100	100	100	500	500	750	750	750
	ENUMERATED PLATE COUNTS (# / drop)	20								1
		30								0
		28								0
	PLATE AVERAGE COUNT (# / drop)	26.00								
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,600,000								
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	8.67E+05								

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	1.0	2.0	3.0	4.0	5.0	6.0	7.0	10.0	
	VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
	ENUMERATED PLATE COUNTS (# / plate)						17			
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)						17			
	PLATE AVERAGE COUNT (# / plate)						17.00			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)						0.094			
Viable Cascade #2	**VIABLE CASCADE SAMPLING TIME (min)**	0.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
	VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
	ENUMERATED PLATE COUNTS (# / plate)									
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)									
	PLATE AVERAGE COUNT (# / plate)									
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)									

Figure 2a: *Staph Trial 1*

Trial Information

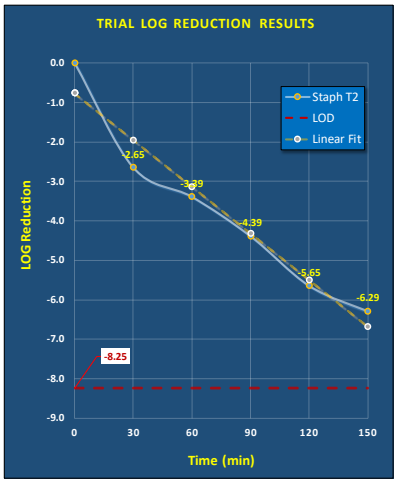
TEST DATE:	Friday, March 12, 2021
TRIAL PERFORMED BY:	WDS
TRIAL NUMBER:	T2
TEST ORGANSIM:	Staphylococcus epidermidis
TRIAL NAME ID (GRAPHS/TABLES):	Staph T2

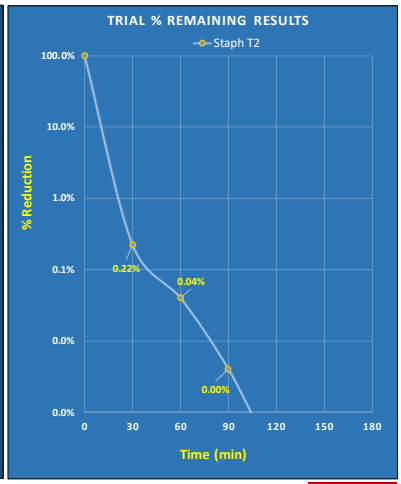
Device Information

MANUFACTURER:	Tadiran
UNIT MODEL:	Air Care O2
FAN SPEED (CFM):	
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	30	60	90	120	150			LOD
IMPINGER USED (y / n)	y	y	y	y	y	y			y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n			n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	5.222E+06	1.167E+04	2.111E+03	2.111E+02	1.167E+01	2.667E+00			2.963E-02
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		25.00%							100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**5.222E+06**	**1.167E+04**	**2.111E+03**	**2.111E+02**	**1.167E+01**	**2.667E+00**			**0.0296**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**0.2234%**	**0.0404%**	**0.0040%**	**0.0002%**	**0.0001%**			**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**99.7766%**	**99.9596%**	**99.9960%**	**99.9998%**	**99.9999%**			**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-2.65**	**-3.39**	**-4.39**	**-5.65**	**-6.29**			**-8.25**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
	IMPINGER FILL VOL (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	10.0
	IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-2**	**-2**	**-1**	**0**	**0**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**500**	**500**	**500**	**750**
	ENUMERATED PLATE COUNTS (# / drop)	14 16 17	20 35 35	6 8 5	7 6 6	17 18	4 4			1 0 0
	PLATE AVERAGE COUNT (# / drop)	15.67	30.00	6.33	6.33	17.50	4.00			0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	15,666,667	30,000	6,333	633	35	8			0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	5.22E+06	1.00E+04	2.11E+03	2.11E+02	1.17E+01	2.67E+00			2.96E-02
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-3**	**-2**	**-1**	**-1**	**-1**	**-1**	**-1**	**0**
	DROPLET SIZE (µl)	**50**	**100**	**50**	**750**	**750**	**750**	**750**	**750**	**750**
	ENUMERATED PLATE COUNTS (# / drop)		4 4 4							1 0 0
	PLATE AVERAGE COUNT (# / drop)		4.00							
	IMPINGER CONCENTRATION (cfu or pfu/ml)		40,000							
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		1.33E+04							

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**10.0**	
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	
	ENUMERATED PLATE COUNTS (# / plate)						22			
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)						22			
	PLATE AVERAGE COUNT (# / plate)						22.00			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)						0.122			
Viable Cascade #2	**VIABLE CASCADE SAMPLING TIME (min)**	**0.5**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	
	VIABLE CASCADE FLOW RATE (lpm)	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	
	ENUMERATED PLATE COUNTS (# / plate)									
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)									
	PLATE AVERAGE COUNT (# / plate)									
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)									

Figure 3a: *Staph Trial 2*

Trial Information

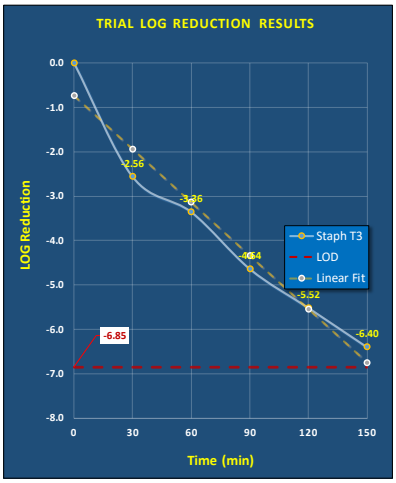
TEST DATE:	Monday, March 15, 2021
TRIAL PERFORMED BY:	WDS
TRIAL NUMBER:	T3
TEST ORGANSIM:	Staphylococcus epidermidis
TRIAL NAME ID (GRAPHS/TABLES):	Staph T3

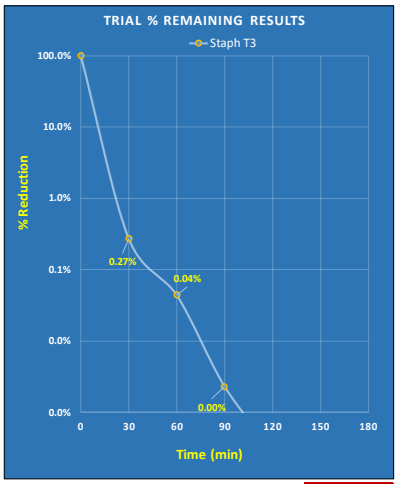
Device Information

MANUFACTURER:	Tadiran
UNIT MODEL:	Air Care O2
FAN SPEED (CFM):	
UNIT SERIAL #:	
FITER ID #:	
FILTER LOT #:	

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 20 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	
TRIAL COMMENTS/NOTES	





BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5	S6	S7	S8	LOD
SAMPLE TIME (min)	0	30	60	90	120	150			LOD
IMPINGER USED (y / n)	y	y	y	y	y	y			y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n			n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.011E+06	2.756E+03	4.444E+02	2.311E+01	3.067E+00	4.000E-01			1.422E-01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)									
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	48.33%	62.22%							100.00%
VIABLE CONSISTENCY CHECKS (% agreement)									
IMP & VIABLE CROSS CHECK (% agreement)									
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.011E+06	2.756E+03	4.444E+02	2.311E+01	3.067E+00	4.000E-01			0.1422
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.2725%	0.0440%	0.0023%	0.0003%	0.0000%			0.0000%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.7275%	99.9560%	99.9977%	99.9997%	100.0000%			100.0000%
LOG REDUCTION FROM T=0 (log_{10})	0.00	-2.56	-3.36	-4.64	-5.52	-6.40			-6.85

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
	IMPINGER FILL VOL (ml)	5.0	5.0	5.0	5.0	5.0	5.0			20.0
	IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	5.0	5.0	10.0			5.0
	IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5	7.5			12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	-4	-2	-2	0	0	0	0	0	0
	DROPLET SIZE (µl)	100	100	100	100	500	500	500	500	750
	ENUMERATED PLATE COUNTS (# / drop)	18	11	4	14	9	4			1
		19	11	2	18	14	2			0
		25	12	4	20					0
	PLATE AVERAGE COUNT (# / drop)	20.67	11.33	3.33	17.33	11.50	3.00			0.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,066,667	11,333	3,333	173	23	6			0
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.89E+05	1.51E+03	4.44E+02	2.31E+01	3.07E+00	4.00E-01			1.42E-01
Dilution Range #1	**DILUTION RATIO (10^x)**	-5	-3	-2	-1	-1	-1	-1	-1	0
	DROPLET SIZE (µl)	50	100	50	750	750	750	750	750	750
	ENUMERATED PLATE COUNTS (# / drop)	1	1							1
		2	3							0
		3	5							0
	PLATE AVERAGE COUNT (# / drop)	2.00	3.00							
	IMPINGER CONCENTRATION (cfu or pfu/ml)	4,000,000	30,000							
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.33E+06	4.00E+03							

Viable Cascade Sampling Conditions ****Statistical Correction Applied Automatically for counts>60**

	SAMPLE TIME (min)	0	30	60	90	120	150			LOD
Viable Cascade #1	**VIABLE CASCADE SAMPLING TIME (min)**	1.0	2.0	3.0	4.0	5.0	10.0	11.0	10.0	
	VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
	ENUMERATED PLATE COUNTS (# / plate)						65			
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)						71			
	PLATE AVERAGE COUNT (# / plate)						71.12			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)						0.237			
Viable Cascade #2	**VIABLE CASCADE SAMPLING TIME (min)**	0.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
	VIABLE CASCADE FLOW RATE (lpm)	30	30	30	30	30	30	30	30	
	ENUMERATED PLATE COUNTS (# / plate)									
	STATISTICALLY CORRECTED PLATE COUNTS (# / plate)									
	PLATE AVERAGE COUNT (# / plate)									
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)									

Figure 4a: *Staph Trial 3*

Appendix B: Calculations

To evaluate the viable aerosol delivery efficiency and define operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination were derived using the following steps:

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) in pfu/mL or cfu/mL, or cfu/g for dry powder.
- Collison 24 jet nebulizer liquid use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 30 psi air supply pressure = 1.0 ml/min.
- Collison 24 jet Generation time (t) = 20 or 30 minutes, test dependent.
- Chamber volume (V_c) = 15,993 Liters
- Nebulizer Generation efficiency (ε) (usually around 10%)

Assuming 100% efficiency, the quantity of aerosolized viable particles (V_P) per liter of air in the chamber for a given nebulizer stock concentration (C_s) is calculated as:

$$\text{Nebulizer: } V_P = \frac{C_s \cdot R_{neb}}{V_c} t \cdot \varepsilon$$

Midget impinger or 47mm filter collection calculation:

- Viable aerosol concentration collection (C_a) = cfu or pfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu or pfu/mL from enumeration of impinger sample or filter sample.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger, or extraction fluid for filter.
- Midget impinger or filter sample flow rate (Q_{imp}) = 12.5 L/min.
- Midget impinger or filter sample time (t) = 5 or 10 minutes, test dependent.

For viable impinger or filter aerosol concentration collection (C_a) = cfu or pfu/L of chamber air:

$$C_a = \frac{\mathrm{C_{Imp}} \cdot \mathrm{I_{vol}}}{\mathrm{Q_{imp}}} \mathrm{t}$$