Efficacy of Radic8 Hextio against Aerosolized MS2 Bacteriophage – 1m^3 Chamber

Sean McLeod [a], Richard Ludwick [a], Jamie Balarashti [a]

[a] Aerosol Research and Engineering Laboratories Inc. Olathe KS

Article Info

Testing Lab:

Aerosol Research and Engineering Laboratories, Inc.
Project #: 10906.50

Keywords:

· *Hextio*
· *MS2 bacteriophage*
· *Bioaerosol Efficacy*

Conflict of Interest:

Aerosol Research and Engineering Laboratories, Inc. have no affiliations with, or involvement in any capacity, with Radic8's financial interests such as; membership, employment, stock ownership, or other equity interest.

Background: This study characterized the decontamination efficacy of the Radic8 Hextio indoor air purifier against aerosolized MS2 bacteriophage which is commonly used as a surrogate for influenza and Sars-Cov-2. The Radic8 Hextio is a device that uses a combination of filtration and UVC, with TiO_2 coated hexagon tubes, to capture and inactivate aerosolized pathogens. The effectiveness of the system was assessed in a 1m^3 bioaerosol chamber for a single stranded RNA virus, MS2, which was tested in triplicate, in addition to a single control trial.

Methods: MS2 bacteriophage was aerosolized into a sealed 1m^3 environmental chamber containing the Radic8 Hextio indoor air purifier. An AGI 30 impinger was 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 concentrations at each sampling point and time. Chamber control trial data was subtracted from Radic8 Hextio trial data to yield net log reduction in the chamber for viable bioaerosol concentration.

Results: Three trials were conducted to evaluate the Radic8 Hextio device's efficacy at removing viable MS2 bacteriophage from the air. The Radic8 Hextio device reduced MS2 bioaerosol by 4.17 +/- 0.22 (99.99%) net log reduction in a 90-minute time period. After 180 minutes the device achieved a total 6.06 +/- 0.28 (99.9999%) net log reduction in the 1m^3 test chamber.

Conclusion: Overall, the Radic8 Hextio system performed very well with a 99.99% net reduction in viable bioaerosol concentration within an 85 minute period. This testing confirms that, in theory, the Radic8 Hextio indoor air purifier should show efficacy at reducing the risk of exposure to similar pathogens.

Overview

This study was conducted to evaluate the efficacy of the Radic8 Hextio at removing viable bioaerosols from room air. The Hextio is a device designed for personal use, to deliver a direct flow of clean air at an individual's desk, counter top, or bedside table. A picture of the device can be found in **Figure 1**.

Testing was conducted in a 1m^3 custom bioaerosol exposure chamber. The device's effectiveness was tested against the single stranded RNA virus, MS2 bacteriophage, in order to evaluate the system's net log reduction of viable bioaerosol within the chamber. Testing was conducted in triplicate trials, plus a control trial, to demonstrate the device's capability for reducing viable bioaerosol concentrations. There were a total of four (4) independent trials in this study.

During the control trial, the Hextio system remained inside the test chamber but remained off. During test trials, the system was turned on after initial chamber concentration sampling and remained operating until the completion of the trial. MS2 bacteriophage was aerosolized into the test chamber and impinger samples were collected at set time points throughout each trial. Trials in which the Hextio device was turned on were compared to control trials to determine net log reduction of viable bioaerosols within the chamber.

Figure 1: Radic8 Hextio 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 Radic8 Hextio device is a sealed 1m^3 environmental chamber constructed of 3/8" Lexan, measuring about 30"D x 30"T x 66"W, and outfitted with all necessary pass-through and sub-systems sampling ports **(Figure 2 below)**. 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 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 in H_2O, 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 an impinger sample port located near the center of the box.

The chamber is 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 then turned off until samples were taken.

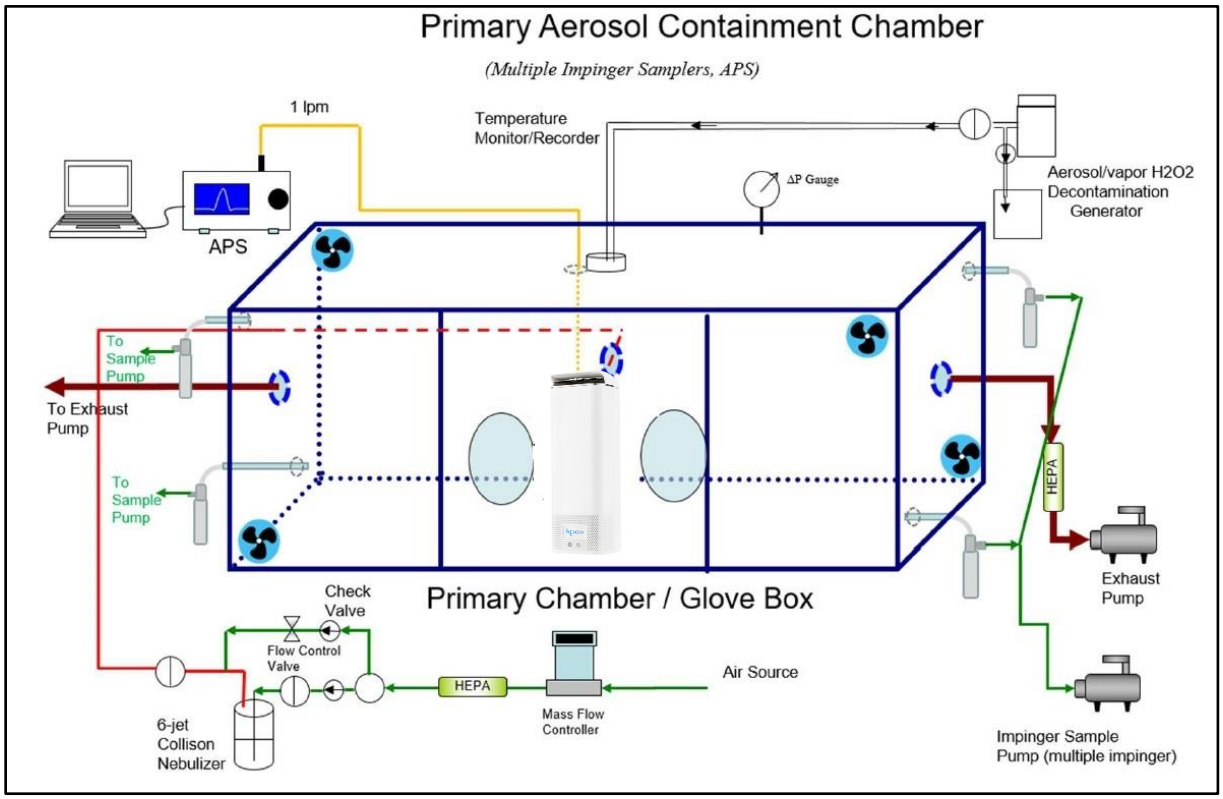

Figure 2: Test Chamber Flow diagram for testing.

Biological Test Matrix										
Trial	Run	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1 2 3 4	Control Challenge Challenge Challenge	*Influenza* , (tentative surrogate for *Sars-cov2*)	MS2 bacteriophage (E. coli phage)	15597-B1	<1.0um	10^4-10^6	180	0, 30, 60, 90, 120, 180	Impingers	all samples in triplicate

Figure 3: Testing matrix for the chamber test.

Bioaerosol Sampling and Monitoring System

An AGI 30 impinger (Ace Glass Inc., Vineland NJ) was used for bioaerosol collection 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 impinger 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 sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter. The impingers were filled with 20 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 virus for proper plate counts. Impingers were taken and pooled for an overall average of chamber concentration.

Test Matrix

To accurately test the Radic8 Hextio device, triplicate challenge trials were performed in the test chamber. Additionally, a control trial was run in order to characterize the device's performance while taking into account the natural losses of the bioaerosol in the chamber. A testing matrix for the device can be found in **Figure 3**.

Species Selection

Species selection was based on Biological Safety Level 1 (BSL1) surrogates for BSL2 and BSL3 pathogenic organisms. MS2 is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus and Sars-Cov-2.

Viral Challenge

MS2 bacteriophage is a viral single-stranded, non-enveloped RNA bacteriophage that has historically been used as a surrogate for influenza viruses. MS2 has also recently been used as a tentative surrogate for SARS-CoV-2 in numerous published bioaerosol studies. The US FDA guidance document, *Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency*, states that lipid enveloped viruses, such as coronaviruses, are the least resistant microorganisms to germicidal chemicals. It is presumed that this susceptibility is similar for other chemical, physical and catalytic methods of destruction.

MS2 is a non-enveloped viruses, which makes it more resistant to disinfection than lipid viruses, and therefore, should represent a "worst case scenario" when compared to actual lipid-enveloped RNA viruses like SARS-CoV-2. **Figure 4** is a graphic from the FDA document, *COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance*, demonstrating resistance to disinfection.

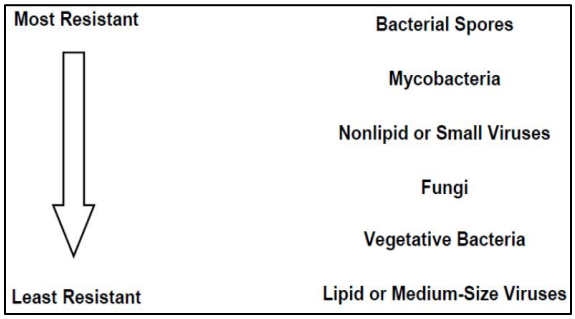

Figure 4: FDA graphic demonstrating general resistance to disinfection for various microorganisms. FDA, Guidance Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers during the Coronavirus Disease 2019 (COVID-19). Pg. 7. March 2009. SAR-CoV-2 (lipid or medium-Sized Virus), MS2 (non-lipid small virus).

For UV-C deactivation, MS2 is also a much more resilient organism than SARS-CoV-2. To achieve a 1 LOG deactivation of MS2 it takes 15.9 mJ/cm^2 of UV irradiation (Wilson et al 1992), coronaviruses such as SARS-CoV-2 takes 3.7mJ/cm^2 (Heßling et al 2020).

These results were obtained by investigations on many different coronaviruses, including SARS-CoV and MERS-CoV, but not SARS-CoV-2. Nevertheless, it can be assumed that they are also applicable for SARS-CoV-2 and all future mutations. RNA mutations might have a strong influence on the pathogenicity of a virus, but they do not result in larger structural differences, especially concerning the UV absorption properties of the RNA, which are the main cause for the antiviral effect of ultraviolet radiation.

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown overnight in Tryptic Soy Broth. The liquid cell suspension was infected during the logarithmic growth cycle with the MS2 bacteriophage. After an appropriate incubation time (approximately 24 hours), the cells were lysed and the cellular debris separated by centrifugation.

MS2 stock yields were greater than 1×10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS to approximately 1×10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collison nebulizer.

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.

Control Testing Method

To accurately assess the Radic8 Hextio unit, test chamber pilot control trials were performed with MS2 bacteriophage 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 Hextio challenge testing and verify that chamber concentrations persisted above the required concentrations over the entire pilot control test period.

Radic8 Hextio Testing Method

For each control and challenge test, the Collison nebulizer was filled with approximately 50 mL of biological stock and operated at 50 psi for a period of 10 minute. 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. Impinger samples were collected for different durations 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 six times 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 180 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.

All samples were plated in triplicate on tryptic soy agar media over a minimum of a 3 log dilution range. Plates were incubated and enumerated for viable plaque forming unit (pfu) 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 killing of the virus.

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.

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. The values depicted on each graph represents the group average at that time point.

Radic8 Hextio Results

The control trial was observed having a 0.64 log reduction of MS2 after 180 minutes of sample collection time using the AGI 30 impingers. When tested, the device showed a steady reduction throughout all of the trials. At the 90 minute time point, the device showed an average 4.17 +/- 0.22 net log reduction. After the 180 minute time point the average total net log reduction was 6.06 +/- 0.28. The reduction capabilities of the device, as well as an average for all the trials, can be found in **Figures 5** and the net log reduction in **Figure 6** below.

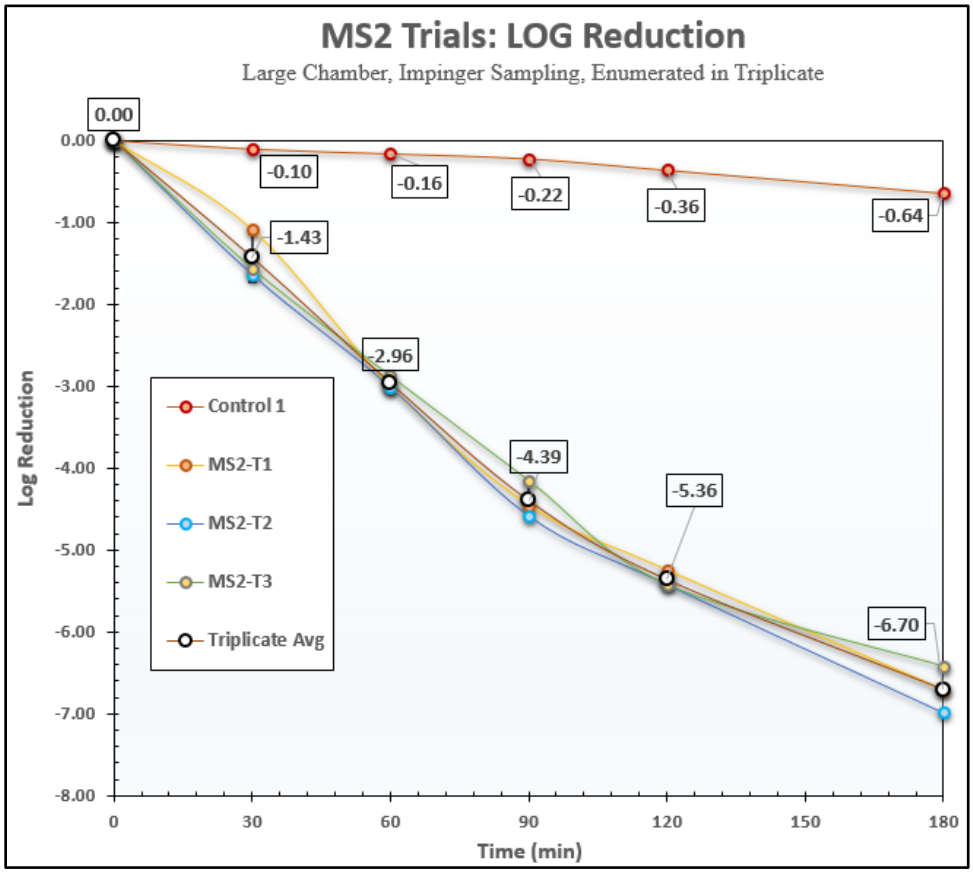

Figure 5: Log reduction of MS2 in control and device challenge trials.

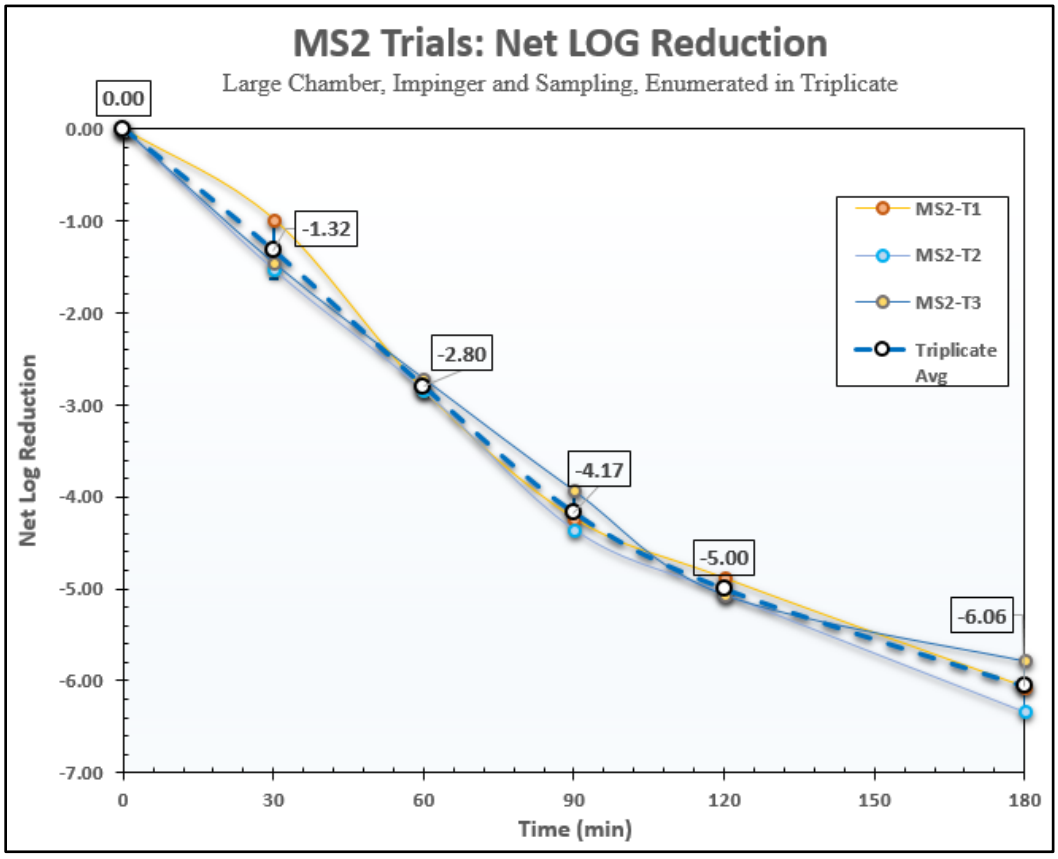

Figure 6: Net log reduction of MS2 in challenge trials.

Summary

The Radic8 Hextio air purifier performed well with a bioaerosol reduction of over 99.99% in 85 minutes. The viable airborne MS2 concentrations started at 1 x 10^7 pfu/L of air in the 1m^3 enclosure. After 180-minutes there was an average 3 pfu/L remaining in the chamber. The results for the trials, including group averages and standard deviations, can be found in a summary table in **Figure 7**.

Radic8 Hextio MS2 Trial Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes) 30	60	90	120	180
Virus	MS2 (RNA E. coli phage)	MS2-T1	*Reduction*	-0.98	-2.84	-4.23	-4.88	-6.07
			Net % Reduction	89.6187%	99.8571%	99.9941%	99.9987%	99.9999%
Virus	MS2 (RNA E. coli phage)	MS2-T2	*Net Log Reduction*	-1.53	-2.84	-4.35	-5.05	-6.34
			Net % Reduction	97.0586%	99.8570%	99.9956%	99.9991%	100.0000%
Virus	MS2 (RNA E. coli phage)	MS2-T3	*Net Log Reduction*	-1.46	-2.71	-3.93	-5.06	-5.78
			Net % Reduction	96.5145%	99.8071%	99.9882%	99.9991%	99.9998%
All Trial Averages			***Net Log Reduction***	**-1.32 +/- 0.3**	**-2.8 +/- 0.08**	**-4.17 +/- 0.22**	**-4.999 +/- 0.1**	**-6.06 +/- 0.28**
			Net % Reduction	***94.3973% +/- 4.1473%***	***99.8404% +/- 0.0288%***	***99.9927% +/- 0.0039%***	***99.999% +/- 0.0002%***	***99.9999% +/- 0.0001%***

Figure 7: Summary Data Table for Net LOG Reduction of the Radic8 Hextio.

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

10906.50

Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories

Conflict of Interest Statement

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Study Director:

Jamie D. Balarashti
Study Director
ARE Labs, Inc.

11/10/2021
Date

Principal Investigator:

Sean McLeod
Principal Investigator
ARE Labs, Inc.

11/10/2021
Date

Appendix A: Raw Data

Trial Information

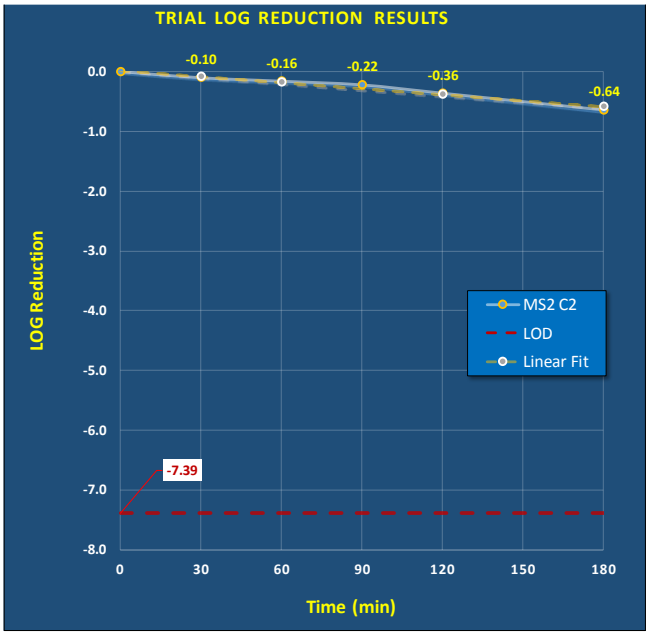
TEST DATE:	Wednesday, September 15, 2021
TRIAL PERFORMED BY:	WDS
TRIAL NUMBER:	C2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 C2

Device Information

MANUFACTURER:	Radic8
UNIT MODEL:	Hextio
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. 5 min neb
SAMPLING METHOD:	AGI 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
SAMPLE TIME (min)	0	30	60	90	120	180
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	3.488E+06	2.752E+06	2.416E+06	2.096E+06	1.525E+06	8.000E+05
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)						
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**3.488E+06**	**2.752E+06**	**2.416E+06**	**2.096E+06**	**1.525E+06**	**8.000E+05**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**78.8991%**	**69.2661%**	**60.0917%**	**43.7309%**	**22.9358%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**21.1009%**	**30.7339%**	**39.9083%**	**56.2691%**	**77.0642%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.10**	**-0.16**	**-0.22**	**-0.36**	**-0.64**

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	30	60	90	120	180
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	5.0	5.0	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-4**	**-4**	**-4**	**-4**	**-4**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	117 114 96	90 82	77 74	67 64	66 40 37	29 27 19
	PLATE AVERAGE COUNT (# / drop)	109.00	86.00	75.50	65.50	47.67	25.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	10,900,000	8,600,000	7,550,000	6,550,000	4,766,667	2,500,000
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**3.49E+06**	**2.75E+06**	**2.42E+06**	**2.10E+06**	**1.53E+06**	**8.00E+05**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-5**	**-5**		**-5**	**-2**
	DROPLET SIZE (µl)	**100**	**100**	**100**		**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)						
	PLATE AVERAGE COUNT (# / drop)						
	IMPINGER CONCENTRATION (cfu or pfu/ml)						
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)						

Figure 1a: *MS2 Control.*

Trial Information

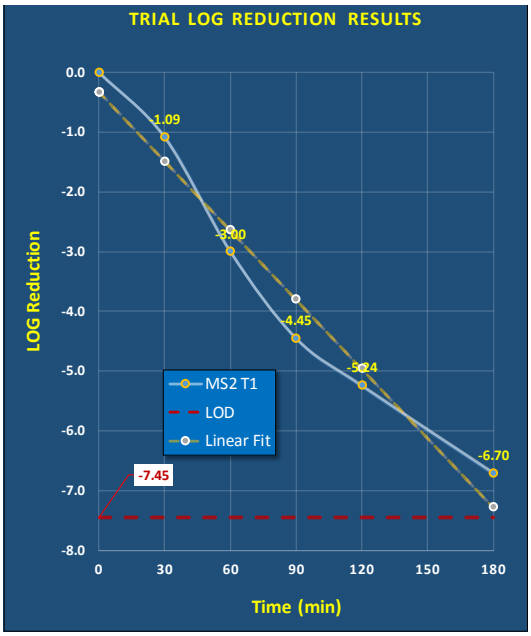
TEST DATE:	Friday, November 5, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T1

Device Information

MANUFACTURER:	Radic8
UNIT MODEL:	Hextio
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
SAMPLE TIME (min)	**0**	**30**	**60**	**90**	**120**	**180**
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	4.053E+06	3.320E+05	4.013E+03	1.427E+02	2.315E+01	8.000E-01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	**31.11%**	**61.67%**	**32.78%**	**12.28%**	**50.34%**	
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**4.053E+06**	**3.320E+05**	**4.013E+03**	**1.427E+02**	**2.315E+01**	**8.000E-01**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**8.1908%**	**0.0990%**	**0.0035%**	**0.0006%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**91.8092%**	**99.9010%**	**99.9965%**	**99.9994%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.09**	**-3.00**	**-4.45**	**-5.24**	**-6.70**

Impinger Sampling Conditions

	SAMPLE TIME (min)	**0**	**30**	**60**	**90**	**120**	**180**
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	5.0	10.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	7 6 5	19 22 28	5 6 7	1 1 3	10 10 9	3 2
	PLATE AVERAGE COUNT (# / drop)	6.00	23.00	6.00	1.67	9.67	2.50
	IMPINGER CONCENTRATION (cfu or pfu/ml)	6,000,000	230,000	6,000	167	97	5
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**4.80E+06**	**1.84E+05**	**4.80E+03**	**1.33E+02**	**3.09E+01**	**8.00E-01**
Dilution Range #1	**DILUTION RATIO (10^x)**	**-4**	**-4**	**-1**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	37 47 40	6 5 7	39 44 38	22 14 21	24	
	PLATE AVERAGE COUNT (# / drop)	41.33	6.00	40.33	19.00	24.00	
	IMPINGER CONCENTRATION (cfu or pfu/ml)	4,133,333	600,000	4,033	190	48	
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**3.31E+06**	**4.80E+05**	**3.23E+03**	**1.52E+02**	**1.54E+01**	

Figure 2a: *MS2 T1.*

Trial Information

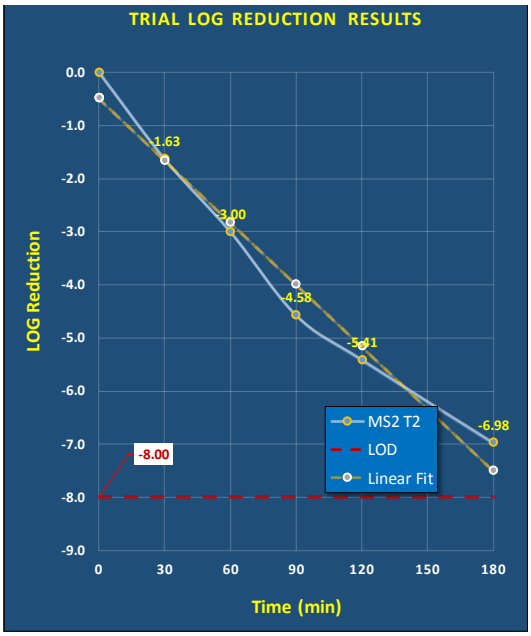
TEST DATE:	Friday, November 5, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T2

Device Information

MANUFACTURER:	Radic8
UNIT MODEL:	Hextio
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
SAMPLE TIME (min)	**0**	**30**	**60**	**90**	**120**	**180**
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.413E+07	3.280E+05	1.400E+04	3.760E+02	5.472E+01	1.493E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		**10.77%**	**38.46%**	**25.93%**	**37.14%**	**44.44%**
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.413E+07**	**3.280E+05**	**1.400E+04**	**3.760E+02**	**5.472E+01**	**1.493E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**2.3208%**	**0.0991%**	**0.0027%**	**0.0004%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**97.6792%**	**99.9009%**	**99.9973%**	**99.9996%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.63**	**-3.00**	**-4.58**	**-5.41**	**-6.98**

Impinger Sampling Conditions

	SAMPLE TIME (min)	**0**	**30**	**60**	**90**	**120**	**180**
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	5.0	10.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	18 16 19	36 35 45	22 25 18	5 4 3	18 27 18	2 10
	PLATE AVERAGE COUNT (# / drop)	17.67	38.67	21.67	4.00	21.00	6.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	17,666,667	386,667	21,667	400	210	12
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.41E+07	3.09E+05	1.73E+04	3.20E+02	6.72E+01	1.92E+00
Dilution Range #1	**DILUTION RATIO (10^x)**	**-5**	**-4**	**-3**	**0**	**0**	**0**
	DROPLET SIZE (µl)	**50**	**100**	**100**	**100**	**500**	**100**
	ENUMERATED PLATE COUNTS (# / drop)		5 2 6	2 1 1	47 56 59	66	1 1 0
	PLATE AVERAGE COUNT (# / drop)		4.33	1.33	54.00	66.00	0.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)		433,333	13,333	540	132	7
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		3.47E+05	1.07E+04	4.32E+02	4.22E+01	1.07E+00

Figure 3a: *MS2 T2.*

Trial Information

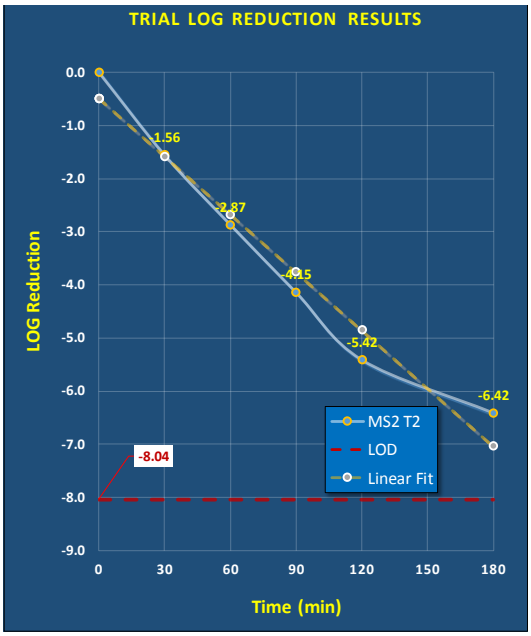
TEST DATE:	Friday, November 5, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T2

Device Information

MANUFACTURER:	Radic8
UNIT MODEL:	Hextio
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
SAMPLE TIME (min)	0	30	60	90	120	180
IMPINGER USED (y / n)	y	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.547E+07	4.253E+05	2.067E+04	1.093E+03	5.888E+01	5.893E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)						
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)		22.78%	17.65%	4.76%	24.76%	0.90%
VIABLE CONSISTENCY CHECKS (% agreement)						
IMP & VIABLE CROSS CHECK (% agreement)						
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.547E+07**	**4.253E+05**	**2.067E+04**	**1.093E+03**	**5.888E+01**	**5.893E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**2.7500%**	**0.1336%**	**0.0071%**	**0.0004%**	**0.0000%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**97.2500%**	**99.8664%**	**99.9929%**	**99.9996%**	**100.0000%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.56**	**-2.87**	**-4.15**	**-5.42**	**-6.42**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	30	60	90	120	180
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	5.0	10.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5	12.5

Dilution Range #1							
	DILUTION RATIO (10^x)	**-5**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	18	50	28	10	22	25
		16	49	24	17	17	12
		24	40	33	15	24	
	PLATE AVERAGE COUNT (# / drop)	19.33	46.33	28.33	14.00	21.00	18.50
	IMPINGER CONCENTRATION (cfu or pfu/ml)	19,333,333	463,333	28,333	1,400	210	37
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.55E+07	3.71E+05	2.27E+04	1.12E+03	6.72E+01	5.92E+00

Dilution Range #1							
	DILUTION RATIO (10^x)	**-5**	**-4**	**-3**	**-2**	**0**	**0**
	DROPLET SIZE (µl)	**50**	**100**	**100**	**100**	**500**	**100**
	ENUMERATED PLATE COUNTS (# / drop)		6	2	1	79	4
			5	2	1		3
			7	3	2		4
	PLATE AVERAGE COUNT (# / drop)		6.00	2.33	1.33	79.00	3.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)		600,000	23,333	1,333	158	37
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)		4.80E+05	1.87E+04	1.07E+03	5.06E+01	5.87E+00

Figure 4a: *MS2 T3.*

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}) = 7.5 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:

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