Efficacy of the YFLife AIR6+ against aerosolized *MS2* bacteriophage in a 16m^3 Chamber

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Background: This in vitro study characterized the efficacy of the YFLife AIR6+ at removing aerosolized MS2 bacteriophage. The AIR6+ device is designed as a home air purifier, and features photocatalytic oxidation (PCO) technology as well as ionization which reduces airborne bacteria, viruses, and fungal spores in order to decrease infections rates from airborne pathogens. For this study, the device was challenged at the highest setting using aerosolized MS2 bacteriophage which has been historically used as a surrogate for numerous respiratory viruses, such as influenza, rhinovirus, and is a tentative surrogate for SARS-CoV-2. This study evaluated the efficacy of the device against aerosolized MS2 bacteriophage, in a stainless steel bioaerosol chamber. The study consisted of three (3) trials on the highest speed setting which is speed 3.

Methods: MS2 bacteriophage were aerosolized into a sealed environmental bioaerosol chamber containing an AIR6+ device. AGI impinger samples were taken at speed setting dependent time intervals from the chamber in order to quantify the reduction speed and capabilities of the AIR6+ device. AGI impingers were used to sample chamber bioaerosol concentrations and 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 YFLife AIR6+ trial data to yield net LOG reduction in the chamber for the bioaerosol challenges.

Results: When tested against the MS2 bacteriophage, the device showed a steady reduction throughout the triplicate trials. After 240-minutes the device yielded a 1.76 +/- 0.04 net LOG reduction. This amount of reduction is equivalent to a 98.2448% +/- 0.1477% reduction above the natural losses seen in the control trial. Considering the size of the device as compared to the size of the chamber this amount of reduction is sizable. The smaller the room the device is used in, the better it would be expected to perform.

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

Introduction

This study was conducted to evaluate the efficacy of the YFLife AIR6+ air purification device at reducing aerosolized MS2 bacteriophage. The YFLife AIR6+ device is an air purification intended for use in small to medium rooms. The unit has three different speed settings ranging from low to high. For this study, testing was conducted on the device on the highest setting (fan speed 3).

The YFLife AIR6+ contains a nano sterilization washable filter to block large particles, 3 dimensional Photocatalytic Oxidation (PCO) structure to purify incoming air, and an ionizer to reduce airborne particles. The test plan incorporated challenging the AIR6+ on the highest fan speed against the MS2 bacteriophage. A picture of the AIR6+ device is shown in **Figure 1,** on the following page.

Study Overview

The effectiveness of the YFLife AIR6+ was evaluated against the MS2 bacteriophage which is a single stranded RNA based non-enveloped virus commonly used as a surrogate for influenza. For more organism information please see species selection section in the body of this report.

Manufacturer:	YFLife
Model:	AIR6+
Notes:	Purify by PCO and ionization

Figure 1: *Picture of YFLife AIR6+ Device*

Testing was conducted to characterize the single AIR6+ unit against MS2 bacteriophage in three (3) independent trials, as well as a single (1) control trial to demonstrate the capability of the YFlife device to reduce viable bioaerosol concentrations.

Bioaerosol Testing Chamber

A large sealed aerosol test chamber was used to replicate a potentially contaminated room environment and to contain any potential release of bioaerosols into the surrounding environment.

The aerosol test chamber is constructed of 304 stainless steel and is equipped with three viewing windows and an air-tight lockable chamber door for system setup and general ingress and egress. The test chamber internal dimensions are 9.1 ft. x 9.1 ft. x 7 ft., with a displacement volume of 562 cubic feet, or 15,914 liters. **Figure 2** shows the bioaerosol chamber used for all testing in this study.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber was equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling, a 1-inch diameter port for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer for the aerosolization of the bacteriophage.

A ¼ inch diameter probe was used for continuous aerosol particle size monitoring via a TSI Aerodynamic Particle Sizer (APS) model 3321. All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects and at a height of approximately 40 inches from the floor. The aerosol sampling and aerosol dissemination probes are stainless steel and bulk headed through the chamber walls to provide external remote access to the aerosol generator and samplers during testing.

Figure 2: *Bioaerosol Test Chamber Exterior*

The test chamber is equipped with two high-flow HEPA filters for the introduction of filtered purified air into the test chamber during aerosol evacuation/purging of the system between test trials and a HEPA filtered exhaust

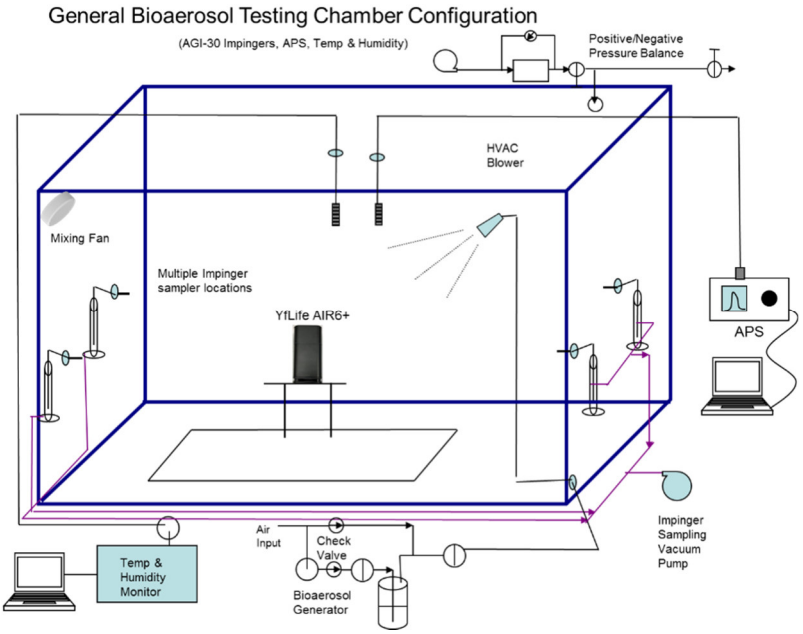

Figure 3*: Bioaerosol Test Chamber Flow Diagram*

blower with a 500 ft^3/min rated flow capability for rapid evacuation of remaining bioaerosols. A Magnehelic gauge with a range of 0.0 +/- 0.5 inch H_2O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 24-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 60 psi, which obtained an output volumetric flow rate of 50-80 LPM with a fluid dissemination rate of approximately 1.25 mL/min. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN). A picture of the Collison nebulizer can be found in **Figure 4**.

Figure 4*: BGI Collison Stainless Nebulizer. (6-Jet version pictured).*

Bioaerosol Sampling and Monitoring System

Two AGI impingers (Ace Glass Inc., Vineland NJ) were used for bio-aerosol collection of all biological aerosols to determine chamber concentration. The AGI-30 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 AGI-30 sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter. Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and AIR6+ trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc., St Paul MN). A general flow diagram of the aerosol test system is shown above in **Figure 3** above. A picture of an AGI-30 impinger can be found in **Figure 5**

Figure 5: *AGI-30 impinger used for bioaerosol sampling during trials*

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	Control	Influenza, (tentative surrogate for Sars-cov2)	*MS2 bacteriophage (E. coli phage)*	15597-B1	<1.0um	10^4-10^6	240	0, 30, 60, 120, 180, 240	APS, Impingers	all samples in triplicate
2	High Speed Challenge									
3	High Speed Challenge									
4	High Speed Challenge									

Figure 6: *Bioaerosol Test Matrices for All Trials*

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for BSL3 pathogenic organisms. MS2 bacteriophage (ATCC 15597-B1) is positive-sense, single-stranded RNA virus that infects the bacterium Escherichia coli and other members of the Enterobacteriaceae family. MS2 is routinely used as a surrogate for pathogenic RNA viruses, such as influenza and a tentative surrogate for coronavirus, SARS-CoV2.

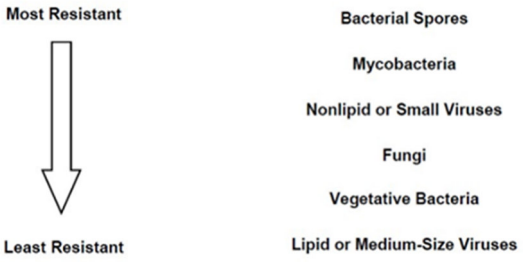

Figure 7: *FDA Graphic showing resistance to disinfection for various organisms.*

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 disinfectants. It is assumed that this trend is similar for other chemical and catalytic methods of kill. MS2 is a non-lipid enveloped virus. This appears to make it more resistant to disinfection than lipid viruses and therefore should be more resistant to kill when compared to SARS-CoV-2. **Figure 7** is a graphic from the FDA document, *COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance, demonstrating resistance to disinfection*.

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media.

The liquid media was infected during the logarithmic growth cycle with the specific bacteriophage. After an appropriate incubation time, the cells were lysed and the cellular debris separated by centrifugation. MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with deionized water to approximately 1 x 10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collision nebulizer.

Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a small drop plaque assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-48 hours and enumerated and recorded.

Bioaerosol Control Testing

To accurately assess the YFLife AIR6+ unit, test chamber pilot control trials were performed over a 240-minute period without the device in operation to characterize the biological challenge aerosol for particle size distribution, aerosol delivery/collection efficiency, and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual efficacy provided from the AIR6+.

During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on 1 minute prior to sampling and remained on during the entire AGI sampling period to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control runs and was also used during YFLife AIR6+ decontamination trials for consistency of method. The two impingers used for bacteriophage sampling were pooled and mixed prior to plating and enumeration to obtain average viable bioaerosol concentrations. A complete test matrix for all bioaerosol trials can be found in **Figure 6**.

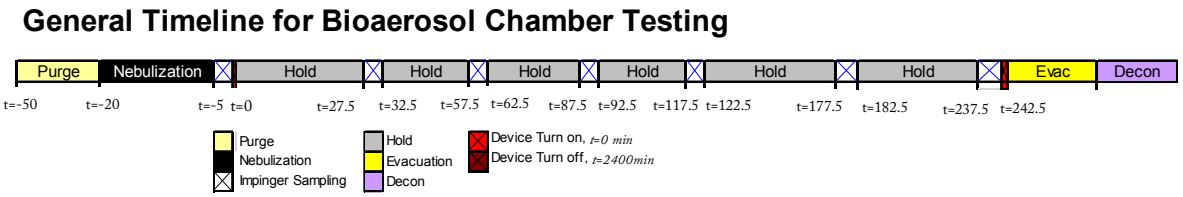

Figure 8: *General Trial Timeline for Bioaerosol Trials.*

YFLife AIR6+ Testing Method

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 50 psi for a period of 25 minutes. For control and AIR6+ trials, 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 microorganisms. The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to taking the first impinger sample.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and AIR6+ test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 2 to 5 minutes and at varying intervals during testing. **Figure 8** shows the general timeline for the bioaerosol challenge trials. Collected impinger chamber samples were pooled and mixed at each sample interval for each test. 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 YFLife AIR6+ biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test. Subsequent impinger samples were taken at set intervals and then samples were enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the YFLife device over time. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 3 log serial dilution range. Plates were incubated for 24 hours and enumerated for viable plaque forming units (pfu) to calculate aerosol challenge concentrations in the chamber for each sample time point.

Post-Testing Decontamination and Prep

Following each test, the chamber was evacuated/purged for a minimum of twenty minutes between tests. The chamber was decontaminated at the conclusion of each trial with aerosol/vaporous hydrogen peroxide (35%). The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 3% 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 for a final rinse.

Bioaerosol Particle Size Data

Aerosol particle size distributions were measured using the APS. The APS has a dynamic measurement range of 0.5 to 20.0 µm. Data was logged in real time to an Acer laptop computer, regressed, and plotted. The aerosol particle size distribution for MS2 is shown in **Figure 9**.

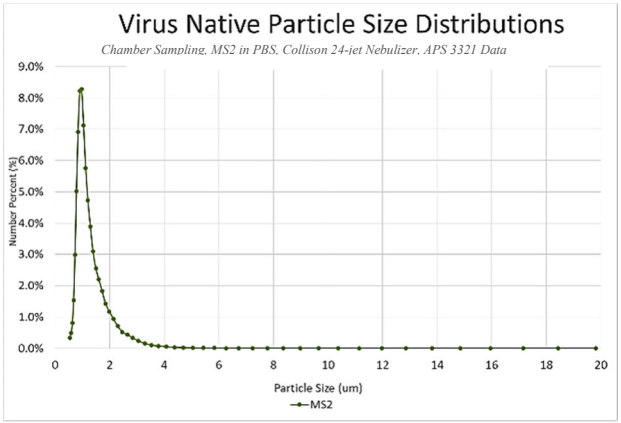

Figure 9: *Viral (MS2) Particle Size Distribution in Test Chamber*

Data Analysis

Results from the control trials were graphed and plotted to show natural viability loss over time in the chamber. These control runs served as the basis to determine the reduction in viable bioaerosol above the natural losses from the control runs. The control and trial runs are plotted showing log reduction in viable bioaerosol for each trial. All data is normalized with time zero (*t=0* minutes) enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time

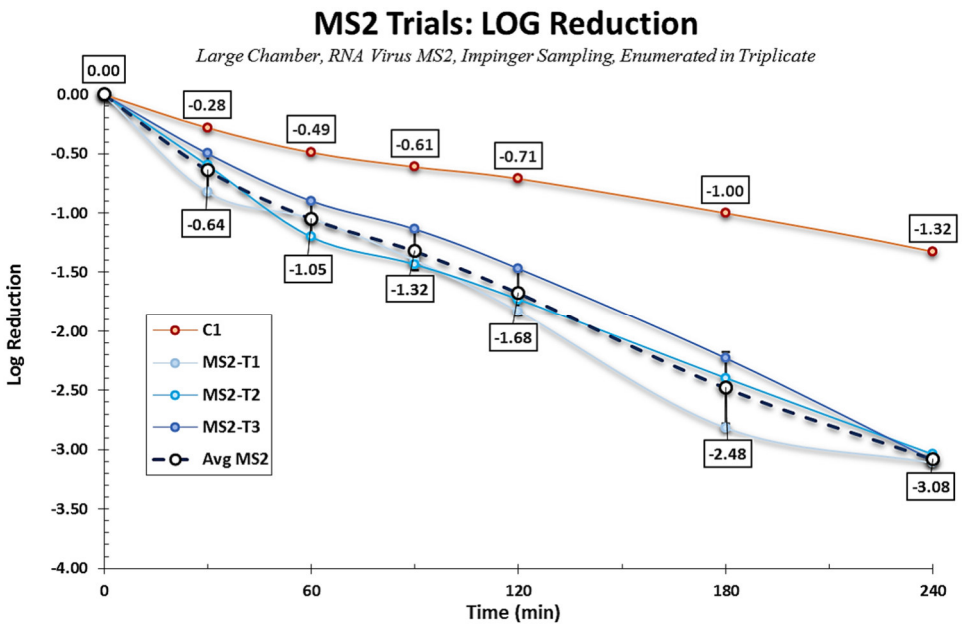

Figure 11: *LOG Reduction of MS2 by the YFlife AIR6+*

YFLife AIR6+ Results

The YFLife bioaerosol chamber trials with the *MS2* bacteriophage showed consistent reduction throughout testing on all three of the trials. At the 60-minute time point the device yielded a LOG reduction average of 1.05. When the natural reduction seen in the control trial is taken into account this equates to a 0.56 net LOG reduction.

At the 120-minute time point the YFLife AIR6+ device showed an average net LOG reduction of 0.97. When converted to net % reduction this amount of reduction is equal to 88.45% above natural losses. A graphical representation of the LOG reduction seen in the trials as well as the values seen in the control trial can be found in **Figure 11.** The net LOG reduction for the trials can be found in **Figure 12.** The data labels on the graphs represent the average of the triplicate trials.

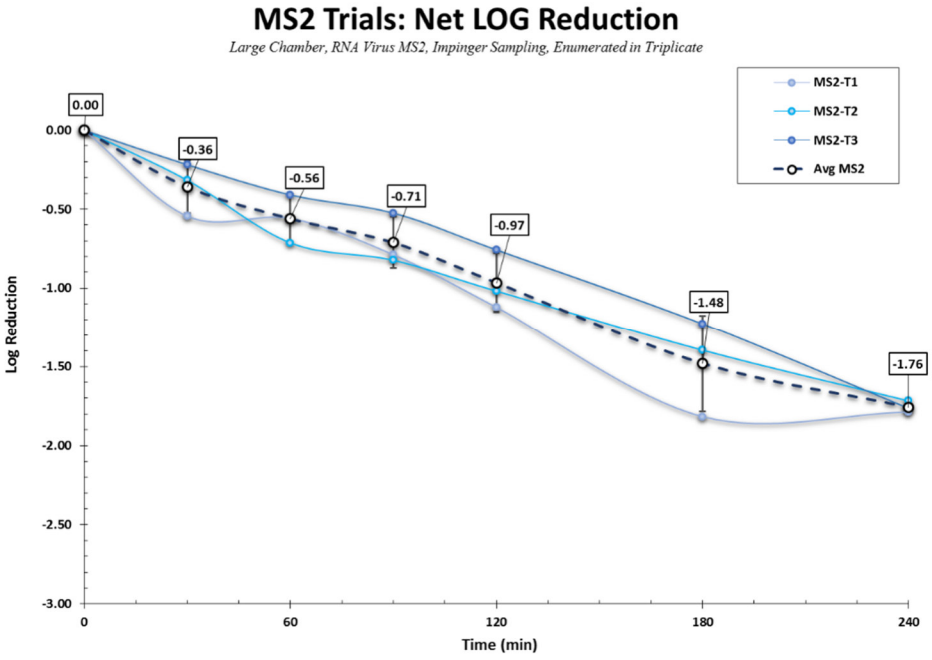

Figure 12: *MS2 YFLife AIR6+ Net LOG Reduction*

Bioaerosol Reduction Summary Table

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)					
				30	60	90	120	180	240
Virus	*MS2* (RNA E. coli phage)	*MS2-T1*	*Net Log Reduction*	-0.54	-0.56	-0.79	-1.12	-1.82	-1.79
			Net % Reduction	71.4181%	72.3077%	83.7048%	92.4359%	98.4750%	98.3684%
Virus	*MS2* (RNA E. coli phage)	*MS2-T2*	*Net Log Reduction*	-0.32	-0.71	-0.82	-1.02	-1.40	-1.72
			Net % Reduction	51.7605%	80.6037%	84.9424%	90.3927%	95.9747%	98.0813%
Virus	*MS2* (RNA E. coli phage)	*MS2-T3*	*Net Log Reduction*	-0.22	-0.41	-0.52	-0.76	-1.23	-1.77
			Net % Reduction	39.4487%	61.0256%	70.1254%	82.5261%	94.0741%	98.2846%
All Trial Averages			***Net Log Reduction***	**-0.36 +/- 0.17**	**-0.56 +/- 0.15**	**-0.71 +/- 0.16**	**-0.97 +/- 0.19**	**-1.48 +/- 0.3**	**-1.76 +/- 0.04**
			Net % Reduction	***54.2091% +/- 16.1247%***	***71.3123% +/- 9.8269%***	***79.5909% +/- 8.2206%***	***88.4516% +/- 5.2323%***	***96.1746% +/- 2.2073%***	***98.2448% +/- 0.1477%***

Figure 13: MS2 bioaerosol testing summary table

Summary of Results

The YFLife AIR6+ showed a considerable reduction when tested against the MS2 bacteriophage in every trial. At the final time point of 240-minutes the device yielded an average net LOG reduction of 1.76 +/- 0.04. This amount of reduction is equivalent to a 98.2448% +/- 0.1477% reduction. When considering the size of the device and the size of the chamber a reduction of this magnitude is good. The smaller the room size the better the device would be expected to perform. A summary table for the testing can be found in **Figure 13**.

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Analytical Testing Facility

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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.

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3/24/2021
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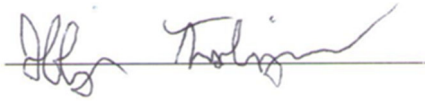

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