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CONFIDENTIAL REPORT

REPORT NO : 12/18-19

IMMUNOLOGY & MICROBIOLOGY REPORT

"Evaluation of CAZ 300 device in reducing airborne *Mycobacterium tuberculosis* (MTB H37Ra) in a laboratory testing facility"

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1 ABBREVIATIONS AND ACRONYMS

ACHe	Equivalent air changes per hour
BMS	Building Management System
BSL	Biological safety level
CADRe	Equivalent clean air delivery rate
CDC	Centres for Disease Control and Prevention
[conc]	Concentration
HCL	Hydrochloric acid
HEPA	High-efficiency particulate air
LOD	Limit of detection
LOQ	Limit of quantification
L/min	Litres per minute
MTB	*Mycobacterium tuberculosis*
MTB H37Ra	Avirulent *Mycobacterium tuberculosis* strain
NHLS	National Health Laboratory Service
NIOH	National Institute for Occupational Health
NIOSH	National Institute for Occupational Safety and Health
O_2	Oxygen
PCR	Polymerase chain reaction
PTFE	Polytetrafluoroethylene
qRT-PCR	Quantitative real time polymerase chain reaction
RH	Relative humidity
TBS	Tris-buffered saline
W	Watt

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2 PURPOSE

The purpose of the laboratory experiments was to evaluate the performance of the CAZ 300 device in reducing airborne *Mycobacterium tuberculosis* (MTB H37Ra) in a laboratory facility using a decay method. The aim is to assess the disinfection performance of the device over a set time period in a laboratory setting as was requested by the client and equate the performance to equivalent air changes per hour.

3 LOCATION OF CHALLENGE EXPERIMENTS

The experiments were conducted in a test laboratory established at the Bioaerosol Unit, NIOH, Johannesburg, South Africa, simulating a healthcare room. The CAZ 300 device was challenged with aerosols of a known concentration of an avirulent strain of *Mycobacterium tuberculosis* (MTB H37Ra). The test room measured 36.48m^3 and has the approximate dimensions of a typical hospital isolation room. The door was kept closed during the experiments and opened only at time intervals of sample collection. The room has a plenum ceiling, one door and sealed observation windows. Sample extraction and analysis was performed in a biosafety level 3 (BSL 3) laboratory.

4 INTRODUCTION

Airborne transmission of *Mycobacterium tuberculosis* and other airborne infectious agents within indoor environments has been a recognized hazard for decades. In congregate settings for example health facilities, correctional facilities, transportation (public and aircrafts), mines, offices etc., the risk of airborne transmission of MTB is a continuing problem, thus TB infection control programmes need to be strengthened (Godfrey-Faussett and Ayles, 2003; Singh & Matuka, 2015; Matuka *et al*, 2015). Droplet nuclei can remain airborne for several hours, can travel over long distances, and be distributed widely throughout buildings. The chain of infection is therefore influenced by the air quality in any particular setting.

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It is believed that airborne transport of microorganisms represents a weak link in the infection transmission route and one where control measures may have the greatest chance of breaking the infection cycle (Nardell *et al*, 2002).

5 INSTRUMENTATION AND METHODS

The following methods and instruments were used for the CAZ 300 instrument's performance evaluation experiments:

5.1 Culture preparations

An avirulent strain of MTB (MTB H37Ra, ATCC 25177) was used during the experiments. MTB H37Ra is the attenuated form of the virulent MTB strain. We used the avirulent strain for the experiments for safety reasons as it is considered a low-level hazard and since it contains the gene sequence that is similar to the MTB virulent strain that can be detected by the PCR method used. It is a suitable surrogate to the wild MTB strains as it is closely related phenotypically and genotypically. In the experiments, we were assessing the performance of Titan theater sterilizer instrument's capability of inactivating the bacilli and not infectivity of MTB where a virulent strain is needed. Known concentrations of fresh cultures of MTB H37Ra strains were prepared in sterile water containing 0.05% Tween to avoid clumping using a 0.7 McFarland standard (~1.5 x 10^6 to 3 x 10^6 cfu/ml) as a reference of the bacterial suspension so that the number of bacteria aerosolised was within a given range (Biosan, 2015).

5.2 Airborne bacteria generation and collection

The stock solution of MTB H37Ra strain concentration of ~1 x 10^6 MTB bacilli/ml was aerosolised using a 6-jet Collision Nebulizer (SKC, USA). The aerosol was generated from the nebuliser discharge port into the test room by a compressed air cylinder containing medical oxygen (O_2) (Afrox, SA) at 275.8 kPa. The nebuliser aerosolised the microorganisms for 45 minutes and was positioned at a height of 0.9 m, mimicking the average hospital bed height. To ensure uniform particle size distribution and a consistent airborne bacterial concentration and physiological state during the experiments (with and without the device operating), a

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fresh bacterial cell suspension was replenished for each sampling session. A ceiling paddle fan was used to ensure adequate mixing. The sampling procedure used was NIOH 0360.

The suspension was aerosolized for 45 minutes, with a paddle fan providing adequate air mixing in the room. After 45 minutes, the aerosolization was stopped to allow decay, and the disinfection device (CAZ 300) was turned on. The first sample was collected using a polytetrafluoroethylene (PTFE) filter and high volume sampling device (SKC, USA) at 20L/min and positioned at a height of 1.5 m above the floor, the average height of a standing individual's breathing zone (e.g. health worker). Air samples were collected at one location in the room (see Figure 1) at 45 minutes sampling intervals: 0 (immediately after initial aerosolization is stopped), 45, 90, 135, 180, 225, 270, 315, 360 and 405 minutes. After the 405th minute sample was taken, the test room was decontaminated by switching on the plant room using the Building Management System (BMS) for 30 minutes at 12 ACH followed by surface decontamination using three step cleaning process according to NIOH0360. Sterile water (lab negative) was used as a negative control in-between ON and OFF tests to rule out any contamination between the tests runs. The OFF test intervals follows the same protocol. The collected samples were quantified using quantitative real time polymerase chain reaction (qPCR) and reported as DNA copies/m^3 where one DNA copy represents one bacterial cell (NIOH0431). The equivalent air changes per hour (ACHe) provided by the device was determined from the slope of the plots of the concentration decay. All negative controls tested were negative for MTB.

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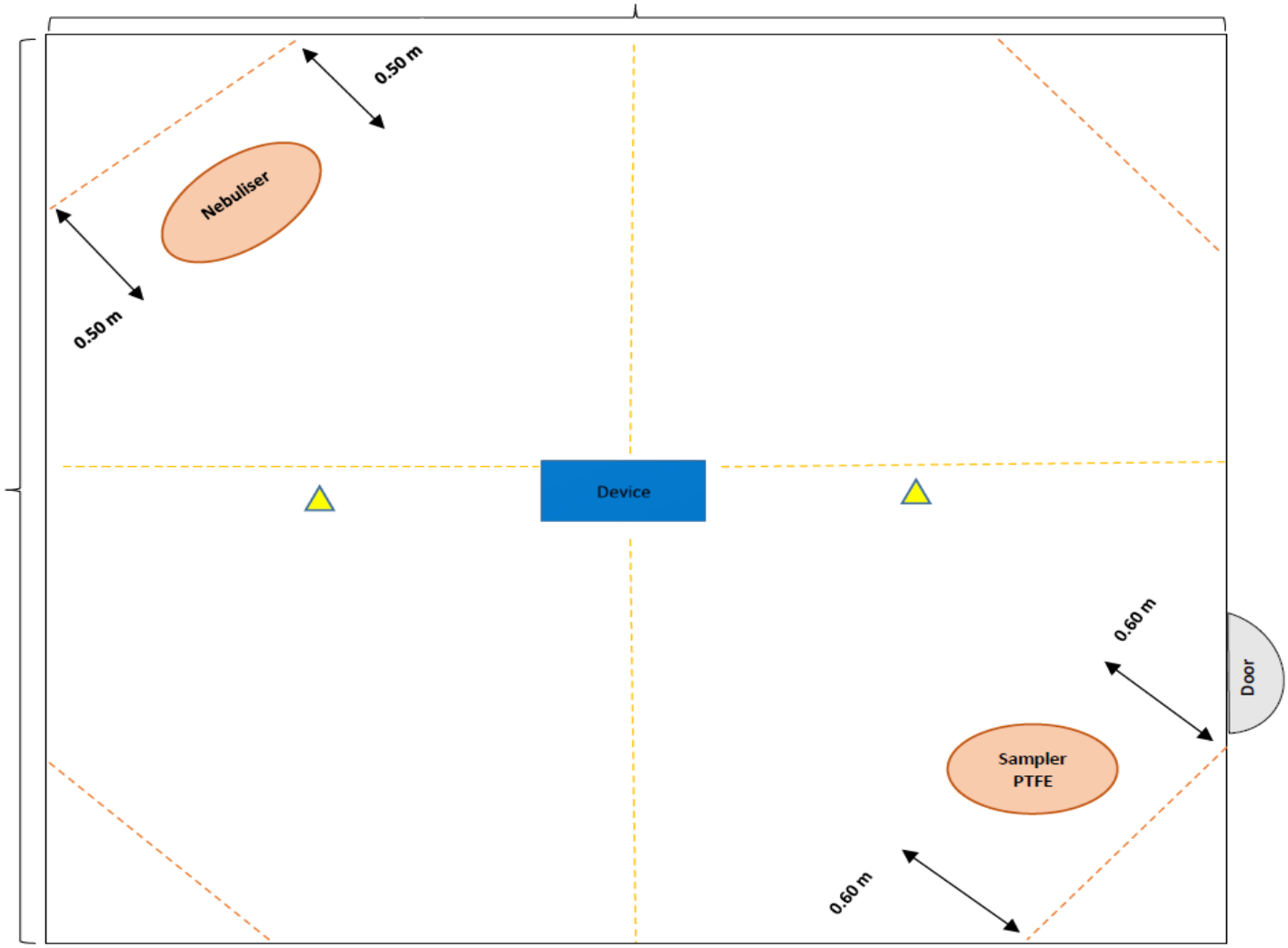

Figure 1 Illustration of the test room (height: 2.4 m) and the positioning of the, nebuliser (height: 0.9 m), sampling device (height 1.2) and the CAZ 300 (blue shading). Yellow beacons indicate positions where the sound measurements were taken.

5.3 CAZ 300 device

According to the manufacturer, the CAZ 300 is green technology (environmentally friendly) using bio oxidation to clean air in all indoor spaces (schools, nursing homes, hospitals, airports). The instrument uses the electrical charge and a cascade of water containing BioCAZ (naturally occurring enzymes) to draw all unwanted airborne pollutants and digest them forming an organic material (figure 2). The supplier also reported that the device can remove particle size of <0.00006 microns which include bacteria, viruses, allergens, odors, cigarette smoke and chemical fumes. However the results contained in this report is only for *M. tuberculosis.*

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5.4 Impaction method: PTFE membrane

Air samples were collected using 37-mm filter cassettes containing Polytetrafluoroethylene (PTFE) membranes (SKC Inc. Eighty Four Pa, USA) at a flow rate of 20 l/min using a Gillian Aircon2 high volume sampling pump (Sensidyne, USA) for 0, 45, 90, 135, 180, 225, 270, 315, 360 and 405 minutes at 1.5m height. The PTFE membranes contain microfibers fused together which allows for the capturing of airborne particulates. The PTFE cassette was removed at the end of each sampling session. The pump was calibrated using a TSI4100 series flow meter (TSI Instruments Ltd, UK) to verify that the air-sampling rate had been constant. If the flow rate was more than 5% below the initial flow rate the test run was repeated [6].

6 ANALYTICAL METHODS

Detection and Quantification of airborne MTB

The samples and lab positive control were analysed in duplicate using the relative quantitative real-time polymerase chain reaction (qPCR) assay (LightMix kit detection of *Mycobacteria genus*/*M. tuberculosis*) and the LightCycler 1.5 instrument (Roche, Germany). Briefly, samples were extracted in the laboratory to obtain the DNA. An approximately 1 kb long fragment within the 16S rRNA gene that is *Mycobacteria genus* specific and also contains a segment that is specific to *M. tuberculosis* complex is amplified. Negative (kit and lab) controls, kit positive and standard control (selected standards concentration of the kit) were included and the analysis were done in triplicate as per NIOH0431). A run was only accepted if all the controls passed and the PCR Efficiency was between 1.8 – 2.2. The CV amongst repeats for all controls and samples was accepted if ≤ 20%.

Water sample analyses

After the CAZ 300 device was filled with the water consisting the BioCAZ, seven water samples were collected from the device at 0 minutes (before MTB aerosolisation),then at 1 hour, 3 hours, 6 hours, 24 hours, 48 hours and 72 hour time intervals during air sampling procedure. Thirty millilitres of water was collected onto a sterile 50 ml tube for each time interval And fifteen millilitres of the samples were sent to the routine TB laboratory for analysis. A loopful from each sample was inoculated into nutrient broth and incubated for 18-24 hrs at 37^{o}C. Following incubation, all contaminated samples (5ml) were decontaminated using the

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Nacetyl-L-Cysteine – Sodium Hydroxide method, followed by inoculation into a BACTEC MGIT tube and incubated at 37°CinaBACTECMGIT960instrument as per SOP MIC0462. All samples were positive between day 5 and 15 days of incubation. Once the samples were detected to be positive for growth of mycobacteria, the Ziehl-Neelsen stain procedure was done to identify Acid Fast Bacilli under light microscopy which would further characterise that indeed the bacteria that was detected was of genus *Mycobacteria*. The seven decontaminated samples underwent qPCR as per NIOH0431 to confirm the presence and quantification *Mycobacteria* species which includes members of the MTB complex.

7 MICROCLIMATIC PARAMETERS

The average temperature, humidity and airflow were set and measured using the automated Building Management Operation System (StruxtureWare). In addition, the device sound levels were also measured using the Quest Technologies: Precision Integrating/Logging Sound Level Meter (Model 1900, SN: CC8090029) with accuracy +/- 0.1 dBA and a calibrator (SN: QE 8100133) was used for the sound level meter. SANS 10103:2008 was used as reference. Measurements were taken in two positions in the laboratory and averaged. According to SANS the indoor measurements of microphone height is between 1.2 and 1.4 metres above the floor and at least 1.2 m away from the walls and other large flat vertical surfaces. The results are displayed in Table 1.

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Figure 2 Photographs of the CAZ 300 device

Noise: The average 8 hour equivalent noise level when the device was off, was 43.3dBA and 66.5.8dBA when the device was on, which is above the noise levels reported by the supplier of 40db. These readings would need to be compared to the recommended maximum allowable sound level (without hearing protection) at varying exposure times in different settings depending on where the device is used.

Room temperature and relative humidity: The performance of the device was tested at average temperatures of 24.64^{o}C when the device was off and 23.46^{o}C when the unit was on. The average relative humidity was 47.49% RH when the device was off and 57.70% RH when the device was on. The performance of the device may vary outside these temperature ranges.

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Air velocity: The average air velocity in the room was 0.71m/s when the device was operational, whereas when the device was off it was 0.53m/s.

Table 1 Microclimatic parameters of the test room with air cleaning device on vs off

Microclimatic parameters			**Date:** 04/12/2018
Noise:	LEQ (Decibel (A) (8 hour equivalent noise))	Min (Decibel (A))	Max (Decibel (A))
Device: OFF	43.3	37.6	52.8
Device: ON	66.5	62.7	71.8
Temperature:	Average T°C	Min T°C	Max T°C
Device: OFF	24.64	22.58	27.66
Device: ON	23.46	21.01	25.02
Humidity:	Average (% RH)	Min (% RH)	Max (% RH)
Device: OFF	47.49	34.64	60.47
Device: ON	57.70	44.41	68.80
Air Velocity:	Average (m/s)	Min (m/s)	Max (m/s)
Device: OFF (room fan on)	0.53	0.25	0.73
Device: ON (room fan on)	0.71	0.39	0.87

8 RESULTS AND DISCUSSION

After aerosolising a concentration of MTB H37Ra (~1x10^6) for 45 minutes (baseline) the average concentration of bacilli detected was 9.02E+05 MTB DNA copies/m^3 and reduced to 3.38E+02 MTB DNA copies/m^3 when the CAZ 300 device was on for 405 minutes (i.e. 6.75 hours). When the CAZ 300 device was off the original aerosolizing concentration reduced slightly to 4.67E+04 MTB DNA copies/m^3 after 405 minutes (Table 2). The latter reduction may be due to environmental stress and gravitational force. When the CAZ 300 device was operational it achieved 1.924 AC/h and CADRe of 19.5 l/s in a room of 36.48 m³. Air changes per hour (AC/h) must still be applied for occupied areas in any building to meet the ventilation requirement for occupant safety. The device's results (spatial averages) appear consistent and reflect the performance of the device in the lab. Figure 3 provides a graphical presentation of the curve of the MTB decay.

The WHO policy on TB infection control strongly recommends the application of defined ventilation rates based on the number of occupants, which is proportional to the risk. This approach is preferred to the

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universal application of a volumetric room air exchange rate. Based on the recommended WHO ventilation rate of 12 ACH (air changes per hour)[2] in airborne precaution rooms, an adjustment for a room volume of 24m³ yields an equivalent ventilation rate of 80l/s/person. This resultant value can be applied to high risk settings with mechanical ventilation. 160l/s/person should be applied to similar rooms relying on natural ventilation (Singh, et al, 2015). If other rooms excluding any of the abovementioned is used, the applicable ACH must be verified in ASHRAE standard 170. Where these recommended ventilation rates cannot be achieved, the implementation of air cleaning device systems with a deactivation capacity equivalent to this ventilation rate should be pursued. The prescriptive design process aims to determine the required number of air cleaning devices for the considered indoor space in accordance with the WHO recommended minimum ventilation rate of 80 l/s per person. If the considered room has an existing and functional mechanical ventilation system, the outside air portion of that system's ventilation rate should be included in the calculation to reduce the number of air cleaning devices required.

Knowing the room volume for an intended installation, the number of devices required for that given space can be calculated. By multiplying the number of devices by the unit's CADRe the total equivalent clean air delivery rate for that given space can be calculated and compared to the WHO recommendation of 80 l/s per person.

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Table 2 Average decay of airborne MTB H37Ra concentration over a period of 405 minutes and the equivalent air changes per hour achieved.

Time	CAZ 300 device (DNA copies/m³)	
	ON (mean)	OFF (mean)
0	9.02E+05	5.98E+05
45	1.13E+04	3.64E+05
90	2.04E+04	2.37E+05
135	1.39E+04	6.54E+05
180	1.09E+04	2.82E+05
225	1.57E+04	1.27E+05
270	9.34E+03	1.13E+05
315	4.00E+03	7.64E+04
360	6.77E+02	4.09E+04
405	3.38E+02	4.67E+04
Outdoor Concentration		0.00
Mean Age of Air		45.64
Nominal Air Change time		27.77
Air Change Rate		2.16
Air Change efficiency %		30.42
Room volume		36.480 m³
CADRe		**19.500 l/s**
ACHe (On - Off)		**1.924 AC/h**

Formula: ACHe = 1/nominal air change time x 60; CADRe = 1000 x (ACHe x room volume) / 3600.

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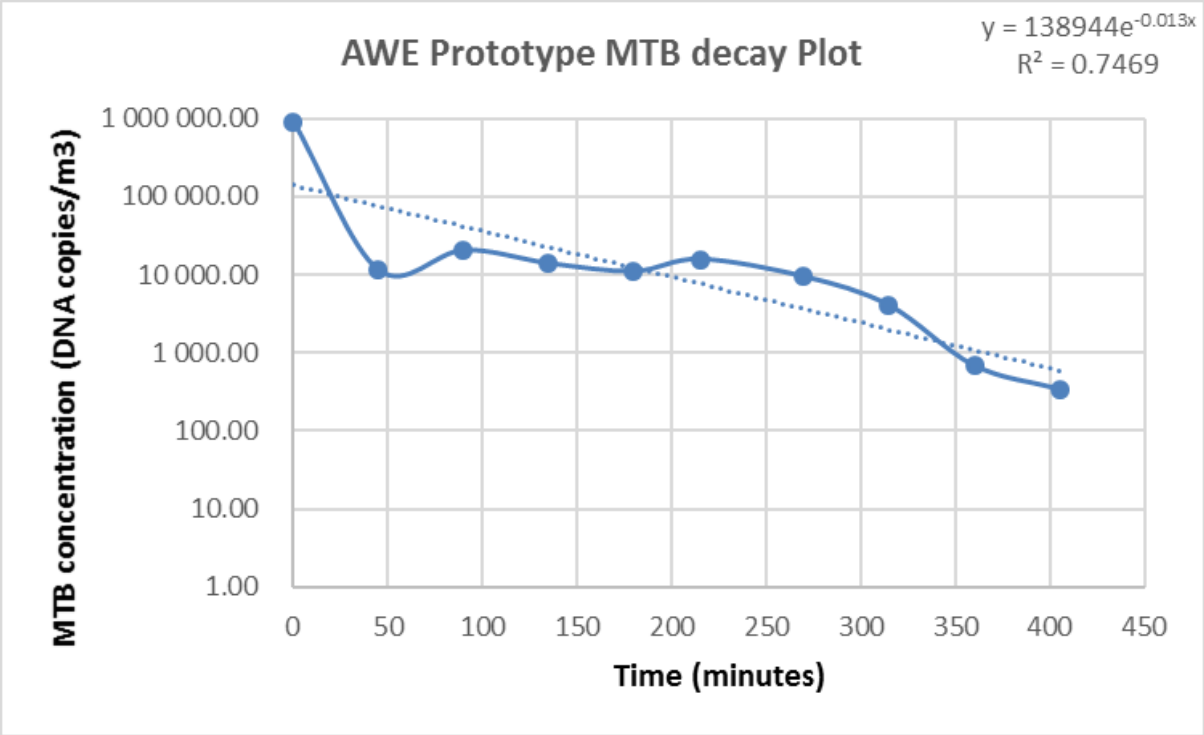

Figure 3 MTB concentration decay curve when the CAZ 300 device was on. The solid line shows the decay of MTB concentration when the device was operational.

All water samples tested were positive for *Mycobacteria* species culture while the Ziehl Neelsen stain also confirmed the presence of bacilli characteristic of *Mycobacteria* species for all samples (Table 3). There is a possibility that the water samples contained bacteria of the *Mycobacteria* genus. Quantitative PCR results demonstrated that the 0 minute water sample contained bacteria of the genus *Mycobacteria*, however the other 6 samples were negative for *Mycobacteria*. Since the 0 minute sample was collected before aerosolisation of MTB in the test room, there is a possibility that the bacteria identified in these samples were genus *Mycobacteria (*melting curve of ~64ºC) from the BioCAZ water solution brought by the supplier or the tap water that was used in the lab to refill the CAZ device during evaporation. Non-tuberculosis mycobacteria (NTM) have been identified in water, therefore could be the source of the isolated genus form the 0 minute sample.

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Table 3 MTB Confirmatory test results in water containing BioCAZ samples from the CAZ 300

LAB INDENTIFICATION	TIME	MTB CULTURE	ZIEHL NEELSEN STAIN	M spp/MTB qPCR
LA00801463	0 MINS	Positive	Acid Fast Bacilli	4.29E+01
LA00801465	1 HOUR	Positive	Acid Fast Bacilli	0.00E+00
LA00801466	3 HOURS	Positive	Acid Fast Bacilli	0.00E+00
LA00801467	6 HOURS	Positive	Acid Fast Bacilli	0.00E+00
LA00801468	24 HOURS	Positive	Acid Fast Bacilli	0.00E+00
LA00801469	48 HOURS	Positive	Acid Fast Bacilli	0.00E+00
LA00801470	72 HOURS	Positive	Acid Fast Bacilli	0.00E+00

9 CONCLUDING REMARKS

The results obtained from the MTB decay test for the CAZ 300 device demonstrates that the device is capable of achieving 1.924 AC/h and CADRe of 19.500 l/s in a room of 36.48 m^3. . The water confirmatory tests results show that the water was contaminated with *Mycobacteria* species at the beginning of the test however was negative for *Mycobacterium tuberculosis* for subsequent test intervals. The source of the *Mycobacteria* could be the original water and the BIO-ox mixture or tap water that was used.

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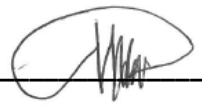
11 SIGNATURES

I, **Thabang Duba**, conducted the air sampling in the test laboratory and hereby declare that the results and findings are a true reflection of conditions encountered during the experiments.

Thabang Duba, BSc(Honours)

MEDICAL SCIENTIST (MSIN 0003123)



_ 21/02/2019_____

I, **Zethembiso Ngcobo**, conducted the PCR analysis and hereby declare that the results and findings are a true reflection of samples analysed in the laboratory.

Zethembiso Ngcobo, MSc

MEDICAL SCIENTIST (MSIN 0003310)

____ 21/02/2019_____

REPORT BY: I, **Onnicah Matuka**, prepared this report and hereby declare that the results and findings are a true reflection of conditions encountered during the experiments.

Onnicah Matuka, MSc (Med)

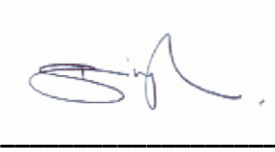
MEDICAL SCIENTIST (MW0010944)

__ 21/02/2019_____

REVIEWED AND APPROVED BY: I, **Tanusha Singh**, accept technical responsibility for the content of this report and hereby approve the report for release.

Tanusha Singh, PhD

SENIOR SPECIALIST SCIENTIST (MW0007994)



_ 25/02/2019