NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH
Division of the National Health Laboratory Service

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

CONFIDENTIAL REPORT

REPORT NO: 002/20-21

IMMUNOLOGY & MICROBIOLOGY REPORT

"Evaluation of Ceiling UVC Air sterilizer device in reducing airborne *Mycobacterium tuberculosis* (MTB H37Ra) in a laboratory testing facility"

DATE OF SAMPLING:	2020/06/01
DATE OF ANALYSIS:	2020/06/02
DATE OF RECEIPT :	2020/05/14
DATE OF REPORT :	2020/06/05, 9:00 am

FOR:

Asher Sharir
Ashur illumination (Pty) Ltd
150 Tenth Rd, Kew
P.O.BOX 82, Bergvlei, 2012

Tel: 011 882 9875
Cell: 0844122692
Email: asher.shahir@gmail.com

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

STATEMENT

This report has been prepared and published by the National Institute for Occupational Health (NIOH) of the National Health Laboratory Service, taking into consideration the Occupational Health and Safety Act (85 of 1993) and other relevant Regulations. The bioaerosol laboratory conducts tests or analysis in accordance with ISO 17025. If published or reproduced by the Client, the report must be reproduced in full, including printed and typed parts of the report and with nothing excluded. Where it is necessary to print or reproduce an abridged form of the report, prior written approval for the publication or reproduction in abridged form must be obtained from NIOH.

The results and findings contained in this report only apply to conditions that existed at the time of the experiment and/or survey in the laboratory setting. Whilst every endeavour is made to ensure that the results and findings contained in this report are representative, it should be noted that the physical conditions during testing might slightly vary due to changes in environmental factors including weather conditions etc. In terms of the South African Occupational Health and Safety Act (85 of 1993), and relevant Regulations, the Client has to keep records of this report for a period of 40 years, depending on the specific term prescribed and if applicable.

LEGAL DISCLAIMER

NIOH/NHLS and its employees and agents assume no liability or responsibility for any loss, damage, injury, disease, cost or expense, and whether direct or consequential, sustained by the Client or any other party, of this reference material, which may in any way, be connected to the Client's usage of, or reliance upon information contained in this report. NIOH/NHLS does not endorse any brand or product. This report is not the equivalence of a compliance certificate but only show the performance of the device at known set points within a controlled environment. In no way must these results be interpreted for any other device except the exact model tested.

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH
Division of the National Health Laboratory Service

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

TABLE OF CONTENTS

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

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
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
W	Watt

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

2 PURPOSE

The purpose of the laboratory experiments was to evaluate the performance of the Ceiling UVC Air sterilizer unit 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 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 Ceiling UVC Air sterilizer was challenged with aerosols of a known concentration of an avirulent strain of *Mycobacterium tuberculosis* (MTB H37Ra). The test room measured 45,60 m^3 and has the approximate dimensions of a typical hospital isolation room. The door was kept closed during the experiments and opened only at specific time intervals for sample collection. The room has a plenum ceiling, one door and sealed 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. 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).

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

5 INSTRUMENTATION AND METHODS

The following methods and instruments were used for the Ceiling UVC Air sterilizer 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 Ceiling UVC Air 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 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 ~1.5 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 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 fresh bacterial cell suspension was replenished for each sampling session. The sampling procedure used was NIOH 0360.

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

The suspension was aerosolized for 45 minutes, with a ceiling paddle fan providing adequate air mixing in the room. After 45 minutes, the aerosolization was stopped to allow decay, and the disinfection device (Ceiling UVC Air sterilizer) was turned on. Samples were collected using polytetrafluoroethylene (PTFE) filters 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 10 minutes sampling intervals: 0 (immediately after initial aerosolization is stopped), 10, 20, 30, 40, 50 and 60 minutes. After the 60 minutes 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 (NIOH0290). 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.

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

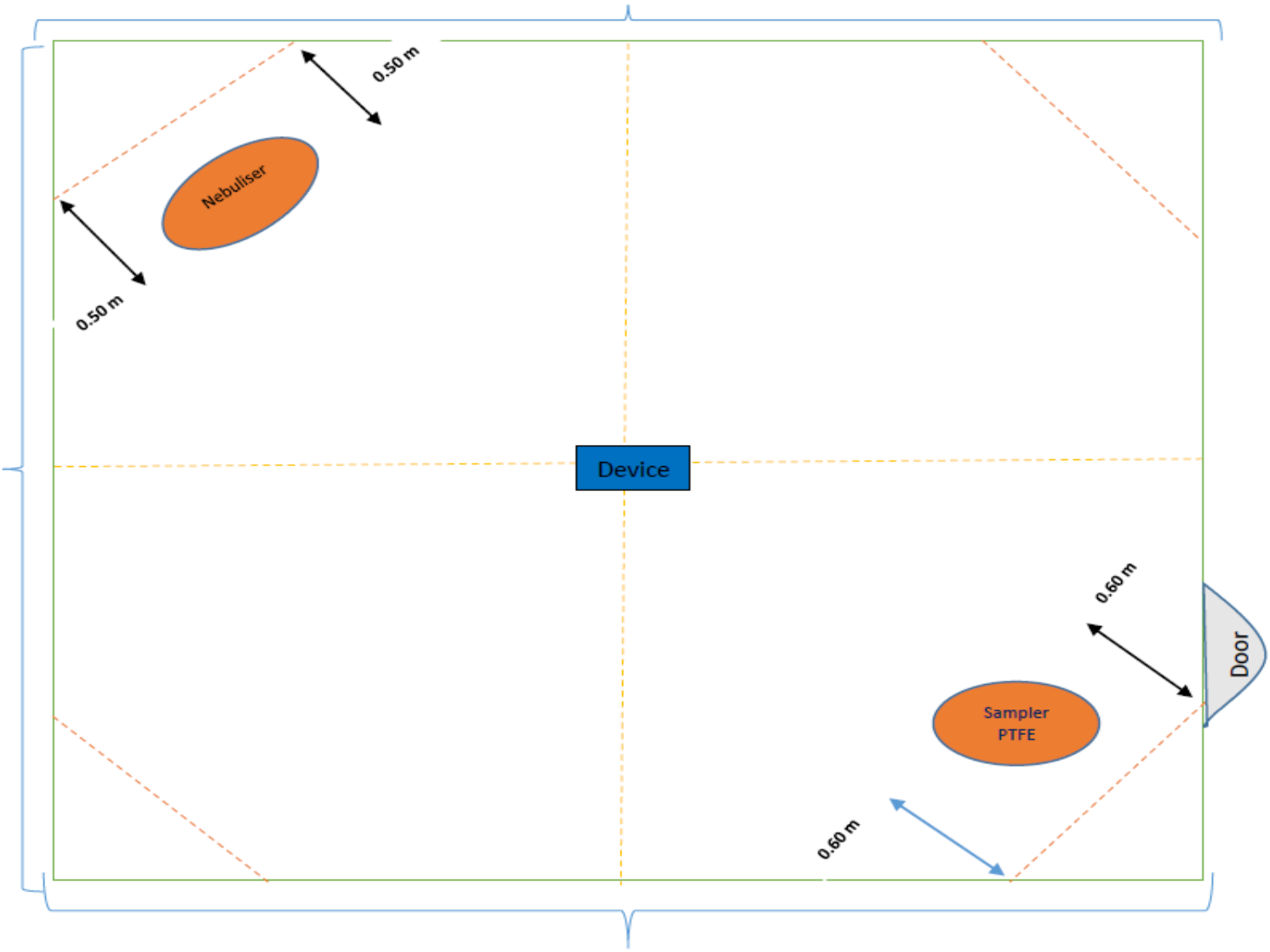
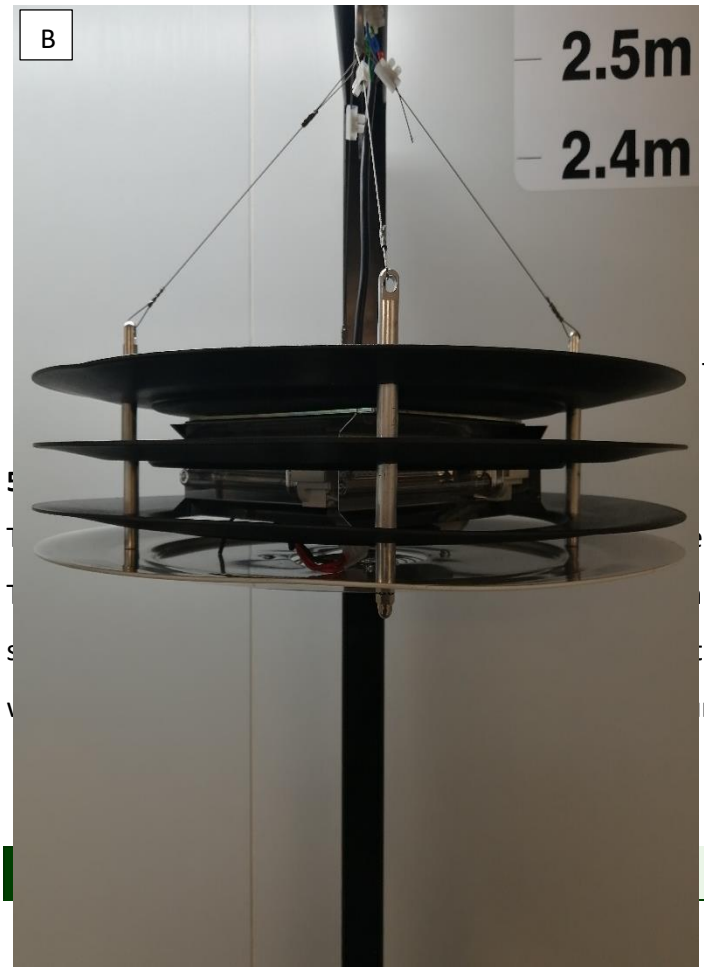


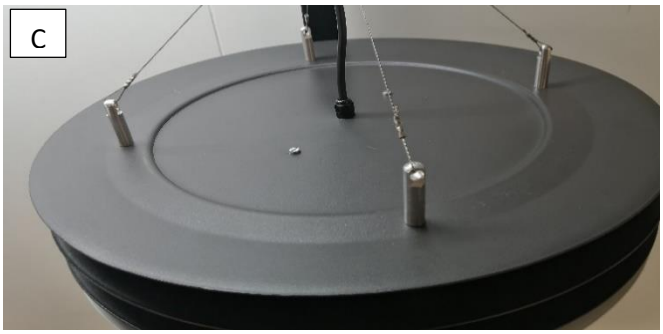
Figure 1 Illustration of the test room (height: 3.0 m) and the positioning of the nebuliser (height: 0.9m), sampling filter (height 1.5m) and the Ceiling UVC Air sterilizer (height 2.0m)

5.3 Ceiling UVC Air sterilizer device

According to the manufacturer, the Ceiling UVC Air sterilizer (SN 4C-230HB) device from Ashur illumination (Pty) Ltd is intended to be used to sterilize airborne pathogens in health care facilities. The instrument uses UVC germicidal lamps (figure 2) to disinfect the airborne pathogens.

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za





top (C) view of the Ceiling UVC Air sterilizer device

r which allows for the capturing of airborne particulates.

pling session. The pump was calibrated using a TSI 4100

the air-sampling rate had been constant. If the flow rate

n was repeated [6].

Detection and Quantification of airborne MTB

The samples were analysed in duplicate using the relative quantitative real-time polymerase chain reaction (qPCR) assay (LightMix kit detection of Mycobacterium spp/*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

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

contains a segment that is specific to *M. tuberculosis* complex is amplified. Negative (kit and lab) controls, kit positive, lab positive and standard control were included and the analysis were done in triplicate as per NIOH 0431. 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%.

7 MICROCLIMATIC PARAMETERS

The average temperature, humidity and airflow were set and measured using the automated Building Management Operation System (StruxtureWare).

Room temperature and relative humidity: The performance of the device was tested at average temperatures of 21,15°C when the device was off and 22,42°C when the unit was on. The average relative humidity was 58,82% RH when the device was off and 52,99% RH when the device was on. The conditions were within the recommended indoor temperature (20-24°C) and humidity (50– 70%) prescribed in the Standard for laboratory testing of UVGI air and surface rate constants. The performance of the device may vary outside these temperature and humidity ranges.

Air velocity: The average air velocity in the room was 0.03 m/s when the device was operational, whereas when the device was off it was 0.02m/s.

Table 1 Microclimatic parameters of the test room with Ceiling UVC Air sterilizer device on vs off

Microclimatic parameters			**Date:** 01/06/2020
Temperature:	Average T°C	Min T°C	Max T°C
Device: OFF	21,84	21,04	22.61
Device: ON	22,43	21,98	22,92
Humidity:	Average (% RH)	Min (% RH)	Max (% RH)
Device: OFF	51,34	49,79	53,03
Device: ON	56,76	54,32	595,84
Air Velocity:	Average (m/s)	Min (m/s)	Max (m/s)
Device: OFF (room fan on)	0,02	0,02	0,03
Device: ON (room fan off)	0,03	0,02	0,03

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

8 RESULTS AND DISCUSSION

After aerosolising a known concentration of MTB H37Ra for 45 minutes (baseline) the average concentration of bacilli detected was 1,88 x 10^6 MTB DNA copies/m^3 and reduced to 1,18 x 10^2 MTB DNA copies/m^3 when the Ceiling UVC Air sterilizer device was on for 60 minutes. When the Ceiling UVC Air sterilizer device was off the original aerosol concentration was 5,07x10^6 MTB DNA copies/m^3 after 60 minutes and reduced to 1,18x10^6 (Table 2). The microclimatic parameters were all within the ranges as stipulated in the Standard for laboratory testing of UVGI air and surface rate constants. When the Ceiling UVC Air sterilizer device was operational it achieved 10,598 ACHe and CADRe of 134,247 l/s in a room of 45,600 m^3. 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 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 24m^3 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). Where these recommended ventilation rates cannot be achieved, the implementation of alternative air cleaning devices with a deactivation capacity equivalent to this ventilation rate should be pursued.

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH
Division of the National Health Laboratory Service

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

Table 2 Average decay of airborne MTB H37Ra concentration over a period of 60 minutes and the equivalent air changes per hour achieved.

Time	Ceiling UVC Air sterilizer device (DNA copies/m³)	
	ON (mean)	OFF (mean)
0	1,88E+06	5,07E+06
10	2,46E+04	4,84E+06
20	3,06E+03	6,28E+06
30	1,48E+03	3,10E+06
40	5,54E+02	4,43E+06
50	2,15E+02	2,36E+06
60	1,18E+02	1,18E+06
Outdoor Concentration		0.00
Mean Age of Air		5,25
Nominal Air Change time		5,16
Air Change Rate		11,63
Air Change efficiency %		49,19
Room volume		45,600 m³
CADRe		**134,247 l/s**
ACHe (On - Off)		**10,598 AC/h**

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

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

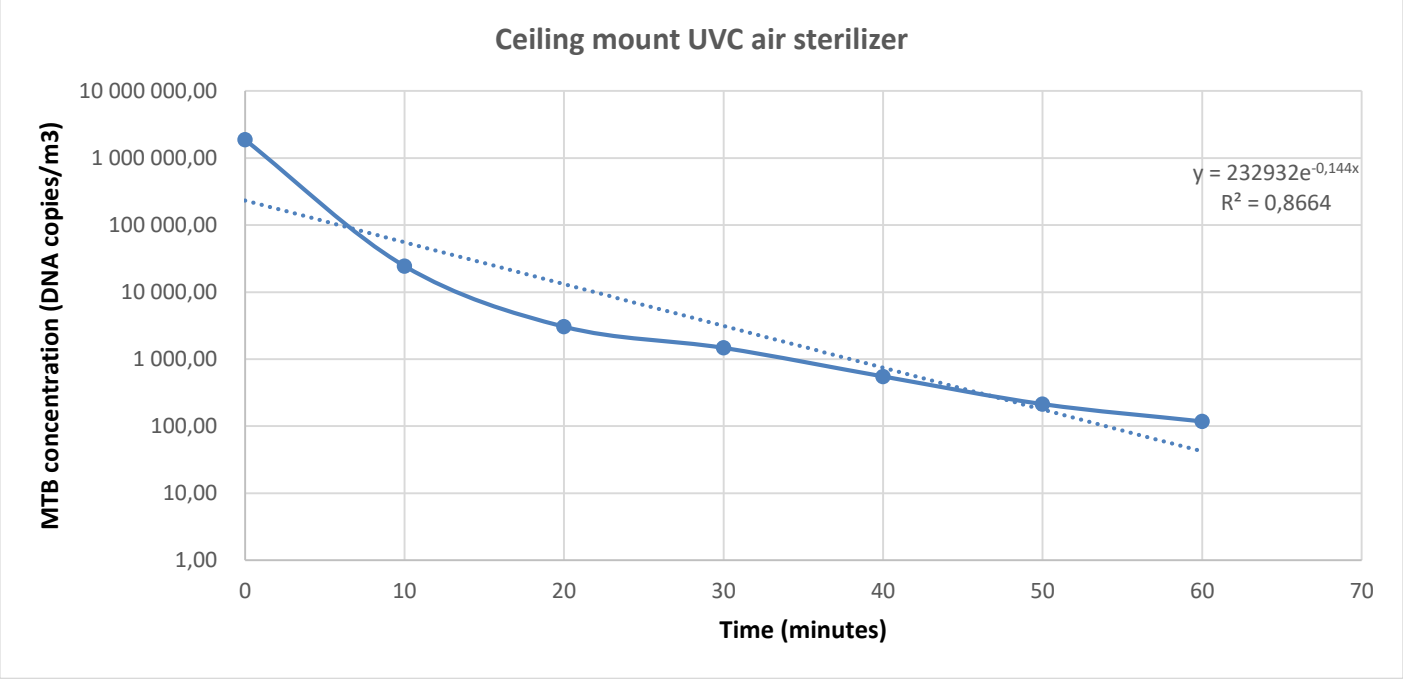


Figure 3 MTB concentration decay curve when the Ceiling UVC Air sterilizer device was on. The solid line shows the decay of MTB concentration when the device was operational.

9 CONCLUDING REMARKS

The results obtained from the MTB decay test for the Ceiling UVC Air sterilizer device demonstrates that the device is capable of achieving 10,598 ACHe and CADRe of 134,247 l/s in a room of 45,600 m^3. The number of devices required for differing room volumes and occupancy can be calculated using CADRe as described in the guideline entitled "UVGI disinfection of room air an evidence based guideline for design, implementation and maintenance".

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

10 REFERENCES

CDC., Environmental Control for Tuberculosis: Basic Upper-Room Ultraviolet Germicidal Irradiation Guidelines for Healthcare Settings. Department of Health and Human Services Centers for Disease Control and Prevention National Institute for Occupational Safety and Health, 2009. DHHS (NIOSH) Publication No. 2009–105.

Dharmadhikari AS, Mphahlele M, Stoltz A, Venter K, Mathebula R, Masotla T, et al. 2012. Surgical face masks worn by patients with multidrug-resistant tuberculosis impact on infectivity of air on a hospital ward. Am J Respir Crit Care Med, 185:1104-9.

Dharmadhikari AS, Basaraba RJ, Van Der Walt ML, Weyer K, Mphahlele M, Venter K, et al. 2011. Natural infection of guinea pigs exposed to patients with highly drug-resistant tuberculosis. Tuberculosis, 91:329-38.

Girdler-Brown BV. NIOH Epistatistics 1 module2 course material, 2007

Godfrey-Faussett P, Ayles H. 2003. Can we control tuberculosis in high HIV prevalence settings? Tuberculosis, 83, 68–76.

Matuka, O., Singh, T.S., Bryce d,E., Yassi e, A., Kgasha, O., Zungu, M., Kyaw, K., Malotle, M., Renton, K. and O'Hara, L. 2015. Pilot study to detect airborne Mycobacteriumtuberculosis exposure in a South African publichealthcare facility outpatient clinic. Journal of Hospital Infection, 89: 192e196.

Memarzadeh, F., R.N. Olmsted, and J.M. Bartley.2010. Applications of ultraviolet germicidal irradiation disinfection in health care facilities: effective adjunct, but not stand-alone technology. Am J Infect Control, 38(5 Suppl 1): p. S13-24.

Nardell, E.A. 2002. Use and misuse of germicidal UV air disinfection for TB in high prevalence settings. Int J Tuberc Lung Dis, 6(8): p. 647-648

Perilla, M.J., Ajello, M.S., Bopp, C., Elliott, J., Facklam, R., Popovic, T. & Wells, J. 2003. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Centre for Disease Control and Prevention: National Centre for Infectious Diseases. 209 – 213.

Schafer, M.P., J.E. Fernback, and P.A. Jensen. 1998. Sampling and analytical method development for qualitative assessment of airborne mycobacterial species of the Mycobacterium tuberculosis complex. Am Ind Hyg Assoc J, 59(8): p. 540-546.

Singh, T. & Matuka, O. 2015. Airborne infection control in healthcare facilities: effecting change. African Newsletter, 25:21-24.

Wells, W.F., Fair, G.M.1935. Viability of B. coli exposed to ultraviolet radiation in air. Science, 82, 280–281.

Biosan Medical-biological Research & Technologies.2017. DEN-1 Densitometer suspension turbidity detector. Operating manual, Version V.2AWKowalski WJ, Nardell ED, Bahnfleth W, Dunn C, Hollander B, Witham D, Speer S, Guzorek S, Clancy ME, Levin B. 2004. IUVA Draft standard IUVA-S06A-2005. Standard for laboratory testing of UVGI air and surface rate constants. Pg 15

Immunology & Microbiology Section
25 Hospital Street, Constitution Hill, Johannesburg, 2000
Tel: +27 (0)11 712 6475 Fax: +27 (0)11 712 6426
tanushas@nioh.ac.za
http://www.nioh.ac.za

11 SIGNATURES

We, **Thabang Duba and Lufuno Muleba**, conducted the air sampling in the test laboratory and the PCR analysis, we hereby declare that the results and findings are a true reflection of conditions encountered during the experiments and samples analysed in the laboratory.

Thabang Duba, MSc
Medical Scientist, MS0003301

Lufuno Muleba, BSc Hon
Medical Scientist, MS0002593

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, MSIN0003310

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

DATE: 05/06/2020