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CONFIDENTIAL REPORT

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IMMUNOLOGY & MICROBIOLOGY REPORT

"Evaluation of HouseIt ARPACK efficacy in reducing airborne *Mycobacterium tuberculosis* (MTB H37Ra) in a laboratory testing facility"

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1 ABBREVIATIONS AND ACRONYMS

BSL	Biological safety level
CDC	Centers 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

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2 PURPOSE

The purpose of the laboratory experiments was to evaluate the performance of the ARPACK instrument in reducing airborne *Mycobacterium tuberculosis* (MTB H37Ra) in a laboratory facility. The device utilises UV-A and titanium dioxide to inactivate microorganisms.

3 LOCATION OF CHALLENGE EXPERIMENTS

The experiments were conducted at the Bioaerosol Unit, NIOH, Johannesburg, South Africa. The ARPACK device was challenged with aerosols of known concentration of an avirulent strain of *Mycobacterium tuberculosis* (MTB H37Ra). The experiments were conducted in a test laboratory established at the Bioaerosol Unit, NIOH, Johannesburg, South Africa, simulating a healthcare room. The test room measured 4.0 m x 4.0 m x 3.5 m (length x width x height) 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 a sealed window. Sample extraction and analysis was performed in a biosafety level 3 (BSL 3) laboratory.

4 INTRODUCTION

Airborne transmission of *Mycobacterium tuberculosis* and other infectious agents within indoor environments has been a recognized hazard for decades. In congregate settings for examples 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). Airborne droplet nuclei can remain airborne for several hours and can travel over long distances and be distributed widely throughout buildings. The chain of infection is therefore very much influenced by the ventilation conditions 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).

Conventional sampling and culture-based methods for the detection of airborne MTB bacilli has had limitations in the past (Schafer, Fernback and Jensen, 1998). Wan et al (2004), Vadrot et al (2004) and Chen and Li (2005) all moved to using filtration technique and PCR to detect airborne TB in contaminated rooms as well as testing releases from TB patients. Various filters were used and numerous positive samples were found by all 3 studies. Flow rates varied, sampling times varied but breathing zone measurements were taken approximately 1 m from the bed and 1.2 to 1.5 m above the floor.

These and other methods of sampling to assess the efficacy of the disinfecting device are noisy, expensive and too complex for routine use (Dharmadhikari et al, 2012; Dharmadhikari et al, 2011). Therefore identifying an effective sampling device that circumvents the disadvantages of previous devices is advantages particularly for under resourced countries.

5 INSTRUMENTATION AND METHODS

The following methods and instruments were used for the ARPACK's performance evaluation experiments:

5.1 Culture preparations

An avirulent strain of MTB (MTB H37Ra) 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 is contains the gene sequence IS6110 which 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 to the wild MTB strains.

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For these experiments we were assessing the performance of the ARPACKs 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 McFarland standard (~1x 10^6cfu/ml) as a reference of the bacterial suspension so that the number of bacteria aerosolised was within a given range (Perilla et al, 2003).

5.2 Airborne bacteria generation and collection

The stock solution of MTB H37Ra strain (~1x 10^8) was diluted to a concentration of ~1 x 10^6 MTB bacilli/ml and 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 40psig or 270kPa. The nebuliser aerolised the microorganisms for 60 minutes and was positioned at a height of 0.9 m, mimicking the average height of a hospital bed height.

Air samples were collected at 6 sampling points (figure 1) at a height of 1.4 m above the floor, approximately the average height of a standing individual's breathing zone. Air samples were collected at four time intervals (1, 4, 6, 12 hours) with all six sampling devices at the same time, respectively. The airborne bacteria were captured using a PTFE filter. The test room was decontaminated with potassium permanganate and a 2% formalin solution between the runs. Negative controls were obtained by aerosolising sterile water instead of MTB H37Ra. The collected samples are quantified using quantitative real time polymerase chain reaction (qPCR) and reported as DNA copies/m^3 where one DNA copies represents one bacterial cell.

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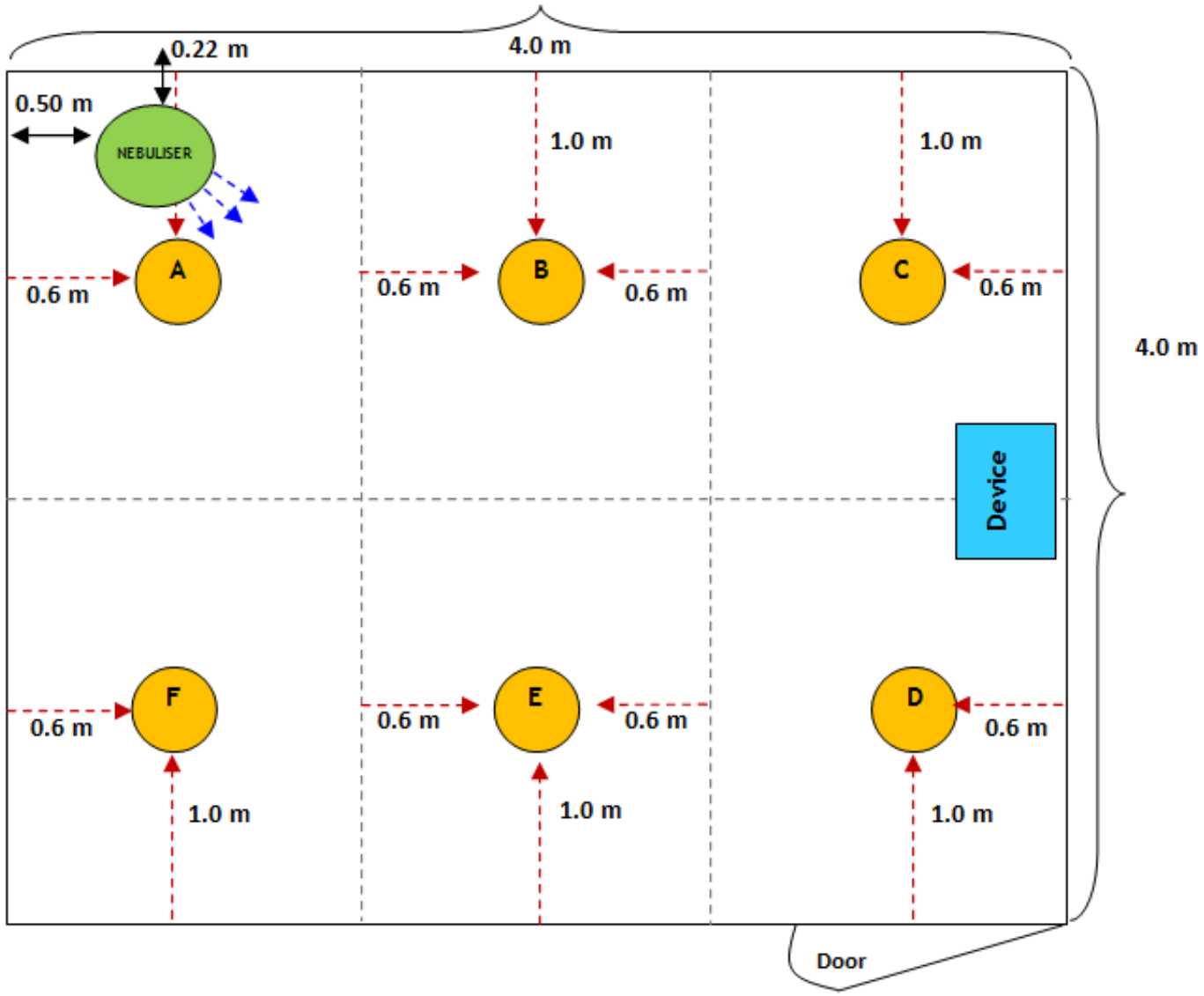

Figure 1 Illustration of the test room (height: 3.5 m) and the positioning of the, nebuliser (height: 0.9 m), sampling devices (A-E, height 1.4 m) and the ARPACK device.

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5.3 Test device: ARPACK

The ARPACK VR400A is reported (product brochure) to be an air sterilising and cleaning system which destroy organic pollutants such as microorganisms through photocatalytic oxidation. The manufacturers claim that 99.9% of any bacteria and viruses are removed from the air for one pass through the ARPACK device. The brochure states that the device oxidizes organic impurities under UV-A exposure on a thin layer of nanocrystalline TiO2, put on a surface of filters in the device.

The ARPACK device was positioned vertically in the test room for the test procedure as shown in Figure 1. MTB cells were aerosolised for one hour (nebuliser switched off) at which point the ARPACK was switched on according to the manufacturers guide. The fan on the back of the apparatus was switched on to level 1.

The tests were performed with the ARPACK ON and OFF for the various sampling times.

5.4 Impaction method: PTFE membrane

Six air samples were collected simultaneously 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 1, 4, 6 and 12 hours at 1.4m height. The PTFE membranes contain microfibres fused together which allows for the capturing of airborne particulates. The PTFE cassettes were 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].

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6 ANALYTICAL METHODS

Detection and Quantification of airborne MTB

The samples were analysed in triplicate using the relative quantitative real-time polymerase chain reaction (qPCR) AMPLICOR *Mycobacterium tuberculosis* test and the LightCycler 1.5 instrument (Roche, Germany). Briefly, samples were extracted in the laboratory to obtain the DNA. The MTB test uses the *Mycobacterium* genus specific primers (KY 18 and KY 75) to define a sequence of approximately 200 base pair fragment within this region (target gene IS6110). A negative control, kit positive and laboratory positive control were included in each analysis run. A run was only accepted if all the controls passed. The total airborne concentration (MTB DNA copies/m^3) was calculated using the number of MTB DNA copies and the sampling time and flow rate sampled. The results obtained are conceived as the total number of DNA copies of MTB with one DNA copy representing one MTB bacilli.

7 RESULTS AND DISCUSSION

After aerosolised a concentration of MTB H37Ra (~1x10^6) for 60 minutes the average concentration of bacilli detected was 2.17 x 10^6 MTB DNA copies/m^3. In the absence of the ARPACK device the concentration reduced from the above concentration to 1.69 x 10^6 MTB DNA copies/m^3 after one hour to and 8.59 x 10^4 DNA copies/m3 after 12 hours (Table 1). The reduction may be due to environmental stress and gravitational force. When the ARPACK device was operational there was a significant reduction in the airborne concentration. There was also a slight difference among the different locations as a result of stagnant areas in the room. The results suggests that the introduction of air mixing will improve the uniformity of the microbial distribution within the room and consistent reduction pattern. The overall

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results demonstrate no contamination during experiments as all the blank samples and the sterile water runs of the room were negative for MTB H37Ra. Based on these results the ARPACK which is referred to as an air sterilisation device was able to significantly reduce counts by >90% (D90) which is the UV dose required to inactivate bacteria for sterilisation devices from one to twelve hours.

Table 1 Average airborne MTB H37Ra concentration detected for different sampling times and locations.

Location[#]	ARPACK OFF ARPACK ON (DNA copies/m^3)							
	1	4	6	12	1	4	6	12
A	1.40E+06	2.84E+05	2.34E+05	9.91E+04	9.81E+03	9.39E+03	3.01E+03	8.82E+02
B	1.56E+06	3.31E+05	1.13E+05	9.17E+04	1.37E+04	1.01E+04	2.81E+03	6.55E+02
C	1.88E+06	3.92E+05	2.87E+05	6.73E+04	8.40E+03	8.79E+03	2.38E+01	3.01E+02
D	1.84E+06	6.40E+05	6.23E+04	5.00E+04	2.23E+04	1.37E+03	1.25E+03	7.71E+02
E	1.48E+06	2.32E+05	2.31E+05	1.03E+05	9.06E+03	8.26E+03	3.12E+03	5.67E+02
F	1.98E+06	5.40E+05	1.94E+05	1.04E+05	1.50E+04	8.78E+03	2.62E+03	4.79E+01

Figure 2 provides a graphical presentation of the results. A gradual decline is observed when the ARPACK is not used and a more rapid decline when the ARPACK device is used.

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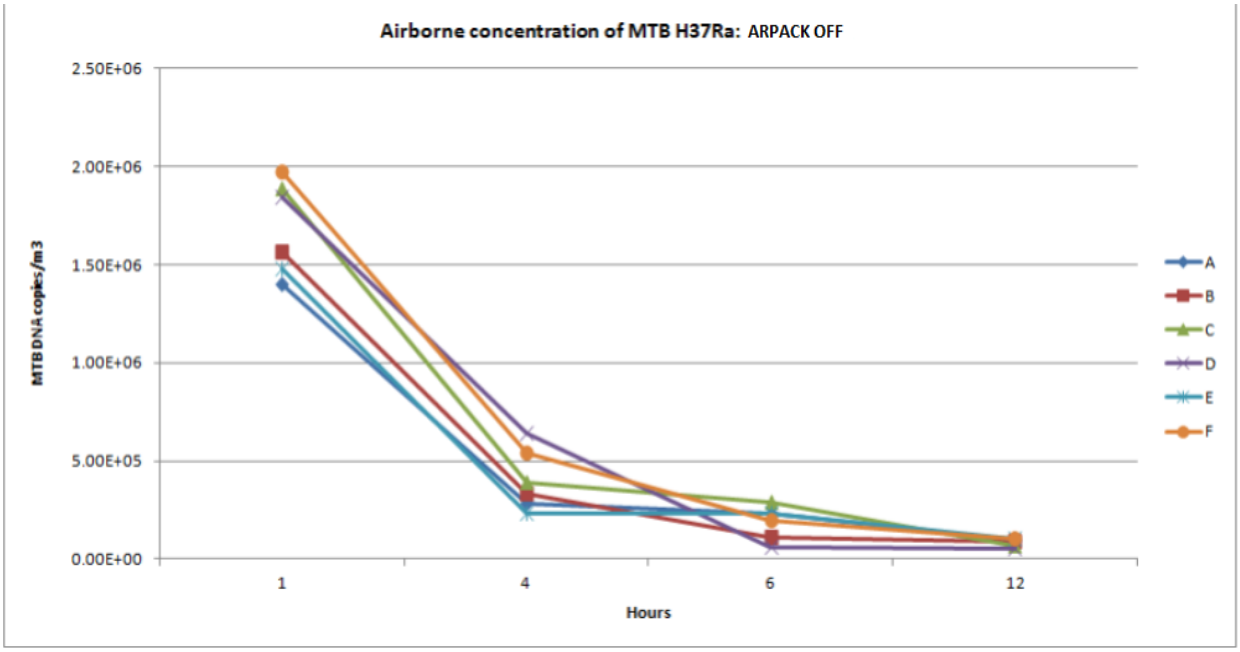

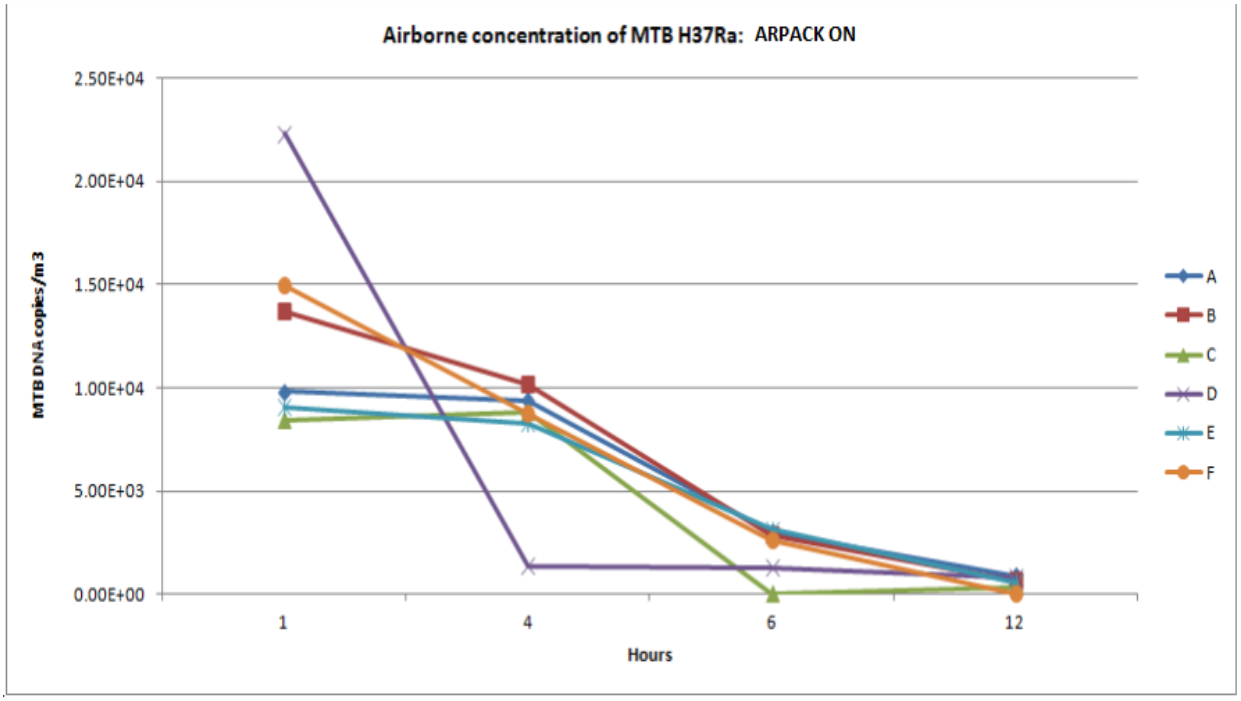

Figure 2 Average airborne MTB H37Ra concentration detected using the ARPACK in the off and on mode.

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The microclimatic parameters were within the indoor occupation comfort limits. The noise level of the machine was 9.9 db (A).

Table 2 Average microclimatic parameters measured during the sampling period.

Sample	Time (hour)	Temperature (°C)	Relative Humidity (%)	Velocity (m/s)
ARPACK off	1	19.8	60.3	0
ARPACK on	1	21.8	59.1	0
ARPACK off	4	20.1	58	0
ARPACK on	4	22.3	51	0
ARPACK off	6	22.6	56.4	0
ARPACK on	6	23.4	60.1	0
ARPACK off	12	21.9	54.1	0
ARPACK on	12	22.8	57.3	0

8 CONCLUDING REMARKS

The results obtained from a limited number of air challenges for the ARPACK device which uses photocatalytic technology (UV-A and TiO_2) demonstrates that the device is effective at sterilising air contaminated with MTB H37Ra in a controlled laboratory setting by >90% from 1 to 12 hours.

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10 SIGNATURES

I, **Buhle Binta**, 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.

Buhle Binta, MSc (Med)

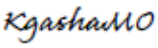
MEDICAL SCIENTIST (MSIN0001880)

_19/09/2014_____

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

Olga Kgasha, MSc (Med)

MEDICAL SCIENTIST (MS0001066)



_ 19/09/2014_____

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)

_19/09/2014_____

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)

_20/09/2014_____