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ORIGINAL ARTICLE

Determination of the Efficacy of the Cold Atmospheric Plasma with Nano Tio_2 Covered in Cathode Towards Enveloped Viruses such as Covid 19 and Influenza in Room Air

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ABSTRACT

In this research, Medwave air and surface disinfection system (model:Klin20)selected to investigation effect , towards aerosolised enveloped viruses in room air. Phi6Pseudomonas syringae phage, a surrogate for coronavirus and influenza, was used in the trials Viral suspensions of Phi6 were aerosolised within the ukas accreditation Campden BRI aerobiology laboratory to achieve initial levels of ~106PFU/m3, representing very heavily contaminated air . Air samples were taken at 15 minute intervals and analysed to determine levels of Phi6 in the room air over a total test period of 135 minutes. On 3 separate days, paired trials were carried out with the Medwave switched onand with the units witched off as a control to determine baseline levels of virus in the air overtime. Trials carried out on the first two test days showed no reduction in viral titre compared with the control. Further investigation revealed that a wiring loom within the test unit had become disconnected during transport and the instrument was therefore not functioning correctly. Results from the trial showed that the level of Phi6 in the room air decreased rapidly from an initial titre of 6.12 log PFU/m3to undetectable levels (<1.78 log PFU/m3) after 45 minutes of operation, representing a log reduction of ≥4.00 logs compared with the control run with the unit switched off. Log reductions of 2.21, 3.30and ≥4.00 logs were observed after 15, 30 and 45 minutes respective to the log PFU/m3countsin the control run

Keywords: Bioaerosols nano-titanium atmospheric cold plasma, covid19.

INTRODUCTION

In recent years, dealing with health problems, ecosystem damage and economic damage caused by microorganisms in the bioaerosols form has become very important. Infectious and viral diseases such as new influenza and COVID-19 due to pathogenic organisms have been spread worldwide [1,2]. Exposure to bioaerosols harms ecosystems and human health; also it causes infectious diseases, allergic diseases, acute toxic effects, respiratory diseases, neurological diseases, and cancer [3, 4]. European Union limit values for bioaerosols in residential areas are determined as 5x103 CFU/m3 for bacteria and 5x103 CFU/m3 for fungi [5]. The maximum limits recommended in the United States are determined to be 1000 CFU /m3 according to the National Institute of Occupational Safety and Health (NIOSH); also the total bacteria number not upper than 500 CFU/m3 according to the American Conference of Governmental Industrial Hygienists Conference (ACGIH) [6, 7].

A helpful and economical purification method is necessary due to the growth of health awareness in living regions, especially in the hospital. With the development of technology, electronic air purification systems are widely used to reduce the damage of bioaerosols. Microorganisms, dust, and similar particles in the inhaled air circulate positively. Nano-plasma is the ion form of the oxygen molecule and neutralizes positive charged microorganisms and particles in the air. Because of this neutralization, particles fall to the ground due to their weight. These operations cause prevent the inhalation of microorganisms and particles that negatively affect our health. Many studies are indicating that air purification devices have killing effects on bacteria and fungi [8, 9].

It is important to consider several factors in selecting the most effective air purification device like long-term performance, use minimum energy, and the minimum amount of unwanted harmful microorganisms and particles [10, 11].

In this study, with Nano-plasma technology by generating negative oxygenation, the rate of elimination of microorganisms in bioaerosol form and their load levels by a passive method in the environment used as a cleanroom in the hospital have been determined.

MATERIAL AND METHOD

In this research, Medwave air and surface disinfection system (model:Klin20)selected to investigation effect , towards aerosolised enveloped viruses in room air. Phi6Pseudomonas syringae phage, a surrogate for coronavirus and influenza, was used in the trials Viral suspensions of Phi6 were aerosolised within the ukas accreditation Campden BRI aerobiology laboratory to achieve initial levels of ~106PFU/m3, representing very heavily contaminated air. Air samples were taken at 15 minute intervals and analysed to determine levels of Phi6 in the room air over a total test period of 135 minutes. On 3 separate days, paired trials were carried out with the Medwave switched onand with the units witched off as a control to determine baseline levels of virus in the air overtime.Trials carried out on the first two test days showed no reduction in viral titre compared with the control. Further investigation revealed that a wiring loom within the test unit had become disconnected during transport and the instrument was therefore not functioning correctly.

Experimental details & conditions: Experimental details and conditions are given in Table 3. On each day of testing,the Campden BRI aerobiology laboratory was prepared and a test unit (Medwave Klin 20) was placed on a table within the room. Phi6 suspension was mixed with 3.0g/L Bovine serum albumen (BSA) at an initial titre

of109to 1010PFU/mLand 30ml was transferred into a Collison nebuliser. The nebuliser was placed into the room (figure 1) at a height of ~1 metre from the ground. A compressed air line was attached to the nebuliser with a regulator and pressure gauge to regulate the pressure to 1.6 bar.3 small electric fans were placed on the floor facing upwards to mix the air within the room and the room cooling system was operated to regulate the room temperature to 20±1°C.The aerobiology laboratory was then sealed and the compressed air feed to the nebuliser was started to commence aerosolization of the test organism. The nebuliser was operated for a period of 10 minutes, then switched off.250L air samples were taken at 15-minute intervals using a Merck Millipore MAS-100 air sampler loaded with NZCYM agar plates. At each sampling interval, the air sampler was loaded with a fresh NZCYM plate and introduced to and retrieved from the aerobiology laboratory via a transfer box (figures2and 3).After the test period had elapsed, the room air was decontaminated by operating HEPA filtered inlet and outlet air supplies until>10 air changes had occurred(approximately 30 minutes), and the test was repeated immediately with Medwave system switched off. The layout and positioning of equipment within the aerobiology lab is shown in figure 1

Table 1. The details of the product tested are shown below along with the sample code.

Campden BRI Sample Code	A273		
Name of the Product	Medwave air and surface decontamination system Model: Klin 20	Product serial number	Not provided
Product Manufacturer	Medwave . Ltd.		
Date of product arrival at Campden BRI	01/09/20		
Condition & appearance of product on receipt	Visual inspection did not reveal any damage		

Table 2: virus tested and bacteria details

Virus Host	**Culture code**	**Passage number**
Phi6 *P.syringae* bacteriophage *Pseudomonas syringae*	DSM 21518 DSM 21482	3

Stock solutions of the Phi6 phage were produced and kept at 5°C

Table 3: Details of experiment

Date of tests	17/09/20 to 23/09/20
Test organisms	Phi 6 *Pseudomonas syringae* phage (surrogate for coronavirus & influenza)
Room capacity	30m^3
Test temperature	20±1°C
Relative humidity in test chamber (%)	58.1
Method of aerosolisation	Collison nebuliser (1.6 bar compressed air); approximately 0.3mL inoculum/minute.
Aerosolisation time	10 minutes
Air sampling intervals	15 minutes (10 sampling intervals)
Air sample volume, method	250L, Merck Millipore MAS-100 air sampler
Inoculum carrier/interfering substance	3g/L BSA in sterile, glass distilled water
Incubation temperature	25°C

Microbiological Analysis: To elute viruses from plate surfaces, 3ml of SM bufferwasadded to the surfaceof each air sampler plate and plates were swirled thenstored at 5°C for 30 minutes, swirled again and eluate wasenumerated for levels of the bacteriophageusing the methodsdetailed in Table 4. Dilutions of the samples were carried out within 15 minutes of the test.

Table 4. Microbiological tests

Organism	Test method	Method Summary*
Phi6 enumeration	Plaque assay (Dawson *et al.* 2005[1]) amended**	Plaque assay with NZCYM 25°C for 18 – 24h

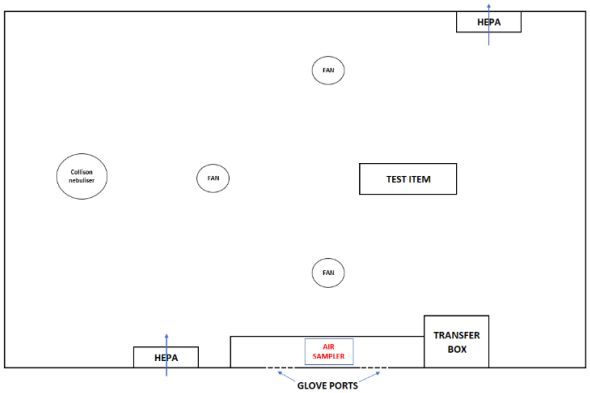

Figure 1. Layout of aerobiology laboratory

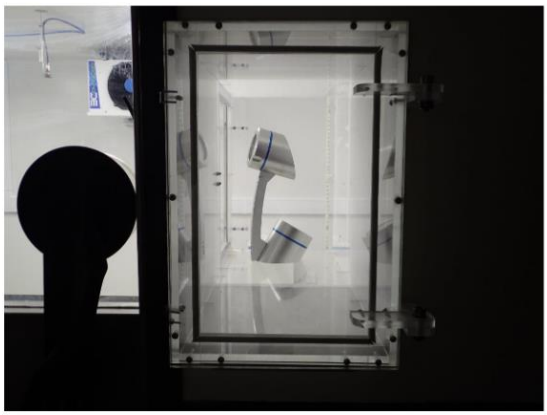

Figure 2 .Airlock sample transfer box–external view

Figure3 .Airlock sample transfer box–internal view

RESULTS AND DISCUSSIONS

Results from the trial showed that the level of Phi6 in the room air decreased rapidly from an initial titre of 6.12 log PFU/m3to undetectable levels (<1.78 log PFU/m3) after 45 minutes of operation, representing a log reduction of ≥4.00 logs compared with the control run with the unit switched off. Log reductions of 2.21, 3.30and ≥4.00 logs were observed after 15, 30 and 45 minutes respective to the log PFU/m3countsin the control run

Table 5-Log reductions achieved towards phi6 phage (enveloped virus)

Sampling time (minutes)	CONTROL RUN Medwave unit switched off (log PFU/m^3)	TEST RUN Medwave unit switched on (log PFU/m^3)	Log reduction (logs)
0	6.19	6.12	0.07
15	7.07	4.86	2.21
30	5.68	2.38	3.30
45	5.78	<1.78	>4.00
60	5.56	<1.78	-
75	5.28	<1.78	-
90	4.61	<1.78	-
105	4.84	<1.78	-
120	4.91	<1.78	-
135	4.33	<1.78	-

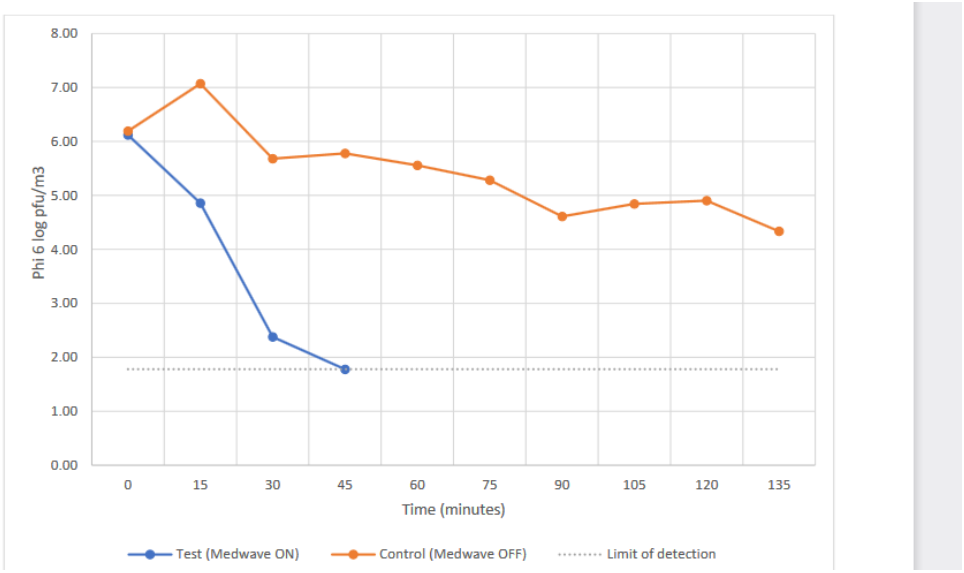

Figure 4: Log counts of Phi6 phagewith and without operation of the Medwave unit(enveloped virus)

CONCLUSION

The product, when operating under the recommended conditions, achieved a 4.00 log reduction in Phi6, a surrogate organism representing enveloped RNA viruses including coronavirus and influenza viruses after 45 minutes of operation. Comparative log reductions of 2.21 and 3.30 logs were observed after 15 minutes and 30 minutes of operation, respectively

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