Public Health England

Assessment of the Efficacy of Hydrogen Peroxide Vapour (HPV) Generated by the Specialist Hygiene Solutions Prototype HPV Unit

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About Public Health England

We work with national and local government, industry and the NHS to protect and improve the nation's health and support healthier choices. We address inequalities by focusing on removing barriers to good health.

We were established on 1 April 2013 to bring together public health specialists from more than 70 organisations into a single public health service.

About Biosafety Investigation Unit

The Biosafety Investigation Unit at Porton Down has been carrying out independent evaluations of infection control interventions in laboratories, health care, containment, workplace and domestic settings for over twenty years. Our expertise is in air and water microbiology applied to nosocomial, pharmaceutical and containment situations. We have developed and offer standard techniques for the determination of the efficacy of filters and air disinfection units, the performance of safety cabinets, sealed centrifuges rotors and air samplers. We are also able to assess liquid and gaseous disinfectants and the microbial air quality of healthcare facilities, workplaces and other environments.

The Biosafety Investigation Unit provides specialist bespoke research, testing and evaluation services for commercial customers that delivers independent analysis and reports. However as a public sector body we are not able to endorse any particular products or recommend them for use by the NHS or others.

Contents

Executive summary

A study was carried out to investigate whether levels of Hydrogen Peroxide Vapour (HPV) generated from the Specialist Hygiene Solutions Prototype HPV unit (supplied by Specialist Hygiene Solutions UK Ltd.) inactivated *Clostridium difficile* (NCTC 11029) inoculated and dried onto stainless steel discs.

Introduction

Gaseous disinfection has been routinely used in microbiology laboratories for many years. There are two main reasons for its use. Firstly, it is used to ensure the inactivation of potentially harmful bacteria and viruses on surfaces in order to prevent the exposure of laboratory and maintenance staff to infectious agents. It is also used to ensure a clean working environment to reduce the chances of contamination of the working area with background agents.

Over the last few decades the problems of diseases caused by environmental microbial contamination has been highlighted in scientific and social media. Therefore rapid, safe methods of decontaminating inhabited areas could help to reduce these levels of infection.

Hydrogen Peroxide Vapour is a relatively new gaseous form of disinfection but is high in popularity due to its natural decay to oxygen and water. A number of systems are available which have been specifically designed to carry out large-scale decontamination including laboratories, cleanrooms and hospital wards. The Specialist Hygiene Solutions Prototype HPV unit involves the use of Deproxin fluid, a 7% concentration formulation of hydrogen peroxide with silver. The Prototype HPV unit uses high frequency ultrasonics (1.8mhz) to create droplets of hydrogen peroxide solution. These droplets are entrained in a regulated air flow which forces and distributes it around the treatment space. Temperature and relative humidity parameters are monitored within the space and control the generation and injection of hydrogen peroxide vapour to ensure the dwell phase does not exceed 'dew point'. At the end of the treatment phase, the main Prototype HPV unit operates a deactivation fan, passing the air in the treatment space through a packed bed reactor of impregnated carbon to catalytically reduce the hydrogen peroxide vapour to water and oxygen.

This study has investigated the efficacy of HPV as a gaseous disinfectant against the micro-organism *Clostridium difficile* (NCTC 11029), dried on metal carriers. The study was carried out in a 21 m^3 room at PHE, Porton Down.

Materials and Method

Room set-up

The Specialist Hygiene Solutions Prototype HPV unit was set-up in the 21 m^3 environmental room (dimension 4 m x 2.3 m x 2.3 m) at PHE, Porton Down. The room is fitted with a filtered extract and supply ventilation system which was active whilst the operator was in the room. The ventilation system was not operated during the testing. The equipment was installed by Specialist Hygiene Solutions' staff and PHE staff were trained on its use.

Hydrogen Peroxide Vapour Generation

The Specialist Hygiene Solutions Prototype HPV unit used to generate the HPV and was operated to the following parameters.

Parameters*	
Injection Delta	Peak RH of 97%
Dwell Time	30 minutes
Aeration Time	60 minutes
H2O2 volume	7%

*Information supplied by Specialist Hygiene Solutions UK Ltd., no printed read out provided.

The experiment was carried out during 2016 at PHE, Porton Down.

Micro-organism

Clostridium difficile

As this organism is a strict anaerobe, all liquid and solid media must be reduced (exposed to a reduced O2, high CO2 atmosphere) for >18 hours prior to use.

Creation of parent stocks

The contents of the lyophilised vial were resuspended in Brain-Heart Infusion (BHI) broth, a portion of which is inoculated onto Fastidious anaerobe agar with Horse Blood (FHB) agar and the second portion added to BHI broth. Both were grown anaerobically at 37°C for 7 days and used to create the parent stock plates. Once resuscitated, *C. difficile* is grown on Clostridium specific media (CLO agar). An anaerobic atmosphere is provided by using 3.5 L sealed, anaerobic jars with the addition of Oxoid AnaeroGen anaerobic sachets and indicators, placed in 37°C incubators.

Creation of high-tire suspensions

A broth-based (CLOSPORE) method was employed to produce a high titre, high purity spore suspension and begins after creation of the parent stocks on FHB. All broths used were grown with lids loosened to allow anaerobic conditions to reach the culture. Fresh, pre-reduced CLO plates were inoculated from the parent stocks and grown anaerobically for 2 days at 37°C. A single colony from these plates was inoculated into 5 ml of pre-reduced Columbia Broth (CB) and incubated anaerobically at 37°C for 48 hours. It was then placed in the fridge until personnel were available to perform the next step (for 6 days). 50 µl of this inoculated CB was added to 20ml of pre-reduced CB in a larger container, which was grown anaerobically for 24 hours at 37°C. Finally, all 20 ml of this inoculum was added carefully, without the generation of bubbles, to 500 ml of pre-reduced Clospore broth and incubated anaerobically for 10 days at 37°C. The spore broth was centrifuged at 10,000 x g (maximum) for 30 minutes in an RC-6 centrifuge with FA-4x1000 LEX buckets and rotor, and all pellets were pooled and washed twice in sterile distilled water. The final spore pellet was weighed (1.12g) and kept in the fridge until the next day. The spore pellet was resuspended in 10ml of 0.1M sodium phosphate buffer and placed back in the fridge for one further day until consumables were available.

The 1.12g of spore pellet was then purified by the addition of lysozyme and trypsin to digest vegetative cells, with 20µg trypsin and 10µg lysozyme added per gram of spore wet weight. Actual weights of enzymes added were: 23.6 µg and 11.8 µg, respectively, and these were dissolved in 25ml of 0.1M Sodium Phosphate buffer, then 20ml of this muix

was added via syringe filtration to the spores. This was then sonicated for 60 seconds on ice, placed on ice for 30 seconds, this was repeated, then it was held at 45°C for 30 minutes. The sonication/ice/water bath step was performed once more, then the spore preparation was washed once with sd water by centrifuging at 3,000 x g for 20 minutes. It was then finally heat shocked at 70°C and washed to remove any remaining vegetative cells and debris. The spore suspension was assayed and assessed for percentage spores as described below. This spore suspension will last for 1 year at 4°C, with a loss in viability of 1 $\log_{10}$ over this time.

Assay of the suspension

The suspension was assayed by 1 in 10 serial dilution in sterile distilled water and spreading onto pre-reduced CLO agar before growth for 5 – 7 days at 37°C and enumeration of colonies. The concentration was 6.90 x 10^8cfu/ml.

Methodology Used

Nine Biological Indicators (BIs) were prepared by drying 10 µl of the microbial suspension onto the surface of sterilised stainless steel discs of 1 cm diameter. The micro-organisms were dried on the discs at 37°C for 1 hour.

The HPV disinfection device was set up by Hygiene Solutions UK.

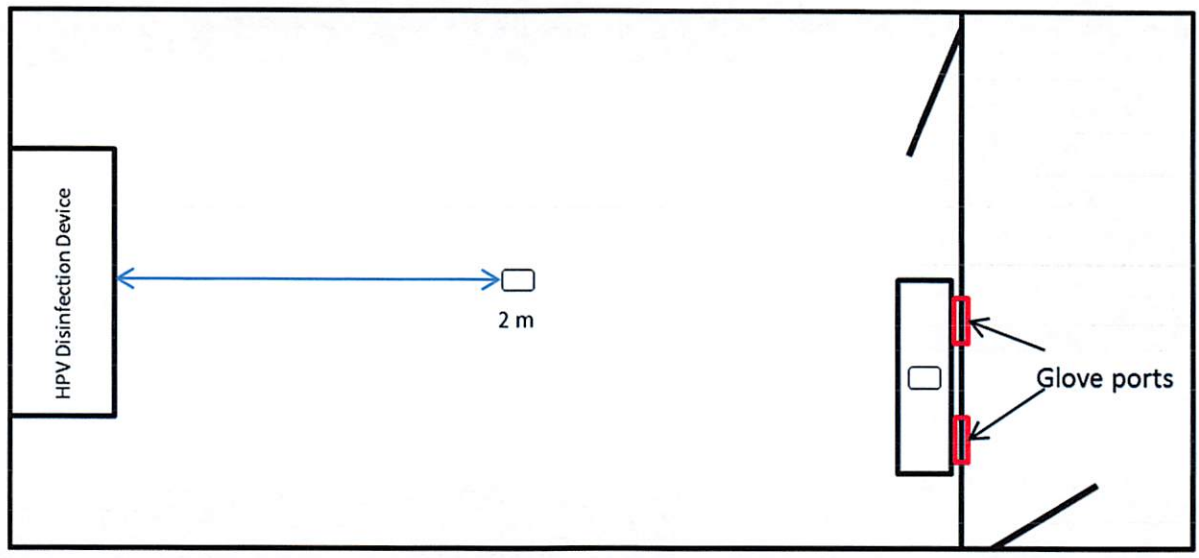
The discs were placed in the environmental room either on the table in front of the glove ports or 2 metres away from the disinfection device, at a height of approx. 1 metre off the floor. Three discs were placed at this location (see figure 1).

During the test procedure the room ventilation was turned off and the Specialist Hygiene Solutions Prototype HPV unit was activated. After completion of the cycle the room was vented for a period of 20 minutes before the operator entered and retrieved the samples. Two of the three discs from each location were assayed quantitatively to give a total viable count (TVC) and the remaining disc was assayed qualitatively. The discs that were assayed quantitatively were placed into individual universals containing 5mls Phosphate Buffered Saline (PBS). The universals were agitated using a mixer then serially diluted,

plated onto suitable agar plates and incubated to observe any growth. Discs that were assayed qualitatively were place into individual universals containing nutrient broth and incubated to show positive or negative growth.

Three positive controls were carried out for the test run. The positive controls were prepared by the same method as the test sample discs but they were not exposed to the disinfection cycle.

Figure 1. Diagram of sampling locations



Results

Test Conditions

Date	20th September 2016	Challenge Micro-organisms	*Clostridium difficile*
Operators	Anna Moy	Suspension Fluid	Sterile Distilled Water
		Concentration	10^8 cfu/ml (10µl)

Results

Location	Coupon No.	Colony Forming Units			Average Total cfu	Log Reduction
		N	1ml	Rest		
2m from device	1	0 , 0	0	0	<1	>5.32
	2	0 , 0	0	0		
	3					
By glove ports	1	0 , 0	0	0	<1	>5.32
	2	0 , 0	0	0		
	3					

Positive Control

Coupon No.	Colony Forming Units			Average Total cfu
	-1	-2	-3	
1	TNTC , TNTC	34, 25	3 , 1	2.13×10^5
2	TNTC , TNTC	48, 63	2 , 4	
3	positive			

Test Conditions

Date	21st September 2016	Challenge Micro-organisms	*Clostridium difficile*
Operators	Anna Moy	Suspension Fluid	Sterile Distilled Water
		Concentration	10^8 cfu/ml (10µl)

Results

Location	Coupon No.	Colony Forming Units			Average Total cfu	Log Reduction
		N	1ml	Rest		
2m from device	1	0 , 0	0	0	<1	>6.12
	2	0 , 0	0	0		
	3					
By glove ports	1	0 , 0	0	0	<1	>6.12
	2	0 , 0	0	0		
	3					

Positive Control

Coupon No.	Colony Forming Units			Average Total cfu
	-1	-2	-3	
1	TNTC , TNTC	TNTC , TNTC	20, 23	1.34×10^6
2	TNTC , TNTC	TNTC , TNTC	34, 30	
3	positive			

Test Conditions

Date	22nd September 2016	Challenge Micro-organisms	*Clostridium difficile*
Operators	Anna Moy	Suspension Fluid	Sterile Distilled Water
		Concentration	10^8 cfu/ml (10µl)

Results

Location	Coupon No.	Colony Forming Units			Average Total cfu	Log Reduction
		N	1ml	Rest		
2m from device	1	0 , 0	0	0	<1	>5.51
	2	0 , 0	0	0		
	3					
By glove ports	1	0 , 0	0	0	<1	>5.51
	2	0 , 0	0	0		
	3					

Positive Control

Coupon No.	Colony Forming Units			Average Total cfu
	-1	-2	-3	
1	TNTC , TNTC	94, 62	4, 12	3.26×10^5
2	TNTC , TNTC	56, 49	9, 9	
3	positive			

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