Investigation of gaseous disinfections systems for use within high containment laboratories

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INTRODUCTION

In the UK, decontamination of laboratories and biological safety cabinets (BS EN 12469:2000 cabinet standard) is still predominantly achieved using formaldehyde. However, there is some uncertainty over it's future use due to the implications of changes in the EU BPR 528/2012 and reclassification as class 1B carcinogen. In addition, future PHE laboratory redevelopment has led to the Biosafety Group investigating alternative gaseous decontamination technologies. The laboratory sector presents some unique challenges for gaseous disinfection systems, such as the use of high titre agents suspended in organic media and these will be used to test the technologies.

Initially a literature review on available gaseous decontamination technologies was completed and a trade off matrix generated to select the most appropriate to use in the laboratory testing phase; Bioquell, Hydrogen Peroxide Vapour, and Steris, Vaporised Hydrogen Peroxide.

Testing has been completed for the Bioquell system, of which the results are shown on this poster.

TEST METHODOLOGY

- HPV cycles were undertaken at containment level 2 (CL2) in Class II and III biological safety cabinets (BSC), environmental chamber (~20 m^3) and laboratory, against panel of hazard group (HG) 1 and 2 spore forming organisms, vegetative cells and bacterial phage. Then at CL3 against HG3 agents.
- Organisms were presented as a worst-case scenario for surface contamination. Stainless steel coupons were inoculated with 10 µl of the agent within its growth medium then dried prior to HPV exposure. As a comparison some agents were centrifuged and resuspended in PBS for comparison.
- Time course and end-point studies were undertaken with coupons assayed quantitatively and qualitatively.
- HG3 pathogens were challenged with the extended cabinet cycle that was shown to be effective against majority of HG2 organisms exposed.
- HPV material compatibility was also assessed against a number of standard lab equipment during laboratory studies.

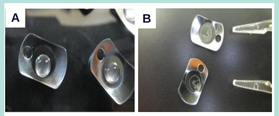


Figure 1. A) 10 µl bacterial suspension on SS coupons, B) dried suspension at 37ºC for 1 hour.

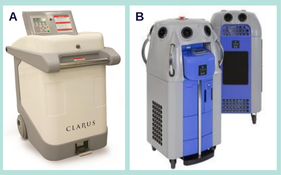


Figure 2. A) Bioquell Clarus C HPV generator for BSCs, B) Bioquell Q-10 & R-30 aeration unit for room decontamination. Courtesy of Bioquell UK ltd.

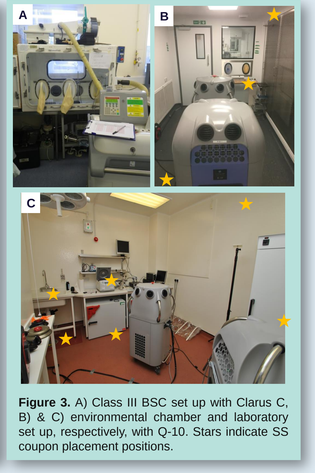
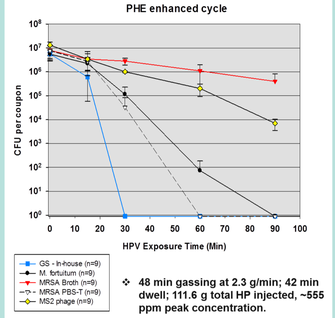


Figure 3. A) Class III BSC set up with Clarus C, B) & C) environmental chamber and laboratory set up, respectively, with Q-10. Stars indicate SS coupon placement positions.

RESULTS

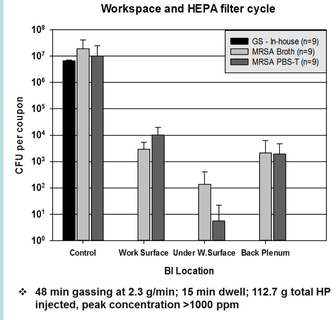
Class III BSC validated HPV Cycle



❖ 48 min gassing at 2.3 g/min; 42 min dwell; 111.6 g total HP injected, ~555 ppm peak concentration.

Figure 4. PHE enhanced Class III BSC HPV cycle efficacy against *G. stearothermophilus* spores (in-house) (□) [cycle validation BIs], *C. difficile* spores (○), MRSA in PBS+Tween (▽), *M. fortuitum* (●), MS2-coliphage (◇) and MRSA in nutrient broth (▼). Error bars represent standard deviation of three replicates in three independent cycles [n=9].

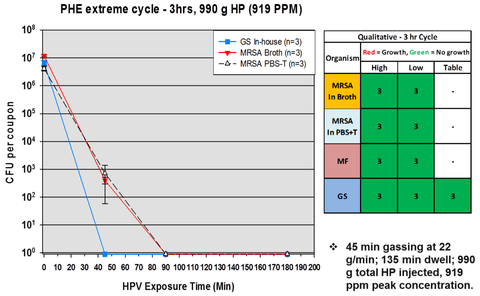
Class II BSC HPV Cycle



❖ 48 min gassing at 2.3 g/min; 15 min dwell; 112.7 g total HP injected, peak concentration >1000 ppm

Figure 5. Class II cabinet HPV fumigation efficacy against BIs: *G. stearothermophilus* spores (in-house) [Black bars] [cycle validation BIs], MRSA in broth [Light grey bars] and MRSA in PBS+Tween [Dark grey bars], positioned in various locations within the cabinet. Error bars represent standard deviation of three replicates in three independent cycles [n=9].

Environmental chamber HPV Cycle

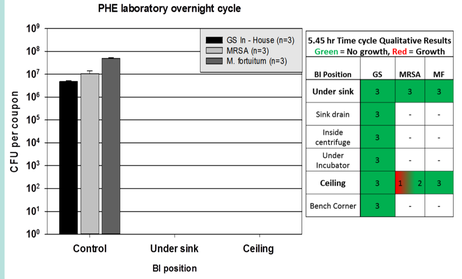


Qualitative - 3 hr Cycle			
Organism	Red = Growth, Green = No growth		
	High	Low	Table
MRSA In Broth	3	3	-
MRSA In PBS+T	3	3	-
MF	3	3	-
GS	3	3	3

❖ 45 min gassing at 22 g/min; 135 min dwell; 990 g total HP injected, 919 ppm peak concentration.

Figure 6. PHE environmental chamber extreme HPV cycle efficacy against *G. stearothermophilus* spores (in-house) (□) [cycle validation BIs], MRSA in nutrient broth (▼) and PBS+Tween (△). Error bars represent standard deviation of three replicates in one cycles [n=3]. Qualitative data shows recovered positive [Red fill] and negative [Green fill] BIs, in triplicate, positioned near ceiling level [High], diagonal floor corner [Low] and in front of the generator [Table].

Laboratory HPV Cycle

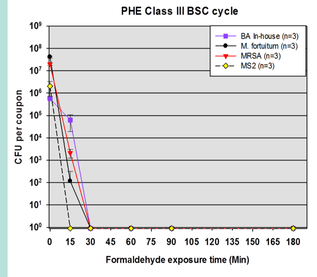


5.45 hr Time cycle Qualitative Results Green = No growth, Red = Growth			
BI Position	GS	MRSA	MF
Under sink	3	3	3
Sink drain	3	-	-
Inside centrifuge	3	-	-
Under Incubator	3	-	-
Ceiling	3	1 2	3
Bench Corner	3	-	-

❖ 48 min gassing at 22 g/min; 300 min dwell; 990g total HP injected, 766ppm peak concentration.

Figure 7. PHE overnight laboratory HPV cycle (5 hr 45 min, excluding aeration) efficacy against *Geobacillus* spores (in-house) [Black bars] [cycle validation BIs], MRSA in broth [Light grey bars] and *M. fortuitum* [Dark grey bars], positioned in various locations within the laboratory. Error bars represent standard deviation of three replicates in one cycles [n=3]. Qualitative data shows recovered positive [Red fill] and negative [Green fill] BIs, positioned under the sink, inside sink drain, inside partially open centrifuge, under a floor incubator, at ceiling level and in obscured bench corner.

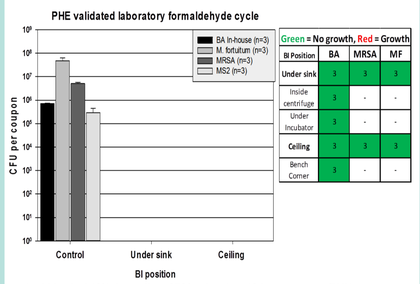
Class III BSC Formaldehyde Cycle



❖ 15 ml 40% formalin + 15 ml water; 3 hr exposure; RH peak range 79.1 % - 99.9 %, temperature peak range 20.4ºC – 26.7ºC. PPM not measured.

Figure 8. PHE Class III BSC formaldehyde cycle (1/2 of 6 hr standard exposure time), against *Bacillus atrophaeus* spores (in-house) (□) [cycle validation BIs], *M. fortuitum* (●), MRSA in nutrient broth (▼) and MS2-coliphage (◇). Error bars represent standard deviation from three replicates in one cycles [n=3].

Laboratory Formaldehyde Cycle



Green = No growth, Red = Growth			
BI Position	BA	MRSA	MF
Under sink	3	3	3
Inside centrifuge	3	-	-
Under Incubator	3	-	-
Ceiling	3	3	3
Bench Corner	3	-	-

❖ 300 ml of 40% formalin + 2700 ml water; 12 hr exposure; RH%, temperature and ppm not measured.

Figure 9. PHE overnight formaldehyde laboratory cycle efficacy against *B. atrophaeus* spores (in-house) [Black bars] [cycle validation BIs], *M. fortuitum* [Light grey bars], MRSA in broth [Dark grey bars] and MS2-coliphage, positioned in various locations within the laboratory. Error bars represent standard deviation of three replicates in one cycles [n=3]. Qualitative data shows recovered positive [Red fill] and negative [Green fill] BIs, positioned under the sink, inside sink drain, inside partially open centrifuge, under a floor incubator, at ceiling level and in obscured bench corner.

Selected panel of HG3 pathogens

- *Bacillus anthracis*
- *Brucella abortus*
- *Burkholderia pseudomallei*
- *Coxiella burnetii*
- *Escherichia coli O157*
- *Francisella tularensis*
- *Mycobacterium tuberculosis*
- *Yersinia pestis*

Exposed to enhanced Class III BSC HPV cycle:

48 min gassing at 2.3 g/min; 42 min dwell; 110.4 g HP per cycle; ~ 365.2 ppm average peak concentration.

Table 1: PHE extended Class III BSC HPV cycle against HG3 pathogens results

HG3 Agent	Initial CFU/coupon	Unexposed control CFU/coupon *	Test recovered CFU/coupon	Qualitative broth results **
Bacillus anthracis (spores)	2 x 10^8 9 x 10^6	2 x 10^8 8 x 10^6	0	No growth
Brucella abortus	5 x 10^6	9 x 10^5	0	No growth
Burkholderia pseudomallei	4 x 10^5	4 x 10^4	0	No growth
Escherichia coli O157	3 x 10^6	1 x 10^6	0	No growth
Yersinia pestis	8 x 10^4	8 x 10^3	0	No growth
Mycobacterium tuberculosis	2 x 10^6	3 x 10^6	1.5 x 10^2	Growth

* Coupons kept in the test BSC during HPV cycle, whilst contained in covered 90 mm petri dishes sealed in double bags to prevent exposure

** Coupons incubated in appropriate growth medium for >7 days and >28 days for *M. tuberculosis*

MATERIAL COMPATIBILITY

Figure 10. Class III BSC HPV fumigation, A) visible HP condensation build up on surfaces, B) BSC's powder coated paint surface deterioration post 3 cycles, C) worsening of surface deterioration

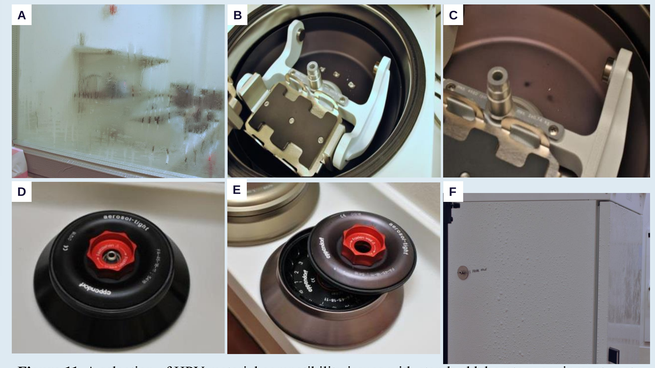


Figure 11. A selection of HPV material compatibility issues with standard laboratory equipment post overnight room cycle required to inactivate MRSA; A) heavily visible HPV condensation on surfaces, B) centrifuge interior before, C) centrifuge interior after exposure showing discolouration around coupon placement obscured surface (black marks), D) Eppendorf rotor before, E) Eppendorf rotor after showing discolouration, and F) LMS fridge/incubator exterior paint damage with bubbling effect.

DISCUSSION AND CONCLUSION

PHE undertook testing using two Bioquell HPV generators, Clarus C for BSCs and Q-10 (Figure 2) for the environmental chamber and laboratory. The cycle used for the Class III BSC needed to be enhanced from a recommended cycle to inactivate *M. fortuitum*, but still failed to inactivate MS2-coliphage and MRSA in broth (Figure 4). However, *G. stearothermophilus* spores, MRSA in PBS+Tween and *C. difficile* spores were inactivated within the 90 min exposure period (Figure 4). The enhanced cycle also led to some material compatibility issues (Figure 10 A and B). In the Class II BSC, MRSA in broth and PBS+Tween survived in all locations whilst *G. stearothermophilus* spores were completely inactivated (Figure 5). Similar inactivation kinetic trends were observed in the environmental and laboratory room fumigations, in which the Bioquell parametric cycles had little effect against MRSA and *M. fortuitum*; requiring longer more aggressive cycles to inactivate MRSA dried from broth (Figure 6 and 7). Comparable formaldehyde cycles following standard, were effective against all HG2 agents in both cabinets (Figure 8) and laboratory (Figure 9) exposures.

At containment level 3, enhanced the HPV cycle was effective against all agents except *M. tuberculosis*, which survived at concentration 50 fold the infection dose (Table 1). For some HG3 agents (*C. burnetii* and *F. tularensis*) drying alone for 90 min on SS coupons resulted in a large reduction in viability. The use of enhanced HPV cycles have been shown to be effective against most of the agents but they have produced material compatibility issues (Figures 10 & 11). These issues affect a range of materials and could lead to the need to replace equipment.

Additional testing will be conducted using vaporised hydrogen peroxide.

This work is currently being prepared for publication.

ACKNOWLEDGEMENTS

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