Reprocessing of PPE Garments for re-use by clinical staff by Vaporised Hydrogen Peroxide

Executive Summary

Background

Single-use PPE garments (gowns or one-piece suits) are known to be in short supply. Usage across UHS is around 1200 garments per day, making any supply line fragile at this high demand. This study was to assess feasibility of reprocessing used garments for further use.

Re-use (ie the wearing of PPE on a separate occasion from when first worn by the same user) of single-use PPE items is outside the intended use of the products and should be avoided wherever possible, however in defined circumstances has been temporarily approved by PHE, published jointly with NHS and MHRA (CAS Alert CEM/CMO/2020/018, 17.04.2020). Reprocessing (ie sanitisation followed by re-issue to a different user) is explicitly outside this guidance, although the guidelines state that separate advice on reprocessing will be issued in due course.

However, it was considered by UHS managers that an immediate contingent stockpile was required in the event of supply-line failure when use beyond the above recommendations might be required (ie if all supplies and stocks of PPE became exhausted, and essential clinical services are at risk as a result). This contingent stockpile would be developed and evaluated, then held in readiness should supply circumstances deteriorate. To be suitable for use, reprocessing must achieve both physical cleaning and microbial decontamination, must not deteriorate the functional effectiveness of the garment, must not introduce further risk to the processor or wearer, and must be able to be defined and controlled for reproducibility.

After initial in-house work to develop cool-temperature laundering of PPE garments to achieve reprocessing, UHS Sterile Services and Microbiology depts engaged Inivos Ltd to develop a process of vaporised hydrogen peroxide (VHP) sanitisation of the laundered garments, in order to assure an effective and reproducible level of sanitisation. Wessex Academic Health Science Network were requested to provide technical and project support. VHP was selected since it is known (Goyal, 2014) to be effective against coronavirus and leaves no toxic residues. Under advice of the UHS Director of Infection Control bacterial cultures were selected for testing decontamination efficacy which in his opinion would be more challenging to destroy than coronavirus SARS-CoV-2.

In principle, a decision to reprocess single-use PPE should be made by the Trust taking into account demand for PPE within the Trust and nationally, anticipated supply-line security, available alternatives, current national guidance, the Trust's understanding of the regulatory position, and the organisation's ability to set up, evaluate and reproducibly operate the process. The process should be the subject of a risk register entry supported by the Director of Infection Control and approved at Trust level; it should be underpinned by documented evaluation results and operating procedures, and accompanied by supporting information to staff.

Process development

- Room rapidly converted by UHS Estates to be suitable for garment reprocessing - all surfaces resistant to VHP and suitable hanging facility to support a defined loading pattern for garments.
- Supply of garments pre-used in clinical work obtained and laundered by UHS Sterile Services dept (brand Biosis Healing, Beijing, China, http://en.biosishealing.com/).
- Loading pattern established to ensure VHP penetration to all surfaces and subsequent extraction, introducing and orientating fans until sufficient garment movement was observed under usage conditions.
- First VHP cycle undertaken using Inivos' proprietary ProXcide automated VHP process, with garments in selected best-case and worst-case positions carrying biological and chemical indicators:
    - Biological indicators were live bacterial cell and spore cultures inoculated at increments of known bioburdens spanning $2x10^3$ to $4x10^7$ CFU, selected as above. Cultures were prepared, applied to the garment surfaces, sampled and incubated post-cycle by UHS Microbiology in their laboratory.
    - Chemical indicators were commercially available colour-change strips indicating presence of VHP and to some extent degree of exposure. These were read immediately the cycle ended.
    - Adjacent office spaces were monitored for hydrogen peroxide in atmosphere during the cycle.
    - At end of cycle residual levels of hydrogen peroxide in atmosphere and garments was measured.

- Second VHP cycle undertaken with more garments, improved loading pattern and wider range of biological indicator organisms, selected as above. For this cycle the study was extended to evaluate the microbial sanitisation achieved by Laundry only, VHP Processing only, and Laundry followed by VHP Processing.
- Following processing, garment integrity was tested using a water spray method developed within UHS.
- All work was undertaken in accordance with a written protocol agreed in advance.

Results

Cycle	Process	Garment Qty	**Biological Indicator result** (no. of samples showing growth/total no. of inoculations)	**Chemical Indicator result** (no. of indicators/total no. used)
1	VHP only	50	0/18 growth (6/18 Env[a])	26/27 fully, 1/23 part[b] exposed
2	Laundry only	78	53/54 growth (no ID[c])	27/27 fully exposed
	VHP only		1[d]/54 growth (7/54 Env[a])	
	Laundry + VHP		0/54 growth (10/54 Env[a])	

Key:-

a) Env – some samples showed growth of the biological indicator on incubation – subsequent robust identification by multiple methods showed these to be of different identity to the inoculating organism, so can be discounted as Environmental contaminants of the sampling process.

b) In Cycle 1 one chemical indicator showed only partial exposure – this was inside a garment leg (worst case).

c) Almost all samples from laundry-only processing showed growth – organisms were not identified.

d) In Cycle 2, VHP-only samples, 1 inoculation with Staph. aureus showed growth with the same organism on incubation. Whilst this may have been a sampling contaminant, it has been taken as positive-growth result.

Conclusion

- Test reprocessing of laundering followed by vaporised hydrogen peroxide exposure achieved satisfactory standards of disinfection, based on limited microbiology testing carried out in UHS Microbiology Laboratory, using organisms considered on current data to be worst-case surrogates for SARS-CoV-2.
- The statement is based on successful removal of heavy inoculum of Staphylococcus aureus, Klebsiella pneumoniae and Bacillus oceanisedemenis spores as part of verification. The UHS Director of Infection Control advises that results provide reasonable assurance this method will therefore remove SARS-CoV-2 from the garments tested. Non-pathogenic environmental organisms identified in the garment samples are of no clinical significance and thought to originate from the sampling process.
- Because of the environment where this process is conducted and post-cycle presence of environmental organisms, it is considered that the method can achieve sufficient decontamination of PPE garments to be used in clinical areas but that these should not be used for sterile process, eg theatres.
- VHP reached all areas of the chamber, indicating the revised loading pattern was suitable.
- No VHP was detected outside the processing room, and effective end-of-cycle removal of hydrogen peroxide residues from environment and garments was demonstrated, indicating process safety.
- Garment integrity was not affected by the process (single cycle).
- Cycle parameters show the process is reproducible.

This process is suitable, subject to Trust approval, to be developed into operating procedures within UHS Sterile Services to reprocess PPE garments, for use in the event of supply-line failure. The process could be transferred to other centres provided prior qualification of local conditions is undertaken – this study provides a basis for designing such qualification (separate guidance issued).

Limitations

- It is recognised that the work undertaken does not constitute validation as it lacks statistical power and only tested one garment type, but it is sufficient to indicate effectiveness of the processes, and establishes a method for testing further garment types.
- With a 3hr cycle time, capacity of the process (estimated to be 450 garments per day max.) is well below the daily demand for PPE garments at UHS.

Further work

- Ongoing testing of garment permeability/integrity is needed if/when repeated cycles are introduced.
- Risk assessments and Risk Register entry should be completed, for both organisational risks of reprocessing and CoSHH risks of the VHP process
- Procedures to cover this process need to be written by UHS and should be assessed for achievability.
- Staff training (internally & by Inivos) against procedures will be necessary.
- Briefing to staff on the evidence to support use of reprocessed PPE will be essential to retain confidence.
- If further testing of different garment types becomes necessary, biological indicator results matched to chemical indicator data from this study should enable subsequent studies to be undertaken with chemical indicators alone, simplifying and shortening the evaluation.

Sharing this work

- A full study report is available, giving details of methods and results.
- It is likely all other UK NHS Trusts are in a similar position of high PPE demand with insecure supply-lines. The study protocol, full report and this executive summary will be shared initially with NHS Trusts in the Wessex and Oxford AHSNs areas participating in a knowledge-sharing group around PPE reprocessing, open to all NHS Trusts in the area. If scope permits it will be shared with any NHS Trust interested in exploring PPE reprocessing.
- To support development of reprocessing methods in other Trusts a 'Suggestions for Implementation' document has been developed from study findings, and will be shared with the other papers.
- All methods, findings and documents will be shared with the joint national NHSE/HSE/PHE working group investigating PPE reprocessing with a view to issuing national guidance.

Study developed and conducted between 21.04.20 and 13.05.20 by:-

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VHP equipment, materials and specialist engineer time on-site provided and funded by:-

Tom Lister, Business Development Manager, Inivos Ltd

Study methods and findings are co-authored by UHS and Inivos Ltd with project support from Wessex AHSN, 13.05.2020.

References

Goyal, S. e. a., 2014. *Evaluating the virucidal efficacy of hydrogen peroxide vapour.* [Online] Available at: https://www.ncbi.nlm.nih.gov/pubmed/24656442 [Accessed 12 May 2020].

Reprocessing of PPE Garments for re-use by clinical staff – Vaporised Hydrogen Peroxide method

REPORT

Background

Single-use PPE garments (gowns or one-piece suits) are known to be in short supply, although at present supply lines for these have not failed at University Hospital Southampton NHS Foundation Trust (UHS). Usage across UHS is around 1200 garments per day, making any supply line fragile at this high demand.

The purpose of this study was to evaluate whether it is technically and organisationally feasible to reprocess PPE garments, and to develop a procedure with risk assessment for Trust approval which could be used to reprocess garments which would only be put into clinical use in the event that supply lines fail, jeopardising clinical services. The results and procedure will be shared nationally for other Trusts to consider.

Reprocessing of PPE masks was not included in this study.

The work was a joint collaboration between UHS and Inivos Ltd with technical support from Wessex Academic Health Science Network

Principles

1. Liability

Re-use of single use items is outside the intended use of the products and should be avoided wherever possible, however re-use in defined circumstances has been temporarily approved by PHE, published jointly with NHS and MHRA (CAS Alert CEM/CMO/2020/018, 17.04.2020); reprocessing (ie sanitisation followed by re-issue to a different user) is explicitly outside this guidance, although the guidelines state that separate advice on reprocessing will be issued in due course.

The Trust carries a liability under CoSHH Regulations (Health And Safety At Work Act 1974) to supply staff with appropriate levels of safe, effective PPE, so this study must demonstrate no reduction in quality or increased risk to the user from reprocessing - risk either from microbial exposure during use or as a result of the reprocessing. If unavoidable after all supplies and stocks of PPE have been exhausted, clinical use of reprocessed garments should only be undertaken on the basis of quantitative evidence that the process is:-

- Safe for those undertaking reprocessing activity
- Effective for garment sanitisation (reduction to zero of viral contamination)
- Safe for garment integrity
- Safe for the staff subsequently using the garments
- Able to be controlled and written in an operating procedure to ensure reproducibility
- Clearly described to assure garment users of this continuity of quality.

2. Sanitisation process options
   - Washing with detergent at 30°C followed by cool tumble drying is thought to be sufficient for viral inactivation, renders garments physically clean, but is unproven and difficult to demonstrate effectiveness given the process variability. It has been found (in a separate study) not to compromise the integrity of garments, providing tumble drying is not excessively hot or lengthy. This facility is available to UHS following the rapid installation of a bank of domestic washing machines and tumble dryers.
   - Washing under the NHS-recommended laundry cycle (HTM 04-01) is expected to be effective at decontamination however experience at UHS and elsewhere indicates at least some single-use garment types will be damaged, affecting permeability – this damage may be invisible. This facility is not available to UHS as the service is contracted out.
   - Exposure to hydrogen peroxide vapour is expected to be highly effective at reducing viral (and other microbiological bioburden) to zero (based on validated processes in other settings[1]), is reproducible, quantifiable and feasible to demonstrate microbiologically, but is less effective if organic matter is present and does not render garments physically clean. See ***Appendix 2*** for Factors affecting efficacy of VHP as a sanitisation agent and controls used.
   - Physical washing in liquid disinfectants whilst expected to be effective is unlikely to be able to be achieved safely for operators (using eg. hypochlorite, hypochlorous acid or hydrogen peroxide concentrates added to the wash).

The combination of physical washing with detergent and tumble drying followed by exposure to hydrogen peroxide was considered to be the most likely to be effective for both viral decontamination and physical cleaning, with scope to be demonstrated robustly and described clearly for both Trust infection control leads and staff using the reprocessed garments. This study therefore investigated vaporised hydrogen peroxide treatment of garments previously subjected to washing with detergent at 30°C and low-temperature tumble drying.

[1] Goyal, SM et al, Evaluating the virucidal efficacy of hydrogen peroxide vapour, J Hosp Infect. 2014 Apr; 86(4):255-9, accessed from https://www.ncbi.nlm.nih.gov/pubmed/24656442 12.05.2020

3. Risks

3 main risks are identified and will be addressed:-

- Exposure to hydrogen peroxide during reprocessing or during wearing of processed garments - risking severe respiratory, eye, skin, mucous membrane irritancy; highly toxic with significant inhaled exposure during processing.
- Failure of sanitisation efficacy – partial or complete – risking viral exposure to users.
- Damage to garments as a result of strong oxidant action of hydrogen peroxide, reducing barrier integrity as PPE in use – risking viral exposure to users.

Study Summary

Investigation	Study questions	Outputs
Pre-study	• What washing and drying process is effective at cleaning without damaging garments? • How can garment integrity be tested?	• Defined wash cycle, detergent & load • Defined dry cycle • Defined post-process integrity test
Study 1 (no VHP)	• What is an effective loading pattern? • Is effective air distribution achieved?	• Defined loading pattern including chemical indicator location

Investigation	Study questions	Outputs
	• What safe practices and controls are required to ensure operator safety?	• Defined use and location of additional fans • Identified safe practices & controls
Study 2 (with VHP) Undertaken in 2 Runs	• Is an effective sanitisation achievable? • What steady state parameters deliver & demonstrate the sanitisation? • How can cycle effectiveness be demonstrated for each batch? • Are the identified safe practices and safety controls effective? • Is garment integrity affected by the process?	• Biological indicators on each type of fabric demonstrate effective sanitisation of the fabric itself. • Defined reported cycle parameters inc time & H2O2 qty. • Identified chemical indicator to use for each batch to demo gas penetration, at identified worst-case locations • Process demonstrated from generator report • Confirmed safe practices • Defined integrity test to use for each batch • Demonstrated integrity compared to control.
Study 3 (with VHP)	• Is the defined process reproducible? • How many processes can safely be conducted without affecting garment integrity?	• Cycle parameter reports and chemical indicator results demonstrate reproducibility • Demonstrated integrity compared to control over defined repeated cycles.

Facilities

An office was selected to be converted for use as the reprocessing chamber, accessed from a quiet corridor and in the area managed by Sterile Services dept, also responsible for PPE garment washing/drying. The space measured 4.1m long (door to windows), 3.5m wide and 3.4m high.

The space was cleared of all furniture and fittings and new vinyl floor covering placed over the existing covering, sealed all round with waterproof duct tape. Opening windows frames were made of powder coated aluminium, existing wall and ceiling finishes were washable paint, the door with inset window was of varnished wood and included key and number-pad locks. Electrical fittings in trunking and light fittings were left in place, ceiling-mounted smoke sensor was electrically isolated and physically covered. There was no false ceiling or ceiling tiles. All surfaces were cleaned of visible dust and debris, including tops of windows and light fittings. All holes in the walls were sealed with duct tape; there were no channels to adjacent offices.

Chains (galvanised steel) were suspended width-wise 2m above the floor, to secure wall fixings, 4 rows in total equally spaced along the length of the space. Rows 2-4 had links approx. 4cm long; row 1 had shorter links approx. 2cm long.

See ***Figure 1*** for details of room layout.

See ***Appendix 3*** for Criteria used to define a suitable VHP reprocessing chamber / room

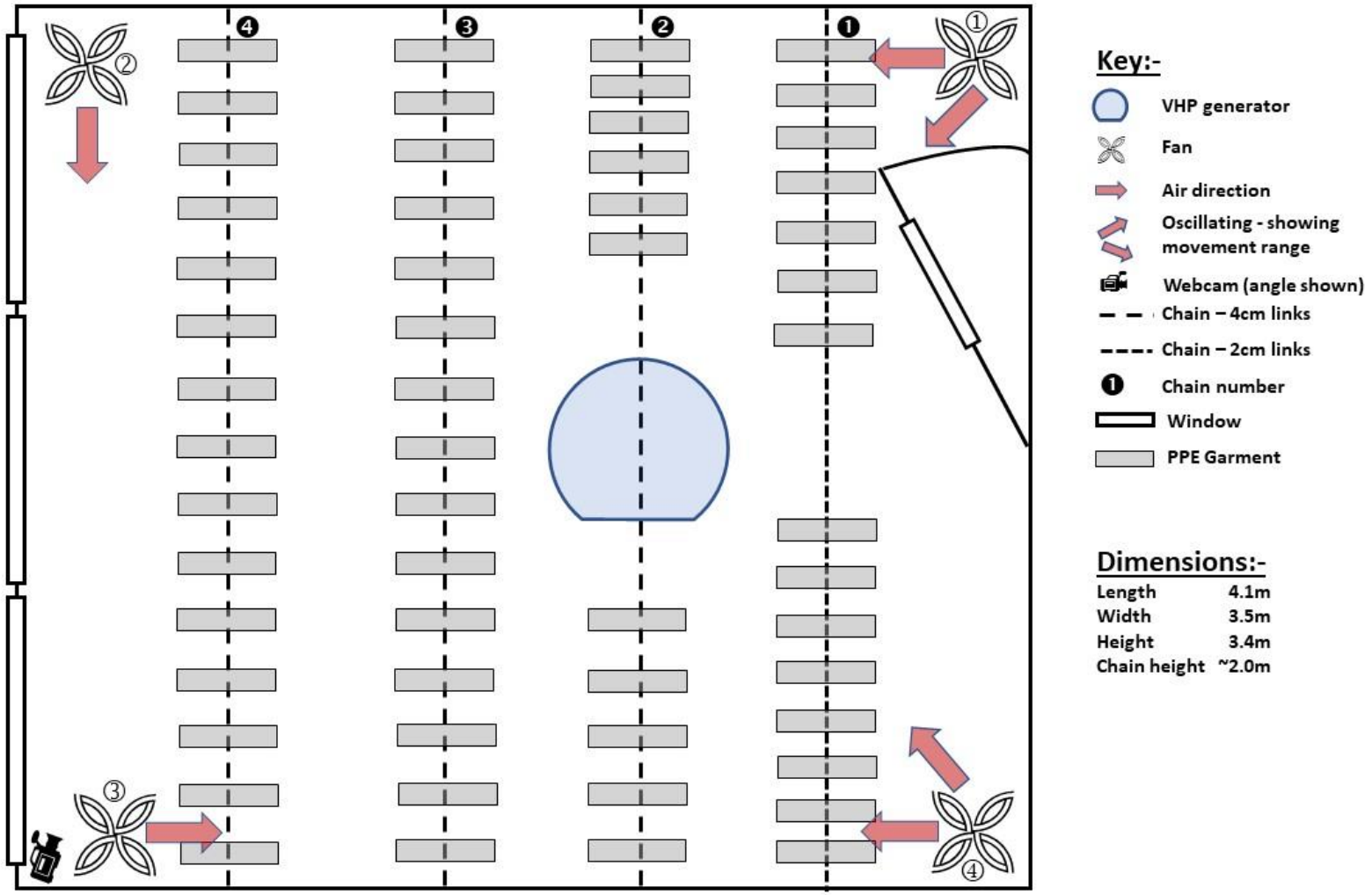

Figure 1 – Room layout for reprocessing chamber, Run 1

Equipment

Vaporised hydrogen peroxide generator: ProXcide, s/n 0017, Hygiene Solutions*, Kings Lynn, UK

Hydrogen peroxide 7.5% solution:	ProXcin, H1303BO Hygiene Solutions*, Kings Lynn, UK
PPE garments:	Biosis Healing, Beijing, China (http://en.biosishealing.com/) 50 garments used in Run 1, 78 garments used in Run 2
Coat hangers:	Various (plastic-coated metal or polystyrene with metal)
Handheld H_2O_2 gas monitor:	ATI Portasens III D16 s/n D16-00-0411 with H_2O_2 sensor, Saddleworth, UK www.atiuk.com
Desk/office fans:	Various: F1 & F4 floor-base and reciprocating, F2, F3, F5, F6, F7, F8 stand-mounted and fixed.
Webcam:	Logitech C922 Pro Stream connected to laptop outside room
Primary Chemical indicators:	Sterrad, ASP, Irvine, USA, product ref 14100, Lot 162911-01, exp 11-Dec-2020, affixed with "Sterrad" indicator tape
Secondary Chemical indicators:	Potassium Permanganate applied to filter paper strips
Biological indicators:	Run 1 - Spore culture of *Bacillus oceanisediminis* prepared by UHS Microbiology dept, see ***Appendix 4***
	Run 2 - *Bacillus oceanisediminis* as Run 1
	Staphylococcus aureus ATCC 29213
	Klebsiella pneumoniae NCTC 11228
	Inoculation levels as Table 1 below

* Hygiene Solutions renamed as Inivos Ltd in early 2020

PPE garments were intended for single use, made of liquid-impermeable coated fabric with elastic cuffs at wrist and ankle, zipped front, blue-taped seams, elasticated hood. 50 garments were tested in Run 1 as this was the only quantity available, and 100 in Run 2 as more were available – this latter is the full capacity of the room.

Secondary Chemical indicators were prepared because the primary indicators were found not to react to liquid hydrogen peroxide, and it was uncertain if they would react to the vapour. Secondary indicators were prepared with 2 different concentrations of potassium permanganate solution applied to filter paper strips approx. 10cm x 1.5cm then dried in warm air; part of the dried solution was covered with clear adhesive tape to act as a control.

See ***Figure 1*** above and ***Figure 2*** below for layout of this equipment.

Method & Results

A full written protocol was followed for all methods (***Appendix 1***, separate document)

This study was undertaken on a Saturday when adjacent offices were unoccupied in case of inadvertent hydrogen peroxide exposure.

Method - Study 1 – to determine loading pattern

1. VHP generator was sited centrally in the chamber – orientated as in Figure 1 (during operation VHP is emitted radially through 270^{o} but no gas emits from the flat side)
2. Garments were hung from coat hangers hooked through the chain loops at the following spacing:-
    a. Row 1 (small chain links) 8-9 links = approx. 15cm – 15 garments in total (8+7) separated with a gap
    b. Row 2 (large chain links) :
        i. Behind generator (lower diag) 6-7 links = approx. 20cm – 5 garments
        ii. In front of generator (upper diag) 4 links = approx. 10cm – 6 garments
    c. Row 3 (large chain links) 6-7 links = approx. 20cm – 15 garments
    d. Row 3 (large chain links) 6-7 links = approx. 20cm – 15 garments

    Garments nearest the walls were spaced approx. 20cm away from walls
3. The generator was set by the engineer in maintenance mode to force the dwell-cycle fan to run at close-to normal performance without VHP injection.
4. With the door and windows shut the hanging garments were observed for movement, with a target as per protocol of "Garments may touch each other gently but have freedom to move with airflow; garments can move or agitate as if in a gentle breeze, allowing all occluded surfaces to periodically move away from each other. Garments in all areas should be seen to move"
5. Observers stood inside the room near the door; garment movement at the far end was observed via the webcam and laptop.
6. Since insufficient movement was observed fans were sited one by one until movement was adequate, located as in Figure 1. Fans 1 & 4 placed on the floor were set to oscillate, fans 2 & 3 mounted on stands were set for a fixed direction.
7. Video was captured from webcam and phone camera from the door to demonstrate movement.
8. Generator fan settings were returned to normal by the engineer and switched off.
9. Gowns were found to be slightly damp from the washing process so door and windows were opened and fans left running for 1 hour to dry them off before moving to Study 2.

Results – Study 1

Movement was demonstrated in line with protocol acceptance criteria. Video was captured and saved. Loading pattern was recorded on paper

Figure 2 shows an image of the fully loaded chamber for Study 1 and Study 2 Run 1

Figure 2 – over-head view of garments in final loading pattern, taken from door (Study 1 & 2 Run 1)

Method - Study 2 – to demonstrate effectiveness of the loading pattern from Study 1

1. Windows were closed, garments fans and generator positioned as in final arrangement in Study 1. All garments were hung with front zips open to optimise gas circulation to inside surfaces.
2. 3 garments were removed and taken to Microbiology lab for inoculation with biological indicators at 6 concentrations ranging from $2.0x10^4$ to $2.0x10^7$, as in ***Table 1*** below (see ***Appendix 4*** for full method), then replaced in the load. Biological indicators were grouped and sited under one arm of each test garment on the outside surface, to represent a worst-case position. Garment placement in the load was considered to be worst-case for gas exposure.
3. Chemical indicators were placed throughout the load, with the following placement criteria:-
    a. 2 at best-case positions as positive controls, facing the generator (A and B)
    b. Others at expected worst-case positions based on garment spacing, movement in air-flow, and distance from the generator (C to W). This included:-
        i. Outer-surfaces facing towards or away from the generator (see ***Figure 5 right)***
        ii. Inside garments (see ***Figure 5 left)***
        iii. Different positions on the garment
    c. One chemical indicator was sited close to the biological indicators on each of the 3 test garments
4. After a final check that all items were arranged correctly and that no extraneous items were left in the chamber, the door was closed, locked and sealed all round with vapour-resistant tape.
5. The VHP gassing cycle was run according to Inivos/Hygiene Solutions procedures, printed and available to hand, including full safety and signage procedures (see ***Figures 3 & 4***).
6. Garment movement was observed through the door window and using the webcam at intervals throughout the cycle to confirm adequate garment movement to the description in Study 1, and video captured to demonstrate this.
7. At the end of cycle the door was cracked open and the hand-held gas analyser used to test the concentration of hydrogen peroxide in the chamber air. If above 2ppm (safe working exposure

limit for 15min in any 8 hours) the door was resealed and a further 15min gas extract cycle run on the generator, then measurement repeated.

8. Once hydrogen peroxide levels were below 2ppm the room was unsealed, entered and external windows opened to improve out-gassing from garments.
9. Using aseptic procedures and sterile equipment the inoculated areas on the 3 biological indicator garments were cut out and placed in individual pre-labelled media containers of Brain Heart broth and incubated at 35°C (for details see ***Appendix 4***).
10. After approx 50min fresh air ventilation 9 garments were sampled externally and internally for residual hydrogen peroxide levels using the handheld gas analyser – for internal areas the probe was placed as far as possible into the garment, for outer surfaces the probe was placed into the scrunched-up fabric. In both cases gas released from the fabric would be detected – experience from the engineer present was that if present in the fabric sufficient would be released to give a quantifiable reading. (see ***Figure 7)***
11. The arrangement of garments with biological and chemical indicators and gas-tested samples is shown in ***Figure 6*** – Sample Layout

Run	Inoculating organism	Inoculation level (CFU)						Vol
1	Bacillus oceanisediminis (spores)	$2.0x10^4$	$2.0x10^5$	$1.2x10^6$	$2.0x10^6$	$1.2x10^7$	$2.0x10^7$	Var.

Table 1 – inoculation levels on test garments for Study 2 Run 1

Figure 3 - VHP generator in operation

Figure 4 – Room locked & sealed with safety signage

Figure 5 – Chemical indicators pre-exposure, showing location inside garment (left) and outside right)

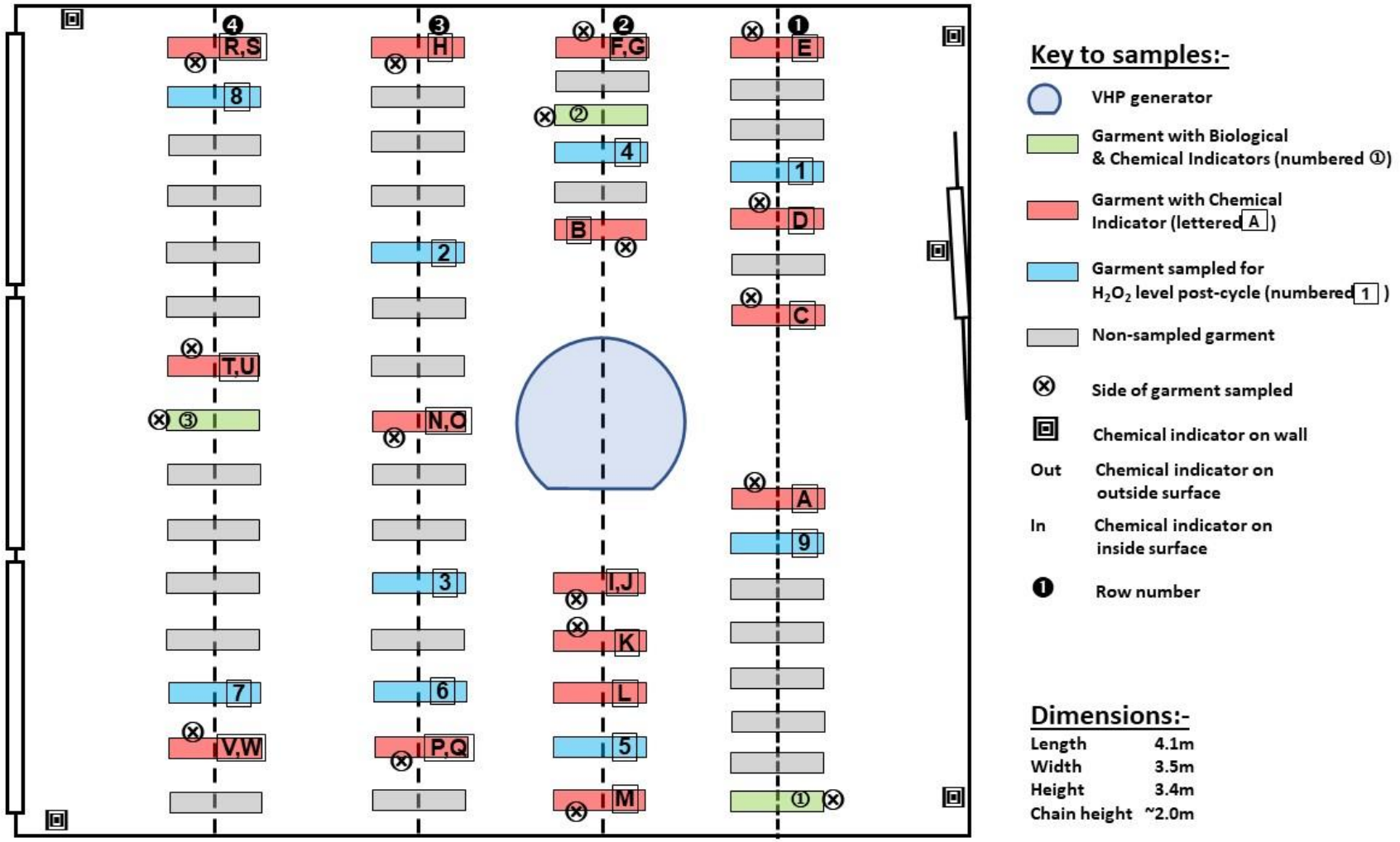

Figure 6 – Sample layout, Study 2 Run 1

Results – Study 2 Run 1

1. Cycle duration & parameters

The cycle completed as expected – see report ref 25420201332 ***Appendix 6***. On-board parameters indicated it had been delivered successfully.

Due to Hydrogen peroxide levels in the room 2 further extract cycles were required, taking the total cycle time to 2hr 58min:-

Process	Time	H_2O_2 measurement
Cycle start	14:32 25.04.20	n/a
Cycle end	16:47	4.1ppm
1st extra extract start	16:52	n/a
Extract end	17:07	3.1ppm
2nd extra extract start	17:15	n/a
Extract end	17:30	1.9ppm

Table 2 – Cycle parameters

2. Chemical indicators

Sample ID	Sample type	Inside or Outside garment	Location on garment	Result
BI 1	Next to Biological Indicator	O	Under arm	Fully exposed
BI 2		O	Under arm	Fully exposed
BI 3		O	Under arm	Fully exposed
A	Positive control	O	Leg	Fully exposed
B	Positive control	O	Leg	Fully exposed
C		O	Front	Fully exposed
D		O	Leg	Fully exposed
E		O	Leg	Fully exposed
F		O	Chest	Fully exposed
G		I	Chest	Fully exposed
H		I	Leg	Fully exposed
I		O	Leg	Fully exposed
J		I	Leg	Fully exposed
K		O	Chest	Fully exposed
L		I	Hood	Fully exposed apart from where occluded by paper strip
M		O	Chest	Fully exposed
N		O	Chest	Fully exposed
O		I	Chest	Fully exposed
I		O	Leg	Fully exposed
Q		I	Leg	Partially exposed
R		O	Chest	Fully exposed
S		I	Chest	Fully exposed
T		O	Neck	Fully exposed
U		I	Neck	Fully exposed
V		O	Chest	Fully exposed
W		I	Chest	Fully exposed
Wall 1	Wall	n/a	n/a	Fully exposed
Wall 2	Wall	n/a	n/a	Fully exposed
Wall 3	Wall	n/a	n/a	Fully exposed
Wall 4	Wall	n/a	n/a	Fully exposed
Wall 5	Wall	n/a	n/a	Fully exposed

Table 3 – Chemical Indicator results

3. Garment gas sampling

Garment	Measured Hydrogen Peroxide gas level at surface (ppm)			
	Inside body	Inside upper leg	Inside lower leg	Outside
1 (post cycle)	1.7	1.8	1.8	0.6
2	0	0	0	0
3	0.5	0.5	0.5	0
4	0.5	0.5	0.5	0
5	0.5	0.5	0.5	0
6	Not tested	Not tested	0.7	0
7	Not tested	Not tested	0.6	0
8	Not tested	Not tested	0.8	0
9	Not tested	Not tested	0.5	0

Table 4 – Garment sampling results

All samples after 50min fresh air ventilation apart from Garment 1 (immediately post cycle)

For garments 6-9 only the inside lower leg was tested since shown in previous samples to be indicative of worst case – see ***Figure 7***.

Figure 7 – sampling garment H_2O_2 levels post-cycle

4. Biological Indicators

Incubated growth media were read at 20 hours, 48 hours and 72 hours. Some growth was visible at 20 hours; samples were left to continue incubation. Further growth was identified at 48 hours and all media showing growth were sub-cultured onto Blood Agar and incubated in 5% CO_2 at 37°C for 16-24 hours. If no growth was observed, the culture was repeated on Chocolate and Blood Agar and incubated in 5% CO_2 at 37C for 16-24 hours. (5% CO_2 ensures growth of the widest range of organisms.) No further samples showed growth at 72 hours. After 24hours of growth on agar sub-cultures were examined morphologically, by gram stain and by MALDI-TOF analyser:

Dilution	CFU per inoculation	Biological Indicator One				Biological Indicator Two				Biological Indicator Three			
		Broth result	ID consistent with inoculating organism?			Broth result	ID consistent with inoculating organism?			Broth result	ID consistent with inoculating organism?		
			Gram stain	Morphology	MALDI TOF		Gram stain	Morphology	MALDI TOF		Gram stain	Morphology	MALDI TOF
Dilution 1	1.2^7	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a
Dilution 2	1.2^6	**POS DAY 1**	**Y**	**N**	**No ID**	NG	n/a	n/a	n/a	**POS DAY 2**	**Y**	**N**	**No ID**
Dilution 3	2.0^7	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a	**POS DAY 2**	**Y**	**N**	**No ID**
Dilution 4	2.0^6	NG	n/a	n/a	n/a	**POS DAY 1**	**Y**	**N**	**No ID**	**POS DAY 2**	**Y**	**N**	**No ID**
Dilution 5	2.0^5	**POS DAY 2**	**Y**	**N**	**No ID**	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a
Dilution 6	2.0^4	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a	NG	n/a	n/a	n/a

Key: **POS DAY #** - Growth seen on day # **NG** - No growth seen by end of study

Table 5 – Preliminary Biological Indicator results

Morphology (physical appearance of the culture, characteristic of its identity) showed all cultures to be different from the inoculated organism. The lack of MALDI-TOF ID is due to the system used

having a limited database of clinically relevant organism IDs, omitting many non-pathogenic environmental organisms.

To confirm results further analysis was undertaken using gene sequencing and matching against a full organism library. This was undertaken by National Biofilms Innovation Centre, Southampton University (see ***Appendix 5*** for method and detailed results). Summary results:-

Sample (original label)	Best identification achieved	Score
Inoculation source	*Bacillus oceanisediminis*	(3/3)
BI one - Dilution 2	*Lysinibacillus fusiformis*	(3/3)
BI one - Dilution 5	Split signal between *Bacillus circulans* and *Lysinibacillus* species.	(2/4)
BI two - Dilution 4	Largely *Bacillus circulans*	(2/3)
BI three - Dilution 2	*Paenibacillus xylanexedens or other Paenibacillus* species. *Bacillus circulans a possibility however.*	(3/4)
BI three - Dilution 3	*Paenibacillus xylanexedens or another unknown Paenibacillus* species	(4/4)
BI three - Dilution 4	*Paenibacillus xylanexedens or another unknown Paenibacillus* species	(4/4)

Table 6 – Confirmed Biological Indicator results

Conclusion of Biological Indicator testing:-

No sample grew the inoculated organism, demonstrating the sanitisation method used was able to effectively sanitise inoculations at all levels.

Growth seen in incubated samples was of environmental organisms entrained during the sampling process – possible sources:-

- Open windows and door to hospital corridor before and during sampling
- Clothing/skin of the microbiologist taking the samples

Conclusion of Chemical Indicator testing:-

Fully exposed indicators correlate to biological sanitisation.

One indicator was not fully exposed, indicating the indicators are sensitive to different levels of hydrogen peroxide exposure (ie do not react immediately at the lowest level) – this was backed-up by in-cycle observation of the indicators visible through the window, which were seen to change gradually throughout the cycle.

They are therefore suitable to be used as a QC indication in each cycle, with a poor exposure indicating a need to repeat the cycle and investigate the cause of failure.

However, the one poor exposure indicated the air distribution in Run 1 was not optimal.

With the uncertainty over Run 1 results before gene-sequencing confirmation was available, and one chemical indicator not fully exposed, Run 2 was designed and scheduled.

Method - Study 2 Run 2 – to confirm effectiveness findings from Run 1

1. The same equipment and methods were used as in Run 1, with the following exceptions
    a. Cycles – 3 different process cycles were evaluated, to identify which had the greatest value in sanitisation:-
        i. Wash/Dry only – garments not exposed to VHP
        ii. VHP only – garments inoculated immediately before VHP processing
        iii. Wash/Dry and VHP – garments inoculated then washed and tumble-dried then processed with VHP
    b. Loading – 78 garments were used, spread evenly on the hanging chains in a similar configuration to ***Figure1***:-
        i. Row 1 – 20 garments
        ii. Row 2 – 14 garments
        iii. Row 3 – 22 garments
        iv. Row 4 – 22 garments
    c. Fans:-
        i. 4 additional fans were obtained and placed at positions 5-8 (for location see ***Figure 8)***
        ii. All 8 fans were set to oscillation mode as this provided greatest movement of the garments, and variation in movement (avoiding conventional movement where all moved together and maintained occlusion)
    d. Biological indicators:-
        i. 3 organisms were inoculated on each test garment, as in ***Table 7*** , chosen to reflect a range of sensitivities to sanitisation agents.
        ii. Inoculations were made on the chest and front of leg of the garments, representing likely highest exposure in clinical use (and therefore highest bioburden for the sanitisation process).
        iii. Improved aseptic precautions were employed to try to prevent environmental contamination during sampling post-VHP; windows and door were kept closed until after sampling, and sample dissection was done in a controlled aseptic environment in the microbiology lab.

Run	Inoculating organism	Inoculation level (CFU)						Vol
1	Bacillus oceanisediminis (spores)	$2.0x10^4$	$2.0x10^5$	$1.2x10^6$	$2.0x10^6$	$1.2x10^7$	$2.0x10^7$	Var.
2	Bacillus oceanisediminis (spores)	$4.9x10^3$	$4.9x10^5$	$4.9x10^6$				10µl
	Staphylococcus aureus ATCC 29213	$4.4x10^3$	$4.4x10^5$	$4.4x10^6$				10µl
	Klebsiella pneumoniae NCTC 11228	$4.6x10^3$	$4.6x10^5$	$4.6x10^6$				10µl

Table 7 – inoculation levels on test garments for Study 2 Run 2

    e. Chemical indicators
        i. Only the commercially available Sterrad indicators were used, as these had been demonstrated effective in Run 1.
        ii. New indicator locations were used, although similar to Run 1
    f. Run took place on a working day as no inadvertent exposure had been detected for Run 1

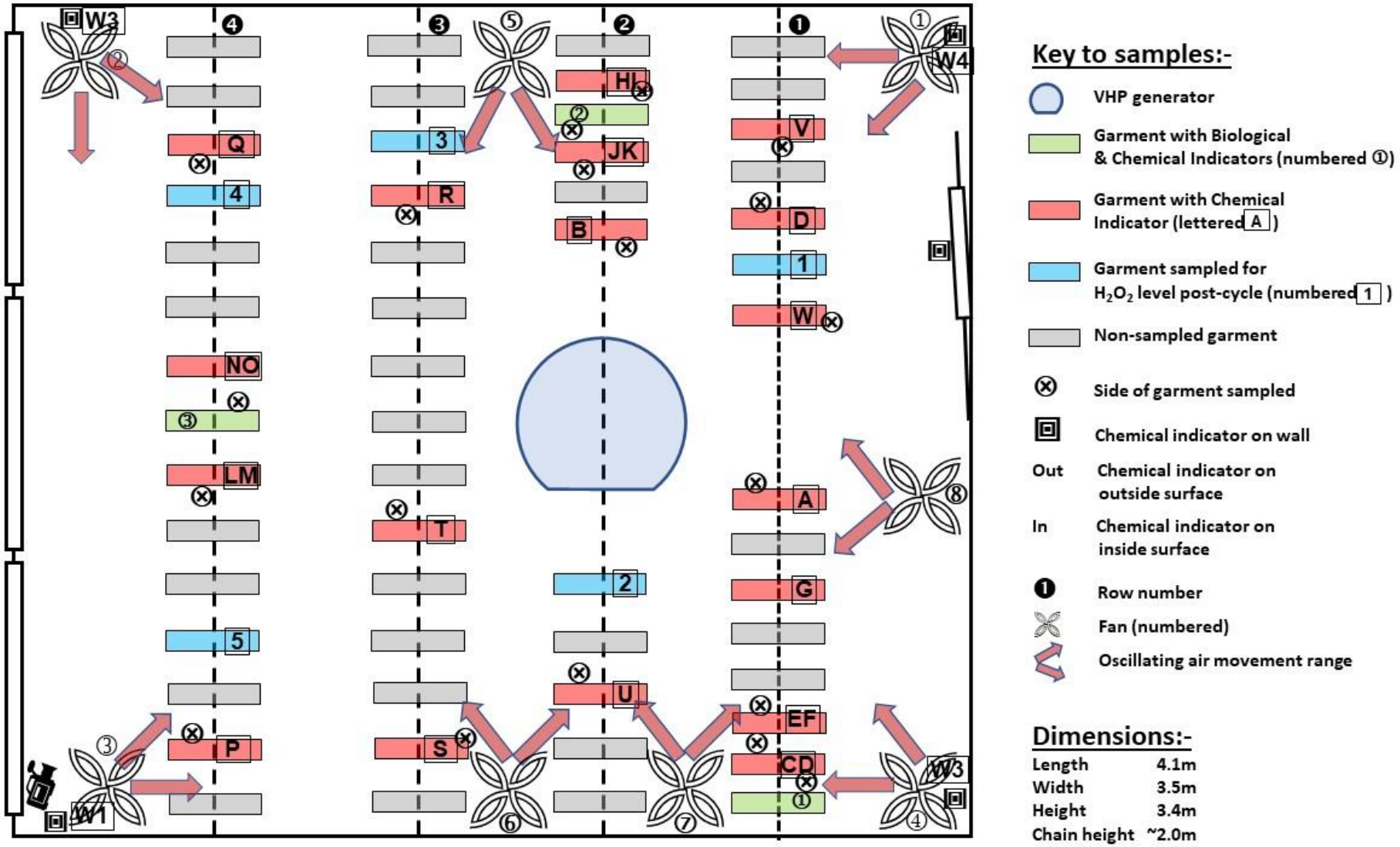

Figure 8 – Sample and Fan layout, Study 2 Run 2

Results – Study 2 Run 2

1. Cycle duration & parameters

The cycle completed as expected – see report ref 28420201401 ***Appendix 7***. On-board parameters indicated it had been delivered successfully. There was a pause at the end of the cycle after which hydrogen peroxide levels in the room were measured. Because the catalytic extract process continues after the main cycle, this took place during the pause, and only 1 further extract cycle was required, taking the total cycle time to 3hr 2min:-

Process	Time	H_2O_2 measurement
Cycle start	15:01 28.04.20	n/a
Cycle end	17:00	Not measured
Pause		n/a
Extract end	17:40	2.2ppm
1st extra extract start	17:48	n/a
Extract end	18:03	1.5ppm

Table 8 – Cycle parameters Run 2

2. Chemical indicators

Sample ID	Sample type	Inside or Outside garment	Location on garment	Result
BI 1	Next to Biological Indicator	O	Chest	Fully exposed
BI 2		O	Chest	Fully exposed
BI 3		O	Chest	Fully exposed
A	Positive control	O	Leg	Fully exposed
B	Positive control	O	Leg	Fully exposed
C		O	Arm	Fully exposed
D		O	Chest	Fully exposed
E		O	Chest	Fully exposed
F		O	Arm	Fully exposed
G		O	Chest	Fully exposed
H		O	Arm	Fully exposed
I		O	Chest	Fully exposed
J		O	Arm	Fully exposed
K		O	Chest	Fully exposed
L		O	Arm	Fully exposed
M		O	Chest	Fully exposed
N		O	Chest	Fully exposed
O		O	Arm	Fully exposed
P		O	Chest	Fully exposed
Q		I	Leg	Fully exposed
R		I	Leg	Fully exposed
S		O	Arm	Fully exposed
T		I	Leg	Fully exposed
U		O	Chest	Fully exposed
V		I	Leg	Fully exposed
W		I	Arm	Fully exposed
Wall 1	Wall	n/a	n/a	Fully exposed
Wall 2	Wall	n/a	n/a	Fully exposed
Wall 3	Wall	n/a	n/a	Fully exposed
Wall 4	Wall	n/a	n/a	Fully exposed

Table 9 – Chemical Indicator results Run 2

Conclusion of chemical indicator testing, Run 2:-

Improved and satisfactory gas distribution was achieved from using the additional fans , overcoming the challenges of the higher load density.

3. Garment gas sampling

Based on findings in run 1 only the inside lower leg was samples, representing worst case

Garment	Measured Hydrogen Peroxide gas level at surface (ppm)			
	Inside body	Inside upper leg	Inside lower leg	Outside
1	Not tested	Not tested	0.9	Not tested
2	Not tested	Not tested	0.6	Not tested
3	Not tested	Not tested	1.1	Not tested
4	Not tested	Not tested	1.8	Not tested
5	Not tested	Not tested	1.1	Not tested

Table 10 – Garment sampling results Run 2

4. Biological Indicators

As with Run 1 there was significant growth across the samples. Growth was too extensive and mixed to subculture for Wash/dry only, but for VHP only and Wash/dry+VHP all were subcultured and subjected to Gram stain, MALDI-TOF analysis. Initial growth results:-

Wash & tumble dry only

	Biological Indicator Garment **One - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

	Biological Indicator Garment **One - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	NG	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

	Biological Indicator Garment **Two - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

	Biological Indicator Garment **Two - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

	Biological Indicator Garment **Three - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

	Biological Indicator Garment **Three - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^5	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE
10^3	TURBID DAY ONE	TURBID DAY ONE	TURBID DAY ONE

VHP only

	Biological Indicator Garment **One - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	TURBID DAY ONE
10^5	TURBID DAY ONE	NG	TURBID DAY ONE
10^3	TURBID DAY ONE	NG	NG

	Biological Indicator Garment **One - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	TURBID DAY ONE	NG
10^5	NG	NG	TURBID DAY ONE
10^3	NG	NG	NG

	Biological Indicator Garment **Two - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	TURBID DAY ONE	TURBID DAY ONE	NG
10^3	NG	NG	NG

	Biological Indicator Garment **Two - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	NG	NG	NG
10^3	NG	NG	NG

	Biological Indicator Garment **Three - Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	NG	NG	NG
10^3	NG	NG	NG

	Biological Indicator Garment **Three - Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	NG	NG	NG
10^3	NG	NG	NG

Wash & tumble dry + VHP

	Biological Indicator Garment **One** - **Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	TURBID DAY ONE	TURBID DAY ONE
10^5	NG	NG	NG
10^3	NG	TURBID DAY ONE	NG

	Biological Indicator Garment **One** - **Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	TURBID DAY ONE	NG	NG
10^5	TURBID DAY ONE	NG	NG
10^3	TURBID DAY ONE	TURBID DAY ONE	NG

	Biological Indicator Garment **Two** - **Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	TURBID DAY ONE	NG	NG
10^3	NG	NG	NG

	Biological Indicator Garment **Two** - **Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	TURBID DAY ONE	NG
10^5	NG	NG	NG
10^3	NG	NG	TURBID DAY ONE

	Biological Indicator Garment **Three** - **Sleeve**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	NG	NG	NG
10^3	NG	NG	NG

	Biological Indicator Garment **Three** - **Chest**		
	B. oceanisediminis	K. pneumoniae	S.aureus
10^6	NG	NG	NG
10^5	NG	NG	NG
10^3	NG	NG	NG

Table 11 – Initial bioindicator results Run 2

Analysis of cultures:-

- Wash/tumble-dry only samples showed such mixed growth that these were not identified.
- Identities from VHP only and Wash+VHP were as follows:-

Sanitisation Method	Bio-indicator number	Gown section	Inoculation	Conc.	Identity from MALDI-TOF analysis	MALDI score	Different organism from inoculation
VHP only	1	Sleeve	B.oceanisediminis	10^5	Bacillus cereus / Staph hominis	1.9 / 2.2	Yes
VHP only	1	Sleeve	B.oceanisediminis	10^3	Bacillus megaterium	2.3	Yes
VHP only	1	Sleeve	S.aureus	10^6	Staph aureus	2.1	**No**
VHP only	1	Sleeve	S.aureus	10^5	Bacillus pumilus	1.8	Yes
VHP only	1	Chest	K.pneumoniae	10^6	Bacillus licheniformis	2.2	Yes
VHP only	1	Chest	S.aureus	10^5	Bacillus megaterium	2.2	Yes
VHP only	2	Sleeve	B.oceanisediminis	10^5	Bacillus megaterium	2.4	Yes
VHP only	2	Sleeve	K.pneumoniae	10^5	Bacillus megaterium	2.1	Yes
Wash & VHP	1	Sleeve	K.pneumoniae	10^6	Bacillus circulans	2.2	Yes
Wash & VHP	1	Sleeve	K.pneumoniae	10^3	Lysinibacillus fusiformis	1.7	Yes
Wash & VHP	1	Sleeve	S.aureus	10^6	Bacillus circulans	2.1	Yes
Wash & VHP	1	Chest	B.oceanisediminis	10^6	Bacillus licheniformis	2.1	Yes
Wash & VHP	1	Chest	B.oceanisediminis	10^5	Bacillus simplex	1.95	Yes
Wash & VHP	1	Chest	B.oceanisediminis	10^3	Bacillus megaterium	2.2	Yes
Wash & VHP	2	Sleeve	B.oceanisediminis	10^5	Bacillus circulans	2	Yes
Wash & VHP	2	Chest	K.pneumoniae	10^6	Bacillus amyloliquefaciens	1.6	Yes
Wash & VHP	2	Chest	S.aureus	10^3	B. amyloliquefaciens / Lysinibacillus spaericus	2.1 / 1.9	Yes

Table 12 – Positive bioindicator identities Run 2

Further, morphological identity of plate cultures of the above all showed significantly different morphologies for all isolates apart from the *Staph. aureus* (row 3), see ***Appendix 4*.**

MALDI scores indicate the probability of an accurate identity – at 2.0 or above the ID is secure to species level, for 1.7-2.0 the ID is secure to genus level and probable for species, decreasing below below 1.7 the ID is progressively less secure.

Conclusion of Biological indicator testing, Run 2:-

For garments with wash+VHP, no inoculated organism was cultured after processing (n=54). Organisms recovered were environmental contaminants from the sampling process. **/cont'd**

For garments only processed with VHP, 1 culture post processing recovered the same organism as inoculated (2%, n=54). Although as a common skin flora this could also be considered an environmental contaminant, this result has been accepted as genotyping was not available for this sample to distinguish from the inoculum. *Further genotyping work is ongoing to identify if this organism originated other than from the inoculum.*

Garments processed only by washing showed substantial contamination which was not identified.

Although inconsistent with Run 1, **this indicates that the only reliable method of sanitisation with UHS facilities and equipment is laundering followed by VHP sanitisation, in line with considered best practice.**

5. Garment integrity

Following the VHP cycle garments were inspected for damage or degradation – none seen. This garment type had previously passed testing for laundering suitability, hence its use in this study.

Since it is unfeasible to test permeability to the original garment standards (EN 14126:2003), integrity against fluid ingress was then tested with a UHS-derived physical test:-

- A staff member wearing cotton scrubs dons the test garment,
- Whilst being worn the garment is liberally sprayed with clean water from a handheld trigger sprayer, See **Figure 9** below.
- The garment is carefully removed and scrubs examined,
- Darker (ie damp/wet) areas indicate permeability failure

Figure 9: Post-cycle garment permeability testing

Study 3

Testing the impact of repeated wear-wash-VHP cycles will only be undertaken if/when garments enter clinical use, and a maximum number of cycles will then be defined and incorporated into the SOP.

Overall summary of results:-

Cycle	Process	Garment Qty	Biological Indicator result (no. of samples showing growth same as inoculum/total no. of inoculations)	Chemical Indicator result (no. of indicators/total no. used)
1	VHP only	50	0/18	26/27 fully exposed 1/23 partially exposed
2	Laundry only	78	53/54 unidentified growth	27/27 fully exposed
	VHP only		1/54	
	Laundry + VHP		0/54	

Conclusion

- Test reprocessing of laundering followed by vaporised hydrogen peroxide exposure achieved satisfactory standards of disinfection, based on limited microbiology testing carried out in UHS Microbiology Laboratory, using organisms considered on current data to be worst-case surrogates for SARS-CoV-2.
- The statement is based on successful removal of heavy inoculum of Staphylococcus aureus, Klebsiella pneumoniae and Bacillus oceanisedemenis spores as part of verification. The UHS Director of Infection Control advises that results provide reasonable assurance this method will therefore remove SARS-CoV-2 from the garments tested. Non-pathogenic environmental organisms identified in the garment samples are of no clinical significance and thought to originate from the sampling process.
- Because of the environment where this process is conducted and post-cycle presence of environmental organisms, it is considered that the method can achieve sufficient decontamination of PPE garments to be used in clinical areas but that these should not be used for sterile process, eg theatres.
- VHP reached all areas of the chamber, indicating the revised loading pattern was suitable.
- No VHP was detected outside the processing room, and effective end-of-cycle removal of hydrogen peroxide residues from environment and garments was demonstrated, indicating process safety.
- Garment integrity was not affected by the process (single cycle).
- Cycle parameters show the process is reproducible.

This process is suitable, subject to Trust approval, to be developed into operating procedures within UHS Sterile Services to reprocess PPE garments, for use in the event of supply-line failure. The process could be transferred to other centres provided prior qualification of local conditions is undertaken – this study provides a basis for designing such qualification (separate guidance issued).

Limitations

- It is recognised that the work undertaken does not constitute validation as it lacks statistical power and only tested one garment type, but it is sufficient to indicate effectiveness of the processes, and establishes a method for testing further garment types.
- With a 3hr cycle time, capacity of the process (estimated to be 450 garments per day max.) is well below the daily demand for PPE garments at UHS.

Further work

Further work

- Ongoing testing of garment permeability/integrity is needed if/when repeated cycles are introduced.
- Risk assessments and Risk Register entry should be completed, for both organisational risks of reprocessing and CoSHH risks of the VHP process
- Procedures to cover this process need to be written by UHS and should be assessed for achievability.
- Staff training (internally & by Inivos) against procedures will be necessary.
- Briefing to staff on the evidence to support use of reprocessed PPE will be essential to retain confidence.
- If further testing of different garment types becomes necessary, biological indicator results matched to chemical indicator data from this study should enable subsequent studies to be undertaken with chemical indicators alone, simplifying and shortening the evaluation.

Sharing this work

- A full study report is available, giving details of methods and results.
- It is likely all other UK NHS Trusts are in a similar position of high PPE demand with insecure supply-lines. The study protocol, full report and this executive summary will be shared initially with NHS Trusts in the Wessex and Oxford AHSNs areas participating in a knowledge-sharing group around PPE reprocessing, open to all NHS Trusts in the area. If scope permits it will be shared with any NHS Trust interested in exploring PPE reprocessing.
- To support development of reprocessing methods in other Trusts a 'Suggestions for Implementation' document has been developed from study findings, and will be shared with the other papers.
- All methods, findings and documents will be shared with the joint national NHSE/HSE/PHE working group investigating PPE reprocessing with a view to issuing national guidance.

Declarations

Study developed and conducted between 21.04.20 and 13.05.20 by:-

Matthew O'Dwyer, Biomedical Scientist, UHS Microbiology
James Austin, Biomedical Scientist, UHS Microbiology
Jean Hedges, Sterile Services Manager and team, UHS
Pam Webb, Business Manager Theatres & Anaesthetics, UHS
Tautvydas Karitonas, Development Engineer, Inivos Ltd
With support from UHS Estates

Study supervised by:-

Dr Nitin Mahobia, Consultant Microbiologist and Director of UHS Infection Prevention Unit
Asa Thorpe, Care Group Manager for Critical Care, Theatres & Anaesthetics, UHS
Peter Rhodes, Programme Manager Industry & Innovation, Wessex Academic Health Science Network

VHP equipment, materials and specialist engineer time on-site provided and funded by:-

Tom Lister, Business Development Manager, Inivos Ltd

Study methods and findings are co-authored by UHS and Inivos Ltd with project support from Wessex AHSN, 15.05.2020.

List of Appendices

Appendix 1: Study protocol (separate document)
Appendix 2: Factors affecting efficacy of VHP as a sanitisation agent and controls used
Appendix 3: Criteria for a suitable VHP reprocessing chamber / room
Appendix 4: Biological indicator Preparation and analysis
Appendix 5: Genotypic Identification of Cycle 1 environmental contamination
Appendix 6: VHP Generator Cycle Report – Run 1
Appendix 7: VHP Generator Cycle Report – Run 2

Document control

Version History:-		
Date	**Version**	**Change**
15.05.2020	1	New document

Appendix 1: Study protocol (separate document)

Appendix 2 - Factors affecting efficacy of vaporised hydrogen peroxide (VHP) as a sanitisation agent and how the study controlled them:-

Factors reducing efficacy	Control
Contact with surfaces occluding gas penetration	Define the loading pattern
Poor distribution of hydrogen peroxide vapour	On-board fan in VHP generator, investigate additional fans
Gas will not penetrate impermeable materials	Loading pattern & gas distribution to ensure all impermeable gowns have a through-flow
Presence of soiling and organic material on surfaces	Cool temperature wash with detergent before VHP process. Chamber construc'n – see below.
Moisture content of materials being exposed	Tumble drying of garments after washing
Contact time at steady state	Process control
Humidity	Process control
Environmental temperature	Allowed for within process control

At the end of VHP processing the generator uses on-board catalysts to convert the hydrogen peroxide (H_2O_2) to water and oxygen (H_2O & O_2). Passive diffusion of the hydrogen peroxide from surfaces into the air can be accelerated by air flow but may still be prolonged. Environmental and surface levels must be reduced below safely limits before the cycle can be considered complete, and demonstrated with electro-chemical sensors before entering the chamber and handling garments. Factors affecting out-gassing time to safe levels:-

Factors reducing out-gassing	Control
Poor distribution of hydrogen peroxide vapour and fresh air	On-board fan in VHP generator, investigate additional fans
Closed system without ventilation	After checking gas levels are below safe limits room can be entered at end of cycle and windows opened to increase outgassing rate from garments
Moisture content of materials being exposed	Tumble drying of garments after washing
Humidity	Process control
Environmental temperature	Allowed for within process control
Absorptive nature of fabric materials and room construction	Unable to control – will define the end-cycle time. May be able to identify some materials which extend time unacceptably. Chamber/ room construction – see below.

Appendix 3 - Criteria for a suitable reprocessing chamber / room:-

- Adequate minimum size for intended load.
- Relatively empty of fixed furniture & fittings – all non-fixed items removed.
- No carpet, or fully covered with new vinyl and sealed round with duct tape.
- Minimum of exposed wood / other organic materials (eg fabrics).
- Impervious surfaces (no exposed concrete, plaster, minimum flaking paint).
- No false ceiling/ceiling tiles (or tiles removed and suitable surfaces above – metal grid can remain).
- Door(s) able to be sealed externally to gas escape, and ideally lockable to prevent entry during cycles (despite warning signage).
- Ideally with opening windows to safe external space; windows able to be sealed airtight.
- Hanging rails or chains to allow garments to be suspended above floor across the room:-
    - height approx. 2000mm from floor,
    - separation approx. 800mm
    - distance from side walls approx. 500mm
- Any inlet or extract ventilation must have air flow isolated and vents sealed over – ideally switchable extract ventilation to external space.
- No channels to other rooms/spaces (or effectively stopped up).
- Multiple electrical sockets for VHP generator & fans, or extension leads.
- Internal lighting
- Socially clean (ie washed to remove dust and dirt)
- Smoke alarm sensors removed and blanked or air-tight covered and isolated permanently at the panel.

Rationale:

Garments should not be considered fully decontaminated after washing, and the VHP chamber may also need to be used for final drying after tumble drying (see 4).

Hydrogen peroxide is rapidly deactivated when in contact with organic materials (eg wood, carpet, fabrics), exposed concrete and plaster, and dirt.

It is quite damaging to some materials on repeated exposure, particularly exposed mild steel (which rusts), fabrics (bleached).

Room size – needs to be big enough to allow sufficient load to be processed (see 2), but NB larger room = longer cycle, although the extra time will be less than an extra cycle per day.

False ceilings will extend cycle time – gas will soak into the tiles (likely to be partly de-activated and needing to out-gas at end) and will collect in space above tiles (taking long to be outgassed)

Extract ventilation or windows (provided this is to a safe external point) will accelerate return to safe gas levels, but must be able to be closed off before cycle start and ideally opened without entering the room. Inlet ventilation should be disabled and closed off to avoid the risk of being switched on during a cycle, forcing gas into surrounding areas. NB the ducts must be physically closed to avoid gas loss.

Hanging rails will need to support the weight of the intended load, allowing for garment separation of about 150mm along the length. Dimensions allow for adequate gas/air circulation around garments, and garment movement. Chains have the advantage of hooking garment hangers through links to stop moving sideways, they can be mounted by securing to padlock staples / rings screwed to the wall. Plastic or galvanised chain will be inert to VHP.

VHP will trigger smoke alarm sensors, and may permanently damage an exposed sensor if left uncovered and in place, may also damage contacts in an exposed socket if removed. Clear instruction to Control team not to return sensor to duty until approved, in case a cycle underway.

Appendix 4 - Biological indicator Preparation and analysis

James Austin & Matthew O'Dwyer, Biomedical Scientists, UHS Dept of Microbiology

Cycle One 25/04/20

Three garments were prepared as biological indicators.

Each garment had 6 inoculation points with the following organism concentrations inoculated onto it, each within an inch square:

1. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 100uL containing 1.2 x10^7 CFU
2. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 10uL containing 1.2 x10^6 CFU
3. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 1000uL containing 2.0 x10^7 CFU
4. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 100uL containing 2.0 x10^6 CFU
5. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 10uL containing 2.0 x10^5 CFU
6. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 10uL containing 2.0 x10^4 CFU

The garments were inoculated under the right arm of the garment as an area of likely occlusion from VHP exposure.

These three gowns had previously undergone a wash and dry cycle. Each inoculation was prepared in the Microbiology laboratory at Southampton General Hospital and was allowed to air dry onto the gown. Once the gowns were dried they were transported in autoclave bags to the Sterile Services Department at Southampton General Hospital and exposed to a VHP gassing cycle as described above.

Following the VHP gassing cycle and suitable period of gas removal (hydrogen peroxide levels <2.0ppm) a trained microbiologist entered the room, and cut out each dilution using scissors and forceps, sterilised using an autoclave cycle in the clean microbiology autoclave and transferred it to an appropriately labelled Brain Heart Infusion broth. The broths were then incubated at 37C. Following incubation the broths were examined for turbidity, indicating bacterial growth. Broths that were turbid were sub-cultured onto solid culture media for identification. Broths that did not indicate any growth were interpreted as negative.

Cycle Two 28/04/20

Nine garments were prepared as biological indicators as follows.

Three garments were exposed to wash and dry cycle only.

Three garments were exposed to VHP cycle only.

Three garments were exposed to the wash and dry cycle, followed by the VHP cycle.

Each garment had an inoculation area on the right sleeve and the chest, as these are the areas most likely to become contaminated during clinical procedures.

Each inoculation area had 9 inoculation points with the following organism concentrations inoculated onto it, each within an inch square:

1. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 100uL containing 4.9 x10^6 CFU
2. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 10uL containing 4.9 x10^5 CFU
3. Bacillus oceanisediminis (following heat shock cycle to encourage sporulation) 10uL containing 4.9 x10^3 CFU
4. Klebsiella pneumoniae NCTC 11228 10uL containing 4.575 x10^6 CFU
5. Klebsiella pneumoniae NCTC 11228 10uL containing 4.575 x10^5 CFU
6. Klebsiella pneumoniae NCTC 11228 10uL containing 4.575 x10^3 CFU
7. S.aureus ATCC 29213 10uL containing 4.425 x10^6 CFU
8. S.aureus ATCC 29213 10uL containing 4.425 x10^5 CFU
9. S.aureus ATCC 29213 10uL containing 4.425 x10^3 CFU

The Bacillus oceanisediminis is a spore forming organism. This isolate was heat shocked at 65C for 30 minutes to encourage sporulation, this was confirmed by observing the presence of spores using a Gram stain. Pre-studies demonstrated that spore formation could be seen at 30, 60 and 90 minutes, however there was signification reduction in viable organisms from subculture of suspensions heat shocked at 60 and 90 minutes. 30 minutes demonstrated the presence of spores, whilst also avoiding any reduction in organism recovery.

Each inoculation was prepared in the Microbiology laboratory at Southampton General Hospital and was allowed to air dry onto the gown. Once the gowns were dried they were transported in autoclave bags to the Sterile Services Department at Southampton General Hospital and processed as described above.

Once the three gowns for wash and dry cycles were complete they were packaged into bags and collected and returned to the Microbiology department to be cultured.

The other six gowns were collected after the VHP gassing cycle was complete and the room had <2.0ppm of hydrogen peroxide. Microbiology staff entered the room and removed the large inoculation areas and transferred them into sterile paper bags, as provided by SSDU. The sterile bags were then transferred down to Microbiology to perform culture.

Each inoculated inch square was aseptically cut off using commercially available sterile scalpels and sterilised forceps and was placed into an appropriately labelled Brain Heart Infusion broth, commercially available pre-prepared. The broths were then incubated at 37C. Broths that did not indicate any growth were interpreted as negative.

A separate garment was inoculated with the same organisms which did not undergo any disinfectant procedure to act as a positive control to demonstrate that the organism can be recovered.

Biological Indicator Results.

Cycle One

The results demonstrate that the VHP cycle completed after a wash and dry cycle enabled a 7 log reduction in spore forming bacteria from the garments as seen in **table 5**.

Other organisms were isolated from the broths, however the morphology of these isolates was inconsistent with Bacillus oceanisediminis, and is therefore a contaminant acquired during the sampling process and is not implicated in the decontamination process.

Cycle Two

The results demonstrate the S. aureus was still viable and was recovered from inoculation point 4 on Biological indicator one from the VHP only cycle. This demonstrates that there is there is a 5 log reduction. See **table 12** and **table A3-1** below.

The inoculating organism was not recovered from any other inoculation point on any other site, demonstrating that in those inoculation points the organisms were completely removed.

Other organisms were isolated from the broths, however the morphology of these isolates was inconsistent with Bacillus oceanisediminis, and is therefore a contaminant acquired during the sampling process and is not implicated in the decontamination process.

The broths from the wash only cycles were heavily contaminated and all sites tested had grown non-pathological environmental organisms; however the target organisms were not recovered from any location.

Table A3-1 Morphological description of isolates

Organism	Colony size	Colony shape	Topography	Colony margin	Colony pigment	Gram stain result
B.oceanisedimenis *(Inoculation ref.)*	Large	irregular	Flat	Undulate	Dark grey	Gram positive bacilli
S.aureus *(Inoculation ref.)*	Small	Circular	Raised	Entire	Golden/Yellow	Gram positive cocci
K. pneumoniae *(Inoculation ref.)*	Large, mucoid	Circular	Convex	Entire	Cream	Gram negative bacilli
Environmental Bacillus sp One	Large	Circular	Flat	Entire	Cream	Gram positive bacilli
Environmental Bacillus sp Two	Medium	Circular	Flat	Entire	Grey	Gram positive bacilli
Environmental Bacillus sp Three	Small	Circular	Flat	Entire	Yellow	Gram positive bacilli
Environmental Bacillus sp Four	Large	Circular	Flat	Curled	Brown	Gram positive bacilli
Environmental Bacillus sp Five	Medium	Circular	Flat	Entire	Cream/Grey	Gram positive bacilli
Environmental Bacillus sp Five	Large	Irregular	Flat	Curled	Translucent	Gram positive bacilli

The table above shows the differences in colonial variation, indicating those organisms are different to the target organisms.

All inoculation points on the positive control garment demonstrated expected growth.

Discussion

The results from cycle one demonstrate that a 7log reduction in spore forming bacteria. This surpasses the acceptance criteria of this study to achieve a 3 log reduction.

The results demonstrate that a 6 log reduction of bacterial cells is achievable using a wash, dry and VHP cycle. The culture results demonstrate that all inoculated bacterial concentrations were eliminated using this method.

The VHP only cycle demonstrated a 5 log reduction of bacterial cells as S. aureus was recovered from the inoculation point on the sleeve of Biological indicator one containing 4.425 x10^6 CFU of S. aureus. S. aureus, or any other inoculating organism was not recovered from any other inoculation point, thus demonstrating at least a 5 log reduction.

The wash only cycles were highly contaminated with environmental organisms due to many factors, including the storage between wash and dry cycles to microbiological testing, aseptic technique not applied in the washing and drying process, tumble dryers produce large amounts of dust, and following tumble drying the garments were not stored in a sterile environment. Whilst the inoculating organisms were not recovered from the broths, due to risk of high level contamination, it would be unlikely to fully appraise the efficacy of the wash and dry cycles alone as the contamination may out compete low levels of target organisms that remained following the wash and dry cycle.

The aim of this study was to demonstrate whether the garments could be disinfected to achieve a 3 log reduction in organism. This study has demonstrated that a wash, dry and VHP cycle a 6 log reduction is achieve, surpassing the expectations outlined at the start of the study. For sites that do not have extensive wash and dry facilities, a VHP cycle demonstrated that a 5 log reduction is achieved, which again is beyond the expectations from the start of the study. It is worth noting that soiled areas should be washed and dried prior to VHP exposure to ensure suitable gas penetration at that site.

Matthew O'Dwyer

James Austin

Appendix 5 – Genotypic Identification of Cycle 1 environmental contamination

Dr. David Cleary; Dr. Joe Parker, Research fellow, University of Southampton National Biofilms Innovation Centre

Aim: Use whole-genome shotgun (WGS) DNA sequencing to identify seven unknown isolates provided by UHS from PPE project, rapidly.

Summary

The overall ID results are presented below:

Sample (original label)	Total sequencing yield, Mbp	Sample	Barcode	Best ID effort	Score
Bac. sp	3.85	A	1	*Bacillus oceanisediminis*	(3/3)
BTI dil 2	8.62	B	2	*Lysinibacillus fusiformis*	(3/3)
BTI Dil 5	9.87	C	3	Split signal between B. circulans and L. spp.	(2/4)
BI2 Dil4	24.64	D	4	Largely B. circulans	(2/3)
BI3 Dil2	28.8	E	5	*Paenibacillus xylanexedens or other P. spp.* *B. circulans a possiblity however.*	(3/4)
BI3 Dil3	20.69	F	6	*Paenibacillus xylanexedens or another unknown Paenibacillus spp.*	(4/4)
BI3 Dil4	60.78	G	7	*Paenibacillus xylanexedens or another unknown Paenibacillus spp.*	(4/4)

Method overview

Wednesday 29th April: Contacted by Rob Hull and asked to ID isolates using sequencing

Thursday 30th April: David Cleary extracted genomic DNA at UHS and quantified using Qubit. DNA concentrations were in the range ~10-40ng/uL, considered sufficient for WGS sequencing.

Friday 1st May: Joe Parker checked DNA for purity using Nanodrop (sample A was the only questionable sample) then prepared and loaded two multiplex sequencing runs on MinION 'Flongle' minature flowcells.

Saturday 2nd May: Sequence read basecalling, demultiplexing, and initial exploration.

Sunday 3rd May:

- Kmer matching with `Kraken`
- BLASTn alignment of (a tiny, random subset of) sequences versus NCBI `nr` database
- Mapping of sequence reads back to likely reference genomes using `guppy_aligner`
- *De novo* genome assembly with `Unicycler` and contig alignment using `BLASTn` to NCBI `'nr'` database

Detail – extraction, sequencing, basecalling and demultiplexing

The sequencing libraries were prepared using the Oxford Nanopore SQK-RAD004 rapid multiplexing kit and barcodes [1-7], according to manufacturer's instructions except that 7.5uL tagmented DNA was taken forward into the adapter ligation (as opposed to 5.0uL recommended).

Two separate runs were operated, for 3.5 and 48hrs respectively. Total sequencing yield was 275Mbp; however, approximately 118Mbp of this could not be demultiplexed reliably with default settings. Optionally this can be refined if desired. Mean fragment size was 4000-13000bp (depending on sample) and maximum single read length was 124,000bp ("Sample G / BI3 Dil4")

Sample (original label)	Total sequencing yield, Mbp	Sample	Barcode
Bac. sp	3.85	A	1
BTI dil 2	8.62	B	2
BTI Dil 5	9.87	C	3
BI2 Dil4	24.64	D	4
BI3 Dil2	28.8	E	5
BI3 Dil3	20.69	F	6
BI3 Dil4	60.78	G	7

Detail – rapid K-mer (short sequence substring) ID via Kraken

An initial rapid identification was attempted using the 'minikraken' 8GB database. This is a reduced-representation database spanning the microbial domains of life with approximately even, but sparse, coverage. This method matches short strings of DNA ('K-mers' of 7-11bp) exactly. It is fast but poor at identifying non-model taxa, or those without published genomes.

Sample NIHR	Label UHS	*Unclassified%*	Family %		Genus %		Species %	
Bac. sp	A	*59.3*	Bacillaceae	40.18	Bacillus	40.06	Bacillus oceanisediminis	39.32
BTI dil 2	B	*58.81*	Bacillaceae	39.68	Lysinibacillus	38.48	Lysinibacillus fusiformis	14.61
BTI Dil 5	C	*88.08*	Bacillaceae	6.29	Bacillus	3.04	Bacillus cereus	0.99
BI2 Dil4	D	*93.71*	Bacillaceae	4.31	Bacillus	3.90	Bacillus oceanisediminis	1.04
BI3 Dil2	E	*87.38*	Paenibacillaceae	8.59	Paenibacillus	8.21	Paenibacillus xylanexedens	1.15
BI3 Dil3	F	*85.76*	Paenibacillaceae	10.23	Paenibacillus	9.57	Paenibacillus xylanexedens	1.01
BI3 Dil4	G	*85.43*	Paenibacillaceae	10.88	Paenibacillus	10.55	Paenibacillus xylanexedens	1.03

Detail – random read subset assignment with BLASTn versus complete NCBI database

Because Kraken relies on short matches (and can therefore give odd results with noisier, longer reads), and was run with a reduced-representation database, BLAST was also used to perform a fuzzier search of available genomes. An exhaustive search of the NCBI complete ('nr') database is still running.

As a stopgap, 30 sequences between 2000-4000bp were selected at random from each sample and aligned to nr using BLASTn with default parameters, wordsize=11 and retaining only the top 10 hits. Results are summarised below:

Sample (NIHR)	Sample (UHS)	BLAST random read subset
Bac. sp	A	+++ B. oceanisediminis
BTI dil 2	B	++ Lysinibacillus fusiformis, or another L. spp like L. varians or L. spaericus
BTI Dil 5	C	++ B. circulans
BI2 Dil4	D	++ All but 1 or 2 of the non-garbage hits: B. circulans
BI3 Dil2	E	+ Mixed signal, with B. circulans, and other odd B. spp., plus Paenibacillus xylanilyticus and other P. spp. etc
BI3 Dil3	F	++ Pretty much all P. spp.
BI3 Dil4	G	++ Pretty much all P. spp., likely xylaniliticus or xylanexedens

Detail – mapping to reference genomes

Kraken and BLAST results were used together to narrow down the list of plausible nearest reference genomes. The raw uncorrected reads were then mapped to each reference genome using guppy_aligner:

	Bacillus oceanisediminis		*Lysinibacillus fusiformis*		*Lysinibacillus SGAir 0095*		*Bacillus circulans*		*Paenibacillus xylanexedens*		*Paenibacillus xylanilyticus*	
sample	**L%**	**MD**	**L%**	**MD**	**L%**	**MD**	**L%**	**MD**	**L%**	**MD**	**L%**	**MD**
A	**38.26**	**0.49**	1.01	0.01	1.21	0.02	2.18	0.03	2.68	0.06	0.55	0.01
B	1.22	0.03	**61.94**	**1.22**	11.34	0.18	2.48	0.04	0.40	0.01	0.65	0.01
C	1.58	0.03	9.35	0.14	**54.57**	**1.08**	2.09	0.04	0.81	0.04	0.73	0.01
D	6.03	0.19	3.33	0.09	3.90	0.11	**63.23**	**2.24**	0.45	0.03	0.97	0.03
E	1.76	0.04	1.64	0.05	1.91	0.05	10.41	0.15	8.56	0.22	10.62	0.25
F	1.18	0.03	1.08	0.03	1.20	0.03	1.37	0.03	8.30	0.17	9.21	0.18
G	1.67	0.09	1.90	0.09	1.85	0.10	1.66	0.09	**11.64**	**0.49**	**13.26**	**0.53**

L% = percentage of the reference genome covered by at least one read.

MD = Average depth of coverage

Detail – *de novo* genome assembly and contig alignment

De novo genome assembly on uncorrected reads was attempted for each sample with SPAdes; canu; minimap2; and Unicycler. Only Unicycler was able to produce meaningful alignments. These contigs were inspected using QUAST and then BLASTed as with uncorrected raw reads:

Sample UHS	Total yield, Mbp	Approx genome coverage	Unicycler length	Unicycler #contigs	Unicycler N50	BLAST	BLAST contig subset 1 random	BLAST contig subset 2 random
Bac. sp	3.85	0.8	FAILED	-	-			
BTI dil 2	8.62	1.8	FAILED	-	-			
BTI Dil 5	9.87	2.1	20455	2	14902	AYBKAUVB014	14kbp, looks like a plasmid, unknown genus	8kbp, Select seq AY325804.1 Lysinibacillus sphaericus plasmid pLG, complete sequence, 24% coverage evalue 1e-165
BI2 Dil4	24.64	5.2	FAILED	-	-			
BI3 Dil2	28.8	6.1	140547	3	52415	AYCNJNKZ014	15.6kbp, Paenibacillus xylanexedens strain PAMC 22703, complete genome at 20%, e=0; or Paenibacillus xylanilyticus strain W4 chromosome, complete genome, 31% at2 e-175	7.9kbp, Paenibacillus xylanilyticus strain W4 chromosome, complete genome at 46%, e=0. P. lautus ALSO a good match
BI3 Dil3	20.69	4.4	36973	1	36973	AYBP5VKZ016	36kbp Paenibacillus cellulositrophicus strain KACC 16577 chromosome, complete genome, 35% query at evalue=0.0	
BI3 Dil4	60.78	12.9	4828974	29	199372	AYCTRJS2014	5.4kbp, P. xylanexedens at 92%(!), e=0	1.4kbp, P. xylanexedens or P. xylanilyticus
-	*118.37*	25.2	4325243	35	162227			

Note that the unclassified barcoding sequences (118Mbp) could be used to greatly improve this assembly given more time if required.

Appendix 6 – VHP Generator Cycle Report – Run 1

ProXcide Decontamination Process Report

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ProXcide System No:	ProXcide 0017
Process ID:	25420201332
Process Location:	garment study 2
Process Start time:	25/04/2020 14:32:48 BST+0100
Process Authorised by:	Tautvydas Karitonas
Process Duration:	2 hours 58 minutes

Decontamination Process Validation

Process Successful

Cycle Parameters

System Safety Check	Environmental Parameter Check	Injection Phase Complete	Dwell Phase Complete	Deactivation Phase Complete
✓	✓	✓	✓	✓

Cycle completed:	Peak RH
Injection time:	38 minutes
Dwell time:	30 minutes
Deactivation time:	60 minutes
Extra deactivation time: -	30 minutes

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Hygiene Solutions
Integrated infection control

Appendix 7 – VHP Generator Cycle Report – Run 2

ProXcide Decontamination Process Report

Hygiene Solutions
Integrated infection control

ProXcide System No:	ProXcide 0017
Process ID:	28420201401
Process Location:	garment study 2 test 2
Process Start time:	28/04/2020 15:01:56 BST+0100
Process Authorised by:	Tautvydas Karitonas
Process Duration:	3 hours 2 minutes

Decontamination Process Validation

Process Successful

Cycle Parameters

System Safety Check	Environmental Parameter Check	Injection Phase Complete	Dwell Phase Complete	Deactivation Phase Complete
				

Cycle completed:	Peak RH
Injection time:	29 minutes
Dwell time:	30 minutes
Deactivation time:	60 minutes
Extra deactivation time: -	15 minutes

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