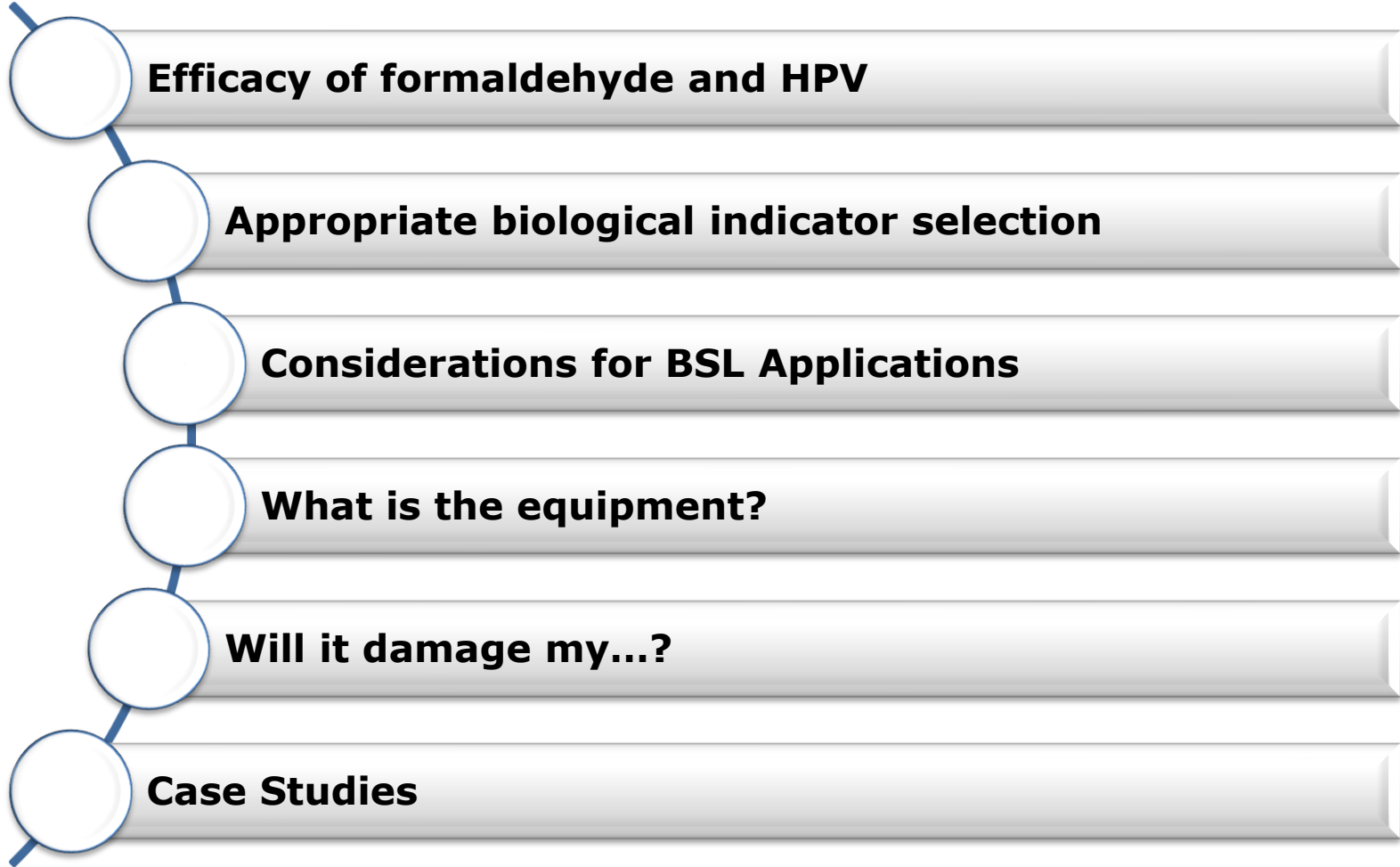


Bioquell RBDS for Biosafety Laboratories

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- Efficacy of formaldehyde and HPV**
- Appropriate biological indicator selection**
- Considerations for BSL Applications**
- What is the equipment?**
- Will it damage my...?**
- Case Studies**



Introduction - Formaldehyde

- Formaldehyde has been the mainstay of decontamination in biosafety applications for many years
- Formaldehyde fumigation has several drawbacks including:
 - Incomplete efficacy data profile
 - Health and safety implications
 - Regulatory issues and bans
- Hydrogen peroxide vapour (HPV) and chlorine dioxide are possible alternatives to formaldehyde
- This presentation will describe outstanding issues related to the use of HPV in biosafety applications



Formaldehyde - Limitations

- Carcinogenicity
- Smell
- Propensity to leak
- Cycle times are typically very long
- Efficacy profile
- Efficacy depends on correct formulation and a range of temperature and humidity values
- Removal of formaldehyde is problematic and, depending on the method used, can result in paraformaldehyde residues that are slow and potentially hazardous to remove
- Rather poor activity of formaldehyde for the inactivation of bacterial endospores (*Geobacillus stearothermophilus*), vegetative bacteria (*Staphylococcus epidermidis*) and viruses (Poliovirus type 3) has been reported¹

Health and safety exposure limits

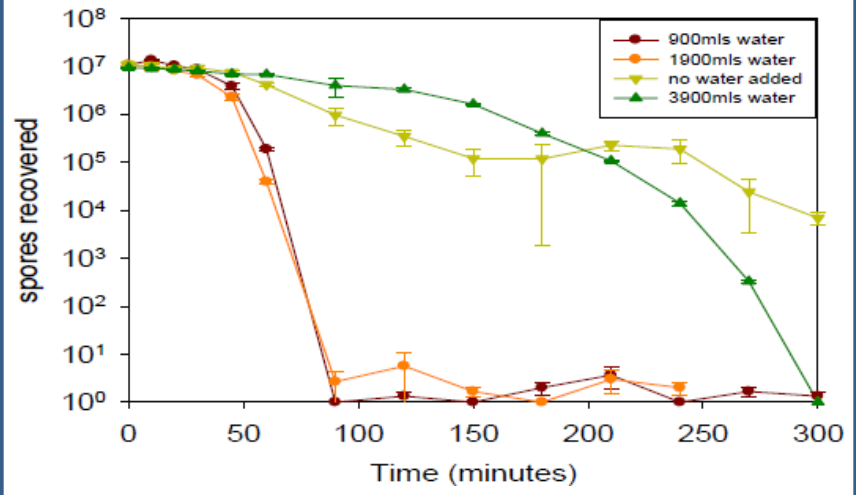
	HPV	Formaldehyde
Hazard	Skin/eye irritant	Human carcinogen
Personal exposure limit / ppm	1.0	0.75
Short term exposure limit / ppm	2.0	0.1
Immediate danger limit / ppm	75	20
Typical room gassing conc. / ppm	100-500	9,000



Formaldehyde – What actually happens?

- We are aware of cases where formaldehyde fumigation has been problematic in practice:
 - One UK mortuary was applying a ludicrously short formaldehyde fumigation cycle, amounting to 15 mins in total.
 - A large pharmaceutical facility has to evaluate the entire facility each time formaldehyde fumigation is required, resulting in significant financial implications

The HPA investigated the effect of boiling off 100mL of formalin in a 20m³ room on the viability of *B. atrophaeus* spores¹



1. Allan Bennett. Assessment of the limitations of gaseous disinfectants for containment and animal laboratories. Epizone.



Is HPV a viable alternative?

In order to be considered as a viable alternative to formaldehyde in biosafety applications, HPV must demonstrate the following properties:

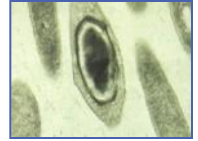
- Effective against organisms of interest in biosafety laboratories in a presentation representative of field conditions
- Safe to use

Some efficacy testing rules of thumb:

- Tests must be representative of actual application
- Most published testing protocols relate to liquid suspension tests and are not suitable for testing dried inocula
- Inoculum, suspending medium and substrate critical
- Vapour must penetrate suspending medium
- Neutralisation difficult
- Particular problems related to virus studies:
 - Cytotoxicity; viral culture medium as a suspending medium; sensitivity to desiccation (enveloped viruses)

Summary of efficacy of HPV?

Bacterial endospores¹ (e.g. *Clostridium difficile*)



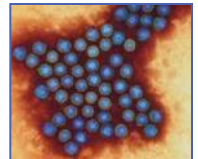
Mycobacteria² (inc. *Mycobacterium tuberculosis*)



Vegetative bacteria³ (e.g. *Staphylococcus aureus*)



Viruses⁴ (e.g. Adenovirus, Norovirus)



Fungi⁵ (e.g. *Aspergillus*)



1. Johnston *et al.* *J Microbiol Methods* 2005;60:403-410.
2. Hall *et al.* *J Clin Microbiol* 2007;45:810-815.
3. Otter *et al.* *J Hosp Infect* 2007;67:182-188.
4. Heckert *et al.* *Appl Environ Microbiol* 1997;63:3916-3918.
5. Hall *et al.* *Med Mycol* 2008;46:189-191.



Sporicidal efficacy

HPV has been shown to inactivate endospores of the following bacteria:

- *Geobacillus stearothermophilus* (used as a biological indicator of Bioquell's HPV services)
- *Bacillus anthracis* (peer reviewed, published testing conducted by US Army and Dstl, Porton Down)¹
- *Clostridium botulinum* (published collaborative investigation with large UK food research company)²
- *Clostridium difficile* (Bioquell *in vitro* studies in collaboration with King's College London)³

1. Rogers *et al.* *J Appl Microbiol* 2005;99:739-748.
2. Johnston *et al.* *J Microbiol Methods* 2005;60:403-410.
3. Otter & French. *J Clin Microbiol* 2009;47:205-207.



Efficacy against *Mycobacterium tuberculosis*

- *Mycobacterium tuberculosis* has a thick, lipid cell wall and is resistant to common disinfectants.
- Several studies have investigated the efficacy of HPV against *M. tuberculosis* or surrogates:
 - *M. tuberculosis* was killed in a biological safety cabinet and in a room, Mayo Clinic (Bioquell)¹
 - Max-Planck Institute, Germany (Steris)²
 - HPA (Bioquell, *M. vaccae*)
- Bioquell performed a collaborative study performed with the Mayo clinic
- Used a low inoculum to simulate a spillage of TB that has been cleaned to remove gross contamination
- A >3-log reduction was demonstrated in a safety cabinet and at various locations around a room.
- A >6-log reduction on *M. tuberculosis* was demonstrated in other studies.



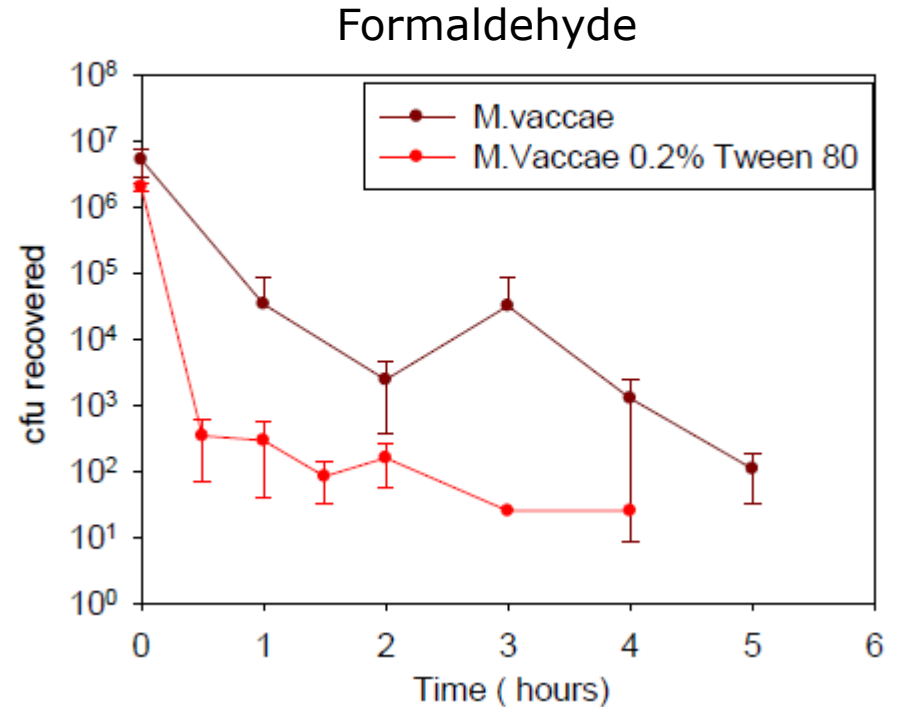
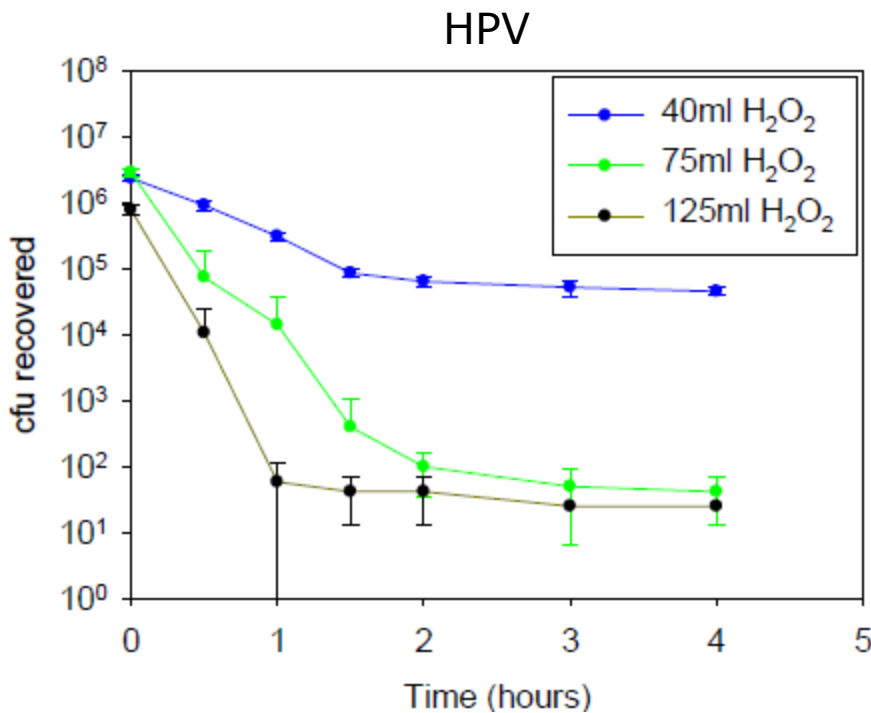
HPV is able to inactivate M. tuberculosis under the conditions tested

1. Hall *et al.* *J Clin Microbiol* 2007;45:810-815.
2. Kahnert *et al.* *Lett App Microbiol* 2005;40:448-452.



Efficacy against *Mycobacterium vaccae*

Recent studies by the HPA investigated the comparative efficacy of various amounts of HPV and formaldehyde for the inactivation of *M. vaccae*:



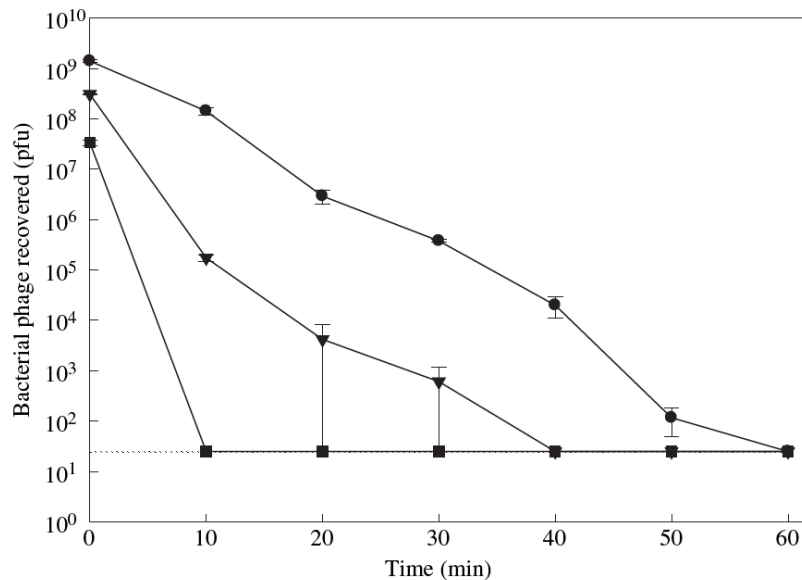
HPV appears to have approximately equal activity against *M. vaccae* compared with formaldehyde



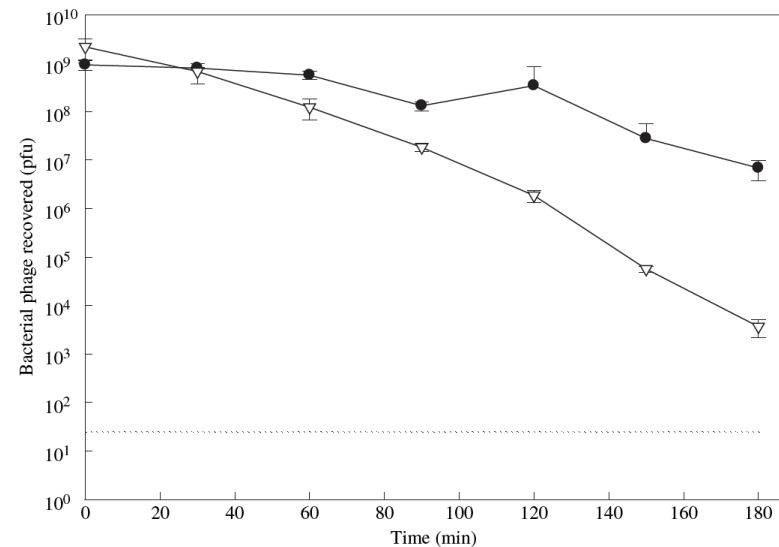
Inactivation of viruses

Recent studies by the HPA investigated the effectiveness of HPV for the inactivation of MS2 coliphage:

Dried in water



Less impact when dried in 10% (triangles) or 50% (circles) blood

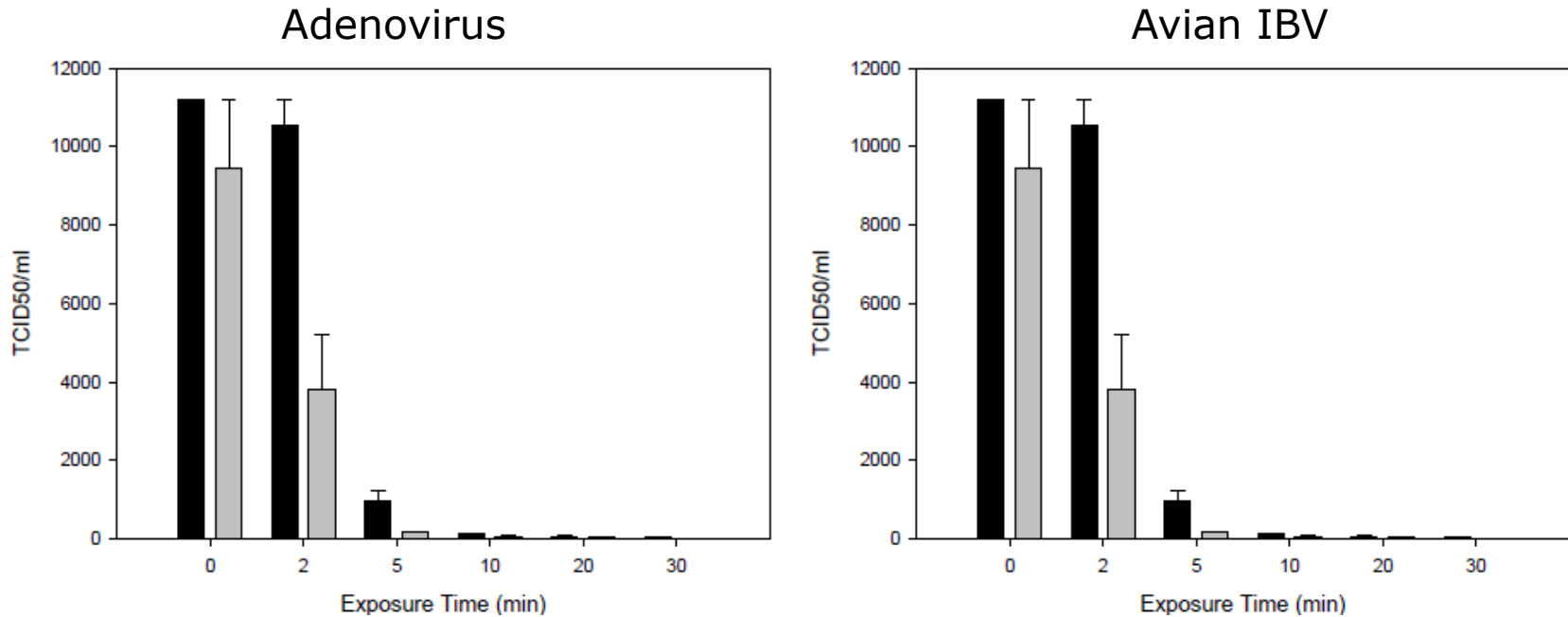


HPV can inactivate phages dried in water, but has less impact when dried in high concentrations of blood



Inactivation of viruses

Recent studies by the HPA investigated the comparative effectiveness of alternate VHP equipment (grey bars) and formaldehyde (black bars) for the inactivation of adenovirus and Avian infections bronchitis virus (IBV):



VHP appears to have approximately equal activity against these viruses compared with formaldehyde



Efficacy against fungi

- A study conducted by the Mayo Clinic in collaboration with Bioquell¹ has demonstrated the *in vitro* efficacy of HPV against a number of medically important dimorphic fungi:
 - *Histoplasma capsulatum*
 - *Blastomyces dermatitidis*
 - *Coccidioides immitis*
- Other studies have demonstrated efficacy against:
 - *Aspergillus niger*
 - *Penicillium* sp.
 - *Alternaria* sp.
 - *Candida albicans*

1. Hall et al. *Med Mycol* 2008;46:189-191.



Future objectives

- Continue to strengthen efficacy data
 - Particular focus on viruses
 - Containment-level 4 viruses
- Investigate the efficacy of HPV for the inactivation of prions
- Study the effect of HPV against DNA
- Continue to respond to efficacy requests and generate organism-specific data in collaboration with clients



Finding and appropriate biological indicator

Ideal features of a biological indicator (BI):

- Safe to handle for the operator
- Will not contaminate the environment / product / experiment
- A sufficient microbiological challenge
- Accepted by the industry
- Not prone to contamination with other organisms (to avoid false positives)

Reasons why *G. stearothermophilus* may be inappropriate

- Not the most resistant organism
 - Any studies to assess which organism is more resistant to HPV must be controlled so that you are testing the resistance of the organism and not the organism + presentation
- Not the most resistant presentation
 - Regardless of underlying innate resistance of the organism, some biosafety challenges in the field will present a challenge that is more difficult to inactivate than a commercially produced BI
- These limitations, particularly relating to presentation, apply equally to formaldehyde.



Finding and appropriate biological indicator

Reasons why *G. stearothermophilus* is the most suitable indicator

- *G. stearothermophilus* is accepted as a BI in most sectors, but the sufficiency of their microbiological challenge is under question in biosafety
- For most biosafety applications, cycles that inactivate *G. stearothermophilus* BIs inactivate challenges with microbes of biosafety interest in their field presentations
- However, there may be occasions when commercially produced BIs will not be the most resistant 'system' (i.e. innate resistance of the organism plus the resilience of the presentation)
- In these situations, *G. stearothermophilus* can be used as a benchmark to calibrate the cycle required for the given application, validated against a specific challenge
- A future alternative may be the use of quantitative Bis that can be calibrated to different challenges – these enzyme based systems are currently under development at the HPA¹
- In the mean time, it is our view that *G. stearothermophilus* BIs are the best available indicator system for biosafety applications

What is it & what do I get?



Room

- Can decontaminate any given enclosure
Provided it can be sealed
- From single rooms to entire buildings

Bio-
Decontamination

- Verifiable 10^6 spore reduction
- Broad efficacy spectrum
- Hydrogen peroxide used so residue free

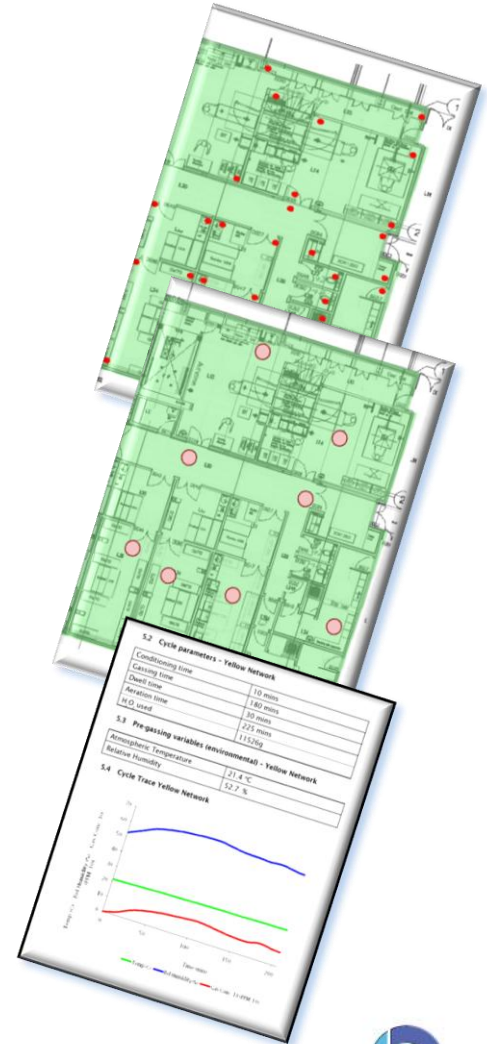
Service

- Self contained including all consumables
- Flexible to fit to your requirements including timescales and operating procedures
- No need for capital equipment purchase



What is it & what do I get?

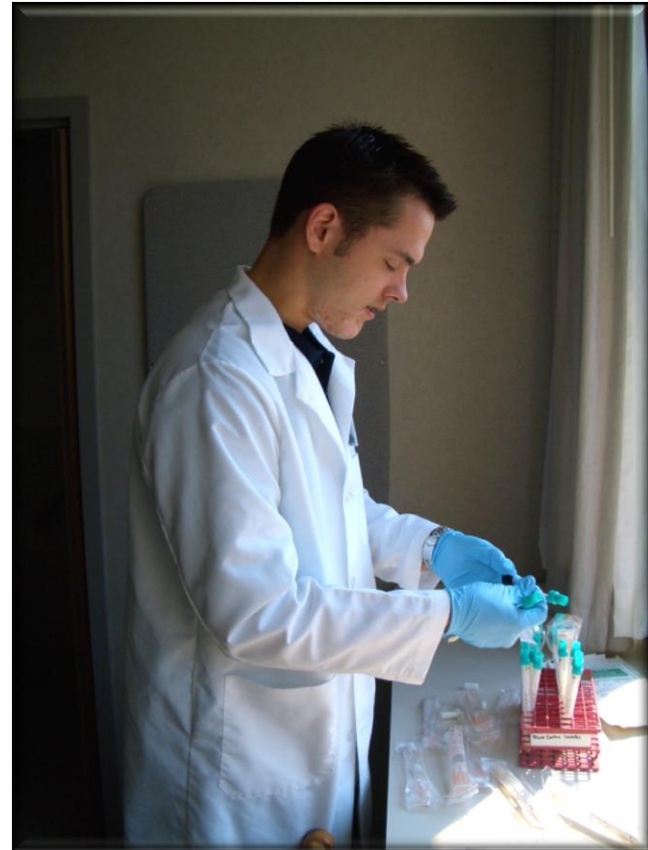
- Flexibility of use and operation;
- Demonstrated biological deactivation in your facility – not reliant on calculations;
- Trained engineers to operate all equipment and ensure SOP's are adhered to;
- All equipment maintained, calibrated and updated as required;
- Full documentation including:
 - Safety briefing;
 - Project plan;
 - Risk assessment and method statements;
 - Biological indicator and equipment maps (including serial number tracking);
 - Calibration certificates of equipment;
 - Detailed final report of decontamination cycle.





Does it work?

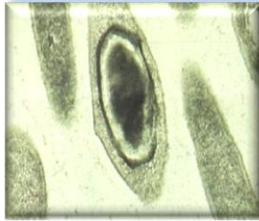
- HPV efficacy now widely accepted
- use 10^6 *Stearothermophilus* indicator
- laid out according to standard matrix & SOP
- additional BIs used when & where requested by customer
- The customer or Bioquell can incubate the Bis
- Over 50,000 successful and safe deployments





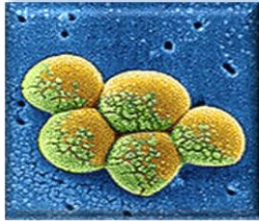
Does it work?

Bacterial
spores



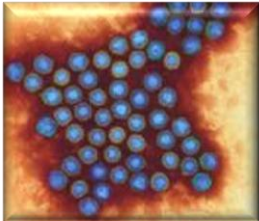
Bacillus stearothermophilus
Bacillus subtilis
Bacillus anthracis (NB anthrax)
Clostridium piliforme (Tyzzer's disease)

Bacteria
"superbugs"



Methicillin-resistant *Staphylococcus aureus*
Vancomycin-resistant *enterococci*
MDR-*Klebsiella*
MDR-*Acinetobacter sp.*

Virus

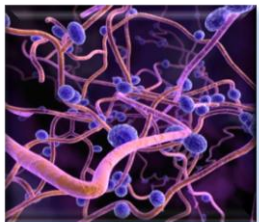


Orthomyxoviridae
Parvovirus
Parainfluenza
Kilhams disease



Bioquell is able to respond to
Microbial bio-deactivation queries
and/or arrange a trial

Fungi



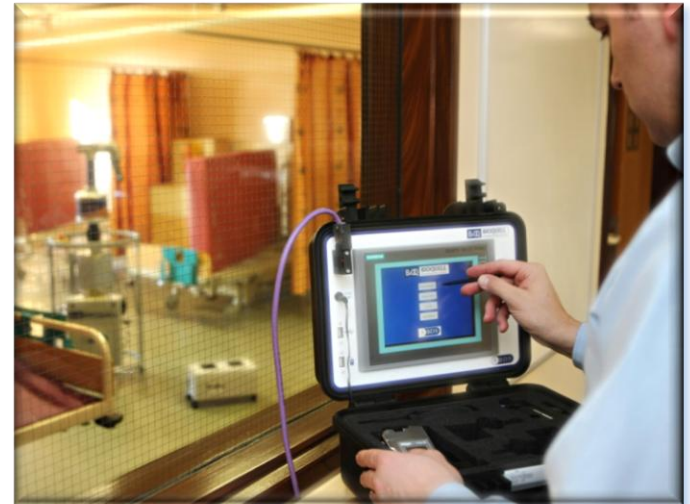
Aspergillus
Candida albicans

Is it Safe?

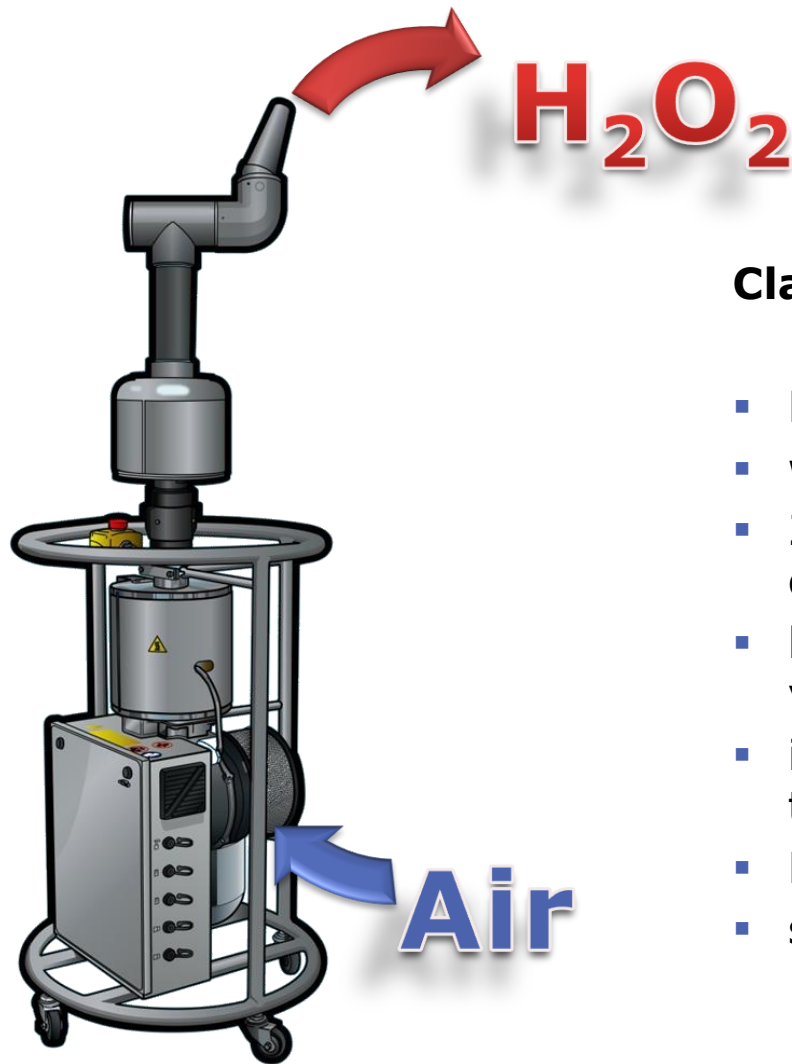


- Calibrated detection equipment – 0.1ppm resolution;
- Strict SOPs on how to operate equipment;
- Continual monitoring during gas injection phase;
- World experts - c. 50,000 rooms decontaminated;

- All equipment constantly monitored;
- Complete control of system from outside the area;
- Emergency shut down (if required);
- If concerned about leakage, cycle performed on a step-by-step basis whilst monitoring.



What is the Equipment.....?



Clarus R vapour Generator

- Located within the enclosure
- weight: 20kg (44lbs)
- 250m³ bio-decontamination volume capability)
- high kinetic energy of vapour (exit velocity 30m/s)
- infinitely scalable ("daisy chain" together)
- Easy to transport and manoeuvre
- self-sterilising

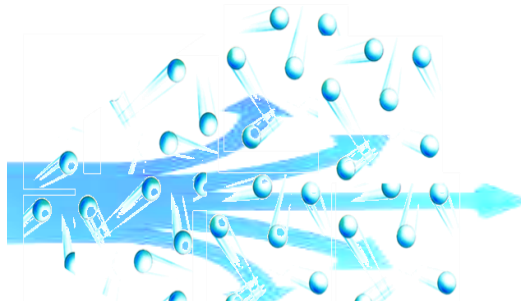
What is the Equipment.....?



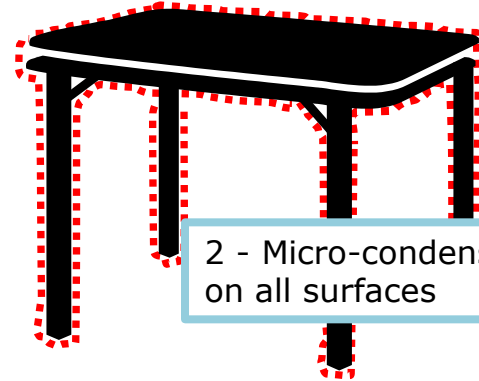
R20 Aeration Unit

- Mobile
- Self sanitising
- Leaves no residue
- 5500m³/hr

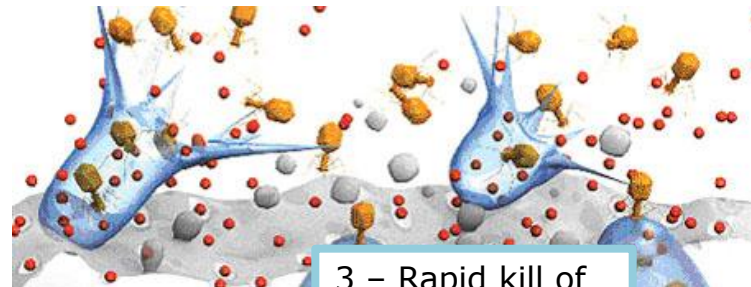
Bioquell's HPV process...



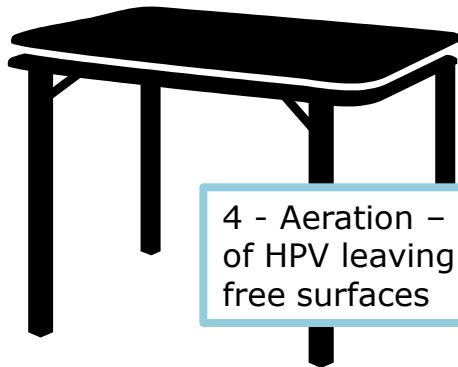
1 - Flash Evaporation forming Hydrogen Peroxide Vapour (HPV)



2 - Micro-condensation on all surfaces



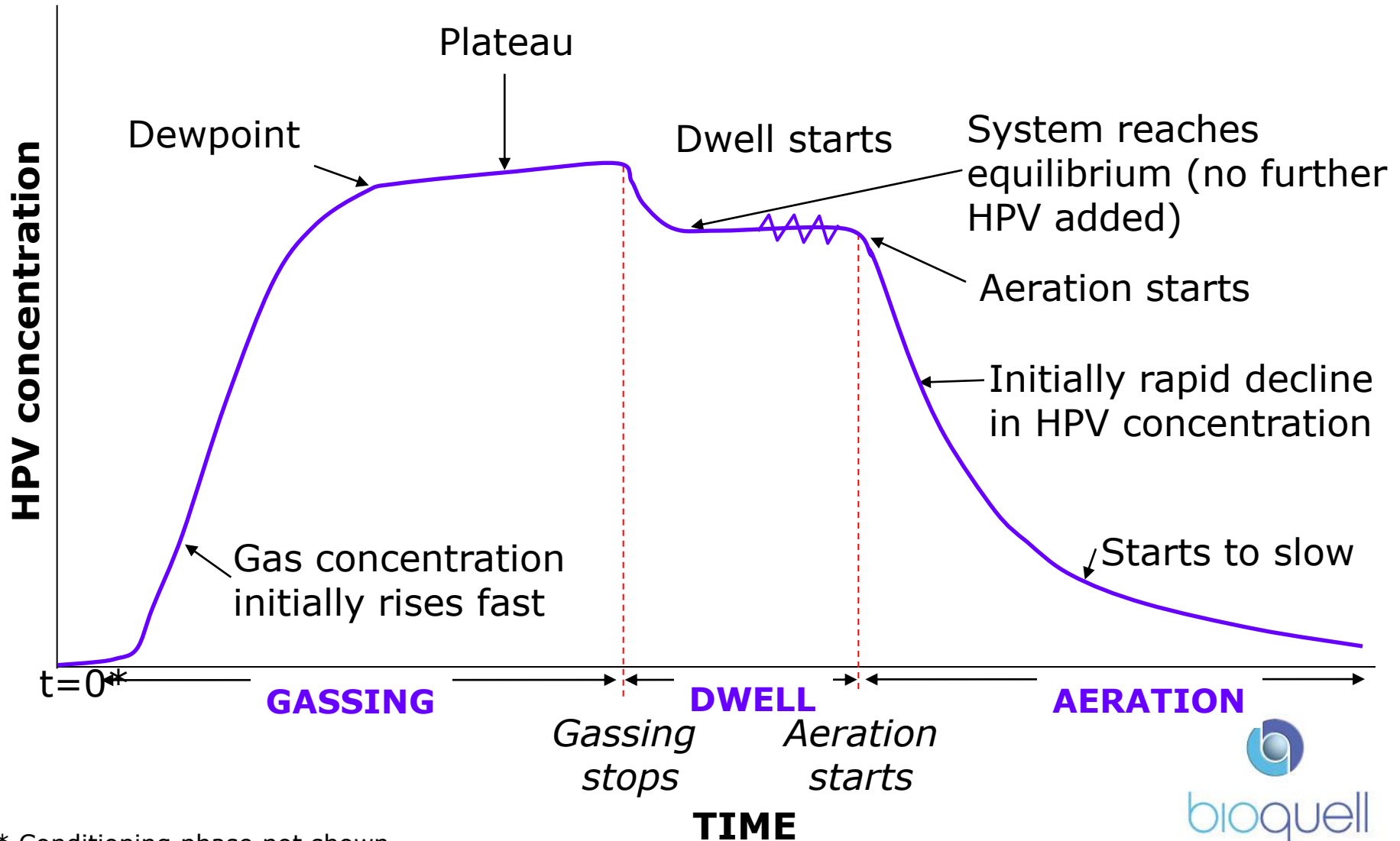
3 - Rapid kill of microorganisms



4 - Aeration - removal of HPV leaving residue free surfaces



Bioquell's HPV process...



* Conditioning phase not shown

Will it damage my.....?



- Materials compatibility data generated over 860 cycles over 4 months
- No electronic failures on any components tested
- Large document listing all materials tested and results
- Further testing on products can be conducted at the customers request.



Will it damage my.....?



Gambro Dialysis Machines



Siemens Servo 300C Ventilator



Tyco Puritan Bennett 800 Ventilator



Siemens Servo 900C Ventilator



Siemens Patient Monitor Systems



Philips Defibrillators



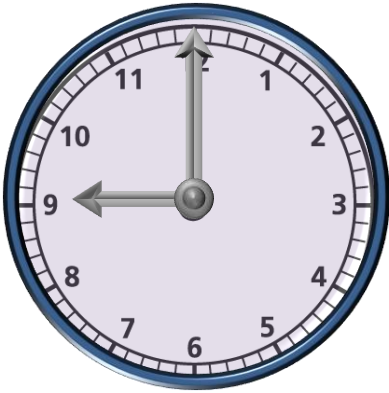
Dinamap NIBP

Case Study 1



- 114 room research facility needing urgent bio-decon after a virus outbreak;
- Large volume of equipment (cage rack systems) needed bio-decontamination;
- 2 story facility with interconnecting stairwells and elevators would all need to be bio-decontaminated.

Case Study 1



Team and equipment arrive

Equipment unloaded & set-up

Final set-up and equipment test

Air-conditioning and fire alarms isolated

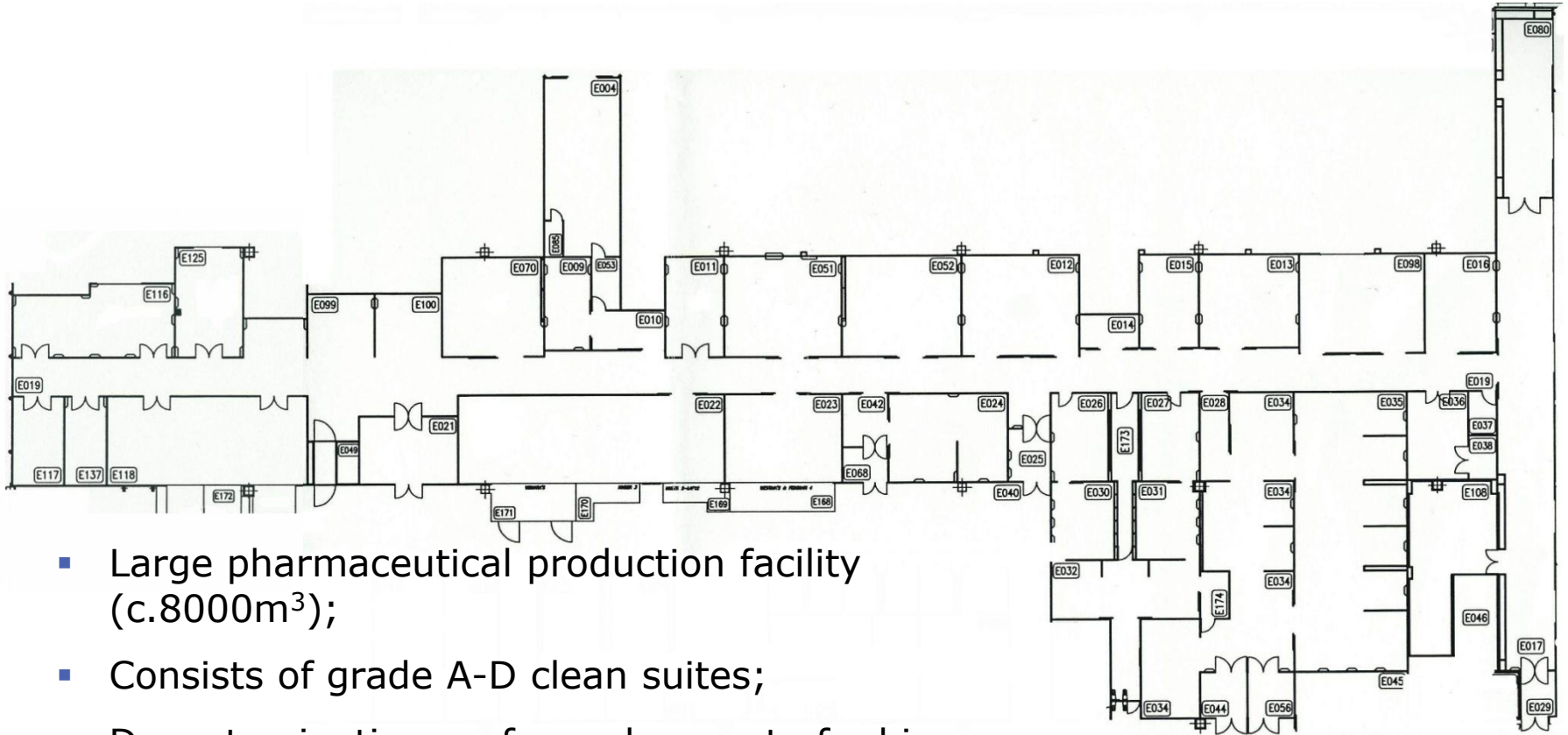
Gassing phase

HVAC re-started

Overnight aeration



Case Study 2

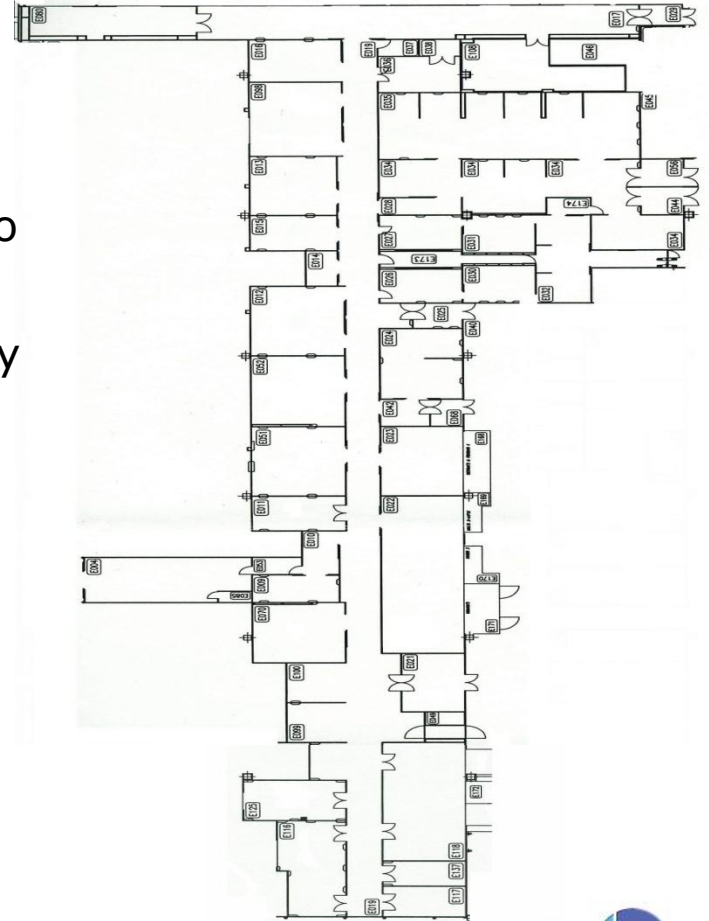


- Large pharmaceutical production facility (c.8000m³);
- Consists of grade A-D clean suites;
- Decontamination performed as part of a bi-annual shutdown;
- Biological indicators and sample plates used to verify cycle.



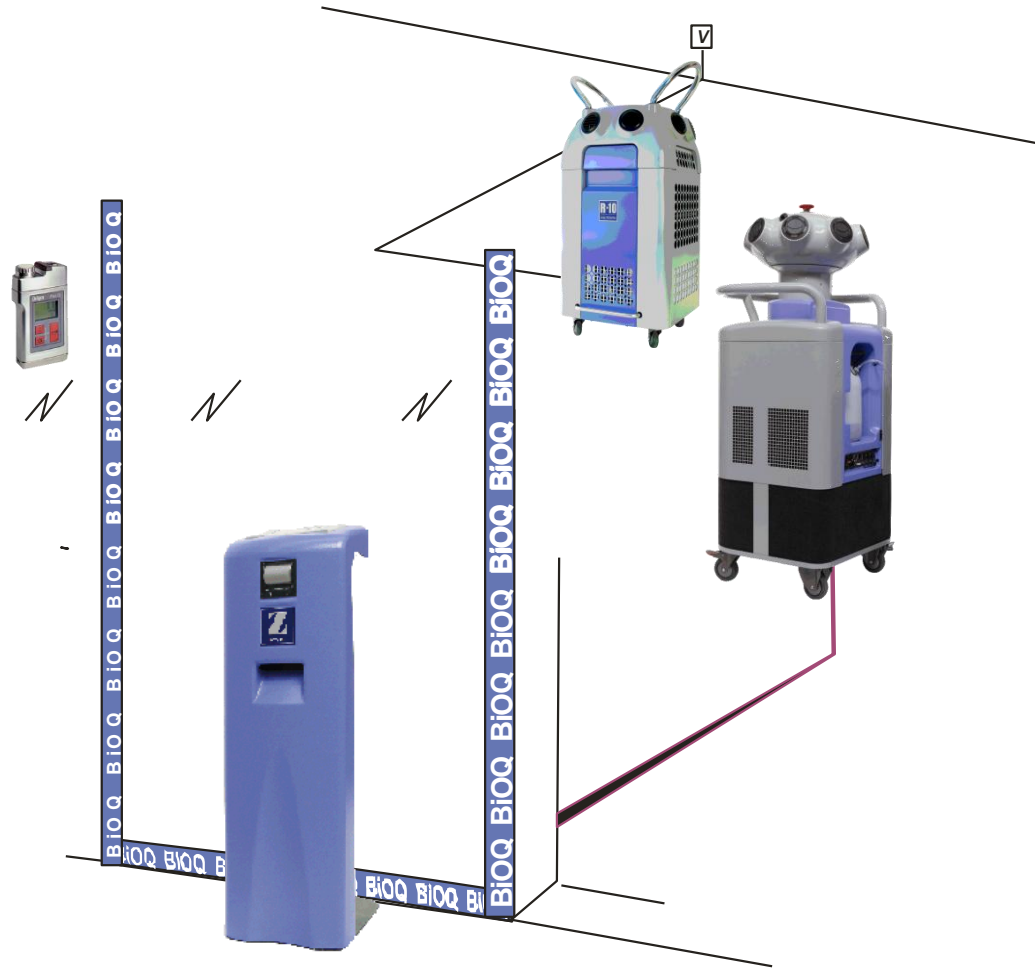
Case Study 2

- Schedule Pre-arranged and agreed by customer;
- Bioquell arrive at 12 noon;
- Equipment “pre-cleaned” prior to entry into Grade D area;
- Team and equipment enter grade C area by 2pm;
- Equipment set-up by 8pm – team leave site;
- 9am decontamination cycle commenced;
- Aeration begins at 12noon;
- Bioquell remove equipment and leave site by 8pm.





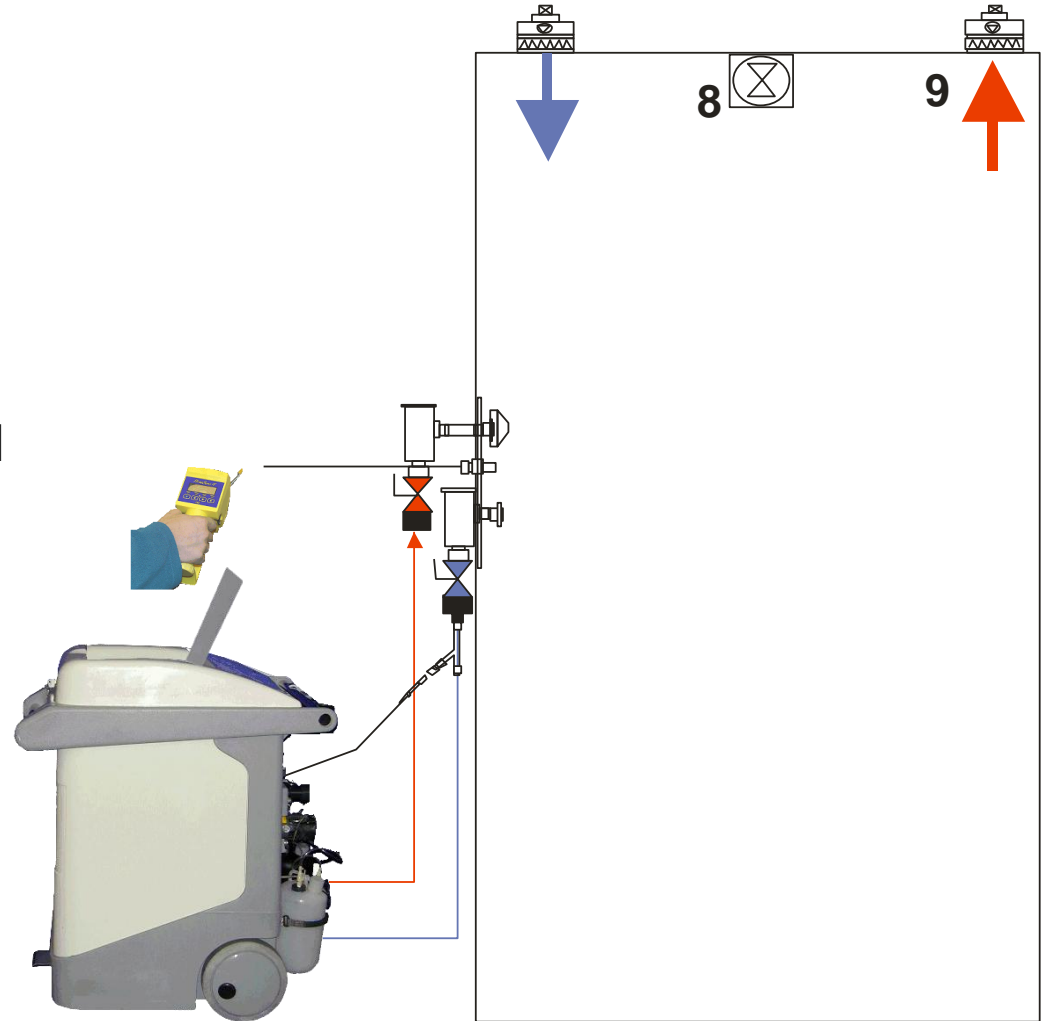
Bioquell Z deployment – Low Risk





Bioquell C fixed system – High Risk

- ❑ Wall plate housing H_2O_2 connections used for the supply and return of HPV inside the room and low level sampling
- ❑ Room fan used to aid distribution of HPV around the room
- ❑ Canister HEPA filters and valves are used to ensure containment



Bioquell | RBDS

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