

Hydrogen Peroxide Vapour (HPV): the new “gold standard” for bio-decontamination in the Biopharmaceutical and Pharmaceutical Industries?

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1. Introduction

Hydrogen Peroxide Vapour (HPV) is fast becoming the bio-decontamination agent of choice for many market leaders in the pharmaceutical and biopharmaceutical industry, which is reflected by the rapid global uptake of the technology. BIOQUELL's HPV patented technology has proven biological efficacy against a wide range of micro-organisms and is considered by many to be the gold standard in bio-decontamination applications. HPV provides a validated 6 log reduction, is infinitely scaleable, residue free and thus offers minimal downtime. This provides numerous benefits over alternative methods of decontamination. This article outlines current knowledge regarding the efficacy of HPV against micro-organisms of interest to the biopharmaceutical and pharmaceutical industries, and discusses the reasons why HPV bio-decontamination is currently one of the hottest topics in these industries.

2. Background

HPV technology has existed for a decade and utilises the microbicidal power of free radicals released by hydrogen peroxide (H_2O_2). BIOQUELL's Clarus[®] technology works by producing HPV through flash evaporation of an aqueous solution of H_2O_2 . The process is effective at ambient temperature and relative humidity - no special pre-conditions are necessary.

HPV is introduced into a room using a dual axis vapour distribution system, which ensures that the HPV is introduced to the room evenly, and that each surface is comparably exposed to the HPV. The equipment lays down approximately 1 micron of H_2O_2 onto exposed surfaces where microorganisms may reside. The microorganisms themselves act as nuclei for the formation of micro-condensation, thus speeding action of the process. The process is controlled from outside the enclosure via a control computer, which provides real time feedback of cycle progress. The room or equipment being exposed to HPV is sealed and monitored by electrochemical hand held HPV sensors to ensure that there is no leakage and that the level of HPV has returned to safe environmental levels after the cycle.

Post decontamination the HPV is catalytically converted into water vapour and oxygen. The HPV can either be completely neutralized in the specified area using powerful aeration units or in conjunction with the building's HVAC system. Either way, unlike formaldehyde, there are no toxic residues.

Geobacillus stearothermophilus spores dried onto stainless steel discs at a 6-log loading and sealed in Tyvek pouch are used as biological indicators to verify the process. Customized testing is also feasible using, for example, contact plates or volumetric air sampling. The *G. stearothermophilus* biological indicators are accepted by industry and are also used to validate the inside of autoclaves, hence bio-decontamination applications using HPV are validated to the same level as the inside of autoclaves!

3. Biological Efficacy

HPV is well established as a bio-decontamination agent due to its wide-ranging biological efficacy and can deactivate micro-organisms faster, and more effectively than many traditional alternative bio-decontamination methods such as formaldehyde. As with any other bio-decontamination agents, HPV has been tested on many individual micro-organisms and classes of organisms. However, because a great number of "common" micro-organisms exist, efficacy testing remains an ongoing process.

Spaulding's classification (Figure 1) of the relative resistance of various micro-organisms to sterilisation and disinfection procedures is widely accepted in the biopharmaceutical and pharmaceutical industries. HPV efficacy has been repeatedly proven against the Spaulding Scale's most resistant organism, bacterial endospores. *Geobacillus stearothermophilus* is a commonly used indicator organism to verify the efficacy of sterilisation and disinfection procedures. A number of studies have demonstrated that HPV is rapidly sporicidal against *G.stearothermophilus* (Johnston et al. 2005; Kokubo et al. 1998). One study showed that *G. stearothermophilus* spores were the most resistant amongst a range of spore-forming bacteria tested, including *Bacillus subtilis* and *B. pumulis*, which are two organisms commonly used to verify other sterilisation and disinfection procedures (Kokubo et al. 1998).

Figure 1: 1972 E.H. Spaulding's classification of the resistance of various micro-organisms to sterilisation and disinfection.

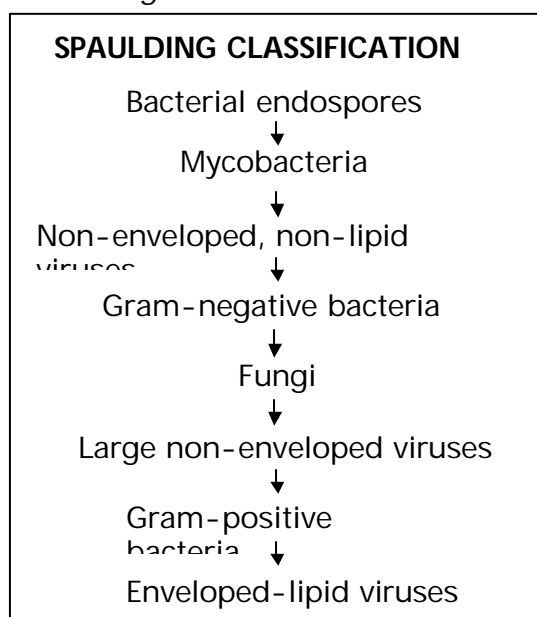
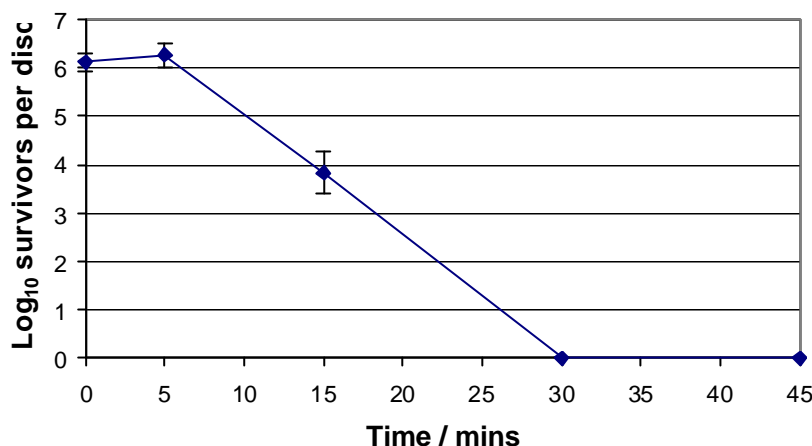


Figure 2 shows data from BIOQUELL's microbiology laboratories into the kill time for *G. stearothermophilus* spores (6-log inoculum, Apex Laboratories, Apex, USA) exposed to HPV in a 100m³ room. Other studies have shown that the kill time inside smaller enclosures, such as biological safety cabinets, is considerably shorter (Johnston et al. 2005).

Figure 2. *Geobacillus stearothermophilus* spores dried onto stainless steel discs exposed to HPV generated by a Clarus R in a 100m³ room. Data points represent a mean of three discs. The Y axis error bars represents the standard deviation.



Studies have been conducted against other spore forming bacteria. Johnston *et al.* (2005) demonstrated that *Clostridium botulinum* spores were killed within 10 minutes exposure to HPV inside a sealed enclosure. Rogers *et al.* (2004) working on behalf of the US Environmental Protection Agency (EPA) investigated the efficacy of HPV against *Bacillus anthracis* spores dried onto various substrates and reported complete killing on non-porous surfaces and a significant log reduction on porous surface, which included industrial grade carpet.

Vegetative bacteria

Vegetative bacteria are a good measure of the microbiological hygiene inside pharmaceutical facilities and their presence in a clean room area is monitored as part of ongoing environmental assessment. HPV has proven biological efficacy against Gram-positive bacteria including staphylococci (French *et al.* 2004), and Gram-negative bacteria including *Escherichia coli* (McDonnell *et al.* 2002) and *Pseudomonas aeruginosa* (Rickloff *et al.* 1989). A room contaminated with a significant bacterial load, which is exposed to a HPV technology, can be proven to be microbiologically clean and free of detectable background contamination (French *et al.* 2004).

Atypical Bacteria

Atypical bacteria such as *Mycoplasma* species occur as both commensals and pathogens in man and animals. The closely associated saprophytic acholeplasmas also occur in animals and widely in the environment. Due to their small size, lack of a cell wall and slow growth rate, these organisms pose a serious contamination risk for the man-made habitat of cell culture. An investigation, conducted collaboratively between BIOQUELL and Mycoplasma Experience Ltd. (Reigate, Surrey) investigated whether HPV can be used to inactivate mycoplasmas contaminating surfaces. Sterile stainless steel carriers were inoculated with a liquid culture of *A.laidawii* and air-dried to achieve a nominal loading of 1.3×10^5 colony forming units (cfu) per carrier. These carriers were exposed to HPV for 40 minutes inside a Class 2

Microbiological Safety Cabinet and transferred to growth media at timed intervals. Further, *A.laidawii* samples were distributed inside the cabinet and removed to growth media at the end of the cycle. Stainless steel carriers inoculated with *G.stearothermophilus* spores to achieve a nominal loading of $>1.0 \times 10^6$ cfu/carrier were included in the same locations as a comparative study. Both microorganisms were fully inactivated within 10 minutes of exposure to HPV and none of the biological indicators distributed inside the cabinet showed any signs of growth. These data indicate that HPV is a suitable method to decontaminate enclosures potentially contaminated with mycoplasmas.

Viruses & Bacteriophage

The most comprehensive study investigating the efficacy of HPV against viruses was conducted by Heckert et al. (1997). Table 1 shows an extract of data from this study. Many of the viruses tested suffered a considerable log reduction when dried onto hard surfaces. It is significant that a 2- \log_{10} reduction only was achieved on the HCV dried in whole blood, in contrast to the complete killing of all other viruses that were suspended in viral culture medium. This demonstrates the importance of the suspending medium in terms of viral resistance to HPV decontamination. Similarly, studies with other microorganisms have shown that bacteria and fungi dried in sterile distilled water are considerably more sensitive to HPV compared to bacteria and fungi dried with 5% bovine serum albumin (BSA).

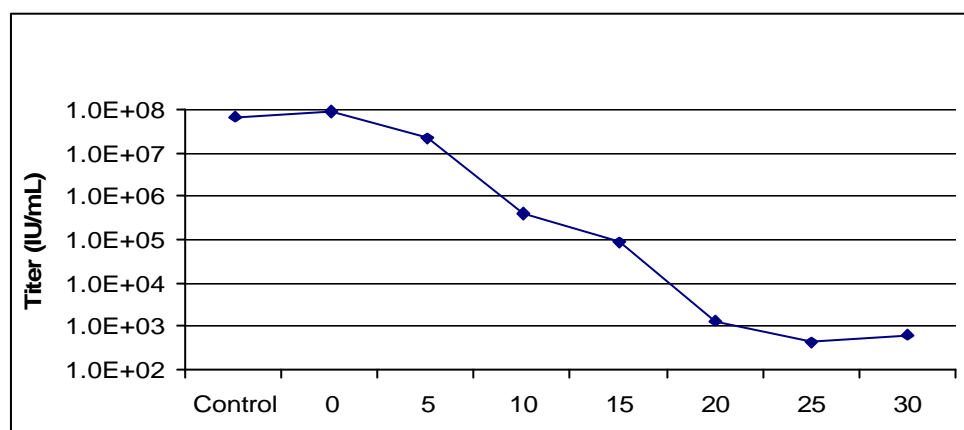
Table 1: Titre (mean \log_{10}/ml) of animal viruses dried onto glass or steel before and after exposure to HPV

	Before HPV exposure		After HPV exposure	
	Glass	Steel	Glass	Steel
AIV	5.68	5.68	<1	<1
ASFV	5.89	6.05	<1	<1
BTV	4.43	4.55	<1	<1
HCV	5.85	5.74	<1	<1
HCV-WB	6.80	6.86	4.18	4.35
NDV	9.14	8.50	<1	<1
PRV	6.10	6.10	<1	<1
SVDV	7.70	8.01	<1	<1
VEV	2.26	2.55	<1	<1
VSV	5.24	4.34	<1	<1

AIV = Avian influenza virus, ASFV = African swine fever virus, BTV = bluetongue virus, HCV = Hog cholera virus, HCV-WB = Hog cholera virus dried in whole blood, NDV = Newcastle disease virus, PRV = Pseudorabies virus, SVDV = Swine vesicular disease virus, VEV = Vesicular exanthema virus, VSV = Vesicular stomatitis virus

A recent study investigating the efficacy of HPV against VRP (inactivated Venezuelan encephalitis virus, VEE) demonstrated a $>5\text{-log}_{10}$ reduction in titre over a 30 minutes exposure period, although not a complete kill (Figure 3). It should be noted that modified count was required on the 20, 25 and 30 min samples due to the low titre, so the chart should be interpreted with caution. This result is impressive micro-biologically and a $>5\text{-log}_{10}$ reduction is the "gold standard" for most viral disinfection tests. Bacterial endospore biological indicators were killed before the end of the 30 minute exposure period. Further trials are planned to increase the exposure period for the VRP, and it is anticipated that a complete kill will be achieved.

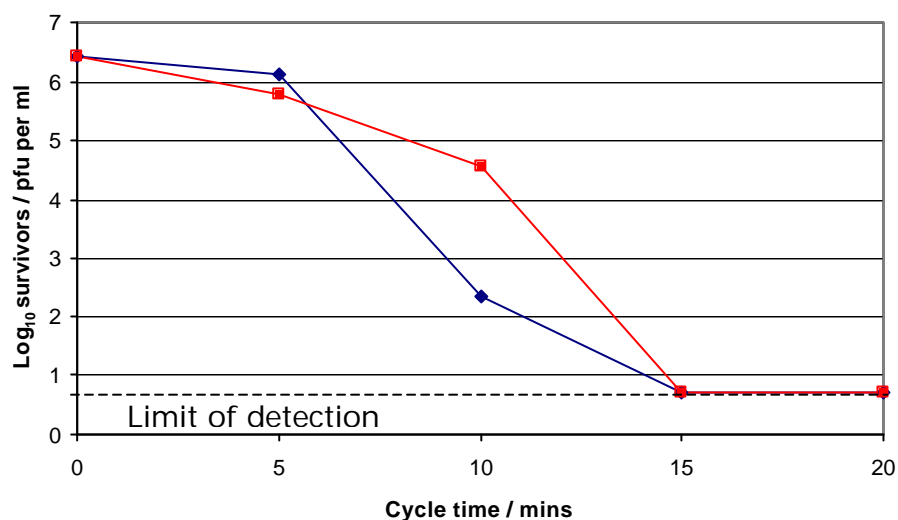
Figure 3: Titre (IU/ml) of VRP exposed to HPV for 30 minutes in a biological safety cabinet.



Bacteriophage

Although not viruses per se, bacteriophage are related to viruses in terms of structure and life cycle. A recent study demonstrated that two strains of bacteriophage were killed rapidly by exposure to HPV (Figure 4). In contrast to the VEE study, the viruses were suspended in water, rather than a nutrient rich culture medium, and in this study the kill time for bacterial endospores was similar to the kill time for the bacteriophage.

Figure 4: Exposure of two strains of bacteriophage to HPV inside a two-glove flexible film isolator connected to a Clarus™ C. A 6-log reduction in both bacteriophage was achieved within 15 mins of being exposed to 30% hydrogen peroxide vapour.



HPV viral deactivation studies can be difficult to interpret and direct comparison with other organisms should be interpreted with caution. Apparent differences in resistance between viruses and other organisms could be explained by differences in presentation of the organisms, often enforced by practical considerations. In reality, actual levels of environmental contamination with viruses will be lower than the experimental inocula used in these studies, ensuring kill will occur more rapidly.

Fungi

Fungi are one of the most common environmental micro-organisms, which can often cause contamination problems in pharmaceutical laboratories and production facilities. Fungal spores are commonly responsible for cross-contamination in laboratories, *Aspergillus sp.* being one of the more frequent culprits. *Aspergillus sp.* are killed when exposed to HPV (HPA, 1995; Rickloff, 1989). Other fungi are also killed when exposed to HPV, including *Penicillium sp.*, *Alternaria sp.* and *Candida sp.* (Rickloff, 1989; Information from Eli Lilly, with Permission).

4. Practical bio-decontamination solutions

BIOQUELL HPV technology has proven, excellent material compatibility. In contrast to alternative disinfection regimes that can cause equipment damage, HPV technology allows clients to leave computer equipment and complex equipment in the room during the duration of the bio-decontamination process. Sensitive electronics, repeatedly exposed to HPV do not sustain any deleterious effects. Contamination control post-HPV is critical - if non-sterile potentially contaminated equipment is re-introduced into a HPV-decontaminated area, this defeats the object of the decontamination!

HPV is non-carcinogenic, used in a low-temperature vapour-phase and "residue free", which is clearly beneficial because there is no need for a post-process residue wipe-down, thus further reducing total downtime and ensuring the safety of staff using the equipment or room.

The latest HPV patented equipment allows multiple HPV generators and aeration units to be linked together creating an HPV technology that is infinitely scaleable. This type of modular system is operated from a single control computer permitting vast spaces such as fermentation suites, purification areas, complete pharmaceutical facilities or entire buildings to be decontaminated with minimal downtime and inconvenience. BIOQUELL's Room Bio-Decontamination Service (RBDS®) can be applied to any size enclosure. Any equipment inside the room, such as incubators and refrigerators will also be decontaminated during the process. By choosing the RBDS service over the purchase of capital equipment, clients can gain access to unique expertise in room bio-decontamination, hire sophisticated patent-protected equipment and there is no need to invest in expensive capital equipment. In addition there is no need for the client to use its own personnel as the service means that the bio-decontamination process can be outsourced to highly trained RBDS engineers. These engineers can be employed to operate this fast, effective process outside normal working hours, which also reduces facility or laboratory operational downtime. Both scheduled/emergency services are available if required. At the end of a room bio-decontamination service clients receive a written report containing verification at the target level that bio-decontamination has been achieved at their site.

HPV technology offers flexibility and an affordable range of products and services to support clients' custom needs from start-up, through scale-up to commercial full-scale production. HPV technology companies have a great deal of experience in working with major biopharmaceutical and pharmaceutical companies. For example BIOQUELL has provided consultancy, validation, bio-decontamination equipment and services in the following areas:

- Commissioning / De-commissioning bio-decontamination
- Eradication of problematic microorganisms from production lines and laboratory areas
- Emergency bio-decontamination for accidental release or spillage of microorganisms
- Regular clean room bio-decontamination
- Robotic equipment
- Isolator Equipment

Prior bio-decontamination, a site survey is often performed to assess the microbiological flora, the topology of the room, the HVAC system, material compatibility, and regulatory issues.

5. Case Studies

Numerous premier pharmaceutical and biopharmaceutical companies have already adopted the BIOQUELL Clarus HPV technology. HPV technology case studies are available to clients to demonstrate the success of the technology in industry. Examples of the application of BIOQUELL's RBDS include the following:

Production

Microbial contamination can be a serious barrier to the production of pharmaceutical products. Bacterial, viral or fungal contamination can halt production lines, and each day of lost production imposes a significant and cumulative financial burden. The rapid deployment of BIOQUELL's RBDS can bio-deactivate the microbial contamination and quickly reinstate the production line, thus preventing further losses and returning production to normal.

Production case study

- Large European contract pharmaceutical manufacturer
- Contamination with *Penicillium* sp. halted production
- RBDS deployed to bio-decontaminate 5000m³ production area comprising 50 rooms (including Class 100 and Class 1000 areas)
- Entire facility bio-decontaminated in a total operational time of 48 hours
- Process verified using 6-log *Geobacillus stearothermophilus* biological indicators, surface swabs, contact plates and particle counts
- Facility repopulated and production reinstated



R&D laboratories

Developing and maintaining a strong product pipeline is a key factor for success in the pharmaceutical industry and efficient R&D is vital for the drug discovery process.

R&D laboratories can become contaminated with microbes and consequently require bio-decontamination services. R&D can involve working with hazardous pathogens in high containment level laboratories, which require regular bio-decontamination. Thus, microbial contamination of a laboratory can cause serious problem by distorting results and contaminating stocks or presenting a hazard to laboratory workers.

R&D laboratory case study

- 350m³ containment Level 3 (CL3) R&D suite in a multinational pharmaceutical company
- Certified, verified, bi-annual bio-decontamination required
- Formaldehyde fumigation viewed as too slow with unacceptable health and safety issues
- RBDS deployed with multiple cycles to reduce total downtime
- Certified, verified, optimised cycle completed within 4 hours



Cleanroom case study



- 250m³ cleanroom in a multi-national media preparation company
- Cleanroom re-furbished and rapid, flexible bio-decontamination service required
- Fully verified service required
- RBDS chosen over competitive technologies
- Bio-decontamination completed in a single 8 hour shift

Information supplied with kind permission of Eli Lilly and Company, Indianapolis, Indiana.

6. Summary

Hydrogen peroxide vapour has been shown to bio-deactivate a wide range of micro-organisms including bacteria, viruses and fungi. For example:

- Endospore-forming bacteria, including;
 - *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*)
 - *Bacillus atrophaeus* (formerly *Bacillus subtilis* var. *niger*)
- Vegetative bacteria
- Atypical bacteria (i.e. Mycoplasma)
- Viruses
 - Bacteriophage
- Fungi (e.g. *Penicillium* sp).

Further studies are necessary to extended the knowledge base for efficacy against viruses, and more unusual micro-organisms such as Cryptosporidium and intestinal nematodes. Efficacy testing remains an on-going process and continues to yield positive results – for example, one recent study suggested that HPV can be used to decontaminate prion-affected medical devices (Fichet *et al.* 2005). Further studies are currently underway to test the efficacy of HPV against *Mycobacterium tuberculosis* and various non-enveloped viruses. In conclusion, the biological efficacy of HPV is wide-ranging and, on the whole, well tested.

HPV technology has proven biological efficacy and excellent material compatibility. BIOQUELL's infinitely scaleable HPV technology achieves a 6-log reduction in bio-burden, is residue free and has excellent material compatibility. Fast, effective, affordable and safe, BIOQUELL's HPV technology has a proven track record in providing the optimum bio-decontamination solutions in the biopharmaceutical and pharmaceutical sectors and therefore this technology is rapidly becoming the gold standard of bio-decontamination.

7. References

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Company Description

BIOQUELL supplies consultancy, validation, equipment and services to premier bio-pharmaceutical and pharmaceutical companies worldwide. BIOQUELL has been in existence for over a century specialising in the design, manufacture and application of complete solutions for decontamination, control of airborne and surface contamination in cGXP environments. BIOQUELL CLARUS® equipment effectively kills micro-organisms using hydrogen peroxide technology whilst avoiding material compatibility issues. The technology achieves a certified, sterile and residue free environment for immediate occupation in minimal downtime.

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