Hydrogen peroxide vapour (HPV) biological efficacy

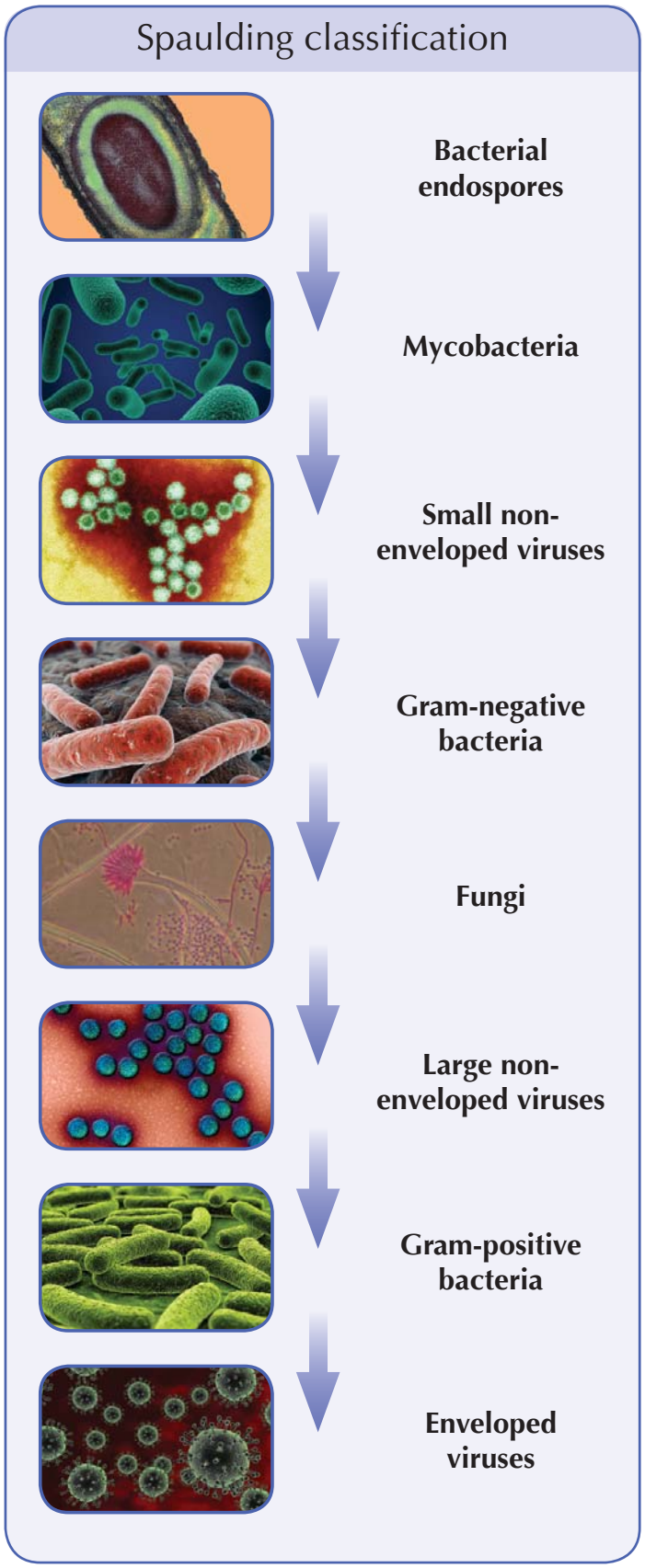

Figure 1. E.H. Spauling classification

Bioquell's hydrogen peroxide vapour (HPV) is well established as a bio-decontamination agent due to its broad spectrum efficacy and its ability to inactivate rapidly the most resilient microorganisms. The residue-free nature of HPV (breaks down to oxygen and water vapour) and low temperature, vapour-phase application increases the practicality of the process. Bioquell's HPV technology has been tested against many organisms and classes of organisms. However, because a great number of 'common' microorganisms exist, efficacy testing remains an ongoing process.

This document outlines the most significant current knowledge that can be attributed to qualified sources. This information can be used not only to look at specific organisms but also the efficacy of HPV against types and groups of organisms.

Figure 1 shows a widely accepted classification of the resistance of various microorganisms to sterilisation and disinfection procedures based on the pioneering work of E.H. Spaulding[1]. This classification can be used as a guide when forming a hypothesis about the efficacy of Bioquell HPV against a particular microorganism.

If a particular organism is not listed here, it does not mean there is no data available or that Bioquell HPV is not effective against it. Therefore, if a specific organism, which is of particular importance is not listed within this document, please contact Bioquell to see if other data (analogous or specific) is available - or if further testing is required.

Bioquell's HPV has been shown to kill a wide range of microorganisms including bacteria, viruses and fungi. The efficacy of HPV has been repeatedly demonstrated against bacterial endospores, which are the most resistant organisms commonly found on environmental surfaces, so are positioned at the top of the Spaulding classification. The organisms listed in this document are divided into broad taxonomic categories (i.e. bacteria, viruses and fungi) and grouped according to their microbiological characteristics. This division allows for an easy comparison of an untested organism with other related organisms that have been tested. The appendix includes the abstracts for the published journal articles.

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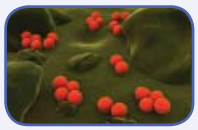
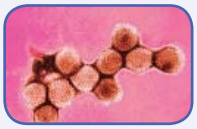
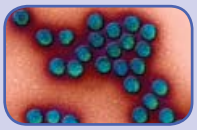
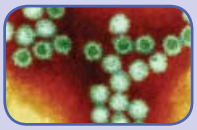
Contents

1. List of tested organisms and source references

1.1 Bacteria and bacterial endospores

Type of organism	Name of organism	Reference
Bacterial endospores **Gram +ve rods** 	*Bacillus anthracis* *Bacillus cereus* *Bacillus circulans* *Bacillus firmus* *Bacillus megaterium* *Bacillus pumilus* *Bacillus subtilis* *Clostridium botulinum* *Clostridium difficile* *Clostridium piliforme* *Clostridium sporogenes* *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*)	(2;3) (4;5) (4) (4) (4) (4;6) (2;4;6-8) (9) (10;11) (12) (6-8) (2;6;7;9;13;14)
Gram +ve rods 	*Mycobacterium smegmatis* *Mycobacterium tuberculosis* *Lactobacillus caesei* *Listeria monocytogenes*	(6) (13) (6) (5)
Gram +ve cocci 	*Enterococcus faecium/faecalis* (inc.VRE) *Staphylococcus aureus* (inc.MRSA) *Staphylococcus epidermidis*	(6;11;15) (11;14-17) (18;19)
Enterobacteriaceae (Enteric Gram–ve rods) 	*Enterobacter cloacae* *Escherichia coli* (inc. O157:H7) *Klebsiella pneumoniae* *Salmonella choleraesuis* *Serratia marcescens* *Yersinia pestis*	(20) (5) (5;11) (5) (6;40) (21)
Gram -ve rods 	*Acinetobacter* sp. *(inc. A. baumannii)* *Legionella* sp. *Pseudomonas aeruginosa*	(11;15;20) (5) (6;7)
Atypical bacteria 	*Acholeplasma laidlawii* (Mycoplasma)	(22)

1.2 Viruses

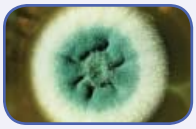
Genome	D/RNA[a]	Family	Name	Reference
DNA (Enveloped) 	Double	Herpesviridae	Herpes Simplex Type 1 Pseudorabies Virus	(23) (24)
	Double	Iridoviridae	African Swine Fever Virus	(24)
	Double	Poxviridae[b]	Vaccinia	(23)
DNA (Non-enveloped) 	Double	Adenoviridae	Adenovirus Canine Adenovirus	(25) (26)
	Single	Parvoviridae	Canine Parvovirus Feline Parvovirus Parvovirus	(26) (26) (27)
RNA (Enveloped) 	Single	Orthomyxoviridae	Influenza A2 Avian Influenza Virus Influenza A (H1N1)	(23) (24) (28)
	Single	Paramyxoviridae	Newcastle Disease Virus	(24;26)
	Single	Rhabdoviridae	Vesicular Stomatitis Virus	(24)
	Single	Flaviviridae	Dengue Virus Hog Cholera Virus	(29) (24)
	Single	Arenaviridae	Lassa Virus	(30)
RNA (Non-enveloped) 	Single	Caliciviridae	Feline Calicivirus Vesicular Exanthema Virus	(31,32) (24)
	Single	Picornaviridae	Rhinovirus 14 Poliovirus Type 1 Swine Vesicular Disease	(23) (23) (23)
	Double	Reoviridae	Bluetongue Virus Avian Reovirus	(24) (26)

a. single = single stranded genome, double = double stranded genome
b. some members of the Poxviridae are non-enveloped

1.3 Viruses continued...(Bacteriophage)

	Name	Reference
	Lactococcal bacteriophage MS2 bacteriophage	(33) (34)

1.4 Fungi

	Name	Reference
 	*Alternaria* sp. *Aspergillus niger* *Candida albicans* *Candida parapsilosis* *Coccidioides immitis* *Blastomyces dermatitidis* *Histoplasma capsulatum* *Penicillium* sp.	(35) (6;7) (19) (6) (36) (36) (36) (35)

1.5 Nematodes and protozoa

	Name	Reference
	*Caenorhabditis elegans* *Syphacia muris**	(37) (38)

* The reference cited is a poster describing a study investigating the efficacy of a HPV systems against *Syphacia muris* (pinworm) eggs. Microscopic destruction was noted on immature, but not on mature eggs. SCID (immuno-deficient) mice exposed to HPV-treated contaminated bedding did not develope pinworm infection whereas mice exposed to non-HPV-treated contaminated bedding did develop pinworm infection. Recent experiments by Bioquell have demonstrated that HPV-treated eggs are able to hatch in a specially formulated hatching medium, so it is possible that exposure to HPV, whilst not preventing *in vitro* hatching, renders pinworm eggs non-infective *in vivo*. Further research is required in this area.

The efficacy of hydrogen peroxide both in liquid and vapour form have been shown against other protozoa including organisms in the genera *Metadinium*, *Eimeria*, *Acanthamoeba*, *Ichthyobodo* and *Cryptosporidium*. For further information, please refer to the efficacy of hydrogen peroxide against protozoa and nematodes document available from Bioquell.

2. Appendix - abstracts / summaries in alphabetical order.

This section includes abstracts from published journal articles. Please contact Bioquell for full copies of these articles and for copies of the other documents that are referenced in this document.

Bates CJ, Pearse R. Use of hydrogen peroxide vapour for environmental control during a *Serratia* outbreak in a neonatal intensive care unit. *J Hosp Infect* **2005;61:364-366.**
Royal Hallamshire Hospital, Sheffield, UK.

The use of hydrogen peroxide vapour (HPV) for environmental control of nosocomial pathogens is receiving much attention. We describe the use of the Bioquell HPV system, combined with other infection control measures, to eradicate *Serratia marcescens* from the neonatal intensive care unit (NICU) at our hospital.

Bentley K, Dove BK, Parks SR, Walker JT, Bennett AM. Hydrogen peroxide vapour decontamination of surfaces artificially contaminated with norovirus surrogate feline calicivirus. *J Hosp Infect* **2012;80:116-21.**
Microbiology Services Division, Health Protection Agency, Porton Down, Salisbury, UK.

BACKGROUND: Noroviruses are a leading cause of gastrointestinal disease and are of particular concern in healthcare settings such as hospitals. As the virus is reported to be environmentally stable, effective decontamination following an outbreak is required to prevent recurrent outbreaks.

AIM: To investigate the use of hydrogen peroxide vapour to decontaminate a number of surfaces that had been artificially contaminated with feline calicivirus (FCV), a surrogate for norovirus. The surfaces tested were representative of those found in hospital wards.

METHODS: FCV was used to contaminate materials representative of a hospital setting (stainless steel, glass, vinyl flooring, ceramic tile and PVC plastic cornering). The carriers were exposed to 30% (w/w) hydrogen peroxide vapour at 5 min intervals over 20 min, after which post-exposure viral titres were measured.

FINDINGS: Hydrogen peroxide vapour reduced the viral titre by 4-$\log_{10}$ on all surfaces tested within 20 min of exposure. The reduction in viral titre took longest to achieve on stainless steel (20 min), and the quickest effect was seen on vinyl flooring (10 min). For glass, plastic and ceramic tile surfaces, the desired reduction in viral titre was seen within 15 min of exposure. Hydrogen peroxide vapour allows for large-scale decontamination of areas following outbreaks of infectious organisms.

CONCLUSION: Hydrogen peroxide vapour is effective against FCV and is active on a range of surfaces. Therefore, it may represent a suitable decontamination system for use following a hospital outbreak of norovirus.

Berrie E, Andrews L, Yezli S, Otter JA. Hydrogen peroxide vapour (HPV) inactivation of adenovirus. *Lett Appl Microbiol* **2011;52(5):555-558.**
Clinical BioManufacturing Facility, University of Oxford, Oxford, UK.

AIMS: Adenovirus contamination can be problematic in various settings including life science laboratories and during pharmaceutical manufacturing processes. Stringent and effective decontamination procedures are necessary to minimise the risk of personnel exposure or product cross contamination in these settings. Hydrogen peroxide vapour (HPV) is sporicidal, tuberculocidal and fungicidal with proven efficacy against some viruses. We investigate the efficacy of HPV for the inactivation of a recombinant adenovirus.

METHODS AND RESULTS: In this study, the survival of a dried recombinant adenovirus (Ad5GFP) was tested before and after HPV exposure to determine the efficacy of HPV at inactivating adenovirus. A >8-$\log_{10}$ $TCID_{50}$ reduction resulted from 45 min exposure to HPV in a microbiological safety cabinet.

CONCLUSIONS: HPV is effective for the inactivation of a recombinant adenovirus.

SIGNIFICANCE AND IMPACT OF THE STUDY: The results suggest that HPV may be useful for adenovirus decontamination in life science laboratories or in manufacturing facilities.

Boyce JM, Havill NL, Otter JA, McDonald LC, Adams NM, Thompson A, Wiggs L, Noble-Wang J. Impact of hydrogen peroxide vapor room bio-decontamination on environmental contamination and nosocomial transmission of *Clostridium difficile*. *Infect Cont Hosp Epidemiol* **2008;29:723-729.**
Hospital of St. Raphael, New Haven, CT, USA / Yale University School of Medicine / CDC / Bioquell.

OBJECTIVE: To determine whether hydrogen peroxide vapor (HPV) decontamination can reduce environmental contamination with and nosocomial transmission of *Clostridium difficile*.

DESIGN: A prospective before/after intervention study.

SETTING: A hospital affected by an epidemic strain of *C. difficile*.

INTERVENTION: Intensive HPV decontamination of 5 high-incidence wards followed by hospital-wide decontamination of rooms vacated by patients with *C. difficile*-associated disease (CDAD). The pre-intervention period was June 2004 through March 2005, and the intervention period was June 2005 to March 2006.

RESULTS: Eleven (25.6%) of 43 cultures of samples collected by sponge from surfaces before HPV decontamination yielded *C. difficile*, compared with 0 of 37 cultures of samples obtained after HPV decontamination (P < .001). On 5 high-incidence wards, the incidence of nosocomial CDAD was significantly lower during the intervention period than during the pre-intervention period (1.28 vs 2.28 cases per 1,000 patient-days; P = .047). The hospital-wide CDAD incidence was lower during the intervention period than during the pre-intervention period (0.84 vs 1.36 cases per 1,000 patient-days; P = .26). In an analysis limited to months in which the epidemic strain was present during both the pre-intervention and the intervention periods, CDAD incidence was significantly lower during the intervention period than during the pre-intervention period (0.88 vs 1.89 cases per 1,000 patient-days; P = .047).

CONCLUSIONS: HPV decontamination was efficacious in eradicating *C. difficile* from contaminated surfaces. Further studies of the impact of HPV decontamination on nosocomial transmission of *C. difficile* are warranted.

Dryden M, Parnaby R, Dailly S, Lewis T, Davis-Blues K, Otter JA, Kearns AM. Hydrogen peroxide vapor (HPV) decontamination in the control of a polyclonal MRSA outbreak on a surgical ward. / *Hosp Infect* **2008;68:190-192.**
Royal Hampshire Country Hospital, Winchester, UK / Bioquell / Health Protection Agency, UK.

We experienced a polyclonal outbreak of meticillin-resistant *Staphylococcus aureus* (MRSA) and reported the findings of our outbreak investigation.

Fichet G, Antloga K, Comoy E, Deslys JP, McDonnell G. Prion inactivation using a new gaseous hydrogen peroxide sterilisation process. *J Hosp Infect* **2007;67:278-86.**
CEA/DSV/DRM/GIDTIP, France / STERIS.

Prions pose a challenge to decontamination, particularly before the re-use of surgical instruments. They have relatively high resistance to standard decontamination methods and require extreme chemical and/or heat-based treatments for devices used in known or suspected cases of disease. This study investigated the effectiveness of a new gaseous hydrogen peroxide sterilisation process for prions as an alternative low-temperature method. Gaseous peroxide, in addition to known antimicrobial efficacy, was shown to inactivate prions both in *in-vitro* and *in-vivo* assays. In contrast to the gas form, liquid peroxide was not effective. The mechanism of action of gaseous peroxide suggested protein unfolding, some protein fragmentation and higher sensitivity to proteolytic digestion. Hydrogen peroxide liquid showed a degree of protein clumping and full resistance to protease degradation. The use of gaseous peroxide in a standard low-temperature sterilisation process may present a useful method for prion inactivation.

French GL, Otter JA, Shannon KP, Adams NMT, Parks MJ, Watling D. Tacking contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination.
J Hosp Infect **2004;57:31-37.** St Thomas' Hospital / King's College London / Bioquell.

The hospital environment can sometimes harbour meticillin-resistant *Staphylococcus aureus* (MRSA) but is not generally regarded as a major source of MRSA infection. We conducted a prospective study in surgical wards of a London teaching hospital affected by MRSA, and compared the effectiveness of standard cleaning with a new method of hydrogen peroxide vapour decontamination. MRSA contamination, measured by surface swabbing was compared before and after terminal cleaning that complied with UK national standards, or hydrogen peroxide vapour decontamination. All isolation rooms, ward bays and bathrooms tested were contaminated with MRSA and several antibiogram types were identified. MRSA was common in sites that might transfer organisms to the hands of staff and was isolated from areas and bed frames used by non-MRSA patients. 74% percent of 359 swabs taken before cleaning yielded MRSA, 70% by direct plating. After cleaning, all areas remained contaminated, with 66% of 124 swabs yielding MRSA, 74% by direct plating. In contrast, after exposing six rooms to hydrogen peroxide vapour, only one of 85 (1.2%) swabs yielded MRSA, by enrichment culture only. The hospital environment can become extensively contaminated with MRSA that is not eliminated by standard cleaning methods. In contrast, hydrogen peroxide vapour decontamination is a highly effective method of eradicating MRSA from rooms, furniture and equipment. Further work is needed to determine the importance of environmental contamination with MRSA and the effect on hospital infection rates of effective decontamination.

Hall L, Otter JA, Chewins J, Wengenack NL. Use of hydrogen peroxide vapour for deactivation of *Mycobacterium tuberculosis* in a biological safety cabinet and a room.
J Clin Microbiol **2007;45:810-815.**
Mayo Clinic, Rochester, MN, USA / Bioquell.

Mycobacterium tuberculosis is an important human pathogen that is routinely cultured in clinical and research laboratories. *M. tuberculosis* can contaminate surfaces and is highly resistant to disinfection. We investigated whether hydrogen peroxide vapor (HPV) is effective for the deactivation of *M. tuberculosis* on experimentally contaminated surfaces in a biological safety cabinet (BSC) and a room. Biological indicators (BIs) consisting of an approximately 3-$\log_{10}$ inoculum of *M. tuberculosis* on stainless steel discs and a 6-$\log_{10}$ inoculum of *Geobacillus stearothermophilus* were exposed to HPV in BSC time course experiments and at 10 locations during room experiments. In three separate BSC experiments, *M. tuberculosis* BIs were transferred to growth media at 15 min intervals during a 180 min HPV exposure period. No *M. tuberculosis* BIs grew following 30 min of HPV exposure. In three separate room experiments, *M. tuberculosis* and *G. stearothermophilus* BIs were exposed to HPV for 90, 120, and 150 min, respectively. BIs for both microorganisms were deactivated in all 10 locations following 90 min of HPV exposure. HPV provides an alternative to traditional decontamination methods, such as formaldehyde fumigation, for laboratories and other areas contaminated with *M. tuberculosis*.

Hall L, Otter JA, Chewins J, Wengenack NL. Deactivation of the dimorphic *fungi Histoplasma capsulatum, Blastomyces dermatitidis and Coccidioides immitis* using hydrogen peroxide vapor. *Med Mycol* **2008;46:189-191.**
Mayo Clinic, Rochester, MN, USA / Bioquell.

Hydrogen peroxide vapor (HPV) has been proposed as an alternative to formaldehyde fumigation for the decontamination of biosafety level (BSL) III laboratories. The aim of this study was to evaluate the efficacy of HPV against the dimorphic fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Coccidioides immitis*. Working inside a class II biological safety cabinet (BSC) within a BSL III laboratory, inocula containing approximately 5-$\log_{10}$ CFU/ml from the mold form of each organism suspended in RPMI medium were deposited on stainless steel discs and allowed to air dry. The organisms were exposed to HPV inside a BSC using a Bioquell Clarus S HPV generator. In three replicate experiments, individual discs were transferred into liquid media at timed intervals during a 105 min HPV exposure period. Control and HPV exposed discs were incubated in RPMI media at 30ºC for 6 weeks to determine if any viable organisms remained. Positive cultures were confirmed using specific nucleic acid hybridization probes. Results indicate that *H. capsulatum*, *B. dermatitidis* and *C. immitis* were killed within 30 min of HPV exposure.

Heckert RA, Best M, Jordan LT, Dulas GC, Eddington DL, Sterritt WG. Efficacy of vaporized hydrogen peroxide against exotic animal viruses. *Appl Environ Microbiol* **1997;63:3916-3918.**
Animal Diseases Research Institute, Canadian Food Inspection Agency, Ontario, Canada.

The efficacy of vapor-phase hydrogen peroxide in a pass-through box for the decontamination of equipment and inanimate materials potentially contaminated with exotic animal viruses was evaluated. Tests were conducted with a variety of viral agents, which included representatives of several virus families (Orthomyxoviridae, Reoviridae, Flaviviridae, Paramyxoviridae, Herpesviridae, Picornaviridae, Caliciviridae and Rhabdoviridae) from both avian and mammalian species, with particular emphasis on animal viruses exotic to Canada. The effects of the gas on a variety of laboratory equipment were also studied. Virus suspensions in cell culture media, egg fluid, or blood were dried onto glass and stainless steel. Virus viability was assessed after exposure to vapor-phase hydrogen peroxide for 30 min. For all viruses tested and under all conditions (except one), the decontamination process reduced the virus titer to zero embryo-lethal doses for the avian viruses (avian influenza and Newcastle disease viruses) or less than 10 tissue culture infective doses for the mammalian viruses (African swine fever, bluetongue, hog cholera, pseudorabies, swine vesicular disease, vesicular exanthema, and vesicular stomatitis viruses). The laboratory equipment exposed to the gas appeared to suffer no adverse effects. Vapor-phase hydrogen peroxide decontamination can be recommended as a safe and efficacious way of removing potentially virus-contaminated objects from biocontainment level III laboratories in which exotic animal disease virus agents are handled.

Jeanes A, Rao G, Osman M, Merrick P. Successful eradication of persistent environmental MRSA. *J Hosp Infect* **2005;61:85-86.**
University Hospital Lewisham, London, UK.

Clinical areas used to care for patients infected or colonised with methicillin-resistant *Staphylococcus aureus* (MRSA) become contaminated, and there is evidence that conventional cleaning methods do not eradicate MRSA. However, environmental hygiene is important for the control of MRSA and other nosocomial pathogens. Here we describe the use of hydrogen peroxide vapour (HPV) decontamination to eradicate MRSA environmental contamination following admissions of MRSA patients and subsequent cross-infection in a surgical ward.

Johnston MD, Lawson S, Otter JA. Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with *Clostridium botulinum* spores. *J Microbiol Methods* **2005;60:403-411.**
Unilever / Bioquell.

The aim of this study was to evaluate the efficacy of hydrogen peroxide vapour (HPV) against spores of *Clostridium botulinum*, for use as a method for decontaminating environments where this pathogen has been handled. Spores were dried onto stainless steel slides and exposed to HPV in a sealed glovebox enclosure, transferred to a quenching agent at timed intervals during the exposure period, before survivors were cultured and enumerated. D-values were calculated from graphs of $\log_{10}$ survivors plotted against time and were found to range from 1.41 to 4.38 min. HPV was found to be effective at deactivating spores of toxigenic *C. botulinum*, non-toxigenic *Clostridium* spp. and *Geobacillus stearothermophilus* dried onto stainless steel surfaces. HPV could be used to decontaminate cabinets and rooms where *C. botulinum* has been handled. The cycle parameters should be based on studies carried out with relevant spores of this organism, rather than based on inactivation data for *G. stearothermophilus* spores, which have been used in the past as a standard biological challenge for disinfection and sterilisation procedures. HPV could provide an attractive alternative to other decontamination methods, as it was rapid, residue-free and did not give rise to the health and safety concerns associated with other gaseous decontamination systems.

Kokubo M, Inoue T, Akers J. Resistance of common environmental spores of the genus *Bacillus* to vapor hydrogen peroxide. *PDA J Pharm Sci Technol* **1998;52:228-231.**
Shibuya Kogyo Company LTD / Process Engineering Organization Kanazawa, Japan.

The use of hydrogen peroxide as an antimicrobial agent has a long history in infection control and contamination prevention. It has long been known that hydrogen peroxide can efficiently and rapidly destroy even highly resistant bacterial spores. In recent years, vapor hydrogen peroxide, commonly called VHP, has come into wide use as a decontaminating or sterilizing agent in the pharmaceutical industry. The most commonly used biological indicator (BI) for VHP sterilization has been *B. stearothermophilus* ATCC #12980. Published studies have indicated that *B. stearothermophilus* is the most resistant organism to VHP. At present, several types of commercial BIs designed specifically for the evaluation of VHP processes are available from vendors. BIs for VHP can be purchased as enveloped packages on various substrates, and as suspension cultures for inoculation onto a carrier or substrate of the user's choice. The purpose of this article is to evaluate and compare the resistance of environmental isolates of wild type organisms of the genus *Bacillus* to that of commercially available BIs. Significantly, when a typical spore suspension of *B. stearothermophilus* ATCC #12980 marketed for use in validating VHP processes was tested under identical conditions and on the same substrate its D value was found to exceed that of the most resistant wild type of our 'bioburden'

organism tested by more than a factor of 10.

McDonnell G, Grignol G, Antloga K. Vapour-phase hydrogen peroxide decontamination of food contact surfaces. *Dairy Food Environ Sanitat* **2002;22:868-873.** STERIS, USA.

Decontamination of food contact surfaces, equipment and general work areas is important for the prevention of transmission of food borne microorganisms. Many liquid-based disinfectants that are widely used for this purpose may not be appropriate for electrical equipment and for relatively large areas. Fumigation with vapour phase hydrogen peroxide (VPHP) is an option in these cases and is discussed in this report. VPHP is a dry and rapidly effective antimicrobial vapour. A typical decontamination cycle consists of four phases in a one-step process that is documented and can be validated for a given application. VPHP has been shown to have potent antimicrobial activity against bacteria, viruses, fungi and bacterial spores. Recently, efficacy has been confirmed against known food borne pathogens, including *Listeria monocytogenes* and *E. coli* O157:H7. Because the VPHP process is dry, it is compatible with many materials, including electronics. In the case study presented, VPHP was shown to be effective in decontaminating a simulative room, including an electrical appliance, in an automated, validated process. VPHP is a possible alternative to liquid-based disinfectants for decontamination of food contact surfaces and equipment.

Otter JA, Budde-Niekiel. Hydrogen peroxide vapour: a novel method for the environmental control of *lactococcal* bacteriophage. *J Food Protect* **2009;72(2):412-4.**
Danisco Deutschland / Bioquell.

Bacteriophage contamination can be problematic, especially in industrial settings. We examined the *in vitro* efficacy of hydrogen peroxide vapour (HPV) for the inactivation of two *lactococcal* bacteriophages dried onto stainless steel discs. A >6-$\log_{10}$ reduction was achieved on both bacteriophages compared with unexposed controls by 50 min HPV exposure in an isolator. HPV may be useful for the environmental control of bacteriophages.

Otter JA, Chewins C, Windsor D, Windsor H. Microbial contamination in cell culture: a potential role for hydrogen peroxide vapour (HPV)? *Cell Biol Int* **2008;32:326-327.**
Mycoplasma Experience, Reigate, Surrey, UK / Bioquell.

Cobo *et al*. highlight the problems caused by microbial contamination in stem cell culture. One of the most common cell culture contaminants identified in their stem cell bank was *Mycoplasma* spp., which remains the single most common cell culture contaminant. Cobo *et al*. (Cell Biol Int 2007;31:991-995) identify the laboratory environment as one of the possible sources of cell culture contamination and other studies have demonstrated indirect transmission of *Mycoplasma* spp. cell culture contamination via contaminated work areas. Thus effective environmental decontamination is good working practice for the prevention of cell culture contamination; indeed Cobo *et al*. attribute their relatively low 12% rate of cell culture contamination to their strict rules of good laboratory practice and recently implemented environmental monitoring program. Hydrogen peroxide vapour (HPV) is a sporicidal vapour-phase method for the decontamination of biological safety cabinets (BSCs), laboratories and other enclosures used increasingly in healthcare, laboratory and pharmaceutical applications. We investigated the *in vitro* efficacy of HPV for the inactivation of *Mycoplasmas*, used here to encompass *Mycoplasma* and *Acholeplasma* species, dried onto surfaces to simulate a liquid spillage.

Otter JA, Cummin M, Ahmad F, van Tonder C, Drabu YJ. Assessing the biological efficacy and rate of recontamination following hydrogen peroxide vapour decontamination. *J Hosp Infect* **2007;67:182-188.**
North Middlesex University Hospital, London, UK / Bioquell.

The inanimate hospital environment can become contaminated with nosocomial pathogens. Hydrogen peroxide vapour (HPV) decontamination has proven effective for the eradication of persistent environmental contamination. We investigated the extent of meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and gentamicin-resistant Gram-negative rod (GNR) contamination in a ward side-room occupied by a patient with a history of MRSA, VRE and GNR infection and colonisation and investigated the impact of HPV decontamination. Fifteen standardised sites in the room were sampled using a selective broth enrichment protocol to culture MRSA, VRE and GNR. Sampling was performed before cleaning, after cleaning, after HPV decontamination and at intervals over the subsequent 19 days on two separate occasions. Environmental contamination was identified before cleaning on 60, 30 and 6.7% of sites for MRSA, GNR and VRE, respectively, and 40, 10 and 6.7% of sites after cleaning. Only one site (3.3%) was contaminated with MRSA after HPV decontamination. No recontamination with VRE was identified and no recontamination with MRSA and GNR was identified during the two days following HPV decontamination. Substantial recontamination was identified approximately one week after HPV decontamination towards post-cleaning levels for GNR and towards pre-cleaning levels for MRSA. HPV is more effective than standard terminal cleaning for the eradication of nosocomial pathogens. Recontamination was not immediate for MRSA and GNR but contamination returned within a week in a room occupied by a patient colonised with MRSA and GNR. This finding has important implications for the optimal deployment of HPV decontamination in hospitals.

Otter JA, French GL. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapour (HPV). *J Clin Microbiol* **2009;47:205-207.**
St. Thomas' Hospital / King's College London / Bioquell.

With inocula of 6 to 7-$\log_{10}$ CFU, most vegetative bacteria and spores tested survived on surfaces for more than 5 weeks, but all were inactivated within 90 min of exposure to hydrogen peroxide vapor in a 100m^3 test room even in the presence of 0.3% bovine serum albumin to simulate biological soiling.

Otter JA, Yezli S, Schouten MA, van Zanten AR, Houmes-Zielman G, Nohlmans-Paulssen MK. Hydrogen peroxide vapor decontamination of an intensive care unit to remove environmental reservoirs of multidrug-resistant gram-negative rods during an outbreak. *Am J Infect Control* **2010;38(9):754-756.**
Bioquell (UK) Ltd., 52 Royce Close, West Portway, Andover, UK.

Multidrug-resistant gram-negative rods (MDR-GNR) are an increasing cause for concern in intensive care units (ICUs). We used hydrogen peroxide vapor (HPV) to decontaminate our entire ICU in an attempt to eradicate undetected environmental contamination during outbreaks of MDR-GNR. Surface sampling identified GNR, including MDR strains, on 10 (48%) of 21 areas cultured after intensive cleaning but before decontamination with HPV, and on no areas after HPV. No new cases of *Acinetobacter* were identified for approximately 3 months after HPV.

Pottage, T., C. Richardson, S. Parks, J. T. Walker, and A. M. Bennett. Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses. *J Hosp Infect* **2010;74:55-61.**
Biosafety Group, Novel and Dangerous Pathogens, Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury SP4 0JG, UK.

This study assessed the efficacy of two commonly used gaseous disinfection systems against high concentrations of a resistant viral surrogate in the presence and absence of soiling. MS2 bacteriophage suspensions were dried on to stainless steel carriers and exposed to hydrogen peroxide vapour (HPV) and vapour hydrogen peroxide (VHP) gaseous disinfection systems. The bacteriophages were also suspended and dried in 10% and 50% of horse blood to simulate the virus being present in a spill of blood/bodily fluids in a hospital ward environment. Carriers were removed from the gaseous disinfectant at regular intervals into phosphate-buffered saline, vortexed and assayed using a standard plaque assay. The effectiveness of both the HPV and VHP systems varied with the concentration of the bacteriophage with HPV resulting in a 6-$\log_{10}$ reduction in 10 min at the lowest viral concentration (10^7 plaque-forming units (pfu)/carrier) and requiring 45 min at the highest concentration (10^9 pfu/carrier). For the VHP system a 30 min exposure period was required to achieve a 6-$\log_{10}$ reduction at the lowest concentration and 60-90 min for the highest concentration. The addition of blood to the suspension greatly reduced the effectiveness of both disinfectants. This study demonstrates that the effectiveness of gaseous disinfectants against bacteriophage is a function of the viral concentration as well as the degree of soiling. It highlights the importance of effective cleaning prior to gaseous disinfection especially where high concentration agents are suspended in body fluids to ensure effective decontamination in hospitals.

Rogers JV, Richter WR, Shaw MQ, Choi YW. Vapour-phase hydrogen peroxide inactivated *Yersinia pestis* dried on polymers, steel, and glass surfaces. *Lett Appl Microbiol* **2008;47:279-285.**
Battelle Memorial Institute, Columbus, OH, USA.

AIMS: This study evaluated the inactivation of virulent *Yersinia pestis* dried on polymers, steel, and glass surfaces using vapour-phase hydrogen peroxide.

METHODS AND RESULTS: A suspension of *Y. pestis* CO92 (1.70 x 10^8 CFU) was dried on 10 different types of test surfaces and exposed to vapour-phase hydrogen peroxide fumigation for a contact time of 2 hrs. A significant reduction in the $\log_{10}$ CFU of *Y. pestis* on all 10 materials was observed between the controls evaluated after a 1 hr drying time and unexposed controls evaluated after the decontamination run. Qualitative growth assessment showed that vapour-phase hydrogen peroxide exposure inactivated *Y. pestis* on all replicates of the 10 test materials as well as biological indicators up to seven days post exposure.

CONCLUSIONS: Virulent *Y. pestis* CO92 is inactivated on polymers, steel and glass surfaces when exposed to vapour-phase hydrogen peroxide without observable physical damage to the test materials.

SIGNIFICANCE AND IMPACT OF THE STUDY: This study provides information for using vapour-phase hydrogen peroxide as a practical process for the decontamination of virulent *Y. pestis* in circumstances where time-dependent attenuation/inactivation or liquid/heat decontamination may not be the most suitable approach.

Rogers JV, Sabourin CL, Choi YW, Richter WR, Rudnicki DC, Riggs KB, Taylor ML, Chang J. Decontamination assessment of *Bacillus anthracis, Bacillus subtilis, and Geobacillus stearothermophilus* on indoor surfaces using a hydrogen peroxide gas generator. *J Appl Microbiol* **2005;99:739-748.**
Battelle Memorial Institute, Columbus, OH, USA.

AIMS: To evaluate the decontamination of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surface materials using hydrogen peroxide gas.

METHODS AND RESULTS: *Bacillus anthracis*, *B. subtilis*, and *G. stearothermophilus* spores were dried on seven types of indoor surfaces and exposed to > or =1000 ppm hydrogen peroxide gas for 20 min. Hydrogen peroxide exposure significantly decreased viable *B. anthracis*, *B. subtilis*, and *G. stearothermophilus* spores on all test materials except *G. stearothermophilus* on industrial carpet. Significant differences were observed when comparing the reduction in viable spores of *B. anthracis* with both surrogates. The effectiveness of gaseous hydrogen peroxide on the growth of biological indicators and spore strips was evaluated in parallel as a qualitative assessment of decontamination. At one and seven days post exposure, decontaminated biological indicators and spore strips exhibited no growth, while the non-decontaminated samples displayed growth.

CONCLUSIONS: Significant differences in decontamination efficacy of hydrogen peroxide gas on porous and non-porous surfaces were observed when comparing the mean log reduction in *B. anthracis* spores with *B. subtilis* and *G. stearothermophilus* spores.

SIGNIFICANCE AND IMPACT OF THE STUDY: These results provide comparative information for the decontamination of *B. anthracis* spores with surrogates on indoor surfaces using hydrogen peroxide gas.

Rudnick, S. N., J. J. McDevitt, M. W. First, and J. D. Spengler. Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations. *Am J Infect Control* **2009;37:813-819.**
Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA.

BACKGROUND: Surfaces in congregate settings, such as vehicles used for mass transportation, can become contaminated with infectious microorganisms and facilitate disease transmission. We disinfected surfaces contaminated with H1N1 influenza viruses using hydrogen peroxide (HP) vapor at concentrations below 100 ppm and triethylene glycol (TEG)-saturated air containing 2 ppm of TEG at 25°C.

METHODS: Influenza viruses in aqueous suspensions were deposited on stainless steel coupons, allowed to dry at ambient conditions, and then exposed for up to 15 min to 10 to 90 ppm of HP vapor or TEG-saturated air. Virus assays were done on the solution used to wash the viruses from these coupons and from coupons treated similarly but without exposure to HP or TEG vapor.

RESULTS: After 2.5 min, exposure to 10 ppm HP vapor resulted in 99% inactivation. For air saturated with TEG at 25 to 29°C, the disinfection rate was about 1.3-$\log_{10}$ reductions per hour, about 16 times faster than the measured natural inactivation rate under ambient conditions.

CONCLUSIONS: Vapor concentrations of 10 ppm HP or 2 ppm TEG can provide effective surface disinfection. At these low concentrations, the potential for damage to even the avionics of an airplane would be expected to be minimal. At a TEG vapor concentration of 2 ppm, there are essentially no health risks to people.

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