Bioquell’s Hydrogen Peroxide Vapor 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 Hydrogen Peroxide Vapor (breaks down to oxygen and water vapor) and low temperature, vapor-phase application increases the practicality of the process. Bioquell’s Hydrogen Peroxide Vapor 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 Hydrogen Peroxide Vapor against types and groups of organisms.

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

If a particular organism is not listed here, it does not mean there is no data available or that Bioquell Hydrogen Peroxide Vapor 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 Hydrogen Peroxide Vapor has been shown to kill a wide range of microorganisms including bacteria, viruses and fungi. The efficacy of Hydrogen Peroxide Vapor 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.
### Contents

1. **List of tested organisms and source references**
   1.1 Bacteria and bacterial endospores
   1.2 Viruses
   1.3 Viruses continued...(Bacteriophage)
   1.4 Fungi
   1.5 Nematodes and protozoa
   1.6 Other
2. **Appendix - abstracts / summaries in alphabetical order**
3. **References**

#### 1.1 Bacteria and bacterial endospores

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Name of organism</th>
<th>Reference</th>
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<tr>
<td>Bacterial endospores</td>
<td></td>
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</tr>
<tr>
<td>Gram-positive rods</td>
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<td></td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td></td>
<td>(2;3;46;48)</td>
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<tr>
<td>Bacillus cereus</td>
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<td>(5)</td>
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<td>Bacillus circulans</td>
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<td>(4)</td>
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<td>Bacillus firmus</td>
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<td>Bacillus megaterium</td>
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<td>Bacillus pumilus</td>
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<td>Bacillus subtilis</td>
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<td>(2;4;6-8;48)</td>
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<td>Bacillus thuringiensis</td>
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<td>Clostridium botulinum</td>
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<td>Clostridium difficile</td>
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<td>(10;11;39;40;42;52;55)</td>
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<td>Clostridium sporogenes</td>
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<td>Gram-positive rods</td>
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<td>Mycobacterium avium</td>
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<td>Mycobacterium smegmatis</td>
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<td>Mycobacterium terrae</td>
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<td>Mycobacterium tuberculosis</td>
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<td>Mycobacterium fortuitum</td>
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<td>Lactobacillus caesei</td>
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<td>Listeria monocytogenes</td>
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<td>Gram-positive cocci</td>
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<td></td>
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<td>Enterococcus faecium/faecalis</td>
<td>(inc. VRE)</td>
<td>(6;11;14;43;51)</td>
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<td>Enterococcus hirae</td>
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<td>Staphylococcus aureus</td>
<td>(inc. MRSA)</td>
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<td>Staphylococcus epidermidis</td>
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<td>Enterobacteriaceae (Enteric Gram-negative rods)</td>
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<td>Brucella suis</td>
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<td>Enterobacter cloacae</td>
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<td>Escherichia coli (inc. O157:H7)</td>
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<td>Francisella tularensis</td>
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<td>Klebsiella pneumoniae</td>
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<td>(5;11)</td>
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<td>Serratia marcescens</td>
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<td>Yersinia pestis</td>
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<td>(20;46)</td>
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<tr>
<td>Gram-negative rods</td>
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<td></td>
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<tr>
<td>Acinetobacter spp. (inc. A. baumannii)</td>
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<td>Legionella sp.</td>
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<td>Pseudomonas aeruginosa</td>
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<td>Atypical bacteria</td>
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<td>Acholeplasma laidlawii (Mycoplasma)</td>
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1.2 Viruses

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<th>Family</th>
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<tr>
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<td>Herpesviridae</td>
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<td>Double</td>
<td>Asfarviridae</td>
<td>African Swine Fever Virus</td>
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<td></td>
<td>Double</td>
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<td>Porcine Parvovirus</td>
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<td>Minute Virus of Mice (MVM)</td>
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<td>RNA (Enveloped)</td>
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<td>Orthomyxoviridae</td>
<td>Avian Influenza Virus</td>
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<td></td>
<td>Influenza A (H1N1)</td>
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<td></td>
<td>Single</td>
<td>Paramyxoviridae</td>
<td>Newcastle Disease Virus</td>
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<td>Rhabdoviridae</td>
<td>Vesicular Stomatitis Virus</td>
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<td>Flaviviridae</td>
<td>Dengue Virus</td>
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<td>Hog Cholera Virus</td>
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<td>Orthomyxoviridae</td>
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<td>Single</td>
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<td>Swine Influenza Virus (H3N2)</td>
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<td>Transmissible gastroenteritis coronavirus (MERS-CoV surrogate)</td>
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<td>RNA (Non-enveloped)</td>
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<td>Feline Calicivirus</td>
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<td>Single</td>
<td>Picornaviridae</td>
<td>Murine norovirus</td>
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<td>Vesicular Exanthema Virus</td>
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<td>Poliovirus Type 1</td>
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<td></td>
<td></td>
<td></td>
<td>Foot and Mouth Disease Virus</td>
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<td></td>
<td></td>
<td>Swine Vesicular Disease Virus</td>
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<tr>
<td></td>
<td>Double</td>
<td>Reoviridae</td>
<td>Bluetongue Virus</td>
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<sup>a. single = single stranded genome, double = double stranded genome </sup>

<sup>b. some members of the Poxviridae are non-enveloped</sup>

1.3 Viruses continued... (Bacteriophage)

<table>
<thead>
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<tr>
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<td>Lactococcal bacteriophage</td>
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<td>MS2 bacteriophage</td>
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1.4 Fungi

<table>
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<tr>
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<td>Alternaria sp.</td>
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<tr>
<td></td>
<td>Aspergillus brasiliensis</td>
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</tr>
<tr>
<td></td>
<td>(formerly Aspergillus niger)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Candida albicans</td>
<td>(7;47)</td>
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<tr>
<td></td>
<td>Candida parapsilosis</td>
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<td></td>
<td>Coccioidoides immitis</td>
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<tr>
<td></td>
<td>Blastomyces dermatitidis</td>
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<tr>
<td></td>
<td>Histoplasma capsulatum</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>(33)</td>
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</table>
Spores were dried onto PVC and laminate carriers at respectively. The carrier materials used, polyvinyl chloride (PVC) and laminate, decontaminate carriers inoculated with three strains of *C. difficile*.

**AIM**: To investigate the use of hydrogen peroxide vapour to contamination contributing to transmission between patient *C. difficile* spores are found in 12-26% of bleach-environment in high numbers by infected patients and are

**BACKGROUND**: *Clostridium difficile* are shed into the *Clostridium difficile*, Hôpital National Reference Laboratory for 2012;80:85-87 *Clostridium difficile* spores. *J Hosp Infect* Barbut F, Yezli S, Otter J. Activity *in vitro* of hydrogen peroxide *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 vapor form have been shown against other protozoa including organisms in the genera *Metazoa*, *Eimeria*, *Acanthamoeba*, *Ichthyobodo* and *Cryptosporidium*. For further information, please refer to the efficacy of hydrogen peroxide against protozoa and nematodes document (document number: CRF001-MKT-044) available from Bioquell.

**2. Appendix - abstracts / summaries in alphabetical order.**

Please contact Bioquell for more information on these articles.


National Reference Laboratory for *Clostridium difficile*, Hôpital Saint-Antoine, Paris, France.

**BACKGROUND**: *Clostridium difficile* are shed into the environment in high numbers by infected patients and are resistant to desiccation and some disinfectants. Studies have shown *C. difficile* spores are found in 12-26% of bleach-treated rooms. There is compelling evidence of environmental contamination contributing to transmission between patient admissions.

**AIM**: To investigate the use of hydrogen peroxide vapour to decontaminate carriers inoculated with three strains of *C. difficile*. The carrier materials used, polyvinyl chloride (PVC) and laminate, were designed to represent healthcare floors and furniture, respectively.

**METHODS**: Spores were dried onto PVC and laminate carriers at mean concentrations of 4.7-6.9-log10 spores/carrier. Three strains were used, including the hyper-virulent ribotype 027/NAP1/BI.

**FINDINGS**: In this study, HPV was effective for complete inactivation of *C. difficile* regardless of strain and/or surface. No statistical difference was observed between the two materials suggesting HPV was equally effective upon both surfaces.


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.


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 5min intervals over 20min, after which post-exposure viral titres were measured.

**FINDINGS**: Hydrogen peroxide vapour reduced the viral titre by 4-log10 on all surfaces tested within 20min of exposure. The reduction in viral titre took longest to achieve on stainless steel (20min), and the quickest effect was seen on vinyl flooring (10min). For glass, plastic and ceramic tile surfaces, the desired reduction in viral titre was seen within 15min 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.


**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-log10 TCID50 reduction resulted from 45min exposure to HPV in a microbiological safety cabinet.

CONCLUSIONS: HPV is effective for the inactivation of a recombiant 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.


Fumigation of high-containment microbiology facilities is an international requirement and in the United Kingdom this process is still commonly undertaken using formaldehyde vaporization. Formaldehyde usage is simple and inexpensive, but concerns exist over its toxicity and carcinogenicity. Alternative fumigants exist, although independent, parallel comparison of these substances is limited. This study determines the level of biocidal efficacy achievable with formaldehyde and compared this with other commonly used fumigants. Three different hydrogen peroxide-based fumigation systems were evaluated (two vapor and one dry-mist methods), along with true gas systems employing ozone and chlorine dioxide. A range of challenge microorganisms was used at different room locations to assess the efficacy, usability and safety of the fumigation equipment. These microorganisms included Clostridium perfringens, C. difficile, Mycobacterium fortuitum and Vaccinia virus. Only chloride dioxide and formaldehyde gave consistently high levels of antimicrobial efficacy across all bacterial challenge tests (typically greater than a 5-log reduction). All systems performed similarly against Vaccinia virus, but variable results were noted for Clostridium, C. difficile and M. fortuitum for the hydrogen peroxide and ozone-based systems. The study also revealed inconsistencies in system reliability and reproducibility, with all fumigant systems aborting mid-cycle on at least one occasion. In contrast, formaldehyde fumigation was confirmed as extremely reliable, largely because of its simplicity (liquid plus hot plate). All the fumigant tested have UK workplace exposure limits of 2 ppm or less, yet residual fumigant was detected for the formaldehyde and hydrogen peroxide systems following cycle completion, even after room aeration.


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.


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


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 sterilization process for prions as an alternative low-temperature method.

Gaseous peroxide, in addition to known antimicrobial efficacy, was shown to inactivate prions both 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 sterilization process may present a useful method for prion inactivation.


ABSTRACT: We report a reduction in the vancomycin-resistant enterococci (VRE) rate from a peak of 1.5 cases per 1,000 admissions (95% confidence interval [CI], 1.0-2.1) in August 2012 to 0.5 per 1,000 admissions (95% CI: 0.3-1.0) by January 2015, associated with a bundle of interventions.


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 antibiotic 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.

**METHOD**

**AIM:** To compare the efficacy, efficiency and safety of hydrogen peroxide-based room decontamination systems.

**BACKGROUND:** This was a head-to-head comparison of two hydrogen peroxide-vapour systems marketed in the USA and UK. AHP was available as Glosair, Advanced Sterilization Products (ASP), Johnson & Johnson Medical Ltd; and hydrogen peroxide vapour (HPV), a vapour-phase disinfection method, for the deactivation of a number of structurally distinct viruses of importance in the healthcare, veterinary and public sectors. The viruses studied were: feline calicivirus (FCV, a norovirus surrogate); human adenovirus type 1; transmissible gastroenteritis coronavirus of pigs (TGEV, severe acute respiratory syndrome coronavirus [SARS-CoV] surrogate); avian influenza virus (AIV); and swine influenza virus (SwIV).

**METHODOLOGY:** The HP was prepared as 10 ml aliquots and exposed to HPV produced by a Clarus L generator (Bioquell, Horsham, PA, USA) in a 0.2 m³ environmental chamber. Three vaporized volumes of hydrogen peroxide were tested in triplicate for each virus: 25, 27 and 33 ml.

**FINDINGS:** No viable viruses were identified after HPV exposure at any of the vaporized volumes tested. HPV was virucidal (>4-log reduction) against FCV, adenovirus, TGEV and AIV at the lowest vaporized volume tested (25 ml). For SwIV, due to low virus titre on the control discs, >3.8-log reduction was shown for the 25 ml vaporized volume and >4-log reduction was shown for the 27 ml and 33 ml vaporized volumes.

**CONCLUSION:** HPV was virucidal for structurally distinct viruses dried on surfaces, suggesting that HPV can be considered for the disinfection of virus-contaminated surfaces.

**FINDINGS:** HPV generally achieved a 6-log reduction, whereas AHP generally achieved less than a 4-log reduction on the BIs and in-house prepared test discs. Uneven distribution was evident for the AHP system but not the HPV system. Hydrogen peroxide leakage during AHP cycles with the door unsealed, as per the manufacturer’s operating manual, exceeded the short-term exposure limit (2ppm) for more than 2 h. When the door was sealed with tape, as per the HPV system, hydrogen peroxide leakage was <1 ppm for both systems. The mean concentration of hydrogen peroxide in the room 2 h after the cycle started was 1.3 [standard deviation (SD) 0.4] ppm and 2.8 (SD 0.8) ppm for the four HPV and AHP cycles, respectively. None of the readings were <2 ppm for the AHP cycles.

**CONCLUSION:** The HPV system was safer, faster and more effective for biological inactivation.

**FINDINGS:** AHP generally achieved a 6-log reduction, whereas HPV generally achieved less than a 4-log reduction on the BIs and in-house prepared test discs. Uneven distribution was evident for the AHP system but not the HPV system. Hydrogen peroxide leakage during AHP cycles with the door unsealed, as per the manufacturer’s operating manual, exceeded the short-term exposure limit (2 ppm) for more than 2 h. When the door was sealed with tape, as per the HPV system, hydrogen peroxide leakage was <1 ppm for both systems. The mean concentration of hydrogen peroxide in the room 2 h after the cycle started was 1.3 [standard deviation (SD) 0.4] ppm and 2.8 (SD 0.8) ppm for the four HPV and AHP cycles, respectively. None of the readings were <2 ppm for the AHP cycles.

**CONCLUSION:** The HPV system was safer, faster and more effective for biological inactivation.
incubated in RPMI media at 30°C 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*, *dermatitis* and *C. immitis* were killed within 30min of HPV exposure.


**OBJECTIVE:** To compare the microbiological efficacy of hydrogen peroxide vapor (HPV) and ultraviolet radiation (UVC) for room decontamination.

**DESIGN:** Prospective observational study.

**SETTING:** 500-bed teaching hospital.

**METHODS:** HPV and UVC processes were performed in 15 patient rooms. Five high-touch sites were sampled before and after the processes and aerobic colony counts (ACCs) were determined. Carrier disks with 10<sup>4</sup> *Clostridium difficile* (CD) spores and biological indicators (BIs) with 10<sup>4</sup> and 10<sup>6</sup> *Geobacillus stearothermophilus* spores were placed in 5 sites before decontamination. After decontamination, CD log reductions were determined and BIs were recorded as growth or no growth.

**RESULTS:** 93% of ACC samples that had growth before HPV did not have growth after HPV, whereas 52% of sites that had growth before UVC did not have growth after UVC (p < .0001). The mean CD log reduction was 16 for HPV and 2 for UVC. After HPV 100% of the 10<sup>4</sup> BIs did not grow, and 22% did not grow after UVC, with a range of 7%–53% for the 5 sites. For the 10<sup>6</sup> BIs, 99% did not grow after HPV and 0% did not grow after UVC. Sites out of direct line of sight were significantly more likely to show growth after UVC than after HPV. Mean cycle time was 153 (range, 140–177) min for HPV and 73 (range, 39–100) min for UVC (p < .0001).

**CONCLUSION:** Both HPV and UVC reduce bacterial contamination, including spores, in patient rooms, but HPV is significantly more effective. UVC is significantly less effective for sites that are out of direct line of sight.


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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 avian 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 30min. For all viruses tested and under all conditions (except one), the decontamination process reduced the virus titer to zero embryolethal 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.


**OBJECTIVE:** To determine whether hydrogen peroxide vapor (HPV) could be used to decontaminate caliciviruses from surfaces in a patient room.

**DESIGN:** Feline calicivirus (FCV) and murine norovirus (MNV) were used as surrogate viability markers to mimic the non-cultivable human norovirus. Cell culture supernatants of FCV and MNV were dried in triplicate 35mm wells of 6-well plastic plates. These plates were placed in various positions in a non-occupied patient room that was subsequently exposed to HPV. Control plates were positioned in a similar room but were never exposed to HPV.

**METHODS:** Virucidal activity was measured in cell culture by reduction in 50% tissue culture infective dose titer for FCV and by both 50% tissue culture infective dose titer and plaque reduction for MNV.

**RESULTS:** Neither viable FCV nor viable MNV could be detected in the test room after HPV treatment. At least 3.65-log reduction for FCV and at least 3.67-log reduction for MNV were found by 50% tissue culture infective dose. With plaque assay, measurable reduction for MNV was at least 2.85-log units.

**CONCLUSIONS:** The successful inactivation of both surrogate viruses indicates that HPV could be a useful tool for surface decontamination of a patient room contaminated by norovirus. Hence nosocomial spread to subsequent patients can be avoided.


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.


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<sup>10</sup> survivors plotted against time and were found to range from 1.41 to 4.38min. HPV was found to be effective at deactivating spores of toxigenic *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.


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.


BACKGROUND: Clostridium difficile spores and multidrug-resistant (MDR) organisms, such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and MDR Acinetobacter baumannii, are important nosocomial pathogens that are difficult to eliminate from the hospital environment. We evaluated the efficacy of hydrogen peroxide vapor (HPV), a no-touch automated room decontamination system, for the inactivation of a range of pathogens dried onto hard non-porous and porous surfaces in an operating room (OR).

METHODS: Stainless steel and cotton carriers containing >4-log10 viable MRSA, VRE, or MDR A baumannii were placed at 4 locations in the OR along with 7 pouches containing 6-log10 Geobacillus stearothermophilus spore biologic indicators (BIs). HPV was then used to decontaminate the OR. The experiment was repeated 3 times.

RESULTS: HPV inactivated all spore BIs (>6-log10 reduction), and no MRSA, VRE, or MDR A baumannii were recovered from the stainless steel and cotton carriers (>4-log10 reduction, depending on the starting inoculum). HPV was equally effective at all carrier locations. We did not identify any difference in efficacy for microbes dried onto stainless steel or cotton surfaces, indicating that HPV may have a role in the decontamination of both porous and non-porous surfaces.

CONCLUSION: HPV is an effective way to decontaminate clinical areas where contamination with bacterial spores and MDR organisms is suspected.


We evaluated the clinical impact of implementing hydrogen peroxide vapour (HPV) for disinfecting rooms vacated by Clostridium difficile infection (CDI) patients. Breakpoint time series analysis indicated a significant reduction (p<0.001) in the rate of CDI that occurred at the time when HPV was implemented, resulting in a reduction in the rate of CDI from 1.0 to 0.4 cases per 1000 patient days in the 2 years before vs. the first 2 years of HPV usage. HPV should be considered to augment the terminal disinfection of rooms vacated by patients with CDI.


Decontamination of food contact surfaces, equipment and general work areas is important for the prevention of transmission of foodborne 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 foodborne 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 simulated 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.

Moy A., Speight S. Assessment of the Efficacy of Vapour Phase Hydrogen Peroxide Generated by the Bioquell Q10 against Mycobacterium avium and Mycobacterium terrae. PHE Report No. 14/006 2014;(1)

EXECUTIVE SUMMARY: A series of tests were carried out to investigate whether vapour phase Hydrogen Peroxide (VPHP) generated from the Bioquell Q10 (supplied by Bioquell) inactivated Mycobacterium avium ATCC 15769 and Mycobacterium terrae ATCC 15755 inoculated and dried onto stainless steel discs.

In all 10 tests with the Mycobacterium avium discs no recovery occurred on any of the 24 discs exposed to VPHP. This represents an average log reduction of >4.88. In the 10 tests with the Mycobacterium terrae discs, the organism was recovered from 3 of the 24 discs exposed to VPHP. The other 21 discs showed no recovery giving an average log reduction of >4.50. These experiments have shown that VPHP generated by the Bioquell Q-10 is capable of inactivating two Mycobacterium species (avium and terrae) on metal carriers at levels of greater than 4-5-logs however the results suggest that the level of soiling may impact on the efficacy of the process and therefore effective pre-cleaning should be carried out.


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-log10 reduction was achieved on both bacteriophages compared with unexposed controls by 50min HPV exposure in an isolator. HPV may be useful for the environmental control of bacteriophages.


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 sporidical 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 Acholera plasma 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 methicillin-resistant Staphylocococcus 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-log10 CFU, most vegetative bacteria and spores tested survived on surfaces for more than 5 weeks, but all were inactivated within 90min of exposure to hydrogen peroxide vapor in a 100m³ test room even in the presence of 0.3% bovine serum albumin to simulate biological soiling.


SUMMARY: Several factors influence the in vitro susceptibility of microbes to disinfectants. We evaluated the impact of various suspending media on the susceptibility of meticillin-resistant Staphylococcus aureus (MRSA) to hydrogen peroxide vapour (HPV) decontamination. From a >6-log10 inoculum, relative susceptibility was 10% bovine serum albumin (BSA) < TSB < 3% BSA < saline < 0.3% BSA ¼ water. MRSA was not recovered after >60min exposure to HPV for all suspensions. These findings indicate that the suspending medium has an effect on the in vitro susceptibility of MRSA to HPV, which may have implications in the case of suboptimal cleaning.


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-log10 reduction in 10min at the lowest viral concentration (10⁶ plaque-forming units (pfu)/carrier) and requiring 45min at the highest concentration (10⁶ pfu/carrier). For the VHP system a 30min exposure period was required to achieve a 6-log10 reduction at the lowest concentration and 60-90min 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.


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 10^9 CFU) was dried on 10 different types of test surfaces and exposed to vapour-phase hydrogen peroxide fumigation for a contact time of 2hrs. A significant reduction in the log10 CFU of Y. pestis on all 10 materials was observed between the controls evaluated after a 1hr 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.


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 =1000ppm hydrogen peroxide gas for 20min. 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.

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.


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 100ppm and triethylene glycol (TEG)-saturated air containing 2ppm of TEG at 25°C.

METHODS: Inactivating influenza viruses in aqueous suspensions were deposited on stainless steel coupons, allowed to dry at ambient conditions, and then exposed for up to 15min to 10 to 90ppm 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 2.5min, exposure to 10ppm HP vapor resulted in >99% inactivation. For air saturated with TEG at 25 to 29°C, the disinfection rate was about 1.3-log10 reductions per hour, about 16 times faster than the measured natural inactivation rate under ambient conditions.

CONCLUSIONS: Vapor concentrations of 10ppm HP or 2ppm 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 2ppm, there are essentially no health risks to people.


17. Health Protection Agency (previously Centre for Applied Microbiology and Research) PDUK. Determination of the effectiveness of VPHP against methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis and Bacillus stearothermophilus. 2001.


55. Information supplied with kind permission of Eli Lilly and Company, Indianapolis, Indiana. 1996.


47. ANSM NF T72-281 Certificate. Contact Bioquell for further details 2015.


49. BluTest Laboratories Ltd. Poliovirus 1 & Adenovirus 5 deactivation investigation. Contact Bioquell for further details 2015.


53. Viral inactivation trials conducted in commercial confidence. Contact Bioquell for further information.
