Efficacité microbiologique de la vapeur de peroxyde d'hydrogène

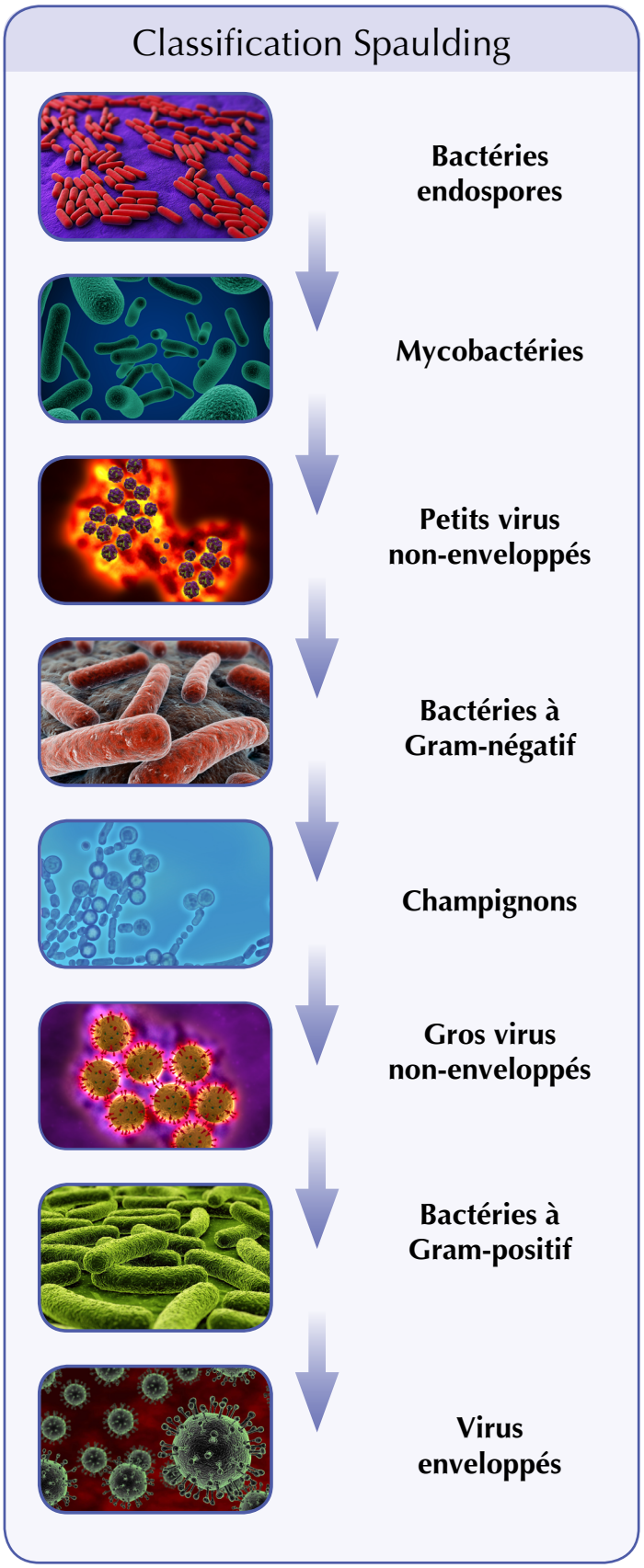

Image 1. Classification de E.H. Spaulding.

Le peroxyde d'hydrogène est un agent biocide reconnu du fait de sa nature « sans résidu » (les seuls résidus sont l'oxygène et l'eau) et de sa capacité à être utilisé sous phase vapeur à basse température. Comme pour tout autre agent désinfectant, la technologie Bioquell a été testé sur de nombreux micro-organismes et classes de micro-organismes. Les tests d'efficacité sont mis à jour en permanence au vu du grand nombre de micro-organismes existants.

Les informations fournies dans ce document sont issues de sources reconnues et peuvent être employées pour visualiser l'efficacité de la vapeur de peroxyde d'hydrogène à l'encontre d'un micro-organisme particulier ou d'un type ou groupe de micro-organismes.

L'image 1 (ci contre) montre une classification de la résistance des différents microorganismes aux procédures de stérilisation et de désinfection basée sur le travail pionnier de E.H. Spaulding (1). Cette classification peut être utilisée en tant que guide lorsqu'une hypothèse est formulée au sujet de l'efficacité de la vapeur de peroxyde d'hydrogène à l'encontre d'un microorganisme particulier.

Si un microorganisme n'est pas listé dans ce document, cela ne signifie pas qu'il n'y a aucune donnée disponible à son sujet ou que la technologie Bioquell n'est pas efficace à son encontre. Dans ce cas de figure, vous pouvez contactez Bioquell pour savoir si d'autres données (analogues ou spécifiques) sont disponibles – ou si des tests complémentaires sont requis.

La technologie Bioquell se révèle capable de désactiver un large spectre de micro-organismes incluant les bactéries, virus et moisissures. L'efficacité de la vapeur de peroxyde d'hydrogène a été démontrée à maintes reprises sur les endospores de bactéries, qui sont typiquement les organismes les plus résistants à toute sorte de stress d'inactivation (voir image 1 – Classification de Spaulding). Les organismes listés dans ce document sont divisés en larges catégories taxonomiques (bactéries, virus et moisissures) et groupés conformément à leurs caractéristiques microbiologiques. Ceci permettra une comparaison simple d'un organisme non testé avec d'autres organismes proches déjà testés.

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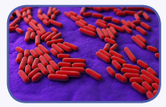
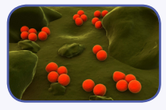
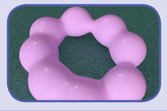
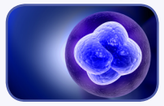
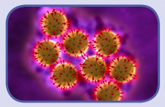
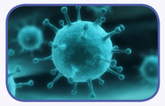
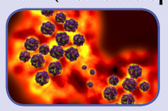
Contenu

1. Liste des organismes testés et références des sources

1.1 Bactéries et endospores de bactéries

Type d'organisme	Nom de l'organisme	Référence
Endospores de bactéries Bâtonnets à Gram-positif 	*Bacillus anthracis*	(2;3;46;48)
	Bacillus cereus	(5)
	Bacillus circulans	(4)
	Bacillus firmus	(4)
	Bacillus megaterium	(4)
	Bacillus pumilus	(4;6)
	Bacillus subtilis	(2;4;6-8;48)
	Bacillus thuringiensis	(48)
	Clostridium botulinum	(9)
	Clostridium difficile	(10;11;39;40;42;52;55)
	Clostridium sporogenes	(6-8)
	Geobacillus stearothermophilus (anciennement *Bacillus stearothermophilus*)	(2;6;7;9;12;13;40; 42;43;48)
Bâtonnets à Gram-positif 	*Mycobacterium avium*	(45;47)
	Mycobacterium smegmatis	(6)
	Mycobacterium terrae	(45;47)
	Mycobacterium tuberculosis	(12)
	Mycobacterium fortuitum	(52)
	Lactobacillus caesei	(6)
	Listeria monocytogenes	(5)
Cocci à Gram-positif 	*Enterococcus faecium/faecalis* (inc. ERV)	(6;11;14;43;51)
	Enterococcus hirae	(47)
	Staphylococcus aureus (inc. SARM)	(11;13-18;40;43;44;47)
	Staphylococcus epidermidis	(17;18)
Entérobactéries (Bâtonnets à Gram-négatif entériques) 	*Brucella suis*	(46)
	Enterobacter cloacae	(19)
	Escherichia coli (inc. O157:H7)	(5;47)
	Francisella tularensis	(46)
	Klebsiella pneumoniae	(5;11)
	Salmonella choleraesuis	(5)
	Serratia marcescens	(6;38)
	Yersinia pestis	(20;46)
Bâtonnets à Gram-négatif 	*Acinetobacter* spp. (inc. *A. baumannii)*	(11;14;19;40;43)
	Legionella sp.	(5)
	Pseudomonas aeruginosa	(6;7;47)
Bactéries atypiques 	*Acholeplasma laidlawii* (Mycoplasma)	(21)

1.2 Virus

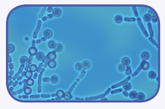
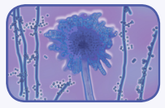
ARN/ADN	Génome[a]	Famille	Nom du virus	Réf.
ADN (Enveloppé) 	Double	Herpesviridae	Maladie d'Aujeszky	(22)
	Double	Asfarviridae	Peste porcine africaine	(22)
	Double	Poxviridae[b]	Virus de la vaccine	(46;52;54)
ADN (Non-enveloppé) 	Double	Adenoviridae	Adénovirus	(23;41;49;47;54)
	Simple	Parvoviridae	Parvovirose de la souris	(24;25)
			Parvovirose porcine	(54)
			Virus minute de la souris (MVMp)	(54)
ARN (Enveloppé) 	Simple	Orthomyxoviridae	Grippe aviaire	(22;41)
			Grippe A (H1N1)	(26)
	Simple	Paramyxoviridae	Maladie de Newcastle	(22)
	Simple	Rhabdoviridae	Virus de la stomatite vésiculaire	(22)
	Simple	Flaviviridae	Virus de la dengue	(27)
			Peste porcine	(22)
	Simple	Arenaviridae	Fièvre de Lassa	(28)
	Simple	Orthomyxoviridae	Grippe porcine (H3N2)	(41)
	Simple	Coronaviridae	Coronavirus de gastro-entérite transmissible (subsitut au MERS-CoV)	(41)
ARN (Non-enveloppé) 	Simple	Caliciviridae	Calicivirus félin	(29;30;41;50)
			Norovirus murin	(47;50)
			Exanthème vésiculeux	(22)
	Simple	Picornaviridae	Poliovirus de Type 1	(49;54)
			Fièvre aphteuse	(53)
			Maladie vésiculeuse du porc	(22)
	Double	Reoviridae	Fièvre catarrhale	(22)

a. simple = brin simple, double = double brin
b. certains membres des Poxviridae sont non-enveloppé

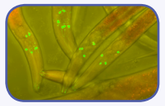
1.3 Bactériophage

Type d'organisme	Nom	Référence
	Bactériophages infectant Lactococcus lactis	(31;47)
	Bactériophage MS2	(32)

1.4 Champignons

Type d'organisme	Nom	Référence
 	*Alternaria sp.*	(33)
	Aspergillus brasiliensis (anciennement *Aspergillus niger)*	(6;7;47)
	Candida albicans	(7;47)
	Candida parapsilosis	(6)
	Coccidioides immitis	(34)
	Blastomyces dermatitidis	(34)
	Histoplasma capsulatum	(34)
	Penicillium sp.	(33)

1.5 Nématodes et protozoaires

Type d'organisme	Nom	Référence
	*Caenorhabditis elegans* *Syphacia muris**	(35) (36)

* La référence mentionnée est un poster décrivant une étude recherchant l'efficacité de la technologie Bioquell contre les oeufs de *Syphacia muris* (oxyure). La destruction microscopique a été remarquée sur des oeufs immatures, mais pas sur des oeufs matures. Des souris immuno-déficientes exposées à une litière contaminée et traitée à la vapeur de peroxyde d'hydrogène n'ont pas développé d'infection d'oxyure tandis que les souris exposées à une litière contaminée non traitée par la technologie Bioquell ont développé l'infection d'oxyure. De récentes expérimentations réalisées par Bioquell ont montré que des oeufs traités par la technologie Bioquell sont capables d'éclore dans un milieu d'éclosion spécialement énoncé, donc il est possible que l'exposition à la vapeur de peroxyde d'hydrogène, alors qu'elle ne prévient pas l'éclosion *in vitro*, rend les oeufs d'oxyure non-infectieux *in vivo*. D'autres recherches sont nécessaires sur ce sujet.

L'efficacité du peroxyde d'hydrogène sous forme liquide et sous forme gazeuse a été démontrée contre d'autres protozoaires dont les organismes parmi les genres *Metadinium, Eimeria, Acanthamoeba, Ichthyobodo* et *Cryptosporidium*. Pour de plus amples informations, merci de se référer à la liste d'efficacité du peroxyde d'hydrogène

1.6 Autres

- Il existe des preuves montrant que la technologie Bioquell inactive des prions (37).

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2. Appendice - abstracts / résumés par ordre alphabétique.

Merci de contacter Bioquell pour plus d'informations sur ces articles.

Barbut F, Yezli S, Otter J. Activity *in vitro* of hydrogen peroxide vapour against *Clostridium difficile* spores. *J Hosp Infect* **2012;80:85-87**.
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-log^{10} 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.

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 5min intervals over 20min, 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 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.

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} TCID50 reduction resulted from 45min 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.

Beswick, Alan J., J. Farrent, C. Makison, J. Gawn, G. Frost, B.Cook, and J. Pride. Comparison of multiple systems for laboratory whole room fumigation. *Applied Biosafety* **16 (2011): 139-157.**
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 *Geobacillus stearothermophilus*, *Clostridium difficile*, *Mycobacterium fortuitum* and Vaccinia virus. Only chlorine 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 *Geobacillus*, *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 fumigants tested have UK workplace exposure limits of 2ppm or less, yet residual fumigant was detected for the formaldehyde and hydrogen peroxide systems following cycle completion, even after room aeration.

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. *J Hosp Infect* **2008;68:190-192**. Royal Hampshire County 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 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 *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.

Fisher, Dale, Long Pang, Sharon Salmon, Raymond T.P. Lin, Cathrine Teo, Paul Tambyah, Roland Jureen, Alex R. Cook, Jonathan A. Otter. A Successful Vancomycin-Resistant Enterococci Reduction Bundle at a Singapore Hospital. *Infection Control & Hospital Epidemiology* **37.01 (2016): 107-109**.
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.

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.

Fu T.Y., Gent P., Kumar V. Efficacy, efficiency and safety aspects of hydrogen peroxide vapour and aerosolized hydrogen peroxide room disinfection systems. *J Hosp Infect* **2012;80:199-205**. Pharmacy Quality Assurance Laboratory, St. George's Hospital, London, UK.
BACKGROUND: This was a head-to-head comparison of two hydrogen peroxide-based room decontamination systems.
AIM: To compare the efficacy, efficiency and safety of hydrogen peroxide vapour (HPV; Clarus R, Bioquell, Andover, UK) and aerosolized hydrogen peroxide (aHP; SR2, Sterinis, now supplied as Glosair, Advanced Sterilization Products (ASP), Johnson & Johnson Medical Ltd, Wokingham, UK) room disinfection systems.
METHOD: Efficacy was tested using 4- and 6-log *Geobacillus stearothermophilus* biological indicators (BIs) and in-house prepared test discs containing approximately 10^6 meticillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and *Acinetobacter baumannii*. Safety was assessed by detecting leakage of hydrogen peroxide using a hand held detector. Efficiency was assessed by measuring the level of hydrogen peroxide using a hand held sensor at three locations inside the room, 2h after the start of the cycles.
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 2h. When the door was sealed with tape, as per the HPV system, hydrogen peroxide leakage was <1ppm for both systems. The mean concentration of hydrogen peroxide in the room 2h 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 <2ppm for the aHP cycles.
CONCLUSION: The HPV system was safer, faster and more effective for biological inactivation.

Goyal S.M., Chander Y., Yezli S., Otter J.A. Evaluating the virucidal efficacy of hydrogen peroxide vapour. *J Hosp Infect* **2014;86:255-259**. Department of Veterinary Population Medicine, USA / Bioquell UK / CIDR UK.
BACKGROUND: Surface contamination has been implicated in the transmission of certain viruses, and surface disinfection can be an effective measure to interrupt the spread of these agents.
AIM: To evaluate the *in vitro* efficacy of hydrogen peroxide vapour (HPV), a vapour-phase disinfection method, for the inactivation 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, a severe acute respiratory syndrome coronavirus [SARS- CoV] surrogate); avian influenza virus (AIV); and swine influenza virus (SwIV).
METHODS: The viruses were dried on stainless steel discs in 20 or 40ml aliquots and exposed to HPV produced by a Clarus L generator (Bioquell, Horsham, PA, USA) in a 0.2m^3 environmental chamber. Three vaporized volumes of hydrogen peroxide were tested in triplicate for each virus: 25, 27 and 33ml.
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 (25ml). For SwIV, due to low virus titre on the control discs, >3.8-log reduction was shown for the 25ml vaporized volume and >4-log reduction was shown for the 27ml and 33ml 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.

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 15min intervals during a 180min HPV exposure period. No *M. tuberculosis* BIs grew following 30min of HPV exposure. In three separate room experiments, *M. tuberculosis* and *G. stearothermophilus* BIs were exposed to HPV for 90, 120 and 150min, respectively. BIs for both microorganisms were deactivated in all 10 locations following 90min 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 105min 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, dermatitidis* and *C. immitis* were killed within 30min of HPV exposure.

Havill, N. L., Moore B. A., Boyce, J. M. Comparison of the Microbiological Efficacy of Hydrogen Peroxide Vapor and Ultraviolet Light Processes for Room Decontamination. *Infect Cont Hosp Epidemiol* **2012;33:5**. Hospital of Saint Raphael.
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 10^6 *Clostridium difficile* (CD) spores and biological indicators (BIs) with 10^4 and 10^6 *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 10^4 BIs did not grow, and 22% did not grow after UVC, with a range of 7%–53% for the 5 sites. For the 10^6 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.

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 30min. 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.

Holmdahl, Torsten, *et al.* Hydrogen Peroxide Vapor Decontamination in a Patient Room Using Feline Calicivirus and Murine Norovirus as Surrogate Markers for Human Norovirus. *Infection Control and Hospital Epidemiology* **37.5 (2016): 561**.
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.

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 4.38min. 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.

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.

Lemmen S., Scheithauer S., Hafner H., Yezil. S., Mohr M., Otter J. A. Evaluation of hydrogen peroxide vapor for the inactivation of nosocomial pathogens on porous and non-porous surfaces. *J Hosp Infect* **2015;43:82-5**. Department of Infection Control and Infectious Diseases, Germany / Bioquell UK / Schulke & Mayr GmbH, Germany / Centre for Clinical Infection and Diagnostics Research UK.
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-$\log^{10}$ viable MRSA, VRE, or MDR *A baumannii* were placed at 4 locations in the OR along with 7 pouched 6-$\log^{10}$ *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-$\log^{10}$ reduction), and no MRSA, VRE, or MDR *A baumannii* were recovered from the stainless steel and cotton carriers (>4-5-$\log^{10}$ 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.

McCord J., Prewitt M., Dyakova E., Mookerjee S., & Otter J. (2016). Reduction in *Clostridium difficile* infection (CDI) associated with the introduction of hydrogen peroxide vapour (HPV) automated room disinfection. *Journal of Hospital Infection*.
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.

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 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 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.

Moy A., Speight S. Assesment 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.

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 50min 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 methicillin-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 90min 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 J. A., Yezli S., French G. L. Impact of the suspending medium on susceptibility of methicillin-resistant *Staphylcococcus aureus* to hydrogen peroxide vapour decontamination. *J Hosp Infect* **2012;82:213-215.** Bioquell UK / CIDR UK.
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-$\log^{10}$ 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.

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.
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 10min at the lowest viral concentration (10^7 plaque-forming units (pfu)/carrier) and requiring 45min at the highest concentration (10^9 pfu/carrier). For the VHP system a 30min exposure period was required to achieve a 6-$\log^{10}$ 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.

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 *Yersini 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 2hrs. A significant reduction in the $\log^{10}$ 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.

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 =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.

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 100ppm and triethylene glycol (TEG)-saturated air containing 2ppm 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 15min to 10 to 90ppm 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 2.5min, 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-$\log^{10}$ 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.

3. References

1. Spaulding EH. Chemical disinfection and antisepsis in the hospital. *J Hosp Res* 1972;9:5-31.
2. Rogers JV, Sabourin CL, Choi YW, Richter WR, Rudnicki DC, Riggs KB, *et al.* Decontamination assessment of *Bacillus anthracis*, *Bacillus subtilis*, and *Geobacillus stearothermophilus* spores on indoor surfaces using a hydrogen peroxide gas generator. *J Appl Microbiol* 2005;99(4):739-48.
3. Defence Science and Technology Laboratory (DSTL) PDUK. *Bacillus anthracis* (Anthrax) deactivation investigation. 2002.
4. 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(5):228-31.
5. McDonnell G, Grignol G, Antloga K. Vapour-phase hydrogen peroxide decontamination of food contact surfaces. *Dairy Food Environ Sanitat* 2002;22:868-73.
6. Rickloff JR, reliski PA. Resistance of various micro-organisms to vaporized hydrogen peroxide in prototype tabletop sterilizer. New Orleans. 1989.
7. Health Protection Agency (previously Centre for Applied Microbiology and Research) PDUK. Cabinet bio-decontamination trial. March 1995.
8. US Environmental Protection Agency (EPA) registered sterilant. EPA registration number 72372-1-86703. 2009.
9. 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(3):403-11.
10. Boyce JM, Havill NL, Otter JA, McDonald LC, Adams NM, Cooper T, *et al.* Impact of hydrogen peroxide vapor room decontamination on *Clostridium difficile* environmental contamination and transmission in a healthcare setting. *Infect Control Hosp Epidemiol* 2008;29(8):723-9.
11. Otter JA, French GL. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. *J Clin Microbiol* 2009;47(1):205-7.
12. Hall L, Otter JA, Chewins J, Wengenack NL. Use of hydrogen peroxide vapor for deactivation of *Mycobacterium tuberculosis* in a biological safety cabinet and a room. *J Clin Microbiol* 2007;45(3):810-5.
13. French GL, Otter JA, Shannon KP, Adams NM, Watling D, Parks MJ. Tackling 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(1):31-7.
14. Otter JA, Cummins 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(2):182-8.
15. Jeanes A, Rao G, Osman M, Merrick P. Eradication of persistent environmental MRSA. *J Hosp Infect* 2005;61(1):85-6.
16. Dryden M, Parnaby R, Dailly S, Lewis T, Davis-Blues K, Otter JA, *et al.* Hydrogen peroxide vapour decontamination in the control of a polyclonal meticillin-resistant *Staphylococcus aureus* outbreak on a surgical ward. *J Hosp Infect* 2008;68(2):190-2.
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.
18. Health Protection Agency (previously Centre for Applied Microbiology and Research) PDUK. Assessment of the efficacy of vapour phase hydrogen peroxide as a room disinfectant. 2003.
19. Otter JA, Yezli S, Shouten MA, van Zanten AR, Houmes-Zielman G, Nohlmans-Paulssen M. Hydrogen peroxide vapor (HPV) 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):754-6.
20. Rogers JV, Richter WR, Shaw MQ, Choi YW. Vapour-phase hydrogen peroxide inactivates *Yersinia pestis* dried on polymers, steel, and glass surfaces. *Lett Appl Microbiol* 2008;47:279-85.
21. Otter JA, Chewins J, Windsor D, Windsor H. Microbiological contamination in cell culture: a potential role for hydrogen peroxide vapour (HPV)? *Cell Biol Int* 2008;32(2):326-7.
22. Heckert RA, Best M, Jordan LT, Dulac GC, Eddington DL, Sterritt WG. Efficacy of vaporized hydrogen peroxide against exotic animal viruses. *Appl Environ Microbiol* 1997;63(10):3916-8.
23. Berrie E, Andrews L, Yezli S, Otter JA. Hydrogen peroxide vapour (HPV) inactivation of adenovirus. *Lett Appl Microbiol* 2011;52(5):555-8.
24. Viral inactivation trials. Conducted in commercial confidence. Contact Bioquell for further details. 2002.
25. McDonnell G, Belete B, Fritz C, Hartling J. Room decontamination with vapour hydrogen peroxide VHP for environmental control of parvovirus. Baltimore, MD. 2001.
26. Rudnick SN, McDevitt JJ, First MW, Spengler JD. Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations. *Am J Infect Control* 2009;37(10):813-9.
27. Investigation into the efficacy of hydrogen peroxide vapour in the bio-deactivation of Dengue virus. Conducted in commercial confidence. Contact Bioquell for further details. 2003.
28. Otter JA, Barnicoat M, Down J, Smyth D, Yezli S, Jeanes A. Hydrogen peroxide vapour decontamination of a critical care unit room used to treat a patient with Lassa fever. *J Hosp Infect* 2010;75(4):335-7.
29. Goyal SM, Chander Y, Yezli S, Otter JA. Hydrogen peroxide vapor (HPV) inactivation of Feline Calicivirus, a surrogate for Norovirus. 2011. Infection Prevention Society Annual General Meeting.
30. 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(2):116-21.
31. Otter JA, Budde-Niekiel A. Hydrogen peroxide vapor: a novel method for the environmental control of lactococcal bacteriophages. *J Food Prot* 2009;72(2):412-4.
32. Pottage T, Richardson C, Parks S, Walker JT, Bennett AM. Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses. *J Hosp Infect* 2010;74(1):55-61.
33. Information supplied with kind permission of Eli Lilly and Company, Indianapolis, Indiana. 1996.
34. 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(2):189-91.
35. Gustin EJ, McDonnell GE, Mullen G, Gordon BE. The efficacy of vapour phase hydrogen peroxide against nematode infestation: the *Caenorhabditis elegans* model. American Association for Laboratory Animal Science (AALAS) Annual meeting San Antonio, Texas, USA 2002.
36. Krause J, Riedesel H. Elimination of pinworm eggs from caging equipment with vapourised hydrogen peroxide. Report from the Max-Planck-Institute for experimental medicine. San Antonio, Texas, USA 2002.
37. 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(3):278-86.
38. 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(4):364-6.
39. Barbut, F., Yezli, S., & Otter, J. A. (2012). Activity *in vitro* of hydrogen peroxide vapour against *Clostridium difficile* spores. *Journal of Hospital Infection*, 80(1), 85-87.
40. Fu, T. Y., Gent, P., & Kumar, V. (2012). Efficacy, efficiency and safety aspects of hydrogen peroxide vapour and aerosolized hydrogen peroxide room disinfection systems. *Journal of Hospital Infection*, 80(3), 199-205.
41. Goyal, S. M., Chander, Y., Yezli, S., & Otter, J. A. (2014). Evaluating the virucidal efficacy of hydrogen peroxide vapour. *Journal of Hospital Infection*, 86(4), 255-259.
42. Havill, N. L., Moore, B. A., & Boyce, J. M. (2012). Comparison of the microbiological efficacy of hydrogen peroxide vapor and ultraviolet light processes for room decontamination. *Infection Control*, 33(05), 507-512.
43. Lemmen, S., Scheithauer, S., Häfner, H., Yezli, S., Mohr, M., & Otter, J. A. (2015). Evaluation of hydrogen peroxide vapor for the inactivation of nosocomial pathogens on porous and nonporous surfaces. *American journal of infection control*, 43(1), 82-85.
44. Otter, J. A., Yezli, S., & French, G. L. (2012). Impact of the suspending medium on susceptibility of meticillin-resistant *Staphylococcus aureus* to hydrogen peroxide vapour decontamination. *Journal of Hospital Infection*, 82(3), 213-215.
45. Moy A., Speight S. Assesment 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).
46. US Environmental Protection Agency. Persistence testing and evaluation of fumigation technologies for decontamination of building materials contaminated with biological agents. EPA/600/R-10/086 August 2010.

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

48. US Environmental Protection Agency. Abbreviated Test Report for Laboratory Validation of Chlorine Dioxide Gas Decontamination, 2002. Contact Bioquell for further details 2015.

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

50. Holmdahl, Torsten, *et al.* "Hydrogen Peroxide Vapor Decontamination in a Patient Room Using Feline Calicivirus and Murine Norovirus as Surrogate Markers for Human Norovirus." *Infection control and hospital epidemiology* 37.5 (2016): 561.

51. Fisher, Dale, *et al.* "A Successful Vancomycin-Resistant *Enterococci* Reduction Bundle at a Singapore Hospital." *Infection Control & Hospital Epidemiology* 37.01 (2016): 107-109.

52. Beswick, Alan J., *et al.* "Comparison of multiple systems for laboratory whole room fumigation." *Applied Biosafety* 16 (2011): 139-157.

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

54. Eterpi M., McDonnell G., & Thomas V. Virucidal activity of disinfectants against parvoviruses and reference viruses. *Applied Biosafety* 15.04 (2010): 165-171.

55. McCord J., Prewitt M., Dyakova E., Mookerjee S., & Otter J. (2016). Reduction in *Clostridium difficile* infection (CDI) associated with the introduction of hydrogen peroxide vapour (HPV) automated room disinfection. *Journal of Hospital Infection*.

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