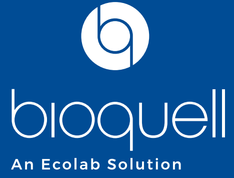
Hydrogen Peroxide Vapour Biological Efficacy



EFFICACY DOCUMENT

Bioquell's hydrogen peroxide vapour 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 vapour (breaks down to oxygen and water vapour) and low temperature, vapour-phase application increases the practicality of the process. Bioquell's 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 vapour against types and groups of organisms.

SPAULDING CLASSIFICATION

Bacterial endospores

Mycobacteria

Small non-enveloped viruses

Gram-negative bacteria

Fungi

Large non-enveloped viruses

Gram-positive bacteria

Enveloped viruses

Figure 1

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

If a particular organism is not listed here, it does not mean there is no data available or that Bioquell technology 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 vapour has been shown to kill a wide range of microorganisms including bacteria, mycobacteria, spores, yeasts, fungi, viruses and phage. The efficacy of hydrogen peroxide vapour has been repeatedly demonstrated against bacterial endospores, which are highly resistant organisms commonly found on environmental surfaces and are positioned at the top of the Spaulding classification. The organisms listed in this document are divided into broad taxonomic categories 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.

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. Reference
3. Appendix – abstracts/summaries in alphabetical order

1. List of tested organisms and source references

1.1. Bacteria and bacterial endospores

Type of organism	Name of organism	Reference
Bacterial endospores Gram +ve rods	*Bacillus anthracis*	(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;47;48)
	Bacillus thuringiensis	(48)
	Clostridium botulinum	(9)
	Clostridium difficile	(10;11;39;40;42;52;55;57;60 61;62;63;68;75)
	Clostridium sporogenes	(6-8)
	Geobacillus stearothermophilus (formerly *Bacillus stearothermophilus*)	(2;6;7;9;12;13;40;42;43;48)
Gram +ve rod	*Mycobacterium avium*	(45;47)
	Mycobacterium smegmatis	(6)
	Mycobacterium terrae	(45;47)
	Mycobacterium tuberculosis	(12)
	Mycobacterium fortuitum	(52)
	Lactobacillus caesei	(6)
	Listeria monocytogenes	(5;58)
	Corynebacterium bovis	(64)
	Burkholderia pseudomallei	(72)
Gram +ve cocci	*Enterococcus faecium/faecalis (inc.VRE)*	(6;11;14;43;51;61;68;76)
	Enterococcus hirae	(47)
	Staphylococcus aureus (inc.MRSA)	(11;13;14;15;16;17;18;40;43;44 47;57;59;65;66;68;76)
	Staphylococcus epidermidis	(17;18)
	Staphylococcus xylesus	(70)
	Staphylococcus warneri	(71)
	Micrococcus luteus	(71)

Enterobacteriaceae (Enteric Gram–ve rods)	*Brucella suis*	(46;72)
	Enterobacter cloacae	(19)
	Escherichia coli (inc. O157:H7)	(5;47; 58;59)
	Francisella tularensis	(46;72)
	Klebsiella pneumoniae	(5;11;57;76)
	Salmonella choleraesuis	(5)
	Salmonella typhimurium	(58)
	Serratia marcescens	(6;38)
	Yersinia pestis	(20;46;72)
Gram -ve rods	*Acinetobacter spp. (inc. A. baumannii)*	(11;14;19;40;43;76)
	Legionella pneumoniae	(5)
	Pseudomonas aeruginosa	(6;7;47;59;76)
Atypical bacteria	*Acholeplasma laidlawii (Mycoplasma)*	(21)

1.2 Viruses

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

D / RNA	Genomea	Family	Name	Reference
DNA (Enveloped)	Double	*Herpesviridae*	Pseudorabies Virus	(22)
	Double	*Asfarviridae*	African Swine Fever Virus	(22)
	Double	*Orthomyxoviridae*	Swine Influenza Virus (H3N2)	(41)
	Double	*Poxviridae*	Vaccinia	(46;52;54)

DNA (Non-enveloped)	Double	Adenoviridae	Adenovirus	(23;41;47;49;54)
	Single	Parvoviridae	Mouse Parvovirus	(24;25)
			Porcine Parvovirus	(47;54)
			Minute Virus of Mice (MVM)	(54)
RNA (Enveloped)	Single	Orthomyxoviridae	Avian Influenza Virus	(22;41)
			Influenza A (H1N1)	(26)
	Single	Paramyxoviridae	Newcastle Disease Virus	(22)
	Single	Rhabdoviridae	Vesicular Stomatitis Virus	(22)
	Single	Flaviviridae	Dengue Virus	(27)
			Hog Cholera Virus	(22)
	Single	Arenaviridae	Lassa Virus	(28)
	Single	Coronaviridae	Transmissible gastroenteritis coronavirus (MERS-CoV surrogate)	(41)
	Single	Filoviridae	Ebola virus	(67)
RNA (Non-enveloped)	Single	Calciviridae	Feline Calcivirus	(29;30;41;50)
			Murine Norovirus	(47;50)
			Vesicular Exanthema Virus	(22)
	Single	Picornaviridae	Poliovirus Type 1	(49;54)
			Foot and Mouth Disease Virus	(53;74)
			Swine Vesicular Disease Virus	(22)
	Double	Reoviridae	Bluetongue Virus	(22)
	Double	Birnaviridae	Infectious Bursal Disease Virus (IBDV)	(77)

1.3 Bacteriophage

Type of organism	Name	Reference
	Lactococcal bacteriophage *MS2 bacteriophage*	(31;47) (32)

1.4 Fungi

Type of organism	Name	Reference
	Alternaria sp. *Aspergillus brasiliensis* *(formerly Aspergillus niger)* *Candida albicans* *Candida auris* *Candida parapsilosis* *Coccidioides immitis* *Blastomyces dermatitidis* *Histoplasma capsulatum* *Penicillium sp* *Sacchromyces cerevisiae*	(33) (6;7;47) (7;47) (56;73) (6) (34) (34) (34) (33) (69)

1.5 Nematodes and protozoa

Type of organism	Name	Reference
	Caenorhabditis elegans *Syphacia muris**	(35) (36)

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

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

1.6 Other

- There is evidence that hydrogen peroxide vapour can inactivate prions (37).
- Biofilms of multi-drug resistant *Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterococcus faecalis* have all been inactivated using hydrogen peroxide vapour (76).

References

Please note, Hydrogen Peroxide Vapour may be abbreviated as HPV in the following References and Appendix section.

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 vapor for environmental control during a Serratia outbreak in a neonatal intensive care unit. *J Hosp Infect* 2005;61(4):346-6.

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. 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).

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 testing data. Contact Bioquell for further details.

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. Petit B.M, Almeida F.C, Urchiyama T.R, Lopes F.O.C, Tino K.H and Chewins J. Evaluating the efficacy of hydrogen peroxide vapour against foot-and-mouth disease virus within a BSL4 biosafety facility. *Lett. Appl. Microbiol.* 2017; 65: 281-284..

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.

56. Abdolrasouli A, Armstrong-James D, Ryan L and Schelenz, S. In vitro efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with *Candida auris*. *Mycoses.* 2017;60: 758–763.

57. Ali S, Muzslay, M, Bruce M, Jeanes A, Moore G and Wilson A.P.R. Efficacy of two hydrogen peroxide vapour aerial decontamination systems for enhanced disinfection of meticillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae* and *Clostridium difficile* in single isolation rooms. *J. Hosp. Infect*. 2016; 93, 70-71

58. Back K, Ha J, Kang D. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella Typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. Food Control 2014; 44: 78-85.

59. Benga L, Benten W.P.M, Englehardt E, Gougoula C, Schulze-Robbecke R and Sager, M. Survival of bacteria of laboratory animal origin on cage bedding and inactivation by hydrogen peroxide vapour. *Lab. Anim*. 2016; 0: 1-10.

60. Cooper T, O'Leary M, Yezli S and Otter J.A. Impact of environmental decontamination using hydrogen peroxide vapour on the

incidence of *Clostridium difficile* infection in one hospital Trust. *J. Hosp. Infect.* 2011; 78: 238-245.

61. Horn K and Otter J.A. Hydrogen peroxide vapor room disinfection and hand hygiene improvements reduce *Clostridium difficile* infection, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and extended-spectrum b-lactamase. *American Journal of Infection Control* 2015; 43: 1354-6.
62. Lawley T.D, Clare S, Deakin L.J, Goulding D, Yen J.L, Raisen C, Brandt C, Lovell J, Cooke F, Clark T.G and Dougan G. Use of Purified *Clostridium difficile* Spores To Facilitate Evaluation of Health Care Disinfection Regimens. *Applied Environ. Microbiol*. 2010; 76: 20, 6895 – 6900.
63. Manian F.A, Griesnauer S and Bryant A. Implementation of hospital-wide enhanced terminal cleaning of targeted patient rooms and its impact on endemic *Clostridium difficile* infection rates. *American Journal of Infection Control*. 2013; 537-41.
64. Miedel E.L, Ragland N.H and Engleman, R.W. Facility-wide Eradication of *Corynebacterium bovis* by using PCR-validated Vaporized Hydrogen Peroxide. *Journal of American Association for Laboratory Animal Sciences*. 2018; 57: 5, 465-476.
65. Murdoch L.E, Bailey L, Banham E, Watson F, Adams N.M.T and Chewins J. Evaluating different concentrations of hydrogen peroxide in an automated room disinfection system. *Lett. Appl. Microbiol*. 2016; 63, 178-182
66. Otter J.A, Davies B, Menson E, Klein J.L, Watts T.L, Kearns A.M, Pichon B, Edgeworth J.D and French G.L. Identification and control of a gentamicin resistant, meticillin susceptible *Staphylococcus aureus* outbreak on a neonatal unit. *J. Infect. Prevention.* 2014; 15: 3, 104-109.
67. Otter J.A, Mepham S, Athan B, Mack D, Smith R, Jacobs M and Hopkins S. Terminal decontamination of the Royal Free London's high-level isolation unit after a case of Ebola virus disease using hydrogen peroxide vapor. *American Journal of Infection Control.* 2016; 44: 233-5.
68. Passaretti C, Otter J.A, Reich N.G, Myers J, Shepard J, Ross T, Carroll K.C, Lipsett P and Perl T.M. An Evaluation of Environmental Decontamination With Hydrogen Peroxide Vapor for Reducing the Risk of Patient Acquisition of Multidrug-Resistant Organisms. *Clinical Infectious Diseases.* 2013; 56: 1, 27-35.
69. Pazienza M, Britti M.S, Carestia M, Cenciarelli O, D'Amico F, Malizia A, Bellecci C, Gaudio P, Gucciardino A, Bellino M, Lancia C, Tamburrini A and Fiorito R. Application of Real-Time PCR to Identify Residual Bio-Decontamination of Confined Environments after Hydrogen Peroxide Vapor Treatment: Preliminary Results. *J. Microb. Biochem. Technol.* 2013; 6:1, 24-28.
70. Ragland N.H, Miedel E.L, Gomez J.M and Engleman R.W. Staphylococcus xylosus PCR-validated Decontamination of Murine Individually Ventilated Cage Racks and Air Handling Units by Using 'Active–Closed' Exposure to Vaporized Hydrogen Peroxide. *Journal American Association Laboratory Animal Science.* 2017; 57: 6, 742-751.
71. Reich R.R and Caputo R.A. Vapor-Phase Hydrogen Peroxide Resistance of Environmental Isolates. *Pharmaceutical Technology.* 2004; Aug, 50-57.
72. Rogers J.M, Richter W.R, Wendling M.Q.S and Shesky A.M. Inactivation of *Brucella suis, Burkholderia pseudomallei, Francisella tularensis,* and *Yersinia pestis* Using Vaporous Hydrogen Peroxide. *Applied Biosafety*. 2010; 15: 1, 25-31.
73. Schelenz S, Hagen F, Rhodes J.L, Abdolrasouli A, Chowdhary A, Hall A, Ryan L, Shackleton J, Trimlett R, Meis J.F, Armstrong-James D and Fisher M.C. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrobial Resistance and Infection Control.* 2016; 5: 35, 1-7.
74. Vannier M and Chewins J. Hydrogen peroxide vapour is an effective replacement for Formaldehyde in a BSL4 Foot and mouth disease vaccine manufacturing facility. *Lett. Appl. Microbiol.* 2019; 69: 237-245.
75. Waqar S, Nigh K, Sisler L, Fanning M, Tancin S, Brozik E, Jones R, Briggs F, Keller L, LaSala P.R, Krautz S and Khakoo R. Multidisciplinary performance improvement team for reducing health care-associated *Clostridium difficile* infection. *American Journal of Infection Control.* 2016; 44, 352-354.
76. Watson F, Keevil C.W, Wilks S.A and Chewins J. Modelling vaporised hydrogen peroxide efficacy against monospecies biofilms. *Nature Scientific Reports* 2018; 8: 12257.
77. Stuart J, Chewins J and Tearle J. Comparing the efficacy of Formaldehyde with Hydrogen Peroxide Fumigation on Infectious Bronchitis Virus. Data on file, contact Bioquell.

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

Please contact Bioquell for full copies of these articles

Abdolrasouli A, Armstrong-James D, Ryan L and Schelenz, S. In vitro efficacy of disinfectants utilised for skin decolonisation and environmental decontamination during a hospital outbreak with *Candida auris*. *Mycoses.* **2017;60: 758–763.**

Department of Microbiology, Royal Brompton Hospital, London, UK.

Candida auris has caused nosocomial infections and transmissions within hospital settings. As little is known about the efficacy of skin and environmental decontamination products to kill *C. auris*, this study investigated the in vitro activity of chlorine, chlorhexidine, iodine povidone and vaporised hydrogen peroxide products against *C. auris*. H_2O_2 vapour showed 96.6%-100% effective killing of *C. auris*. All isolates were inhibited by chlorhexidine gluconate concentrations at 0.125%-1.5% and for iodinated povidone at 0.07%-1.25%. Other species of *Candida* were also killed at 1000 ppm chlorine except *C. parapsilosis* which failed to be killed at 3 minutes contact time. We conclude that chlorhexidine gluconate, iodinated povidone, chlorine and H_2O_2 vapour demonstrate effective killing activity against *C. auris* at concentrations used in clinical practice.

Ali S, Muzslay, M, Bruce M, Jeanes A, Moore G and Wilson A.P.R. Efficacy of two hydrogen peroxide vapour aerial decontamination systems for enhanced disinfection of meticillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae* and *Clostridium difficile* in single isolation rooms. *J. Hosp. Infect.* **2016; 93, 70-71**

University College London Hospitals NHS Foundation Trust, London, UK

BACKGROUND: Hydrogen peroxide vapour (HPV) disinfection systems are being used to reduce patients' exposure to hospital pathogens in the environment. HPV whole-room aerial disinfection systems may vary in terms of operating concentration and mode of delivery.

AIM: To assess the efficacy of two HPV systems (HPS1 and HPS2) for whole-room aerial disinfection of single isolation rooms (SIRs).

METHODS: Ten SIRs were selected for manual terminal disinfection after patient discharge. Test coupons seeded with biological indicator (BI) organisms [~10^6 colony-forming units (cfu) of meticillin-resistant *Staphylococcus aureus* (MRSA) or *Klebsiella pneumoniae*, or ~10^5 cfu *Clostridium difficile* 027 spores] prepared in a soil challenge were placed at five locations per room. For each cycle, 22 high-frequency-touch surfaces in SIRs were sampled with contact plates (~25 cm2) before and after HPV decontamination, and BIs were assayed for the persistence of pathogens.

FINDINGS: Approximately 95% of 214 sites were contaminated with bacteria after manual terminal disinfection, with high numbers present on the SIR floor (238.0 - 352.5 cfu), bed control panel (24.0 - 33.5 cfu), and nurse call button (21.5 - 7.0 cfu). Enhanced disinfection using HPV reduced surface contamination to low levels: HPS1 [0.25 cfu, interquartile range (IQR) 0 - 1.13] and HPS2 (0.5 cfu, IQR 0 - 2.0). Both systems demonstrated similar turnaround times (~2 - 2.5 h), and no differences were observed in the efficacy of the two systems against BIs (*C. difficile* ~5.1 log^{10} reduction; MRSA/K. pneumoniae ~6.3 log^{10} reduction). Despite different operating concentrations of hydrogen peroxide, MRSA persisted on 27% of coupons after HPV decontamination.

CONCLUSION: Enhanced disinfection with HPV reduces surface contamination left by manual terminal cleaning, minimizing the risks of cross-contamination. The starting concentration and mode of delivery of hydrogen peroxide may not improve the efficacy of decontamination in practice, and therefore the choice of HPV system may be based upon other considerations such as cost, convenience and logistics.

Back K, Ha J, Kang D. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella Typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Control* **2014; 44: 78-85.**

In this study, the efficacy of hydrogen peroxide vapor (HPV) for reducing *Salmonella Typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce was investigated as well as its effect on lettuce quality. Lettuce was inoculated with a cocktail containing three strains of each pathogen then treated with vaporized hydrogen peroxide for 0, 2, 4,6, 8 and 10 min.

The concentrations of hydrogen peroxide used were 0, 1, 3, 5 and 10%. With increasing treatment time and hydrogen peroxide concentration, HPV treatment showed significant ($P < 0.05$) reduction compared to the control (0%, treated with vaporized distilled water). In particular, vaporized 10% hydrogen peroxide treatment for 10 min was the most effective combination for reducing the three pathogens on lettuce. The reduction levels of *S. Typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* on lettuce were 3.12, 3.15 and 2.95 log10 CFU/g, respectively. Furthermore, there were no significant ($P > 0.05$) quality changes (color and texture) of lettuce among all tested samples, and hydrogen peroxide residues were not detected after 36 h storage time in any of the treated samples. These results suggest that HPV treatment could be an alternative method for reducing *S. Typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* on fresh produce.

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*, Hopital 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

Benga L, Benten W.P.M, Englehardt E, Gougoula C, Schulze-Robbecke R and Sager, M. Survival of bacteria of laboratory animal origin on cage bedding and inactivation by hydrogen peroxide vapour. *Lab. Anim.* **2016; 0: 1-10.**

Institute for Medical Microbiology and Hospital Hygiene, University Hospital, Heinrich-Heine University, Dusseldorf, Germany

This study aims to determine the ability of laboratory animal bacteria to resist desiccation and inactivation by hydrogen peroxide vapour (HPV) on paper bedding pieces. Bedding pieces were saturated with bacterial suspensions in water or 2% (w/v) bovine serum albumin (BSA) in water, and held in a mouse facility. Viable counts showed variable survival rates over time for the bacterial species used *([Pasteurella] pneumotropica, Muribacter muris, Pseudomonas aeruginosa, Acinetobacter redioresistens, Escherichia coli, Klebsiella oxytoca, Bordetella bronchiseptica, Bordetella hinzii, Enterococcus faecalis, b-haemolytic Streptococcus spp., Staphylococcus aureus and Staphylococcus xylosus).* Overall, BSA increased bacterial survival in the bedding pieces. The survival rates of *Bacillus safensis* were not influenced by BSA but depended on sporulation. When bedding pieces and Petri dishes inoculated with *E. coli*, *P. aeruginosa* and *S. aureus* were subjected to HPV disinfection, all bacterial species on the bedding pieces inoculated with bacterial suspensions in water were readily inactivated. By contrast, *S. aureus* and *P. aeruginosa*, but not *E. coli* cells survived HPV treatment in high numbers when inoculated on bedding pieces as a BSA suspension. Notably, all three bacterial

species were readily inactivated by HPV even in the presence of BSA when smeared on smooth surfaces. In conclusion, the suspension medium and the carrier can influence the environmental survival and susceptibility of bacterial species to HPV. Our results may help to develop standard protocols that can be used to ensure the microbiological quality of experimental rodent housing.

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 which had been artificially contaminated with Feline Calicivirus (FCV), a surrogate for norovirus. The surfaces tested were representative of those found in hospital wards.

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

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

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

Berrie E, Andrews L, Yezli S, Otter JA. Hydrogen peroxide vapour (HPV) inactivation of Adenovirus. *Lett Appl Microbiol* **2011;52(5):555-558.**

Clinical BioManufacturing Facility, University of Oxford, Oxford, UK.

AIMS: Adenovirus contamination can be problematic in various settings including life science laboratories and during pharmaceutical manufacturing processes. Stringent and effective decontamination procedures are necessary to minimise the risk of personnel exposure or product cross contamination in these settings. Hydrogen peroxide vapour (HPV) is sporicidal, tuberculocidal and fungicidal with proven efficacy against some viruses. We investigate the efficacy of HPV for the inactivation of 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 45 min exposure to HPV in a microbiological safety cabinet.

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

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

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.

Cooper T, O'Leary M, Yezli S and Otter J.A. Impact of environmental decontamination using hydrogen peroxide vapour on the incidence of *Clostridium difficile* infection in one hospital Trust. *J. Hosp. Infect.* **2011; 78: 238-245.**

South London Healthcare NHS Trust, Sidcup, UK

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 Country Hospital, Winchester, UK / Bioquell / Health Protection Agency, UK.

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

Eterpi M, McDonnell G and Thomas V. Virucidal Activity of Disinfectants Against Parvoviruses and Reference Viruses. *Applied Biosafety.* **2010; 15: 4, 165-171.**

STERIS R&D, Fontenay-aux-Roses, Hauts-de-Seine, France

Some virus species can resist surprisingly harsh environmental conditions, being able to survive on surfaces for long periods and often presenting high resistance to disinfection. Viruses can be transmitted to susceptible hosts via these contaminated surfaces. This is a major concern not only in healthcare and research but also in manufacturing settings, with recent occurrences reporting viral contamination of non-human cells used to produce therapeutic enzymes in bioreactors. Consequently, a need to validate the virucidal efficacy of products used to clean and decontaminate surfaces in these facilities exists. In this study we tested two alkaline cleaners (CIP-100 and ProKlenz-One), two liquid disinfectants (SporKlenz and a peracetic-based formulation), and a gaseous hydrogen peroxide-based aerial decontamination process for their capacity to inactivate several viruses, including parvoviruses in the presence of organic soils. We demonstrate that these decontamination methods offer virucidal efficacy and can consequently ensure a very high level of protection against viral contamination.

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 meticillinresistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and *Acinetobacter baumannii.* Safety was assessed by detecting leakage of hydrogen peroxide using a handheld detector. Efficiency was assessed by measuring the level of hydrogen peroxide using a hand-held sensor at three locations inside the room, 2 h 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 (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.

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 40-mL aliquots and exposed to HPV produced by a Clarus L generator (Bioquell, Horsham, PA, USA) in a 0.2-m^3 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.

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-log10 inoculum of *M. tuberculosis* on stainless steel discs and a 6-log10 inoculum of *Geobacillus stearothermophilus* were exposed to HPV in BSC time course experiments and at 10 locations during room experiments. In three separate BSC experiments, *M. tuberculosis* BIs were transferred to growth media at 15 min intervals during a 180 min HPV exposure period. No *M. tuberculosis* BIs grew following 30 min of HPV exposure. In three separate room experiments, *M. tuberculosis* and *G. stearothermophilus* BIs were exposed to HPV for 90, 120, and 150 min, respectively. BIs for both microorganisms were deactivated in all 10 locations following 90 min of HPV exposure. HPV provides an alternative to traditional decontamination methods, such as formaldehyde fumigation, for laboratories and other areas contaminated with *M. tuberculosis*.

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

Mayo Clinic, Rochester, MN,USA / Bioquell.

Hydrogen peroxide vapor (HPV) has been proposed as an alternative to formaldehyde fumigation for the decontamination of biosafety level (BSL) III laboratories. The aim of this study was to evaluate the efficacy of HPV against the dimorphic fungi *Histoplasma capsulatum, Blastomyces dermatitidis* and *Coccidioides immitis*. Working inside a class II biological safety cabinet (BSC) within a BSL III laboratory, inocula containing approximately 5-log10 CFU/ml from the mold form of each organism suspended in RPMI medium were deposited on stainless steel discs and allowed to air dry. The organisms were exposed to HPV inside a BSC using a Bioquell Clarus S HPV generator. In three replicate experiments, individual discs were transferred into liquid media at timed intervals during a 105 min HPV exposure period. Control and HPV exposed discs were incubated in RPMI media at 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, dermatitidis* and *C. immitis* were killed within 30 min 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 104 BIs did not grow, and 22% did not grow after UVC, with a range of 7%–53% for the 5 sites. For the 106 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 30 min. For all viruses tested and under all conditions (except one), the decontamination process reduced the virus titer to zero embryo-lethal doses for the avian viruses (avian influenza and Newcastle disease viruses) or less than 10 tissue culture infective doses for the mammalian viruses (African swine fever, bluetongue, hog cholera, pseudorabies, swine vesicular disease, vesicular exanthema, and vesicular stomatitis viruses). The laboratory equipment exposed to the gas appeared to suffer no adverse effects. Vapor-phase hydrogen peroxide decontamination can be recommended as a safe and efficacious way of removing potentially virus-contaminated objects from biocontainment level III laboratories in which exotic animal disease virus agents are handled.

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 35-mm wells of 6-well plastic plates. These plates were placed in various positions in a nonoccupied 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.

Horn K and Otter J.A. Hydrogen peroxide vapor room disinfection and hand hygiene improvements reduce *Clostridium difficile*

infection, methicillin-resistant *Staphylococcus aureus,* vancomycin-resistant enterococci, and extended-spectrum b-lactamase. *American Journal of Infection Control* **2015; 43: 1354-6.**

Flagstaff Medical Center, Flagstaff, AZ

We report a statistically significant reduction in *Clostridium difficile* infection (from 1.38 to 0.90 cases per 1,000 patient days), vancomycin-resistant enterococci (from 0.21 to 0.01 cases per 1,000 patient days), and extended-spectrum b-lactamase producing gram-negative bacteria (from 0.16 to 0.01 cases per 1,000 patient days) associated with the introduction of hydrogen peroxide vapor for terminal decontamination of patient rooms and improvements in hand hygiene compliance.

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

University Hospital Lewisham, London, UK.

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

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

Unilever / Bioquell.

The aim of this study was to evaluate the efficacy of hydrogen peroxide vapour (HPV) against spores of *Clostridium botulinum*, for use as a method for decontaminating environments where this pathogen has been handled. Spores were dried onto stainless steel slides and exposed to HPV in a sealed glovebox enclosure, transferred to a quenching agent at timed intervals during the exposure period, before survivors were cultured and enumerated. D-values were calculated from graphs of log^{10} survivors plotted against time and were found to range from 1.41 to 4.38 min. HPV was found to be effective at deactivating spores of toxigenic *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.

Lawley T.D, Clare S, Deakin L.J, Goulding D, Yen J.L, Raisen C, Brandt C, Lovell J, Cooke F, Clark T.G and Dougan G. Use of Purified *Clostridium difficile* Spores To Facilitate Evaluation of Health Care Disinfection Regimens. *Applied Environ. Microbiol.* **2010; 76: 20, 6895 – 6900.**

Microbial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Cambridge, UK.

Clostridium difficile is a major cause of antibiotic-associated diarrheal disease in many parts of the world. In recent years, distinct genetic variants of *C. difficile* that cause severe disease and persist within health care settings have emerged. Highly resistant and infectious *C. difficile* spores are proposed to be the main vectors of environmental persistence and host transmission, so methods to accurately monitor spores and their inactivation are urgently needed. Here we describe simple quantitative methods, based on purified *C. difficile* spores and a murine transmission model, for evaluating health care disinfection regimens. We demonstrate that disinfectants that contain strong oxidizing active ingredients, such as hydrogen peroxide, are very effective in inactivating pure spores and blocking spore-mediated transmission. Complete inactivation of 10^6 pure *C. difficile* spores on indicator strips, a six-log reduction, and a standard measure of stringent disinfection regimens require at least 5 min of exposure to hydrogen peroxide vapor (HPV; 400 ppm). In contrast, a 1-min treatment with HPV was required to disinfect an environment that was heavily contaminated with *C. difficile* spores (17 to 29 spores/cm2) and block host transmission. Thus, pure *C. difficile* spores facilitate practical methods for evaluating the efficacy of *C. difficile* spore disinfection regimens and bringing scientific acumen to *C. difficile* infection control.

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 nonporous 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 methicillinresistant *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 nonporous 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 nonporous surfaces.

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

Manian F.A, Griesnauer S and Bryant A. Implementation of hospital-wide enhanced terminal cleaning of targeted patient rooms and its impact on endemic *Clostridium difficile* infection rates. *American Journal of Infection Control.* **2013; 537-41.**

Department of Infection Control, Mercy Hospital, St Louis, USA.

BACKGROUND: Implementation of a hospital-wide program of terminal cleaning of patient rooms revolving around hydrogen peroxide vapor

(HPV) technology and evaluation of its impact on endemic nosocomial *Clostridium difficile*-associated diarrhea (CDAD) have not been previously reported.

METHODS: This was a retrospective quasi experimental study involving a 900-bed community hospital. During the pre intervention period (January 2007-November 2008), rooms vacated by patients with CDAD or on contact precautions for other targeted pathogens underwent 1 or more rounds of cleaning with bleach. During the intervention period (January-December 2009), targeted newly evacuated rooms underwent "enhanced cleaning" consisting of use of bleach followed by HPV decontamination utilizing a priority scale based on the pathogen and room location. Rooms vacated by patients with CDAD but for which HPV decontamination was not possible the same day underwent 4 rounds of cleaning with bleach instead.

RESULTS: During the intervention period, 1,123 HPV decontamination rounds were performed involving 96.7% of hospital rooms. Of 334 rooms vacated by patients with CDAD (May-December 2009), 180 (54%) underwent HPV decontamination. The rate of nosocomial CDAD rate dropped significantly from 0.88 cases/1,000 patient-days to 0.55 cases/1,000 patient-days (rate ratio, 0.63; 95% confidence interval: 0.50-0.79, $P < .0001$).

CONCLUSION: A hospital-wide program of enhanced terminal cleaning of targeted patient rooms revolving around HPV technology was practical and was associated with a significant reduction in CDAD rates.

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

Miedel E.L, Ragland N.H and Engleman, R.W. Facility-wide Eradication of *Corynebacterium bovis* by using PCR-validated Vaporized Hydrogen Peroxide. *Journal of American Association for Laboratory Animal Sciences.* **2018; 57: 5, 465-476.**

H. Lee Cancer Center & Research Institute, University of South Florida, Tampa, Florida

Facility-wide *Corynebacterium bovis* eradication was established using vaporized hydrogen peroxide (VHP) decontamination guided by *C. bovis* PCR surveillance. Prior attempts limited to culling PCR-positive mice and decontaminating affected rooms were ineffective in preventing

recurrence. Because research aims often require trafficking to and use of procedural cores, a 12-mo facility-wide *C. bovis* PCR surveillance of 2064 specimens was performed and documented that, despite the presence of few clinically hyperkeratotic mice, 35% of the murine housing and use space was contaminated by *C. bovis*. The airways of IVC racks and air-handling units (AHU) provided a substantive niche for *C. bovis* survival, comparable to the primary enclosure, with 26% of murine and 22% of airway specimens PCR-positive for *C. bovis*. Equipment airway VHP sterilization in a 'flex room' required an 'active–closed' setting with the IVC rack connected to the AHU set to the VHP cycle, because 12% of specimens from 'static–open' VHP-exposed airways remained PCR-positive for *C. bovis*, whereas 0% of specimens from active–closed VHP exposures were positive. VHP decontamination of the 29,931-ft2 facility was completed in 2 mo. *C. bovis* PCR testing of IVC exhaust plenums for 200 d in previously *C. bovis*-affected rooms confirmed that none of the 259 specimens tested were PCR-positive for the organism. Monthly surveillance identified a single recurrence during June 2017 (month 9), ensuring rapid culling of *C. bovis* PCR-positive mice and acute VHP decontamination of equipment and rooms. Molecular persistence of *C. bovis* was resolved in procedural and personnel areas, and no murine or housing specimens tested *C. bovis* PCR-positive during study months 11 and 12. Furthermore, since the conclusion of the 12-mo study, none of the 452 additional murine, cell biologic, environmental, and monthly equipment surveillance specimens tested were *C. bovis* PCR-positive, documenting an 11-mo period of facility-wide *C. bovis* eradication to date. Study invalidation due to *C. bovis* can be avoided through PCR.

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.

Murdoch L.E, Bailey L, Banham E, Watson F, Adams N.M.T and Chewins J. Evaluating different concentrations of hydrogen peroxide in an automated room disinfection system. *Lett. Appl. Microbiol.* **2016; 63, 178-182**

Wickham Laboratories Limited, Hoeford Point, Gosport, UK.

A comparative study was made on the efficacy of 5, 10 and 35% weight by weight (w/w) hydrogen peroxide solutions when applied using an automated room disinfection system. Six-log biological indicators of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Geobacillus stearothermophilus* were produced on stainless steel coupons and placed within a large, sealed, environmentally controlled enclosure. Five percent hydrogen peroxide was distributed throughout the enclosure using a Bioquell hydrogen peroxide vapour generator (BQ-50) for 40 min and left to reside for a further 200 min. Biological indicators were removed at 10-min intervals throughout the first 120 min of the process. The experiment was repeated for 10 and 35% hydrogen peroxide solutions. Five percent and 10% hydrogen peroxide solutions failed to achieve any reduction of MRSA, but achieved full kill of *G. stearothermophilus* spores at 70 and 40 min respectively. Thirty-five percent hydrogen peroxide achieved a 6-log reduction of MRSA after 30 min and full kill of *G. stearothermophilus* at 20 min. The concentration of 5% hydrogen peroxide within the enclosure after the 200-min dwell was measured at 9.0 ppm. This level

exceeds the 15-min Short Term Exposure Limit (STEL) for hydrogen peroxide of 2.0 ppm. Users of automated hydrogen peroxide disinfection systems should review system efficacy and room re-entry protocols in light of these results.

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

Danisco Deutschland / Bioquell.

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

Otter JA, Chewins J, 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 J.A, Davies B, Menson E, Klein J.L, Watts T.L, Kearns A.M, Pichon B, Edgeworth J.D and French G.L. Identification and control of a gentamicin resistant, meticillin susceptible *Staphylococcus aureus* outbreak on a neonatal unit. *J. Infect. Prevention.* **2014; 15: 3, 104-109.**

Centre for Clinical Infection and Diagnostics Research (CIDR), Department of Infectious Diseases, King's College London and Guy's and St Thomas NHS Foundation Trust, London, UK.

We describe the identification and control of an outbreak of gentamicin resistant, methicillin susceptible *Staphylococcus aureus* (GRMSSA) on a 36-bed neonatal unit (NNU) in London. Control measures included admission and weekly screening for GR-MSSA, cohorting affected babies, environmental and staff screening, hydrogen peroxide vapour (HPV) for terminal disinfection of cohort rooms, and reinforcement of hand hygiene. Seventeen babies were affected by the outbreak strain over ten months; seven were infected and ten were asymptomatic carriers. The outbreak strain was gentamicin resistant and all isolates were indistinguishable by pulsed-field gel electrophoresis. The outbreak strains spread rapidly and were associated with a high rate of bacteraemia (35% of 17 affected patients had bacteraemia vs. 10% of 284 patients with MSSA prior to the outbreak, p=0.007). None of 113 staff members tested were colonised with GR-MSSA. GR-MSSA was recovered from 11.5% of 87 environmental surfaces in cohort rooms, 7.1% of 28 communal surfaces and 4.1% of 74 surfaces after conventional terminal disinfection. None of 64 surfaces sampled after HPV decontamination yielded GR-MSSA. Recovery of GR-MSSA from two high level sites suggested that the organism could have been transmitted via air. Occasional breakdown in hand hygiene compliance and contaminated environmental surfaces probably contributed to transmission.

Otter JA, French GL. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapour (HPV). *J Clin Microbiol* **2009;47:205-207.**

St. Thomas' Hospital / King's College London / Bioquell.

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

Otter J.A, Mepham S, Athan B, Mack D, Smith R, Jacobs M and Hopkins S. Terminal decontamination of the Royal Free London's high-level isolation unit after a case of Ebola virus disease using hydrogen peroxide vapor. *American Journal of Infection Control.* **2016; 44: 233-5.**

Centre for Clinical Infection and Diagnostics Research, Department of Infectious Diseases, King's College London & Guy's and St. Thomas' NHS Foundation Trust, London, UK

We report the decontamination of the high-level isolation unit at the Royal Free London after the discharge of a patient with Ebola virus disease, who was repatriated from West Africa. Hydrogen peroxide vapor (HPV) was used to decontaminate the patient care isolators and the rooms housing them. HPV decontamination was completed without incident and allowed the unit to be returned to service more quickly than the previous protocol of using formaldehyde.

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 meticillinresistant *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 >60 min 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.

Passaretti C, Otter J.A, Reich N.G, Myers J, Shepard J, Ross T, Carroll K.C, Lipsett P and Perl T.M. An Evaluation of Environmental Decontamination With Hydrogen Peroxide Vapor for Reducing the Risk of Patient Acquisition of Multidrug-Resistant Organisms. *Clinical Infectious Diseases.* **2013; 56: 1, 27-35.**

Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

BACKGROUND: Admission to a room previously occupied by a patient with certain multidrug-resistant organisms (MDROs) increases the risk of acquisition. Traditional cleaning strategies do not remove all environmental MDROs. We evaluated the environmental and clinical impact of hydrogen peroxide vapor (HPV) room disinfection.

METHODS: We performed a 30-month prospective cohort intervention study on 6 high-risk units in a 994-bed tertiary care hospital. Following a 12-month pre-intervention phase, HPV was implemented on 3 units to decontaminate the rooms of patients known to be infected or colonized with epidemiologically important MDROs, following their discharge. Monthly environmental samples for MDROs were collected on all study units for 3 pre-intervention and 6 intervention months. The risk of MDRO acquisition in patients admitted to rooms decontaminated using HPV was compared with rooms disinfected using standard methods.

RESULTS: The prior room occupant was known to be infected or colonized with an MDRO in 22% of 6350 admissions. Patients admitted to rooms decontaminated using HPV were 64% less likely to acquire any MDRO (incidence rate ratio [IRR], 0.36; 95% confidence interval [CI], .19–.70; P < .001) and 80% less likely to acquire VRE (IRR, 0.20; 95% CI, .08–.52; P < .001) after adjusting for other factors. The risk of acquiring *Clostridium difficile*, methicillin resistant *Staphylococcus aureus*, and multidrug-resistant gram-negative rods individually was reduced, but not significantly. The proportion of rooms environmentally contaminated with MDROs was reduced significantly on the HPV units (relative risk, 0.65, P = .03), but not on non-HPV units.

CONCLUSION: HPV decontamination reduced environmental contamination and the risk of acquiring MDROs compared with standard cleaning protocols.

Pazienza M, Britti M.S, Carestia M, Cenciarelli O, D'Amico F, Malizia A, Bellecci C, Gaudio P, Gucciardino A, Bellino M, Lancia C, Tamburrini A and Fiorito R. Application of Real-Time PCR to Identify Residual Bio-Decontamination of Confined Environments after Hydrogen Peroxide Vapor Treatment: Preliminary Results. *J. Microb. Biochem. Technol.* **2013; 6:1, 24-28.**

Department of Industrial Engineering, University of Rome "Tor Vergata", Rome, Italy

This study was conducted to assess the effectiveness of Hydrogen Peroxide Vapor (HPV) to remove biological contamination in a confined environment and to evaluate real-time PCR assay as a technique for the evaluation of the decontamination efficiency. Decontamination after the dispersion of biological aerosol is a main issue from a civilian, public health and military perspective. Despite the effectiveness of aggressive substances, eco-friendly but still efficient methods for decontamination are a relevant demand and Hydrogen Peroxide Vapor (HPV) is among the most recent and promising technologies in this field. Another related issue is: when an environment can be considered fully decontaminated? The answer clearly depends on the objectives of the

decontamination and this will affect the choice of the methodology.

Furthermore, classical microbiological and molecular biology techniques are commonly used to identify biological contamination and residual contamination, but many of them are time consuming and require advanced training for the operators who perform the analysis. This may represent a bottleneck, especially when a quick response to an emergency is needed (i.e. during an unconventional event like CBRNe ones). In this work, a combination of commercially available equipment for detection, identification and decontamination, was evaluated in partnership between the Italian Army, the Department of Industrial Engineering and the School of Medicine and Surgery of the University of Rome "Tor Vergata". The purpose of this work was to find a setup for equipment and methodologies for detection, identification and decontamination, to implement in case of biological events. Preliminary results show that, despite the death of the microorganisms, nucleic acids are not completely degraded by HPV treatment and, as a consequence, that real-time PCR may be the adequate, quick and easy method to verify the efficiency of bio decontamination when nucleic acid degradation represent the final objective.

Petit B.M, Almeida F.C, Urchiyama T.R, Lopes F.O.C, Tino K.H and Chewins J. Evaluating the efficacy of hydrogen peroxide vapour against foot-and-mouth disease virus within a BSL4 biosafety facility. *Lett. Appl. Microbiol.* **2017; 65: 281-284.**

INOVA Biotecnologia, Juatuba, Minas Gerais, Brazil

An evaluation was made of the efficacy of 35% hydrogen peroxide vapour (HPV) against foot-and-mouth disease virus (FMDV) in a biosafety facility. Biological indicators (BIs) were produced using three serotypes of FMDV, all with a titre of $\geq 10^6$ TCID50 per ml. Fifteen BIs of each serotype were distributed across five locations, throughout a 30-m^3 airlock chamber, producing a total of 45 BIs. Thirty-five percent HPV was generated and applied using a Bioquell vaporization module located in the centre of the chamber. After a dwell period of 40 min, the HPV was removed via the enclosures air handling system and the BIs were collected. The surfaces of the BIs were recovered into Glasgow's modified Eagle's medium (GMEM), cultivated in BHK21 Cl13 cell culture and analysed for evidence of cytopathic effect (CPE). No CPE was detected in any BI sample. Positive controls showed CPE. The experimentation shows that FMDV is susceptible to HPV decontamination and presents a potential alternative to formaldehyde.

Pottage, T., C. Richardson, S. Parks, J. T. Walker, and A. M. Bennett. Evaluation of hydrogen peroxide gaseous disinfection systems to decontaminate viruses. *J Hosp Infect* **2010;74:55-61.**

Biosafety Group, Novel and Dangerous Pathogens, Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury SP4 0JG, UK.

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

Ragland N.H, Miedel E.L, Gomez J.M and Engleman R.W. *Staphylococcus xylosus* PCR-validated Decontamination of Murine Individually Ventilated Cage Racks and Air Handling Units by Using 'Active–Closed' Exposure to Vaporized Hydrogen Peroxide. *Journal American Association Laboratory Animal Science.* **2017; 57: 6, 742-751.**

Department of Comparative Medicine, H Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, Florida, USA.

Vaporized hydrogen peroxide (VHP) is used to decontaminate clinical, biocontainment, and research animal rooms and equipment. To assist with its implementation in a murine facility, we developed a safe and effective method of VHP sterilization of IVC racks and air handling units (AHU). Safety of VHP decontamination was assessed by ensuring VHP levels dissipated to less than 1 ppm in the room prior to personnel reentry and inside the primary enclosure prior to the return of mice; this condition occurred at least 18 h after the VHP cycle. Efficacy of VHP sterilization was assessed by using chemical indicators, biologic indicators, and PCR testing for *Staphylococcus xylosus*, a commensal organism of murine skin and an opportunistic pathogen, which was present in 160 of 172 (93%) of specimens from occupied IVC racks and the interior surfaces of in-use AHU. Neither mechanized washing nor hand-sanitizing eradicated *S. xylosus* from equipment airway interiors, with 17% to 24% of specimens remaining PCR-positive for *S. xylosus*. 'Static–open' VHP exposure of sanitized equipment did not ensure its sterilization. In contrast, 'active–closed' VHP exposure, in which IVC racks were assembled, sealed, and connected to AHU set to the VHP cycle, increased the proportion of chemical indicators that detected sterilizing levels of VHP inside the assembled equipment, and significantly decreased PCR-detectable *S. xylosus* inside the equipment. Supplementing bulk steam sterilization of the primary enclosure with VHP sterilization of the secondary housing equipment during room change-outs may help to mitigate opportunistic agents that jeopardize studies involving immunodeficient strains.

Reich R.R and Caputo R.A. Vapor-Phase Hydrogen Peroxide Resistance of Environmental Isolates. *Pharmaceutical Technology.* **2004; Aug, 50-57.**

The authors examine the vapor-phase hydrogen peroxide resistance of microbial isolates recovered from the controlled environments of pharmaceutical and/or medical device companies and compared them with commercially available biological indicators under various test conditions.

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 2 hrs. A significant reduction in the log^{10} CFU of *Y. pestis* on all 10 materials was observed between the controls evaluated after a 1 hr drying time and unexposed controls evaluated after the decontamination run. Qualitative growth assessment showed that vapour-phase hydrogen peroxide exposure inactivated Y. pestis on all replicates of the 10 test materials as well as biological indicators up to seven days post exposure.

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

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

Rogers J.M, Richter W.R, Wendling M.Q.S and Shesky A.M. Inactivation of *Brucella suis, Burkholderia pseudomallei, Francisella tularensis*, and *Yersinia pestis* Using Vaporous

Hydrogen Peroxide. *Applied Biosafety.* **2010; 15: 1, 25-31.**

Battelle Memorial Institute, Columbus, Ohio, USA.

This study evaluated the inactivation of *Brucella suis, Burkholderia pseudomallei, Francisella tularensis*, and *Yersinia pestis* on glass, Hypalon® rubber glove, and stainless steel using vaporous hydrogen peroxide fumigation of a ~15 m^3 chamber. A suspension of approximately 1 x 10^8 colony forming units (CFU) of each organism was dried on coupons of each type of test surface and exposed to vaporous hydrogen peroxide. A significant reduction in the log^{10} CFU of each organism on all test materials was observed between the controls evaluated after a 1-hour drying time and unexposed controls evaluated after decontamination. For all organisms, qualitative growth assessments showed that vaporous hydrogen peroxide exposure completely inactivated bacterial viability on all replicates of the test materials incubated up to 7 days post-exposure. In parallel, all *Geobacillus stearothermophilus* biological indicators (BI) exposed to vaporous hydrogen peroxide exhibited no growth after 1 and 7 days incubation. This study provides information on using a combination of quantitative and qualitative growth assessments to evaluate vaporous hydrogen peroxide for the surface decontamination of *B. suis, B. pseudomallei, F. tularensis*, and *Y. pestis* within a large-scale chamber.

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

Battelle Memorial Institute, Columbus, OH, USA.

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

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

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

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

Rudnick, S. N., J. J. McDevitt, M. W. First, and J. D. Spengler. Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations. *Am J Infect Control* **2009;37:813-819.**

Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA.

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

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

RESULTS: After 2.5 min, exposure to 10 ppm HP

vapor resulted in 99% inactivation. For air saturated with TEG at 25 to 29°C, the disinfection rate was about 1.3-log[10] reductions per hour, about 16 times faster than the measured natural inactivation rate under ambient conditions.

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

Schelenz S, Hagen F, Rhodes J.L, Abdolrasouli A, Chowdhary A, Hall A, Ryan L, Shackleton J, Trimlett R, Meis J.F, Armstrong-James D and Fisher M.C. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrobial Resistance and Infection Control.* **2016; 5: 35, 1-7.**

Department of Microbiology, Royal Brompton Hospital, London, UK

BACKGROUND: *Candida auris* is a globally emerging multidrug resistant fungal pathogen causing nosocomial transmission. We report an ongoing outbreak of *C. auris* in a London cardio-thoracic center between April 2015 and July 2016. This is the first report of *C. auris* in Europe and the largest outbreak so far. We describe the identification, investigation and implementation of control measures.

METHODS: Data on *C. auris* case demographics, environmental screening, implementation of infection prevention/control measures, and antifungal susceptibility of patient isolates were prospectively recorded then analysed retrospectively. Speciation of *C. auris* was performed by MALDI-TOF and typing of outbreak isolates performed by amplified fragment length polymorphism (AFLP).

RESULTS: This report describes an ongoing outbreak of 50 *C. auris* cases over the first 16 month (April 2015 to July 2016) within a single Hospital Trust in London. A total of 44 % (n = 22/50) patients developed possible or proven *C. auris* infection with a candidaemia rate of 18 % (n = 9/50). Environmental sampling showed persistent presence of the yeast around bed space areas. Implementation of strict infection and prevention control measures included: isolation of cases and their contacts, wearing of personal protective clothing by health care workers, screening of patients on affected wards, skin decontamination with chlorhexidine, environmental cleaning with chorine based reagents and hydrogen peroxide vapour. Genotyping with AFLP demonstrated that *C. auris* isolates from the same geographic region clustered.

CONCLUSION: This ongoing outbreak with genotypically closely related *C. auris* highlights the importance of appropriate species identification and rapid detection of cases in order to contain hospital acquired transmission.

Stuart J, Chewins J and Tearle J. Comparing the efficacy of Formaldehyde with Hydrogen Peroxide Fumigation on Infectious Bronchitis Virus. Data on file, contact Bioquell.

Vannier M and Chewins J. Hydrogen peroxide vapour is an effective replacement for Formaldehyde in a BSL4 Foot and mouth disease vaccine manufacturing facility. *Lett. Appl. Microbiol.* **2019; 69: 237-245.**

Boehringer Inglelheim, Pirbright, Surrey, UK

An evaluation of the efficacy of 35% hydrogen peroxide vapour (HPV) against two strains of FMDV was conducted over a period of 6 months. FMDV biological indicators were produced on-site using strains obtained from a commercial FMDV vaccine manufacturing process. FMDV biological indicators were distributed within a BSL4 laboratory and exposed to short duration hydrogen peroxide cycles. Variations in titre, support matrix (soiling), temperature and humidity were evaluated in a series of 16 exposures using over 200 individual FMDV indicators. Additional verification testing was performed in an operational material transfer lock to replicate real-world use. HPV was found to be efficacious in inactivating FMDV strains; the inoculum titre influenced the level of reduction achieved with the specified cycle.

Waqar S, Nigh K, Sisler L, Fanning M, Tancin S, Brozik E, Jones R, Briggs F, Keller L, LaSala P.R, Krautz S and Khakoo R. Multidisciplinary performance improvement team for reducing health care-associated *Clostridium difficile* infection. *American Journal of Infection Control.* **2016; 44, 352-354.**

Department of Infectious Diseases, Robert C. Byrd Health Sciences Center, West Virginia University, West Virginia, USA.

Clostridium difficile is the most frequent cause of health care-associated diarrhea and is a significant cause of morbidity and mortality. It is also associated with a considerable financial burden. A concerted multidisciplinary approach is required for prevention.

Watson F, Keevil C.W, Wilks S.A and Chewins J. Modelling vaporised hydrogen peroxide efficacy against monospecies biofilms. *Nature Scientific Reports* **2018; 8: 12257.**

Environmental Healthcare Unit, Centre for Biological Sciences, University of Southampton, Southampton, UK.

This pilot study investigates a novel approach towards efficacy testing of antimicrobial cleaning agents; focusing primarily on hydrogen peroxide vapour (HPV). Contaminated surfaces are recognised modes of pathogen transmission within healthcare environments and increase the risk of pathogen acquisition in newly admitted patients. Studies have shown these pathogens can survive on surfaces for extended periods of time in spite of cleaning. This resilience is characteristic of biofilm formation and recent publications have identified their presence in hospitals. In this study, biofilm models comprised of multidrug-resistant organisms (MDROs) were generated using a drip flow reactor and exposed to HPV decontamination. The MDROs included *Acinetobacter baumannii*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Upon exposure, samples were periodically removed and enumerated to generate kill curves for each species. Consequently revealing any inherent resistances; such as catalase-producing organisms which expressed reduced susceptibility. Epifluorescence microscopy revealed an abundance of viable and non-viable microcolonies before and after decontamination, respectively. Greater than 6-Log^{10} reduction was achieved within a 100 minutes exposure time. This pilot study puts forward a potential methodology for testing antimicrobial agents against biofilms and supports the efficacy of HPV.

UK Headquarters
Bioquell UK Ltd
T: +44 (0)1264 835 835

The Americas
Bioquell Inc
T: +1 215 682 0225

France
Bioquell SAS
T: +33 (0)1 43 78 15 94

Germany
Bioquell GmbH
T: +49 (0) 221 168 996 74

Ireland
Bioquell Ireland
T: +353 (0)61 603 622

Singapore
Bioquell Asia Pacific Pte Ltd
T: +65 6592 5145

China
Bioquell Shenzhen
Technology Company Ltd
T: +86 755 8635 2622

Disclaimer: Please note that this document comprises marketing literature and is for summary information purposes only; customers or potential customers must not rely upon the contents of this document. Bioquell UK Ltd. or its affiliates, distributors, agents or licensees (together 'Bioquell') reserve the right to make changes to the contents of this document at any time and without prior notification. Use Bioquell systems and products safely. Always read the label and product information before use.

Bioquell is a registered trademark of Bioquell. © (2020). All rights reserved. BQ001-MKT-011 Rev 4