Journal of Microbiology and Infectious Diseases / JMID
2012; 2 (3): 93-99
doi: 10.5799/ahinjs.02.2012.03.0051

ORIGINAL ARTICLE

Validation of peracetic acid as a sporicide for sterilization of working surfaces in biological safety cabinets

Sandra Regina B.R. Sella [1,2], Belquis P. Guizelini [2], Hindy Ribeiro [3]

[1] *Production and Research Centre of Immunobiological Products, Parana State Department of Health, Piraquara-PR, Brazil*
[2] *Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Curitiba-PR, Brazil*
[3] *Pharmacy Department, Federal University of Paraná, Curitiba-PR, Brazil*

ABSTRACT

Objectives: This study aimed to validate the use of peracetic acid as sporicide agent to decontaminate the working surface of a laminar flow biological safety cabinet (BSC), as an alternative to glutaraldehyde, including the selection of the disinfecting agent, the method of application, and the contact time.

Materials and methods: The test organism was the spore-forming bacterium *Bacillus atrophaeus* ATCC 9372, which is a surrogate for an important infectious agent. Spore cultures were prepared from *B. atrophaeus* and used them to test the sporicidal efficacy of peracetic acid on a BSC stainless steel surface. The performance of the sterilant was assessed by determining minimal inhibitory concentration (MIC) and by microbial challenge in conditions that mimicked surface contamination. It was used 2.0% glutaraldehyde solution as the control.

Results: The range of MICs was 0.6-1.1% for the control and 0.003-0.006% for the 0.2% peracetic acid solution. The 0.2% peracetic acid was an effective sterilant against *B. atrophaeus* spores (6-7 log spores, under defined conditions of use) after 40 min contact time, which was double that recommended on the product label.

Conclusion: It was conclude that while the results of official methods can help to evaluate how products will perform, they are not usually reproducible in real-life user conditions and environments. Validation tests must be carried out to ensure the efficacy and safety of surface decontamination procedures. *J Microbiol Infect Dis 2012; 2(3): 93-99*

Key words: Peracetic acid, sporicidal activity, sterility, *Bacillus atrophaeus* spores, glutaraldehyde

Perasetik Asit'in sporosit olarak Biyolojik Güvenlik Kabinlerinde çalışma yüzeylerinin sterilizasyonunda validasyonu

ÖZET

Amaç: Bu çalışma, dezenfektan ajanın seçilmesi, uygulama metodu ve temas süresi dahil, perasetik asidin bir laminer akım biyolojik güvenlik kabinin (BGK) çalışma yüzeyi dekontaminasyonunda, gluteraldehite alternatif olarak, sporosit ajan olarak kullanılmasının validasyonu için yapıldı.

Gereç ve yöntem: Test organizması önemli bir enfeksiyon ajanı olan *Bacillus atrophaeus* ATCC 9372 sporları idi. *B. atrophaeus* sporlarıdan kültür hazırlandı ve bunlar bir BGK'nin paslanmaz çelik yüzeyinde perasetik asitin sporosidal etkinliğini test etmekte kullanıldı. Sterilleyicinin performansı, minimal inhibisyon konsantrasyonu (MİK) ve oluşturulan yüzey kontaminasyonu şartlarındaki mikrobiyal engellemesinin belirlenmesi ile değerlendirildi. Kontrol olarak % 2,0'lık gluteraldehit çözeltisi kullanıldı.

Bulgular: MİK değerleri kontrol için % 0,6-% 1,1 arasında ve % 0,2 perasetik asit çözeltisi için % 0,003 ile % 0,006 arasında değişmekteydi. Yüzde 0,2 perasetik asit çözeltisi *B. atrophaeus* sporlarına karşı, ürün etiketinde tavsiye edilenin yarısı kadar bir zamanda, 40 dakikalık temasla etkili bir sterilleyici idi (tanımlanan kullanma şartlarında 6-7 log spor).

Sonuç: Genel kabul gören metotlar ürünlerin etkinliğini araştırmada yardımcı olsalar bile genellikle gerçek hayattaki kullanıcıların şartlarında ve ortamında tekrarlanamazlar. Validasyon testleri yüzey dekontaminasyon prosedürlerinin etkinlik ve güvenilirliğini teyit etmede yerine getirilmelidirler.

Anahtar kelimler: Perasetik asit, sporosidal aktivite, sterilite, *Bacillus atrophaeus* sporları, gluteraldehit

Correspondence: Sandra R.B.R.Sella, Production and Research Centre of Immunobiological Products, Parana State Department of Health, Piraquara-PR, Brazil Email sella.sandra@gmail.com
Received: 21 April, 2012 Accepted: 17 September, 2012

INTRODUCTION

Biological Safety Cabinets (BSCs) are the primary means of containment developed for working safely with infectious microorganisms.[1] In order to achieve good laboratory practices (GLP), good manufacturing practices (GMP), and other regulatory requirements, a validated cleaning and sanitization program for laminar flow BSCs is essential.[2-4]

Liquid disinfectants are routinely used to decontaminate the working surfaces and other easily accessible internal parts of BSCs.[1] However, the selected disinfectant must be validated as being effective for this use. In particular, the sporicidal activity of a disinfectant must be validated when BSC use involves spore-forming organisms such as *Bacillus* sp. and *Clostridium* sp., as spores are highly resistant to disinfectants. An appropriate biological indicator for testing sporicidal efficacy is *Bacillus atrophaeus*, which forms bacterial endospores. Bacterial spores are highly resistant to physical and chemical agents[5,6] and only a few antibacterial agents are active sporicides.[7]

Activated glutaraldehyde solution is a powerful sporicidal agent. It is frequently used in the health care sector to disinfect equipment that cannot be heat sterilized. Booth and McDonald showed that solutions of 1 and 2% glutaraldehyde destroyed *Bacillus anthracis* spores more rapidly than did 4% formaldehyde.[8] However, glutaraldehyde has toxic effects on humans. Chronic inhalation affects the nose and respiratory tract, and lesions become severe with prolonged duration of exposure.[9] Therefore, the UK Health and Safety Commission has recommended substantial reductions in the use of glutaraldehyde, and the use of peracetic acid instead.[10]

Peracetic acid is mixture of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) in an aqueous solution. It is an oxidative agent commonly used as a disinfectant and sanitizer for contact surfaces, processing plants, and processing equipment.[11-13] It has a broad spectrum of activity, inactivating gram-positive and gram-negative bacteria, fungi, and yeasts. Peracetic acid acts rapidly, effectively removes organic matter, and leaves no residues. The main advantage of its use is the low toxicity of its decomposition products (acetic acid, water, oxygen, and hydrogen peroxide). It remains effective in the presence of organic matter and at low temperatures, but its use has been limited because of its corrosive properties.[14]

The efficacy of disinfectants is usually determined using microorganism suspensions. However, this does not reproduce the bacterial conditions on surfaces where the agents are required to inactivate the microbes.[15] In the standard test methods, biocontamination is carried out by depositing known concentrations of bacteria on glass or steel carrier surfaces.[16,17] Neither of these methods reproduces the conditions of a BSC. In this context, it is important to validate the sporicidal ability of a disinfectant in the user environment and conditions that most closely resemble the real surface contamination.[18,19]

This study aimed validates the use of peracetic acid as a sporicidal agent to effectively clean the surfaces of BSCs, as an alternative to glutaraldehyde.

MATERIALS AND METHODS

Bacterial strain

B. atrophaeus ATCC 9372, Batch-1403349, was obtained from Instituto Nacional de Controle de Qualidade em Saúde (INCQS/MS, Brazil).

Spore preparation

For inoculum production, spores (10^6 CFU) were inoculated into 30.0 mL tryptone soy broth (TSB) and then incubated for 18 h at 36°C, until the log-phase of growth. The sporulation culture was grown in agar as described by Sella et al. [20] The spore suspensions were subjected to heat treatment of 80°C for 10 min, which is lethal to vegetative cells but not to spores, and were stored at 4°C. Viable spore counts were evaluated by the drop counting method. Serial decimal dilutions in distilled sterile water were submitted to heat treatment of 80°C- 85°C for 10 min and 50 µL of each dilution was placed on the surface of a tryptone soy agar (TSA) plate, in duplicate. Plates were incubated overnight. Three different batches of spore suspensions were produced.

The resistance condition of the spores was evaluated by a dry-heat resistance test.[21] For this experiment, 300 strip vials of biological indicators (BI) with 10^6 CFU.unit^{-1} were produced using each batch of spore suspension. The composition of the recovery medium was 30.0 g L^{-1} TSB, 0.18 g

L^{-1} $CaCl_2$ $6H_2O$, 1.0 g L^{-1} soluble starch, and 0.02 g L^{-1} bromothymol blue. The D value is defined as the time taken, under specified conditions, to reduce the spore population by 90% or 1 log. The D value was assessed by fraction negative analysis-the limited Spearman-Kaber method.[21] Dry-heat exposure conditions were 160°C at 25, 30, 35, 40, 45, and 50 min in a tabletop circulating air oven. The germination and growth of the spores were observed at 24-48 h of incubation by visual identification of a color change (green to yellow) and turbidity of the substrate medium.

Liquid sterilants

The chemical products evaluated were as follows: 2.0% glutaraldehyde solution activated with 0.3% sodium bicarbonate (Glutalabor™-Glicolabor, Ribeirão Preto, Brazil), and 0.2% peracetic acid consisting of 0.2% peracetic acid, 7% hydrogen peroxide, acetic acid, stabilizer, and water (Sterilife™-Lifemed, São Paulo, Brazil). In this study, the products tested were evaluated based on the product label claims.

Minimum inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by using the two-fold broth dilution method. Starting from a pure chemical agent solution, serial dilutions were prepared in TSB and then inoculated with 100 µL test spore suspension before incubation at 36°C. Two tubes were used for the positive (TSB + 100 µL of inoculum) and negative (TSB + 1.0 mL disinfectant) controls (Fig. 1). The MIC was identified as the lowest concentration of the chemical agent that inhibited growth of the tested microorganism after 48 h of optimal incubation conditions. The MIC is expressed as a percentage. Each batch of spores produced was tested in triplicate (nine tests in total).

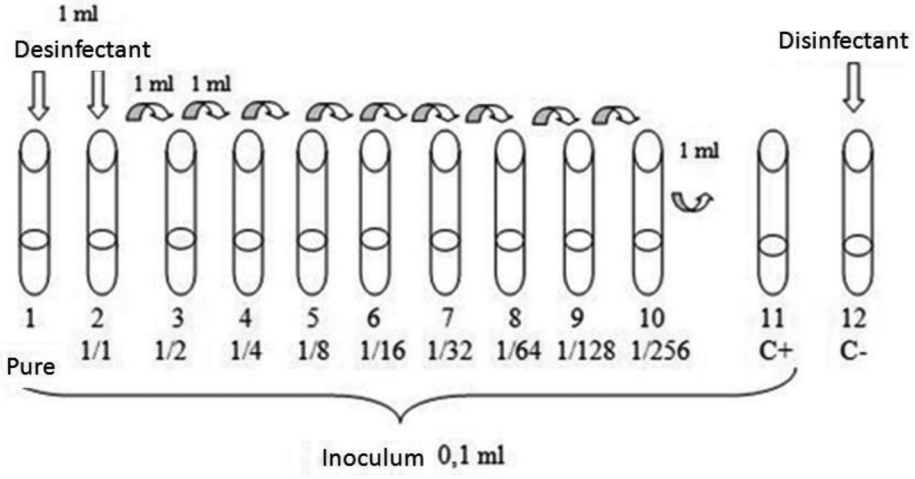

Figure 1. Method of determining minimal inhibitory concentration (MIC)

Surface challenge test

A spore suspension (6-7 log spores) was diluted to give concentrations of 10^6 to 10^7 CFU 50 μL^{-1} and this volume of each dilution was spread in a circle (6.5 cm diameter) on a clean, sterile, delimited BSC stainless steel working surface and then allowed to dry for 15 min.

Log reduction determination

Once the inoculum had dried on the surface, the surface was wiped with towelettes pre-saturated with the test sterilant and then allowed to stand for the labeled contact time (20 min). Surface bacterial samples were taken with TSA contact plates (Rodac™, BD, São Paulo, Brazil) (Fig. 2). The contact plates were incubated at 36°C for 48 h and then growth on the plate was evaluated.

Contact time determination

The surface with the dried inoculum was wiped with pre-saturated product wipes and allowed to stand for various contact times: 10, 20, 40, 60, and 120 min. Surface bacterial samples were taken with TSB culture-swabs. The media were incubated at 36°C for 48 h to 7 d. The turbidity of the suspension indicated a positive culture-swab result.

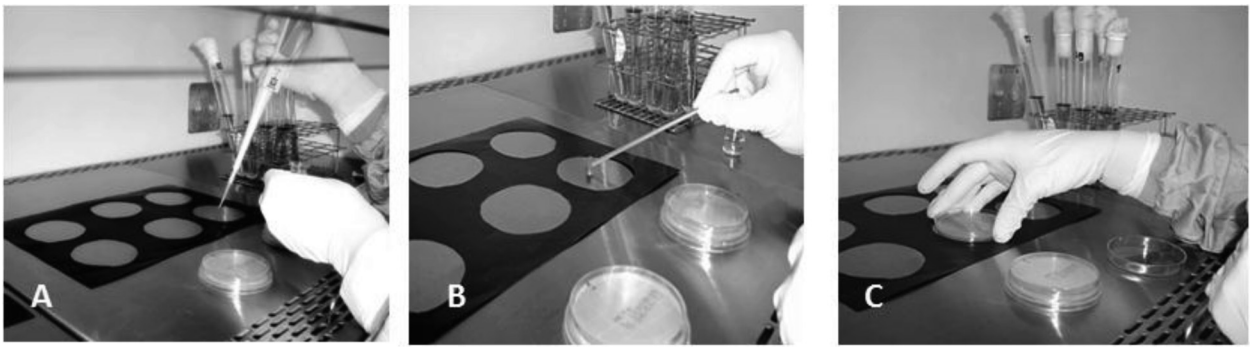

Figure 2. Sequence for determining log reduction: Inoculation of spores (A), spreading of spores (B) and sampling after various periods of exposure to sterilant using Rodac plates (C)

General conditions

At least three different batches of spore suspensions were evaluated. The assays were carried out in triplicate. Microbiological assays were conducted under aseptic conditions in a GMP-certified laboratory ISO 5 clean room[22]. Control experiments were conducted in the same conditions without the sterilant and with the addition of the same volume of sterile water. After the tests the BSC surface was cleaned at the follow order: disinfected with 0.2% peracetic acid for 60 minutes, rinsed twice with sterile water, brushing with neutral soap and rinsed twice with sterile water again. The results shown are average values. All experiments were carried out at room temperature (22±2°C). The validation was carried out following modified European regulatory agencies procedures (EN 13704[23] and USP guidelines[24]).

RESULTS

Spore preparation and resistance

Three batches of *B. atrophaeus* spores with concentrations ranging from 8.0 x 10^7 to 3.5 x 10^8 CFU mL^{-1} were produced. These spores' suspensions allowed dilutions of 6-7 log for the challenges. The dry-heat resistance value was $D_{160°C}$ = 5.2 ± 0.2 min for biological indicators (10^6 UFC/unit) produced from the three batches of spores.

Minimum inhibitory concentration

For the glutaraldehyde solution, the MIC ranged from 0.6 to 1.1%. The 0.2% peracetic acid solution showed an MIC range of 0.003-0.006% (Fig. 3).

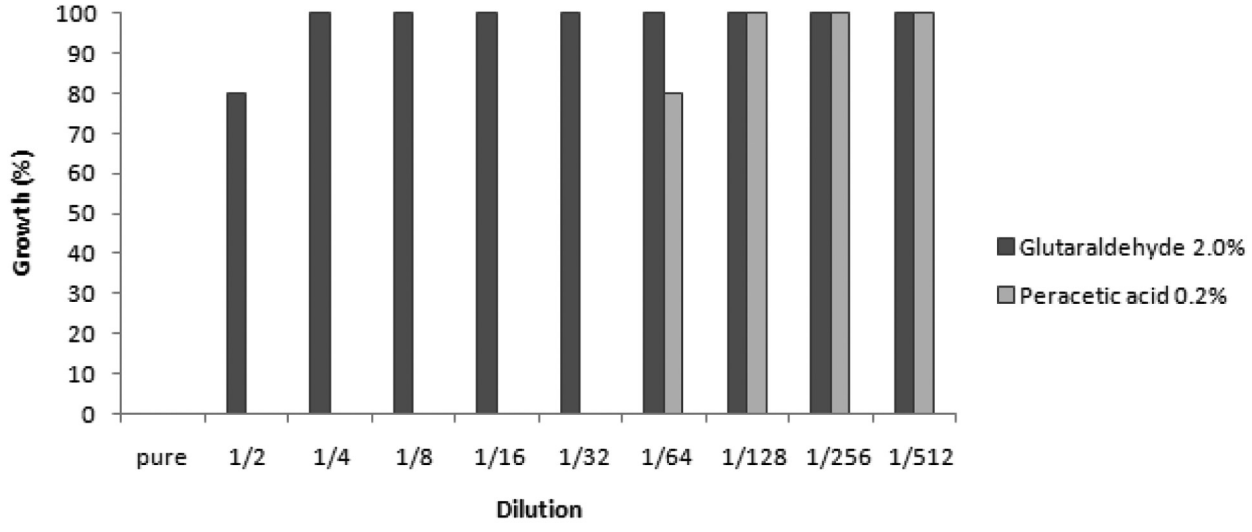

Figure 3. MIC results for glutaraldehyde (2.0%) and peracetic acid (0.2%) against *B. atrophaeus* spores

Surface challenge test

In the first surface test, the log reduction in spore count was tested with the recommended contact time of the disinfectant (20 min), the results (Table 1) demonstrated that contact plates were able to recover 100% of the inoculum when only sterile water was used, and that the disinfectant reduced the spore count by only 4 log at the tested concentration. The 0.2% peracetic acid contact time determination indicated that 40 min is the minimum exposure time; that is, the time after which there was no microbial growth in any of the tests (Table 2).

Table 1. Surface test results showing log reduction of *Bacillus atrophaeus* spores after 20 min contact time with 0.2% peracetic acid solution.

Challenge (CFU spores)	10^7	10^6	10^5	10^4	10^3	10^2	10^1
Test (CFU)	>300	176	No growth	No growth	No growth	No growth	No growth
Control (CFU)	>300	>300	>300	>300	>300	143	36

Table 2. Effects of peracetic acid contact time on growth of *Bacillus atrophaeus* spores.

Spore	Contact time (min) per challenge				
concentration	10	20	40	60	120
1.8±0.1 10^7 CFU	Growth	Growth	No growth	No growth	No growth
1.5±0.2 10^7 CFU	Growth	No growth	No growth	No growth	No growth
4.0±0.5 10^6 CFU	Growth	No growth	No growth	No growth	No growth

DISCUSSION

Chemical sterilants are used to destroy or eliminate all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores. Spores are considered to be the most difficult form of microorganism to destroy.[25] *B. atrophaeus* spores were chose as the test microorganism in this study, because they are highly resistant to chemical disinfectants, they are widely distributed in the environment, they substitute for *B. anthracis* in development and validation of biosafety methods, and they are produced safely.[26-28] Oie et al.[29] tested five bacterial species, and demonstrated that *B. atrophaeus* spores were the most resistant to disinfection by chemical agents, followed by *B. anthracis* and *Clostridium tetani*.

Sporulation conditions may affect spore resistance.[30] The dry-heat resistance result was higher than the typical D value (~ 3.0 min) for commercially supplied bioindicator systems, indicating its suitability to be used as challenge.[31]

The MIC method does not allow analog comparisons among the activities of different chemical agents. The glutaraldehyde solution was used only as a control test in the MIC determination. The glutaraldehyde solution' MIC (0.6 to 1.1%) were greater than that reported by Mazzolla et al. (0.3-0.4% for *B. subtilis* spores) and Serry et al.[33] (0.35% for *B. atrophaeus* ATCC 9372), for 10^6 spores used as the challenge in both studies. However, this result showed that the labeled concentration of glutaraldehyde showed sporicidal efficacy against the spores of the tested strain.

The 0.2% peracetic acid solution' MIC (0.003-0.006%) are approximately 50-fold lower than that of the commercial solution (0.2%), indicating that this solution is highly effective at inhibiting bacterial growth. Penna et al.[34] reported an MIC range of 0.9-1.9% to reduce populations of more than 10^9 *B. subtilis* and gram-negative bacteria for a 3.0% peracetic acid solution. No studies have reported on the efficacy of a 0.2% peracetic acid solution, as used in these experiments.

At present, there are two main methods for validating a disinfection process: the first is to apply the disinfectant to a dried contaminated surface and then test the surface, and the second is to demonstrate efficacy with a kill versus time procedure.[17,23,24] The first one demonstrates the efficacy of the disinfectant to decontaminate the working surfaces of equipment. The kill time pro-

cedure is a valuable tool for determining the time required achieving an acceptable log reduction or total kill, if that is desirable. Sporicidal activity is defined as the ability of a product to achieve at least a 6-log reduction in the number of bacterial spores. In the conditions used in the present experiments, the product did not meet the requirements of a sterilant; for a sterilization claim, there can be no surviving spores. Surfaces can provide favorable conditions for growth and adherence of microorganisms, and irregularities on the surface may protect them from contact with the chemical, so more time may be required for the chemical to kill the entire microbial population. Although two of the three samples showed no growth after 20 min exposure time to peracetic acid, the 40 min exposure time ensured sterilization of the surface. This difference in the kill time among the experiments may be because of the natural heterogeneity of the spores, non-uniformity of spore distribution, or non-uniformity of the tested surfaces. However, all of these factors must be considered as normal factors, because they exist in the normal working conditions of BSCs. This confirms the importance of real-life simulations for microbial challenge tests.

The tested product stipulated on its label that 20 min exposure time is sufficient for sterilant activity, at the recommended concentration. This sterilant action in 20 min was not confirmed in the present experiments, demonstrating that there are differences between official regulatory methods used for product registration and evaluations in real-life conditions. In addition, Kitis concluded that the mode of inoculation was just as influential a parameter as the characteristics of the solid substrate for determinations of spore decontamination efficiency.[13]

Concluding, it was demonstrated that 0.2% peracetic acid, under defined conditions of use, served as a surface sterilant against *B. atrophaeus* 6-7 log spores after 40 min contact time, double the contact time recommended on its label. Companies must register disinfectants and sterilants to support the directions for use given on the label. While such directions provide valuable information, they are not necessarily optimal for use of the chemical in a manufacturing environment, as demonstrated in this study. The procedures to validate disinfection processes are cost-effective and simple, and therefore, can be carried out by most microbiological control laboratories. These tests better reflect in-use conditions, and should be used to determine the optimum contact times and dilutions of the chemical, and to test surface properties. Such tests may provide new parameters for monitoring and improving decontamination processes, ensuring efficacy and safety. Maintenance and regular microbiological monitoring of BSCs is very important to ensure safe and accurate operations in manufacturing and health care settings.

Acknowledgments

The authors thank Elza Retka and Neuza Araujo for laboratory support. This research was financially supported by the Department of Health of the State of Parana, Brazil.

REFERENCES

1. U.S. Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th edn. U.S. Government Printing Office: Washington, 2007.
2. Luftman HS, Regits MA, Lorcheim P, Lorcheim K, Daniel Paznek D. Validation study for the use of chlorine dioxide gas as a decontaminant for biological safety cabinets. Appl Biosaf 2008; 13: 199-212.
3. World Health Organization -WHO. Quality assurance of pharmaceuticals: A compendium of Guidelines and Related Materials. Good manufacturing practices and inspection, vol 2. WHO: Geneva, 2007.
4. World Health Organization- WHO. Handbook: Good laboratory practice (GLP): quality practices for regulated non-clinical research and development, 2nd ed. WHO: Geneva, 2009.
5. Kort R, O'Brien AC, van Stokkum IH, et al. Assessment of heat resistance of bacterial spores from food product isolates by fluorescence monitoring of dipicolinic acid release. Appl Environ Microbiol 2005; 71: 3556-3564.
6. Setlow P. Spores of *Bacillus subtilis*: their resistance to radiation, heat and chemicals. J Bacteriol 2006; 101:514-525.
7. Russel AD. Bacterial spores and chemical sporicidal agents. Clin Microbiol Rev 1990; 3: 99-119.
8. Booth NH, Mcdonald LE. Veterinary Pharmacology and Therapeutics. Iowa State University Press: Ames, 1982.
9. Takigawa T, Endo Y. Effects of glutaraldehyde exposure on human health. J Occup Health 2006; 48:75-87.
10. Cowan RE. Cleaning and disinfection of equipment for gastrointestinal endoscopy. Gut- J Gastroen Hepatol 1988; 42:585-593.
11. Baldry MGC, Fraser JAL. In: Payne KR. Industrial biocides. Wiley: New York, 1988.
12. Block, SS. In: Kirk-Othmer. Encyclopedia of chemical technology. Wiley: New York, 1992.
13. Kitis M. Disinfection of wastewater with peracetic acid: a review. Environ Int 2004; 30:47-55.
14. Coleman WH, Yong-Qing Li DC, Cowan AE, Setlow P. How moist heat kills spores of *Bacillus subtilis*. J Bacteriol 2007; 189: 8458-8466.
15. Wirtanen G, Salo S. Disinfection in food processing - efficacy testing of disinfectants. Rev Environ Sci Biotechnol 2003; 2:293-306.

16. ASTM- American Society for Testing and Materials. Standard quantitative carrier test method to evaluate the bactericidal, fungicidal, mycobactericidal, and sporicidal potencies of liquid chemical germicides. ASTM: West Conshohocken, PA, USA, 2000.
17. European Committee of Standardization. EN14561: Chemical disinfectants and antiseptics- Quantitative carrier test for the evaluation of the bactericidal activity for instruments used in the medical area .Test method and requirements. British Standards Institute, London, UK, 2006.
18. Grand I, Bellon-Fontaine MN, Herry JM, Hilaire D, Moriconi FX, Naïtali M. The resistance of *Bacillus atrophaeus* spores to the bactericidal activity of peracetic acid is influenced by both the nature of the solid substrates and the mode of contamination. J Appl Microbiol 2010; 109:1706-1714.
19. Sagripanti J-L, Carrera M, Insalaco J, Ziemski M, Rogers J, Zandomeni R. Virulent spores of *Bacillus anthracis* and other *Bacillus* species deposited on solid surfaces have similar sensitivity to chemical decontaminants. J Appl Microbiol 2007; 102:11-21.
20. Sella SRBR, Guizelini BP, Vandenberghe LPS, Medeiros ABP, Soccol CR. Bioindicator production with *Bacillus atrophaeus*' thermal-resistant spores cultivated by solid-state fermentation. Appl Microbiol Biotechnol 2009; 82:1019-1026.
21. International Standard ISO- 11138-4. Sterilization of health care products-Biological indicators Part 4: Biological indicators for dry heat sterilization processes. ISO: Geneva, 2006.
22. International Standard ISO 14644-1. Clean rooms and associated controlled environments-part 1: classification of air cleanliness. ISO: Geneva, 1999.
23. European Committee for standardization. European standard EN 13704: Chemical disinfectants - Quantitative suspension test for evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1). British Standards Institute: London, UK, 2002.
24. USP-United States Pharmacopeia XXXI. Second Supplement, General Information, 1072 Disinfectants & Antiseptics. United States Pharmacopoeia Convection: Rockville, MD, 2008:3792.
25. EPA- U. S. Environmental Protection Agency. What Are Antimicrobial Pesticides? http://www.epa.gov/oppad001/ad_info.htm. Accessed 15 Dec 2010.
26. Driks A. The dynamic spore. Proc Natl Acad Sci USA 2003; 100:3007-3009.
27. McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clin Microbiol Rev 1999:12:147-179.
28. Mohan A, Dunn J, Hunt MC, Sizer CE. Inactivation *Bacillus atrophaeus* spores with surface-active peracids and characterization of formed free radicals using Electron Spin Resonance Spectroscopy. J Food Sci 2009; 74: M 411-M417.
29. Oie S, Obayashi A, Yamasaki H, et al. Disinfection methods for spores of *Bacillus atrophaeus*, *B. anthracis*, *Clostridium tetani*, *C. botulinum* and *C. difficile*. Biol Pharm Bull 2011; 34:1325-9.
30. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 2000; 64: 548-572.
31. Food and Drug Administration-FDA. Guidance for industry and FDA Staff - Biological Indicator (BI) Intended to Monitor Sterilizers used in Heath Care Facilities: remarket Notification [510(k)] Submissions, 2007. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071261.htm. Accessed 28 Jun 2011
32. Mazzola PG, Jozala AF, Novaes LCL, Moriel P, Penna TCV. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. Braz J Pharm Sci 2009; 459:241-248.
33. Serry FM, Kadry AA, Abdelrahman AA. Potential biological indicators for glutaraldehyde and formaldehyde sterilization processes. J Ind Microbiol Biotechnol 2003; 30:135-140.
34. Penna TCV, Mazzola PG, Martins AM. The efficacy of chemical agents in cleaning and disinfection programs. BMC Infec Dis 2001; 1:16-21.