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REVIEW

Testing Standard for Sporicides

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Summary

Sporicidal products are of considerable importance in healthcare environments due to the requirement for products that are capable of dealing with contamination with *Clostridium difficile* spores. Sporicidal testing standards to validate the claims of sporicidal activity are an important tool in the evaluation of commercial sporicides. Within Europe there are a number of sporicidal testing standards which are often used to validate the claims of commercial sporicides. However, the extent to which these standards reflect the practical application of sporicides in healthcare setting is limited since they employ long contact times (≥30 minutes) and do not involve surface contamination. Alternative international standards are available which employ contaminated carriers rather than spore suspensions, and the Organisation for Economic Co-operation and Development (OECD) is currently developing a unified set of standards which are more realistic in their design than the currently available European standards.

This paper reviews the currently available testing standards for sporicides, highlighting the key procedural differences between them and the extent to which they reflect the practical application of sporicidal products. Some of the common problems and errors associated with the application of the European sporicidal standard methods are also highlighted and discussed. Finally gaps in the currently available testing standards are identified and discussed.

Introduction

The emergence of *Clostridium difficile* as a major cause of nosocomial infections in the UK[1,2] and worldwide[3], has resulted in considerable focus on sporicidal disinfection products. Although a number of sporicidal chemical agents have been known for some time, the emergence of a wide range of products claiming sporicidal performance and/or activity against *C. difficile* has emphasised the need for standard methods to allow claims validation.

The situation is complicated in the UK by the lack of any regulatory position on disinfection performance. This contrasts greatly with the position in the United States where the Environmental Protection Agency (EPA)[4] considers spores to be the most difficult form of microorganism to destroy and consequently considers the term sporicidal to be synonymous with steriliser, a steriliser being an “antimicrobial pesticide that destroys or eliminates all forms of microbial life in the inanimate environment”[5]. This has led to a relatively small number of products with a limited number of active ingredients being considered sporicidal in the United States[6] and clear guidance on the testing methodology and performance criteria required to demonstrate activity against C*. difficile* spores[7]. The US EPA has also taken steps to prevent products claiming activity against *C. difficile* based on tests against vegetative cells

rather than spores[8], pointing out that this is misleading since it is the spore rather than the vegetative cell that is the infective agent.

In the absence of any UK regulatory position on sporicides the definition of a sporicides is left to the Technical Committee (CEN/TC 216 "Chemical disinfectants and antiseptics") of the European Committee for Standardisation which defines a sporicide as a *product which kills dormant bacterial spores of relevant test organisms under defined conditions*[9]. A definition which leads to different specifications of sporicidal performance depending on which European standard method is applied[9,10]. For the purposes of this review a sporicide is an agent which renders a bacterial spore no longer able to germinate and produce viable, vegetative bacterial cells. It is not an agent which kills vegetative cells immediately after they have emerged from a germinated spore. This is an important distinction since one of the common sources of false positive results in sporicidal testing is bactericidal agents that adhere to spores and then kill vegetative cells on spore germination.

This paper reviews the available standard methods for the determination of sporicidal activity, discusses key aspects of these standards and emphasises some of the shortcomings of those standards currently available.

Sporicidal Performance Requirement
When considering sporicidal agents for the disinfection of healthcare environments and associated surfaces it is possible to conceive of a range of desirable performance criteria. Namely:

- fast acting (<5 minutes);
- able to deal with high levels of contamination (e.g. a $\log_{10}$ 6.0 reduction[11]);
- able to deal with realistic levels of organic contamination;
- compatible with construction materials; and,
- safe to use.

It may not be possible for any one product to meet all these criteria, however this list does provide a useful guide against which available products may be assessed.

Test Requirement
An effective standard test method needs to meet a number of criteria. Firstly it needs to be accurate, and that any inherent inaccuracy is conservative. That is that the chance of false negatives is greater than the chance of false positives. Tests also need to be reproducible, generating the same results under the same conditions when repeated both within laboratories and between laboratories. Finally an effective standard needs to be as close to realistic conditions as practically possible.

Testing Standards

General Approaches

The majority of quantitative disinfection tests involve a number of common procedures, i.e.:

- preparation of a test suspension of the target organism (e.g. bacterial spore) to a known concentration;
- contact between the test suspension and the disinfectant under test for a known contact time at a designated temperature in the presence or absence of an interfering substance;
- after the specified contact time the inactivation of the disinfectant using either a chemical neutraliser or via filtration;
- determination of the number of target organisms surviving contact with the biocide over the specified contact time;
- the calculation of some form of reduction factor.

Disinfection tests fall into one of three categories, i.e. suspension tests, carrier tests or surface tests[12], all of which have advantages and disadvantages. Suspension tests are the simplest form of quantitative test involving the mixing of a known volume of test suspension with a known volume of product for a specified contact time, in some cases an interfering substance is also added. For commercial products the use of suspension tests results in a dilution of the product, 20% in the case of many European standard tests. The advantage of these tests is that there is good mixing between product and test suspension which improves the reproducibility of the test. The European standard sporicidal test BS EN 13704 is an example of a suspension test[10]. In carrier tests[12,13], inanimate carriers (e.g. porcelain, steel, glass, silk) are contaminated by submersion in the test suspension and allowed to dry. Once dry the contaminated carriers are submersed in the disinfectant under test for a specified contact time, after which the carriers are transferred to a neutraliser followed by a further transfer to a nutrient broth with both neutralisation and culture tubes being incubated. The presence or absence of growth indicates the efficacy of the product under test. The advantage of this kind of test is that large numbers of carriers (e.g. 30 to 60[7]) can be used per test to increase the sensitivity of the test. For example for registration with the US EPA as a sporicide/steriliant a product must return zero growth on 720 carriers utilising two organisms and two carrier types[13]. The final testing approach, surface tests, involves the drying of a known test suspension e.g. 0.05 ml, onto an inanimate surface e.g. 2cm diameter steel disc, which once dry is challenged with a surface application of the biocide under test e.g. 0.1ml. After the specified contact time the number of organisms surviving the treatment is recovered from the surface using and appropriate neutraliser. Surface tests are more commonly used to determine bactericidal[14,15,16] as well as sporicidal activity[16,17]. The transition from suspension test to carrier test to surface test represents a reduction in the ratio of product to contaminant and a progression towards test conditions that more closely resemble actual product usage[11].

Test Organisms

A range of test organisms are specified in various national and international standards. European standards[9,10] specify the use of *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 12826) and *Clostridium sporogenes* (CIP 7939) as a candidate anaerobe, whereas the US AOAC and ASTM tests employ different strains of the same species[13,17]. In terms of C. difficile the US EPA[7] recommends the toxigenic strains ATCC 700792, ATCC 43598 and ATCC 43599, although there is some evidence that *C. difficile* spores are more sensitive to some sporicides that candidate spores such as *B. subtilis*[18]. In other studies however, the relative sensitivity of *C. difficile, B. subtilis* and *C. sporogenes* changed with the sporicide under test and the culture conditions used to generate the spores investigated[19].

The majority of sporicidal testing standards contain instructions regarding the preparation of the spores required[9,10,13,17,20,21]. Key issues with the generation of spores are achieving the numbers required, ensuring only spores are present in the suspension and that the spore stock does not contain significant amounts of organic material originating from the growth media that may interfere with some sporicides such as chlorine release products. The carryover of media constituents has been implicated in variations in sporicidal performance observed between *Bacillus* and *Clostridium* test species[11]. The generation of an appropriate spore inoculums is particularly an issue when *C. difficile* is being used as the test organism since achieving the required spore loading e.g. 10^6 cfu/ml[10] can be difficult with some strains[19]. This may actually be due to spores clumping rather than low levels of sporulation.

Interfering Substances

Interfering substances are added to disinfection standards to provide a reproducible simulation of organic contamination. Given the origins of *C. difficile* contamination in healthcare settings[22] the effective assessment of a sporicide against *C. difficile* needs to consider the presence of organic contamination alongside the bacterial spores. The European sporicidal suspension tests are run either in the absence of interfering substance[9] or under simulated clean conditions[10] with low levels (0.3g/l) of bovine serum albumin (BSA) used to simulate organic contamination. Other standards[17] use more complex simulated “soils” which incorporate other proteins such as tryptone and mucin and in some cases milk[16]. It is not clear whether or not the level of organic contamination simulated in the standard European sporicidal tests accurately simulates soil levels associated with *C. difficile* contamination. However, the sporicidal activity of some commercial disinfectants can be significantly reduced when tested under simulated dirty conditions (3.0g/l BSA) as specified in the European standard tests for bactericidal activity[23] (Figure 1).

Neutralisation

Effective neutralisation is an essential component of biocide testing since it ensures that the specified contact time is adhered to. However, neutralisation can be a significant challenge in biocide testing in general since there are no generic neutralisers and the selection of neutralisers is often specific to the active ingredient under consideration[24]. The need for effective neutralisation is particularly the case in sporicidal tests since ineffective neutralisation can lead to false positives due to carryover of the active ingredient into to the culture media. This carry over results in the test organism being killed post germination giving the impression of a successful test. This is particularly an issue with products designed to adhere to surfaces to provide residual biocidal activity; this characteristic results in the biocide adhering to the spore surface making neutralisation more challenging. The carryover of biocide into the culture media can be detected by diluting and plating out the test suspension. If biocide carryover has occurred a characteristic pattern emerges as the biocide is diluted to a sub-inhibitory concentration (Figure 2). Problems with neutralisation can be avoided by using membrane filtration as an alternative to dilution neutralisation, an approach which is specified in some standard methods[10,11,25].

Performance Criteria

There are a range of performance criteria specified by individual sporicidal tests. These criteria generally consist of a required reduction in the spore load, within a specified contact time in the presence or absence of an interfering substance (Tables 2 and 3). Or as seen in the US carrier tests where there is a requirement for the complete removal of spores from a specified number of carriers within a specified contact time[7] (Table 3). In the European testing standards relatively long contact times are specified (Table 2) that have limited relevance to the application of sporicidal products in environmental decontamination. This specification of long contact times is not necessarily a problem since these tests are easily modified to accommodate shorter more relevant contact times. Problems arise however, when products aimed at environmental disinfection are able to report compliance with European standards[9,10] by virtue of the extended contact times these tests specify.

European Testing Standards

Disinfection testing in Europe was subject to rationalisation in the 1990's[12] with the formation by the European Committee for Standardisation (CEN) of the Technical Committee CEN/TC 216 for Chemical disinfectants and antiseptics. This saw the establishment of a range of standard disinfection tests drawn from those already in use within member states[12,26,27], the

relationship between these tests being outlined in BS EN 14885[27]. These tests are arranged within a structured framework based on their field of application (medical, veterinary and food, industrial, domestic and institutional areas (FIDI), Figure 3), and applied in a hierarchical manner which aims to reproduce a progressive increase in complexity and practicality. This hierarchical structure has three stages[27]:

- Phase 1: suspension tests for the basic activity of the product;
- Phase 2 step 1; suspension tests under conditions representative of practical use;
- Phase 2 step 2; other laboratory tests e.g. handwash, handrub and surface tests simulating practical conditions;
- Phase 3; field tests under practical conditions.

BS EN 14885[27] identifies two sporicidal standards (BS EN 14347 and 13704) at phase 1 and phase 2.1 respectively (Table I), neither of which are specified for products for use in medical areas; with BS EN 14347 required for products for use in veterinary areas and BS EN 13704 for application to products for use in FIDI areas. It should be noted that Phase 1 tests are not required to support claims for products designed for medical or FIDI applications[27]. Both of these standards are suspension tests with different protocols and performance criteria (Table II, Table III), with BS EN 14347 requiring the greater level of sporicidal activity and BS EN 13704 including low levels of interfering substance. Both of these standards specify relatively long contact times (Table II).

Although there are no CEN sporicidal surface tests, there are bactericidal surface tests aimed at veterinary and FIDI applications[14,15], which have been applied to the evaluation of sporicidal performance[28]. There are also French national standards which have similarities to the CEN bactericidal surface test[15] and employ glass, steel or plastic surfaces to evaluate the efficiency of liquid[16] and gaseous[29] sporicides against dried bacterial spores utilising contact time specified by the product manufacturers (Table III).

International Standards

The USA EPA[7] recommends four sporicidal test procedures to underpin efficacy claims against *C. difficile*, namely AOAC methods 966.04[13] (2006 version) AOAC 2008.05[21] and the ASTM standards 2197-02[17] and 2414-05[20]. These tests are carrier tests, with the exception of 2197-02[17] which is a surface test (Table III). In addition to these recommended standards there is also the ASTM sporicidal surface test E2111-05[30]. AOAC 966.04[13] is a classical carrier test where success is based on presence or absence of growth in multiple tubes containing carriers which have been treated with the sporicide under test. AOAC 2008.05[21] and ASTM 2414-05[20] are three step carrier tests where surviving spores are recovered from the carriers through a sequence of physical recovery steps. This focus on carrier and surface tests

contrast markedly with CEN sporicidal suspension tests[9,10], suggesting that the US tests may be closer to realistic conditions than the available European tests. The AOAC[13,21] tests employ clean conditions where as the ASTM tests include assessment under simulated dirty conditions[17,20]. The contact times for four of the five tests is left to the manufacturers recommendations, with the AOAC 966.04 covering a range of contact times from 2 to 20 minutes (Table III). This again contrasts with the CEN sporicidal tests where the obligatory minimum contact times are 30 and 60 minutes (Table II), although shorter contact times can be and often are utilised. Comparisons between AOAC 966.04[13], the quantitative three step methods AOAC 2008.05[21] and ASTM E 2111-05[30] demonstrated the ability of these tests to produce equivalent results when testing the same products[31].

The Organisation for Economic Co-operation and Development (OECD) is developing a range of testing standards for biocides to be used on non-porous, hard surfaces. The general approach being to produce a unified testing approach for all target microorganisms i.e. bacteria, fungi and viruses, draft guidelines are publically available for bacteria, fungi, mycobacterium and viruses[32-35], but not for sporicidal activity, although such testing against *B. subtilis* spores was included in the associated validation programme[36]. The proposed OECD tests are based on[36] the second tier of the ASTM sporicidal carrier 2197-02[17] and have similarities with the European surface test BS EN 13697[15]. This approach has been chosen because it is considered to "give a better indication of the potential of a given microbicide to perform under field conditions"[32].

The general approach of the OECD tests is the contamination of 1 cm diameter brushed steel discs with the test organism (5.5 to 6.5 $\log_{10}$ per carrier) in the presence of a simulated organic soil (BSA, mucin and yeast extract). Contaminated carriers are then challenged with 50 µl of the test product for the required contact time. Alongside these test coupons control coupons receiving 50 µl of phosphate buffered saline are also prepared. After the prescribed contact time the test product is neutralised using a validated neutraliser and the number of surviving organisms recovered from the carrier via vortexing and membrane filtration. Membrane filtration is used because it allows the complete recovery of organisms in a sample[32]. The performance of the product is determined by calculating a $\log_{10}$ reduction factor using the number of organisms recovered from the test discs and the number recovered from the control discs. A key aspect of this test approach is the recovery of the test organisms from the control discs to allow a $\log_{10}$ reduction to be calculated. Highly variable control results were observed in inter laboratory comparison studies on sporicidal performance carried out for the OECD validation programme[36]. The successful recovery of spores from carrier surfaces can be a significant issue with this approach to sporicidal testing (Figure 4). Peracetic acid inactivation curves[37] constructed as part of the OECD validation programme also showed considerable variation between laboratories.

Closing Comments

The currently available European standard test methods[9,10] for sporicidal performance do not reflect the current demands placed on sporicidal products in healthcare environments. The current standards are suspension tests performed over extended time periods in the presence of minimal organic loading. However, the practical application of sporicides is against surface contamination, where rapid sporicidal activity is desirable and significant organic contamination may be present. This discrepancy could potentially lead to the application of sporicidal products that do not meet the performance needs of current healthcare environments. Although European surface tests are available for bactericidal activity[14,15] there does not appear to be any plans to extend these tests to sporicides[27]. The proposed unified OECD biocide efficacy tests may fill this gap if the sporicidal standard becomes available alongside the DRAFT tests currently available[32-35]. The emergence in recent years of wipe based sporicides has generated additional demands on the available testing standards. Currently, there are no testing standards that reflect the manner in which wipes are used to disinfect surfaces, making it difficult to determine the true efficacy of wipe based sporicides.

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References

1. Office of National Statistics. Deaths involving *Clostridium difficile*: England and Wales 2008.*Statistical Bulletin* 2009; l-l3.
2. Office of National Statistics. Deaths involving *Clostridium difficile*: England and Wales,1999–2004. *Health Statistics Quarterly* 2004; **30:** 56-60.
3. Thomas C, Stevenson M, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile*-associated diarrhoea: a systematic review. *J. Antimicrob Chemother* 2003; **51**: 339-1350.
4. US EPA. *What are Antimicrobial Pesticides?* US Environmental Protection Agency Office of Pesticide Programs, USA 2006.
5. US EPA. *DIS/TSS-9 Efficacy Data Requirements*. US Environmental Protection Agency Office of Pesticide Programs, USA 2008.
6. US EPA. *List A: EPA's Registered Antimicrobial Products Registered with the EPA as Sterilizers*. US Environmental Protection Agency Office of Pesticide Programs, USA 2009.
7. US EPA. *Guidance for the Efficacy Evaluation of Products with Sporicidal Claims against Clostridium difficile.* Antimicrobial

Registration Policy Documents, US Environmental Protection Agency Office of Pesticide Programs, USA 2009.
8. US EPA. *Clostridium difficile product labelling.* US Environmental Protection Agency Office of Pesticide Programs, USA 2008.
9. BSI. *BS EN 14347:2005, Chemical disinfectants and antiseptics - Basic sporicidal activity - Test method and requirements (phase 1, step 1).* British Standards Institute, London, UK 2005.
10. BSI. *BS EN 13704:2002, Chemical Disinfectants-Quantitative suspension test for the 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.
11. Springthorpe VS, Sattar SA. Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. *J. AOAC Int* 2005; **88**: 182-201.
12. Reybrouck G. The testing of disinfectants. *International Biodeterioration and Biodegradation*, 1998; **41**(3-4): 269-272.
13. AOAC. *Official Method 966.04. Sporicidal Activity of Disinfectants*. AOAC International, Maryland, USA 2006.
14. BSI. *BS EN 14349:2007, Chemical disinfectants and antiseptics-Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on non-porous surfaces without mechanical action — Test method and requirements (phase 2, step 2).* British Standards Institute, London, UK 2007.
15. BSI. *BS EN 13697:2001:*Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2/step 2). British Standards Institute, London, UK 2001.
16. AFNOR. *NF T72-190. Water-miscible contact disinfectant used in liquid state. Germ-carrier method determining bactericidal, fungicidal and sporicidal action*. AFNOR, Paris, France 1989.
17. ASTM. *ASTM E2197-02 Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides*. ASTM International, PA, USA 2002.
18. Votava M. Slitrová B. Comparison of susceptibility of spores of *Bacillus subtilis* and Czech strains of *Clostridium difficile* to disinfectants. *Epidemiol Mikrobiol Imunol* 2009; **58**(1): 36-42.
19. Perez J, Springthorpe VS, Sattar SA. Activity of selected oxidizing microbicides against the spores of *Clostridium difficile*: Relevance to environmental control. *Am J Infec Control* 2005; **33**(6): 320-325.
20. ASTM. *E 2414-05: Standard Test Method for Quantitative Sporicidal Three-Step Method (TSM) to Determine sporicidal Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surfaces*. ASTM International, PA, USA 2005.
21. Tomasino SF, Pines RM and Cottrill MP. Determining the efficacy of liquid sporicides against spores of Bacillus subtilis on a hard nonporous

surface using the quantitative Three Step Method: collaborative study. *J AOAC Int* 2008; **91**(4): 833-852.
22. Vonberg RP, Kuijper EJ, Wilcox MH *et al*. Infection control measures to limit the spread of Clostridium difficile. *Clin Microbiol Infect* 2008; **14** (5): 2–20.
23. BSI. *BS EN 1276:1997, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas — Test method and requirements (phase 2, step 1*). British Standards Institute, London, UK 1997.
24. Russell AD. Factors influencing the efficacy of antimicrobial agents. In: Fraise AP, Lambert PA, Maillard J-Y, Eds. *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection Preservation and Sterilisation*, 4th edn, Blackwell Publishing, Oxford, UK 2004; 98-127.
25. AFNOR. *NF T72-230.Water –miscible and neutralisable antiseptics and disinfectants used in liquid state – Determining sporicidal action – Filtering diaphragm method*. AFNOR, Paris, France 1988.
26. AFNOR. *NF T72-231.Water –miscible and neutralisable antiseptics and disinfectants used in liquid state – Determining sporicidal action – Dilution – Neutralisation Method*. AFNOR, Paris, France 1988.
27. BSI. *BS EN 14885:2006, Chemical disinfectants and antiseptics-Application of European Standards for chemical disinfectants and antiseptics.* British Standards Institute, London, UK 2006.
28. Block C. The effect of Perasafe® and sodium dichloroisocyanurate (NaDCC) against spores of *Clostridium difficile* and *Bacillus atrophaeus* on stainless steel and polyvinyl chloride surfaces. *J Hosp Infect* 2004; **57**(2): 144-148.
29. AFNOR. *NF T72-281.Methods of airborne disinfection of surfaces – determination of bactericidal, fungicidal, yeasticidal and sporicidal activity*. AFNOR, Paris, France 2008.
30. ASTM. *E2111-05. Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Microbicides*. ASTM International, PA, USA 2005.
31. Tomasino SF, Hamilton MA. Comparative Evaluation of Two Quantitative Test Methods for Determining the Efficacy of Liquid Sporicides and Sterilants on a Hard Surface: A Precollaborative Study. *J AOAC Int* 2007; **90**(2): 456-464.
32. OECD. *Quantitative Method for Evaluating Bactericidal Efficacy of Biocides Used on Hard Surfaces. Draft OECD Guideline for the Testing of Chemicals*. Organisation for Economic Co-operation and Development, Paris, France 2009.
33. OECD. *Quantitative Method for Evaluating virucidal Efficacy of Biocides Used on Hard Surfaces. Draft OECD Guideline for the Testing of Chemicals.* Organisation for Economic Co-operation and Development, Paris, France 2009.
34. OECD. *Quantitative Method for Evaluating Mycobactericidal Efficacy of Biocides Used on Hard Surfaces. Draft OECD Guideline for the Testing of Chemicals*, Organisation for Economic Co-operation and Development, Paris, France 2009.

35. OECD. *Quantitative Method for Evaluating Fungicidal Efficacy of Biocides Used on Hard Surfaces. Draft OECD Guideline for the Testing of Chemicals*, Organisation for Economic Co-operation and Development, Paris, France 2009.
36. Uhlig S, Simon K, Antoni S, Kunath K. *Validation of efficacy methods for antimicrobials used on hard surfaces*. DRAFT Report , 2009-11-20, Quo data, Gesellschaft fur Qualitatsmanagement und Statistik mbH, Dresden, Germany 2009.
37. Majcher MR, Bernard KA, Sattar SA (2008) Identification by quantitative carrier test of surrogate spore-forming bacteria to assess sporicidal chemicals for use against *Bacillus anthracis*. *Appl Environ Microbiol 2008;* **74**(3): 676–681.

Figure 1. Impact of organic contamination on the sporicidal activity of chlorine based sporicides. Sporicidal activity was assessed using the approach outlined in BS EN 13704:2002[10] with the clean and dirty conditions outlined in BS EN 1276:1997[23].

Figure 2. Impact of product carryover in sporicidal suspension tests. Sporicidal activity was assessed using the approach outlined in BS EN 13704:2002[10].

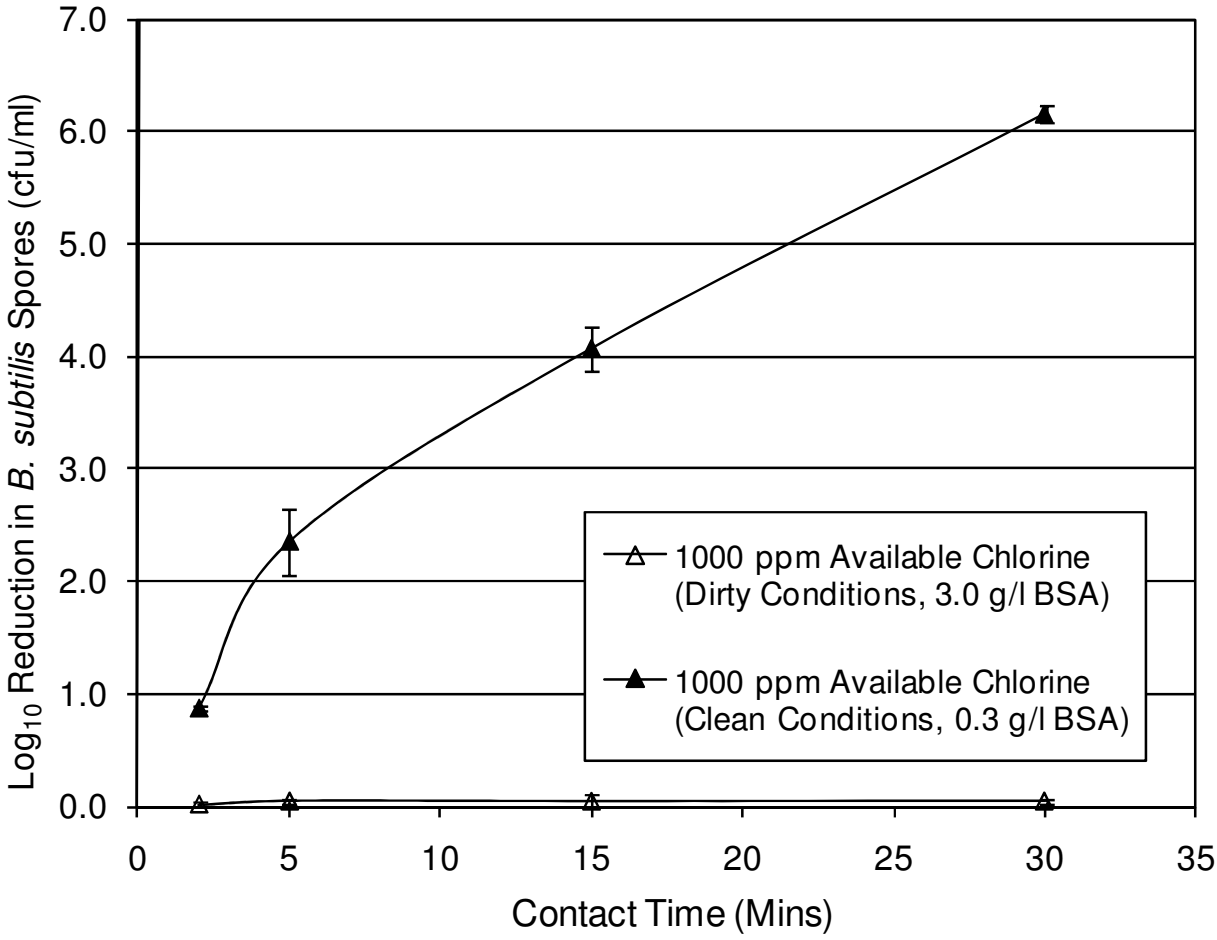
Figure 3. European Disinfection Testing Framework[27].

Figure 4. SEM images of brushed steel test coupons: a) after cleaning but before contamination with *B. subtilis* spores, b) after unsuccessful recovery of *B. subtilis* spores using the technique outlined in BS EN 13697:2001[14].

Table I. Status of European sporicidal tests according to EN 14885:2006[27].

Table II. Comparison of the two European Standard sporicidal tests BS EN 14347[9] and 13704[10].

Table III. International sporicidal standards



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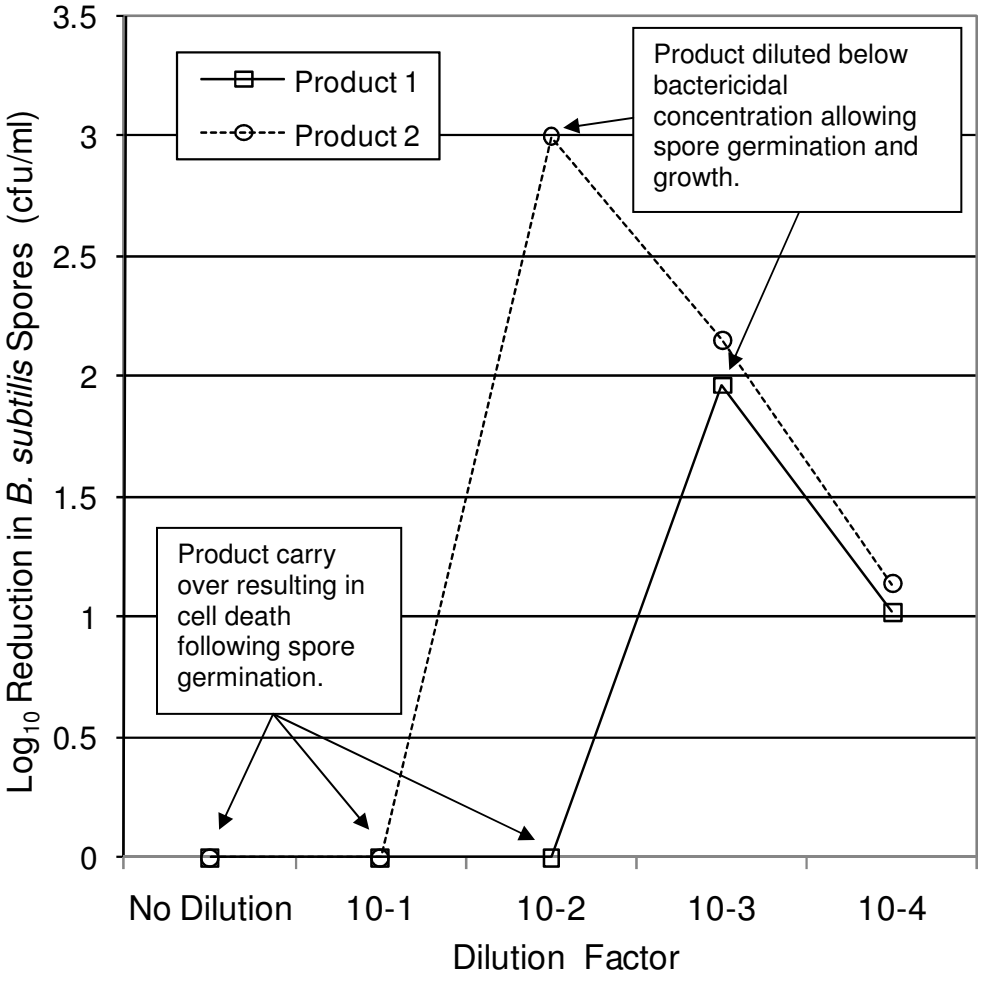

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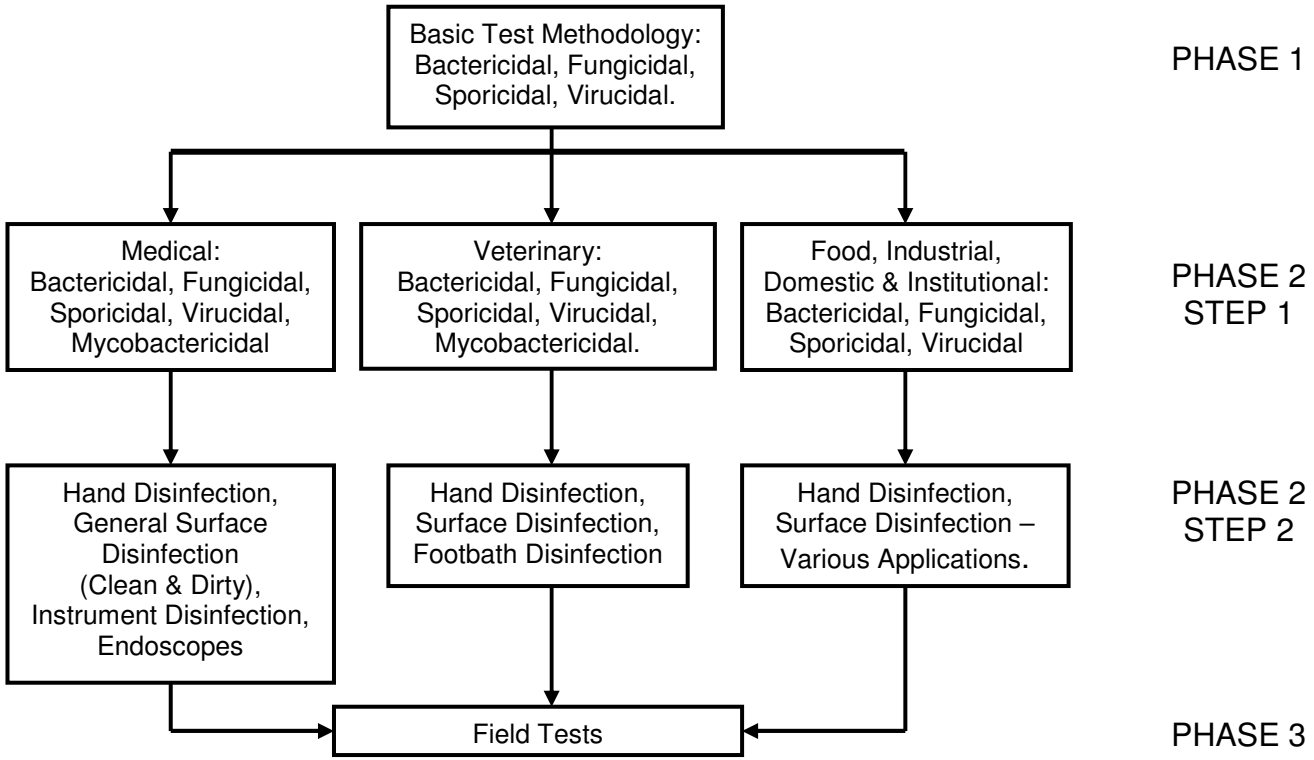

Figure 3. European Disinfection Testing Framework[27].

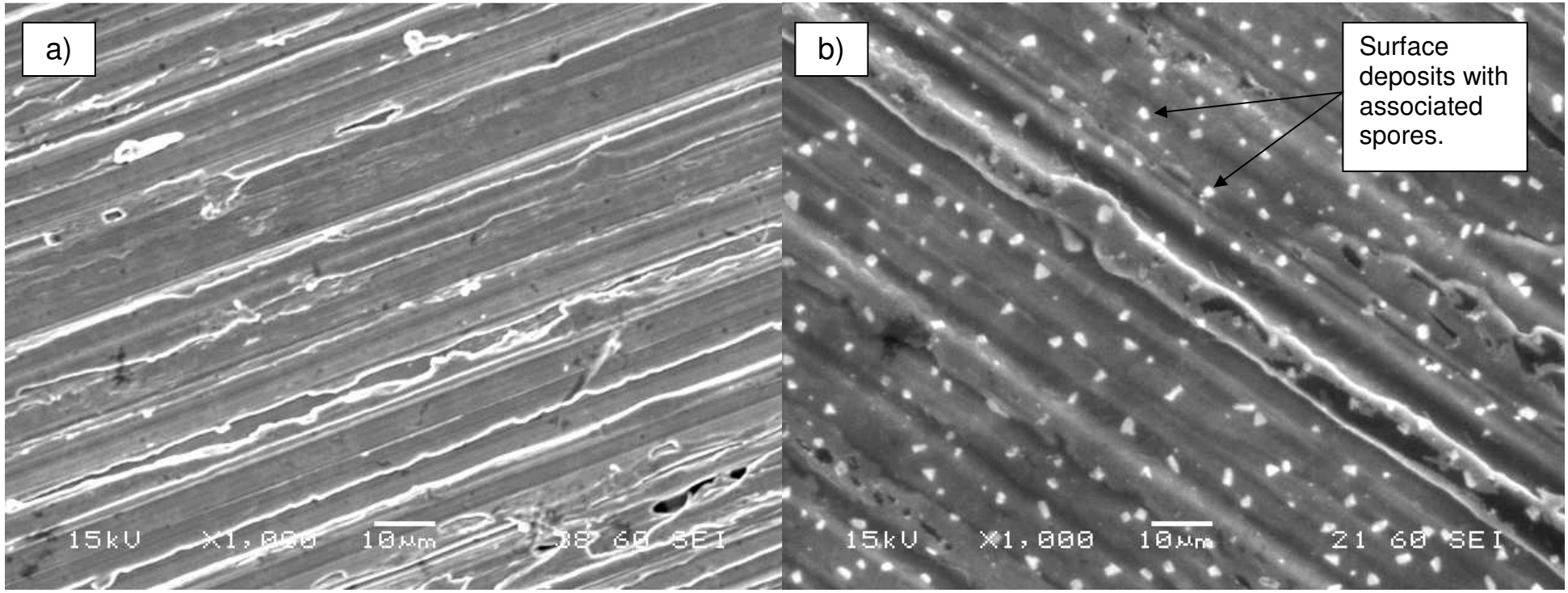

Figure 4. SEM images of brushed steel test coupons: a) after cleaning but before contamination with *B. subtilis* spores, b) after unsuccessful recovery of *B. subtilis* spores using the technique outlined in BS EN 13697:2001[14].

Field of Application	Type and purpose of product	Phase	Status of Test			
			No test anticipated	Test may be developed	Test under development	Test developed
Medical	Surface disinfection	2.1		✓		
		2.2				
	Instrument disinfection	2.1			✓	
		2.2				
	Water treatment	2.1	✓			
		2.2				
Veterinary	Surface disinfection (clean/dirty)	1				EN14347
		2.1			✓	
		2.2		✓		
	Contaminated objects	1				EN14347
		2.1			✓	
		2.2		✓		
Food, industrial, domestic and institutional	Surface disinfection (clean/dirty)	2.1				EN13704
		2.2	✓			
	Cleaning in place	2.1	✓			EN13704
	Wipes	2.1	✓			
		2.2	✓			

Table I. Status of European sporicidal tests according to EN 14885:2006[27].

Standard	Inoculum	Species	Contact Time /mins	Soil	Pass Level
BS EN 14347	3.0×10^{8} to 1.0×10^{9}	*Bacillus subtilis* *Bacillus cereus*	30, 60 or 120	None	$\geq 10^{4}$
BS EN 13704	1.5×10^{6} to 5.0×10^{6}	*Bacillus subtilis* (option for *Bacillus cereus* & *Clostridium sporogenes)*	60	Clean 0.3g/l BSA	$\geq 10^{3}$

Table II. Comparison of the two European Standard sporicidal tests BS EN 14347[9] and 13704[10].

	European					American				
	AFNOR			CEN		AOAC		ASTM		
Standard	T72-190[16]	T72-230/ 231[25,26]	T72-281[29]	14347[9]	13704[10]	966.04[13]	2008.05[21]	E2197-02[17]	E2414-05[20]	E2111-05[30]
Phase	2.2	1	2.2	1	2.1	2.2	2.2	2.2	2.2	2.2
Contact Time/ Minutes	MS	60	MS	30, 60 or 120	60	2,5,10,20	MS	MS	MS	MS
Approach	Surface	Suspension	Surface	Suspension		Carrier Test		Surface	Carrier Test	Surface
Clean/ Dirty?	Dirty	Clean	Clean	Clean		Clean	Clean	Dirty	Clean or Dirty	Clean or Dirty
Surface?	Steel, Glass, Plastic	N/A	Steel	N/A		Suture/ Dacron Loops, Porcelain cylinders	Glass	Steel	Steel, Glass, Rubber	Glass
MS = Manufacturer's Specification, N/A = Not Applicable.						US EPA Recommended?				
						Yes	Yes	Yes	Yes	No

Table III. International sporicidal standards