
 **STERIS**  
Life Sciences Group

**Disinfection Processes**

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 **STERIS**  
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## Agenda

- Current Industry Trends
  - Cleaning and Disinfection
  - Current Warning Letters & Industry Guidance

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## VALIDATION – Microorganism Selection



	Microorganism	Examples
	Prions	Scrapie, Creutzfeld-Jacob disease, Chronic wasting disease
	Bacterial Spores	<i>Bacillus</i> , <i>Geobacillus</i> , <i>Clostridium</i>
	Protozoal Oocysts	<i>Cryptosporidium</i>
	Helminth Eggs	<i>Ascaris</i> , <i>Enterobius</i>
	Mycobacteria	<i>Mycobacterium tuberculosis</i> , <i>M. terrae</i> , <i>M. chelonae</i>
	Small, Non-Enveloped Viruses	Poliovirus, Parvoviruses, Papilloma viruses
	Protozoal Cysts	<i>Giardia</i> , <i>Acanthamoeba</i>
	Fungal Spores	<i>Aspergillus</i> , <i>Penicillium</i>
	Gram negative bacteria	<i>Pseudomonas</i> , <i>Providencia</i> , <i>Escherichia</i>
	Vegetative Fungi and Algae	<i>Aspergillus</i> , <i>Trichophyton</i> , <i>Candida</i> , <i>Chlamydomonas</i>
	Vegetative Helminths and Protozoa	<i>Ascaris</i> , <i>Cryptosporidium</i> , <i>Giardia</i>
	Large, non-enveloped viruses	Adenoviruses, Rotaviruses
	Gram positive bacteria	<i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Enterococcus</i>
	Enveloped viruses	HIV, Hepatitis B virus, Herpes Simplex virus

***Bacillus cereus* / *sphaericus***

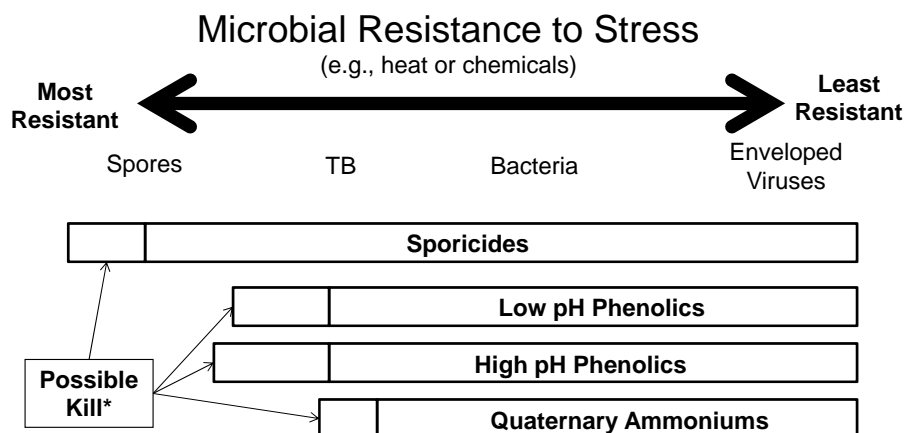
***Bacillus subtilis* / *G. stearothermophilus***

***Clostridium spp***

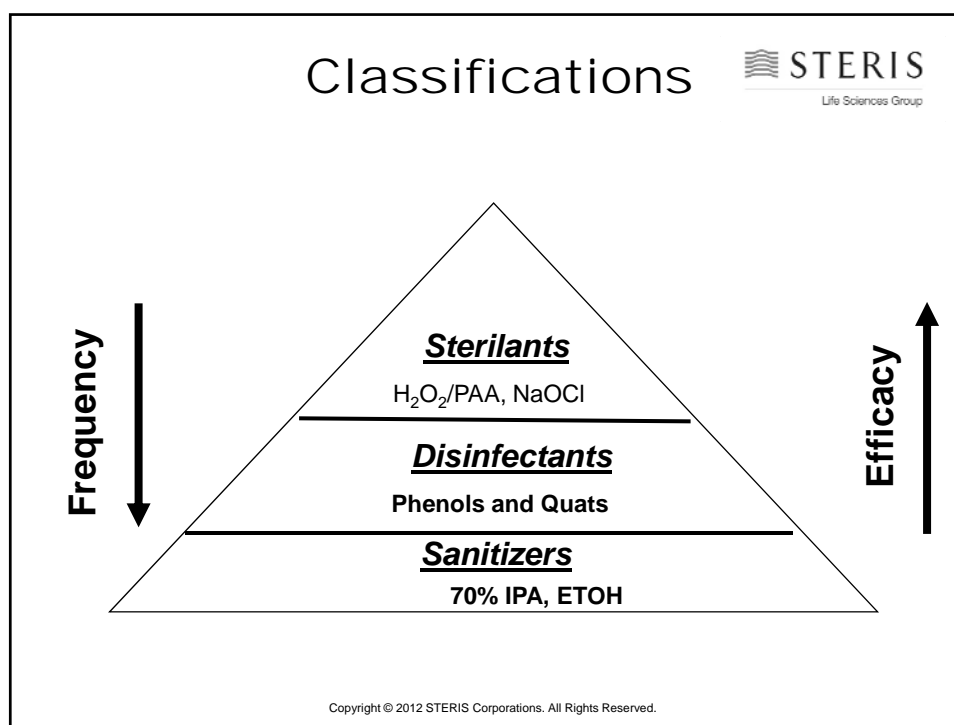
From McDonnell, "Antisepsis, Disinfection, and Sterilization: Types, Action, and Resistance" 2007, ASM Press

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## Contamination Control



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## Disinfectant components

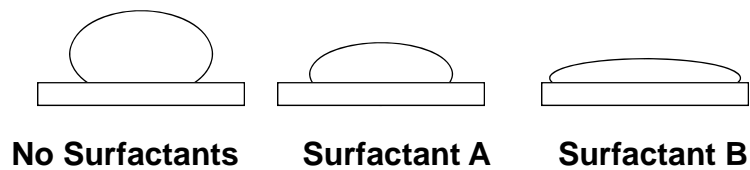


Component	Function in disinfectant
• Water	<b>Solvent</b>
• Solvents	<b>Solubilization and stabilization</b>
• Antimicrobials	<b>Kill, reduce microbes</b>
• Oxidants	<b>Oxidize, kill microbes</b>
• Chelants oxidants,	<b>Tie up Calcium, Iron, Stabilize</b>
• Bases	<b>Potentiate antimicrobial action</b>
• Acids	<b>Alkalinity source (NaOH, KOH)</b>
• Surfactants	<b>Acidity source (HCl, Phosphoric Acid)</b>
	<b>Emulsification, Wetting</b>

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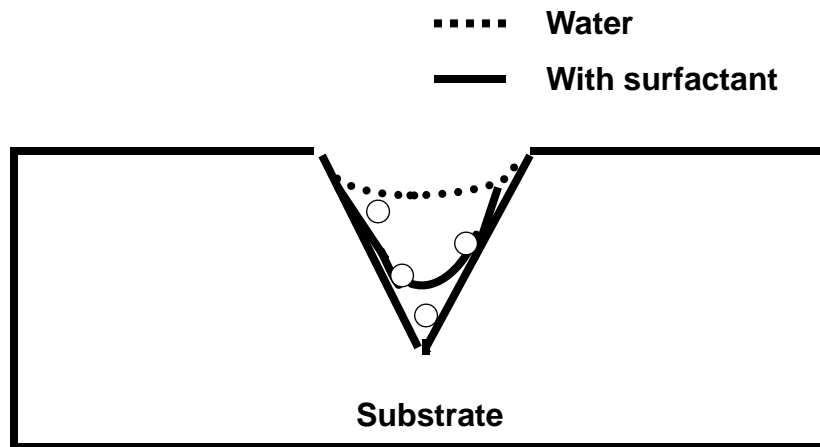
## Effect of Surfactants

- Influence of Surfactants on Wetting
  - Ability to displace particles
  - Penetrate soil and surface irregularities
  - Better contact



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## Access to microbes



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## Molds common to cleanrooms and coldrooms



- *Aspergillus* species
- *Penicillium* species
- *Stachy bottrus*
- *Candida albicans*
- *Cladosporium*
- *Mucor*
- *Scopulariopsis*
- *Trychophyton*

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## Bacterial Spores in Operations



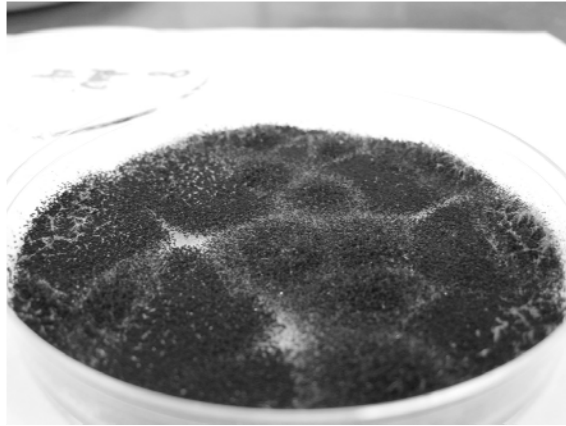
- *Bacillus subtilis*
- *Bacillus cereus*
- *Bacillus pumilus*
- *Bacillus licheniformis*
- *Bacillus sphaericus*
- *Bacillus thuringiensis*
- *Paenibacillus polymyxa*
- *Geobacillus*
- *Clostridium difficile*

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## Aspergillus niger



Courtesy Jim Polarine



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## Cleanroom Fungi



Courtesy Dan Klein

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## Bacterial Endospore

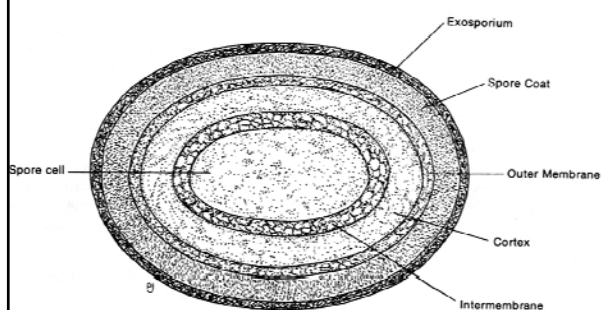
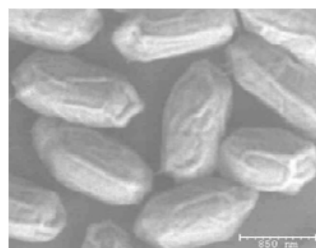


Fig. 8.1. Endospore

Courtesy Dan Klein

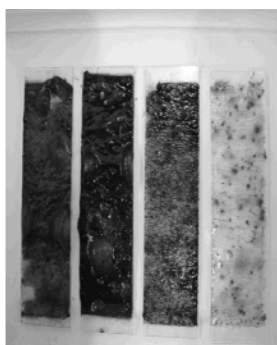


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## Biofilm



Courtesy Google Image Library



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## New Mopping Systems

- The Mop King (<http://www.am-king.com/mopking.htm>)
- The Micron Swep (<http://www.youtube.com/watch?v=qTWaYQIX2IY>)

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## Application Techniques

- ☐ Most critical areas to least critical areas
- ☐ Apply disinfectant to wiper or spray on the surface (garden variety sprayer)
- ☐ Changing out the use dilutions (2-3 Bucket Routines) ref. Anne Marie Dixon
  - ✓ 600 square feet (56 square meters) in ISO-5 (Grade A & B)
  - ✓ 1,000 square feet in (93 square meters) ISO-6, 7, 8 (Grade C & D)
- ☐ Grid (Blueprint of the Room)
- ☐ Pull and lift
- ☐ Overlapping strokes (by 20%)
- ☐ Figure 8 (also called figure S) or Unidirectional mopping strokes

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## Two Bucket System

- ☐ Sterilant (Disinfectant) in front bucket, optional to put some sterilant (Disinfectant) in waste bucket (bucket beneath the ringer)
- ☐ Dip mop head into front bucket, let excess liquid drain off, apply to the surface.
- ☐ When mop head appears to be dragging on the surface, dip into waste bucket, then wring out. Go back to front bucket and dip mop head, let excess liquid drain off and apply to the surface.
- ☐ Repeat above steps
- ☐ Other Mopping Systems: Single Bucket, Triple Bucket, MicronSweep System by Aramark.

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## Two and Three Bucket Systems



Courtesy Perfex Corp.

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## Cleanroom Curtain Devices



### •Surfaces

- Floors
- Walls
- Isolators
- Lyophilisers
- Cabinets
- Tanks
- Curtains/Softwalls



Courtesy Micronova Mfg.

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## Bucket Systems & Sterile Liners



- Adaptable
- Resistant
- Dedicated

Courtesy Micronova Mfg.



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## Mop Heads for Cleanrooms



Courtesy Micronova Mfg.

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## Spills and Leaks



- Disinfectant applicator
- Choice of widths
- Universal
  - Floors/Walls
  - Large/small areas



Courtesy of Micronova Mfg.

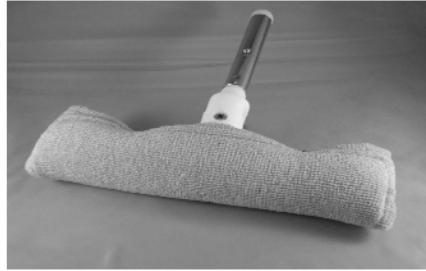
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## Commonly Used Equipment



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Courtesy Micronova Mfg.



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## Sprayers and Foamers



Life Sciences Group

### Acid Sprayer

- Compatible with oxidizers, phenolics, quats, and other acids

### Cart

- Carts designed for 5 gal pail

### Sanitation Sprayer

- Sprayer Attachment
- Has rinse cycle

### Foam Master

- Plant Air powered



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## Controlled Areas

- Hallways and Floors ---Mop daily ---Rinse as needed
- Walls and Ceilings---Mop monthly---Rinse as needed
- Equipment (carts, racks, trash receptacles, etc.)---Wipe weekly---Rinse as needed
- Rinsing is based on visual observation and safety.

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## Class 100000 - D



### Class 100,000 Closed Processes – Recommendations

(solution prep, fermentation, purification, media prep, wash bays, raw material weigh area, stopper prep, packaging inspection)

Surface	Method	Cleaning Agent	Frequency	Rinse
<b>Floors</b> <ul style="list-style-type: none"> <li>• around drains</li> <li>• foot traffic paths</li> <li>• spill areas</li> <li>• access ports</li> </ul>	mop	high-level disinfectant with surfactants	daily at shutdown, between process changeover	not necessary
<b>Walls, Ceilings</b> <ul style="list-style-type: none"> <li>• general</li> </ul>	wipe, mop	high-level disinfectant with surfactants followed with sporicide	monthly	not necessary
<ul style="list-style-type: none"> <li>• doors, handles, high-traffic areas</li> </ul>	mop	high-level disinfectant with surfactants	daily	
<b>Equipment</b> <ul style="list-style-type: none"> <li>• adjacent to access ports</li> </ul>	spray, wipe	high-level disinfectant with surfactants	daily during processing	as needed to remove residue buildup
<ul style="list-style-type: none"> <li>• surface upstream airflow path to process opening</li> </ul>	spray, wipe		weekly	
<b>Other Surfaces</b> <ul style="list-style-type: none"> <li>• sinks</li> <li>• benches</li> <li>• trash containers</li> </ul>	wipe	high-level disinfectant with surfactants	daily	not necessary

**Class 10000 - C****Class 10,000 – Outside Laminar Flow Hoods, Rooms, and Halls – Recommendations**

Surface	Method	Cleaning Agent	Frequency	Rinse
<b>Floors</b> • normal traffic paths	mop	high-level disinfectant with surfactants	daily after transfers	not necessary
• proximity to open process or transfer areas		high-level disinfectant with surfactants followed by a sporicide	weekly or monthly, if necessary	
<b>Walls</b> • general	wipe	high-level disinfectant with surfactants followed by a sporicide, if necessary	weekly or monthly	not necessary
• door plate	mop	high-level disinfectant with surfactants	daily	
<b>Equipment</b> • shelving • portable tanks • processing items	spray or wipe	high-level disinfectant with surfactants	before and after use	WFI rinse as necessary to address residue buildup
• carts (wheels)		sporicide		
<b>Furniture and Other</b> • chair (wheels)	spray or wipe	high-level disinfectant with surfactants	daily	not necessary
		sporicide		

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**Class 100 - A & B****Class 100 – Laminar Flow Hoods and Aseptic Filling Suites – Recommendations**

Surface	Method	Cleaning Agent	Frequency	Rinse
<b>External Hoods</b> • back, sides, top	wipe	sterile high-level disinfectant with surfactants	daily	not necessary
• door, sliding panel	wipe	sterile high-level disinfectant with surfactants	daily	
		sporicide	weekly or in response to microbial monitoring	
<b>Inside Hood or Curtain</b> • work surface • sidewalls • apparatus/critical surfaces	wipe	sterile high-level disinfectant with surfactants	daily, pre-use and post-use	WFI as necessary in product contact areas, or to address residue buildup
		sporicide	weekly or in response to microbial monitoring	
• curtains	wipe	sterile high-level disinfectant with surfactants	daily	
		sporicide	weekly or in response to microbial monitoring	
<b>Adjacent Flooring and Walls</b>	mop	sterile high-level disinfectant with surfactants	daily, between lots and shifts	not necessary
		sterile high-level disinfectant with surfactants followed by a sporicide, as necessary	weekly or in response to microbial monitoring	

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## Sporicidal Agent Application

### ✓ Rationale

#### ✓ Spore control vs. chemical exposure

#### ✓ Corrosivity and Irritation

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## Application Frequency

### ☐ Sporicidal agent

#### ☐ Rationale

##### ☐ Weekly

##### ☐ Monthly

##### ☐ Quarterly

#### ✓ Should be written in SOP's

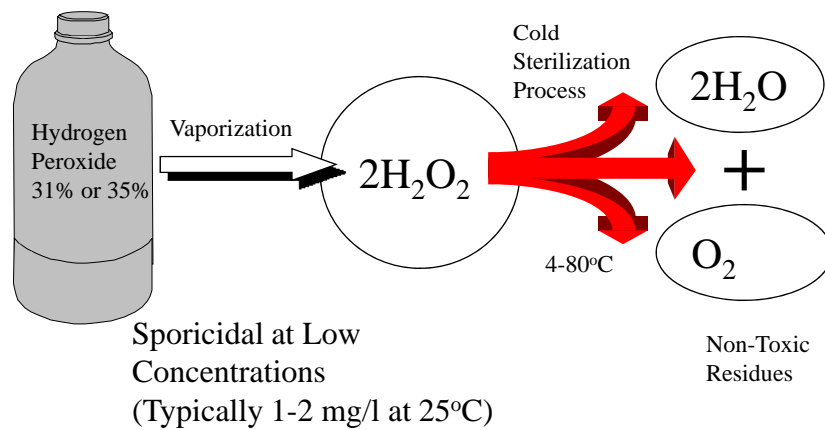
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## Alternative Technique

- ☐ Foaming
- ☐ VHP
- ☐ Spraying (also known as fogging)
  - ✓ Aerosolizes disinfectant
- ☐ Fumigation
  - ✓ Vaporizes disinfectant
- ☐ Full immersion
  - ✓ Disinfectant soak

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## Hydrogen Peroxide Vapor



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## Room Decontamination



### Walk-In Refrigerator Type Construction

Oklahoma  
Medical Research  
Foundation,  
Oklahoma City,  
OK



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## Fogger / Dynafogger



- ✓ Room Size
- ✓ Effectiveness
- ✓ Material Compatibility
- ✓ Contact Time
- ✓ Re-entry Time



Courtesy of Microclean-

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## What is Rotation?

### ☐ Alternation of antimicrobial actives

- ✓ Two disinfectants in sequence, regular rotation, with sterilant as needed
- ✓ One disinfectant daily, with sterilant weekly or monthly

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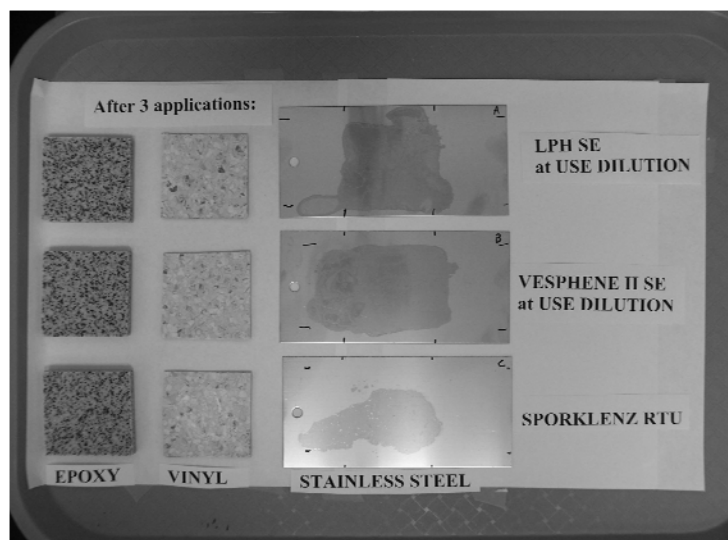


## Rotation Guidance

- ✓ USP 35 <1072>
- ✓ Annex I (2007- Orange Guide)
- ✓ Aseptic Processing Guide (2004)
- ✓ Conner & Eckman Studies
- ✓ FDA, MHRA, IMB, French and Japanese, & EMA Expectations
- ✓ Industry Articles (Ex. Scott Sutton, Jose Martinez, Richard Prince)
- ✓ USP 35 <797>

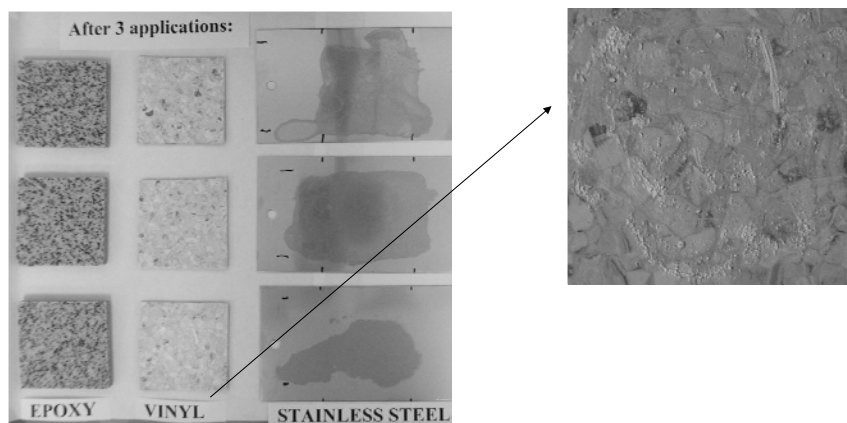
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## Residues on Substrates:



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## Surface Type and Topography



Photos provided by Brook Meadows

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## Rinsing Frequency

### ☐ Rinsing

### ☐ Guidance USP 35 <1072>

- ✓ 70% IPA or Water for Injection
- ✓ Cleaners (Acidic, Neutral, Basic)
- ✓ As needed to control residue
  - ✓ Aesthetic
  - ✓ Safety Risk (Sticky, Tacky, Slippery)
  - ✓ Particulate Issues
  - ✓ Functional
  - ✓ Microbial Issue (Hiding Microbes & Food Sources)
  - ✓ Product risk (Flaking of residues into filled products)

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## Case Study on Substrates

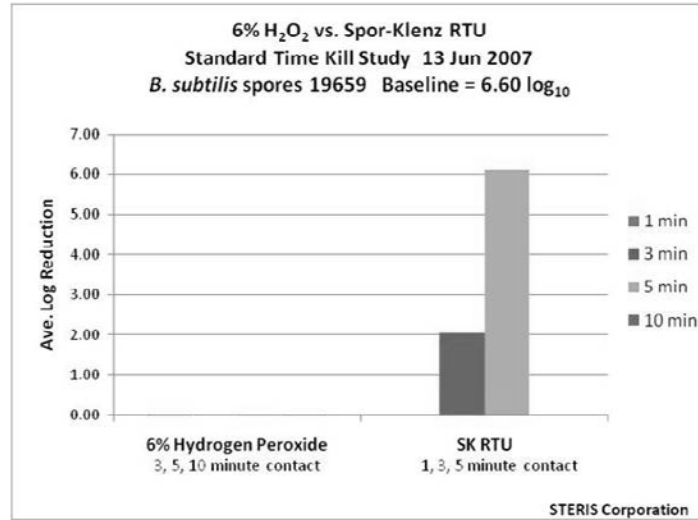
Table 3. Efficacy (log reduction) of Low pH phenolic; (1:256 Dilution) against test microorganisms on representative surfaces

Surface	<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Corynebacterium glutamicum</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Penicillium chrysogenum</i>
Stainless Steel	6.62	>6.10 <sup>b</sup>	4.18	>4.31 <sup>b</sup>	<3.00 <sup>c</sup>	4.95
Glass	6.85	6.42	5.26	>5.80 <sup>b</sup>	2.98	5.11
Aluminum	6.35	5.69	5.14	>3.93 <sup>b</sup>	<3.00 <sup>c</sup>	3.48
Epoxy	4.36	4.45	4.48	3.19	<3.00 <sup>c</sup>	<3.00 <sup>c</sup>
Enamel	>6.05 <sup>b</sup>	>5.72 <sup>b</sup>	5.45	>3.92 <sup>b</sup>	<3.00 <sup>c</sup>	2.83
Acrylic	4.53	6.06	4.49	2.92	<3.00 <sup>c</sup>	<3.0 <sup>c</sup>
Miplan	4.36	3.87	4.29	4.37	<3.00 <sup>c</sup>	3.25
Vinyl	4.08	3.68	3.93	2.61	<3.00 <sup>c</sup>	2.1
Hardwood	5.18	>4.54 <sup>b</sup>	5.26	3.2	<3.00 <sup>c</sup>	2.59
Melamine Covered Wood	>5.38 <sup>b</sup>	>5.64 <sup>b</sup>	>5.09 <sup>b</sup>	>5.12 <sup>b</sup>	3.65	3.95
Plastic	>5.73 <sup>b</sup>	>5.32 <sup>b</sup>	>5.05 <sup>b</sup>	>4.04 <sup>b</sup>	<3.00 <sup>c</sup>	2.44
Plexiglas	>5.90 <sup>b</sup>	5.62	4.83	>4.40 <sup>b</sup>	<3.00 <sup>c</sup>	3.85
Print	5.85	5.86	5.74	4.51	<3.00 <sup>c</sup>	3.38
Chromium	6.55	5.95	6.63	4.08	<3.00 <sup>c</sup>	2.61

<sup>a</sup> Disinfectant Efficacy = (Log MSP<sub>(positive control)</sub> - Log MSP<sub>(test coupons)</sub>), where MSP<sub>(Positive Control)</sub> = Mean surviving population on positive control coupons; MSP<sub>(test coupon)</sub> = Mean surviving population on test coupons after disinfectant treatment; <sup>b</sup> Each of triplicate coupons showed no growth after disinfectant treatment; <sup>c</sup> Each of triplicate coupons showed TNTC growth

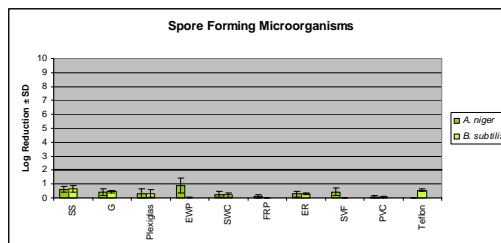
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# Spore Testing



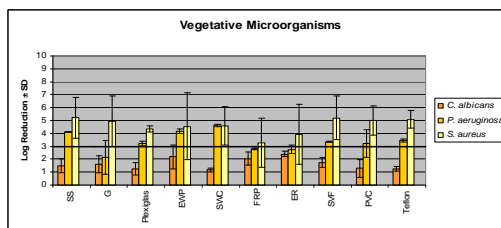
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## ATCC: Hydrogen Peroxide 6%



CT = 9 min.

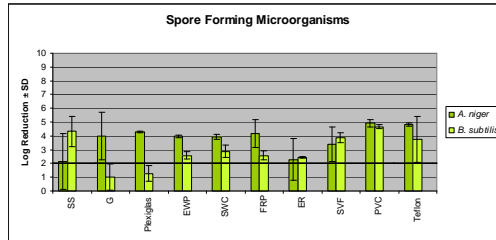
2 Log Reduction Target



3 Log Reduction Target

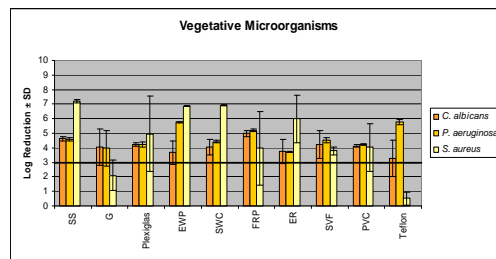
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## ATCC: H2O2/PAA RTU



CT = 5 min.

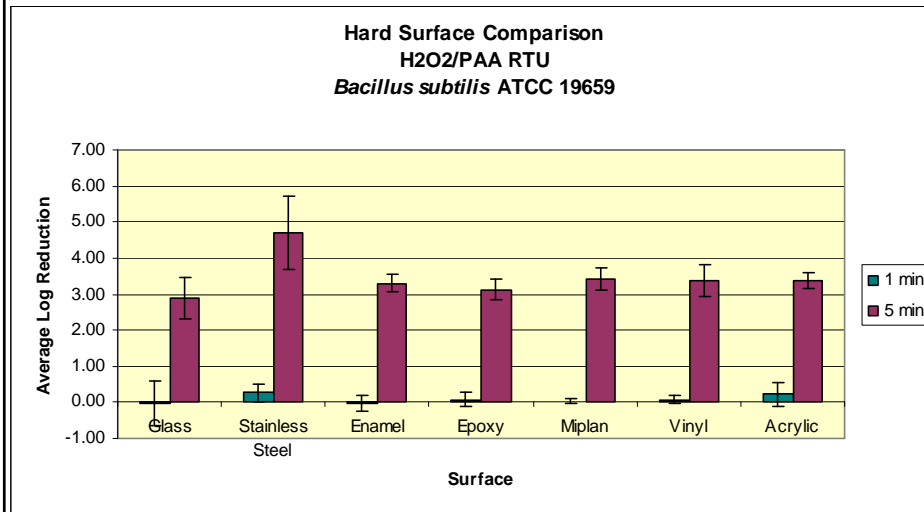
2 Log Reduction Target



3 Log Reduction Target

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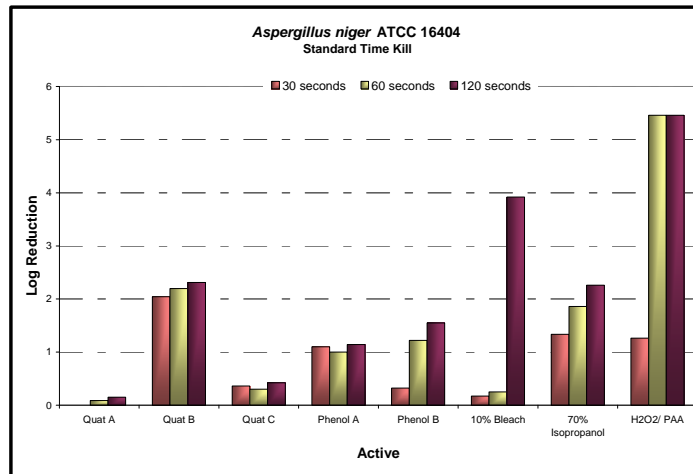
## Hard Surface Tests



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## Efficacy Comparison with STERIS Fungi

Life Sciences Group



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## Agenda



- Current Industry Trends
  - Cleaning and Disinfection
  - Current Warning Letters & Industry Guidance

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## Current Guidance on Disinfectants



- USP 35 General Chapter <1072>
- ISO /DIS 14698-parts1-3
- The Orange Guide 2007
- Aseptic Processing Guide 2004
- Current Industry Articles (Jose Martinez, Scott Sutton, Richard Prince)
- USP 35 <797>
- Several Industry Books (Seymour S. Block)

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## 483 Observation



- “The records do not include specific documentation on the time employed in the cleaning and disinfection of the separate areas in order to make a better assessment about the procedures executed and if they are consistently followed.” March 1, 2012

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## 483 Observation

- “The firm enlisted the services of a contract firm to conduct cleaning and disinfection and inspection reports lacked adequate scientific justification and/or rationale documentation to support conclusions or actions to handle OOS results.” March 15, 2012

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## Current Warning Letter

- “Your firm has not established procedures designed to prevent microbiological contamination of drug products purporting to be sterile” Warning Letter February 22, 2012

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## Current Warning Letter



“Your Disinfectant qualification for (b) (4) and (b) (4) bi-spore disinfectants documented that the log reduction criteria (Bacteria>4, Fungi>3) was not met when challenged with multiple organisms in variety of surfaces. After disinfection you recovered *Micrococcus luteus* on vinyl, (b) (4), stainless steel, glass and wall laminate and *Enterobacter cloacae*, *Rhodococcus* sp, *Burkholderia cepacia*, *Pseudomonas aeruginosa* on glass. However your procedures for routine cleaning of the aseptic manufacturing area continue to require the use of unqualified disinfectants during days (b) (4) through (b) (4) of your disinfection program” Warning Letter October 7, 2011.

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## Current Warning Letters



“The materials that were tested in the Disinfectant Efficacy study were not representative of all the surfaces present in the Aseptic Processing Area.” “The stainless steel coupon tested did not represent these damaged surfaces” Warning Letter May 25, 2011

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## Current Warning Letter

- “Furthermore, we evaluated your environmental data from 2008 to 2010 and are concerned with the lack of comprehensive investigations when mold and bacteria were identified in your aseptic filling facility that exceeded action levels.”  
Warning Letter February 10, 2011

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## Current Warning Letter

- “We note that the cGMP violations listed in this letter include similar violates to those cited in the previous February 2008 inspection including failure to adequately conduct disinfectant efficacy studies” Warning Letter July 14, 2011.

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## Current Warning Letter

- “The inspection documented mold contamination in the Class 100 production room and visible black mold on the wall” “Your firm did not establish a schedule for cleaning with an agent designed to kill spores, although mold continued to be found in the class 10,000 area.” Warning Letter October 29, 2010

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## Warning Letter

- “Systematic facility cleaning for mold was not initiated in a timely manner. Systematic cleaning was initiated after several months of environmental excursions for mold throughout the manufacturing areas, including aseptic areas.” Warning Letter March 28, 2008.

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## Warning Letter



- “Your firm does not ensure that a \_\_\_\_\_ system is employed, or that the disinfectant is rendered sterile prior to use.”
- However your response to our FDA-483 is inadequate because the following were not addressed: Effectiveness of \_\_\_\_\_ solution at the dilution used, and 2) effectiveness of \_\_\_\_\_
- throughout the shelf life (up to the expiry date).”
- Warning Letter March 2009.

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## Questions & Answers

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