

Control of Fungal and Bacterial Spores in Cleanrooms and Controlled Environments



Jim Polarine
Senior Technical
Service Manager

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Science & Solutions for Life

Agenda

➤ **Common sources of Bioburden**

- Disinfectant Qualification Studies
- Bringing the Cleanroom Online



Review - Microflora in Cleanrooms (U.K.)

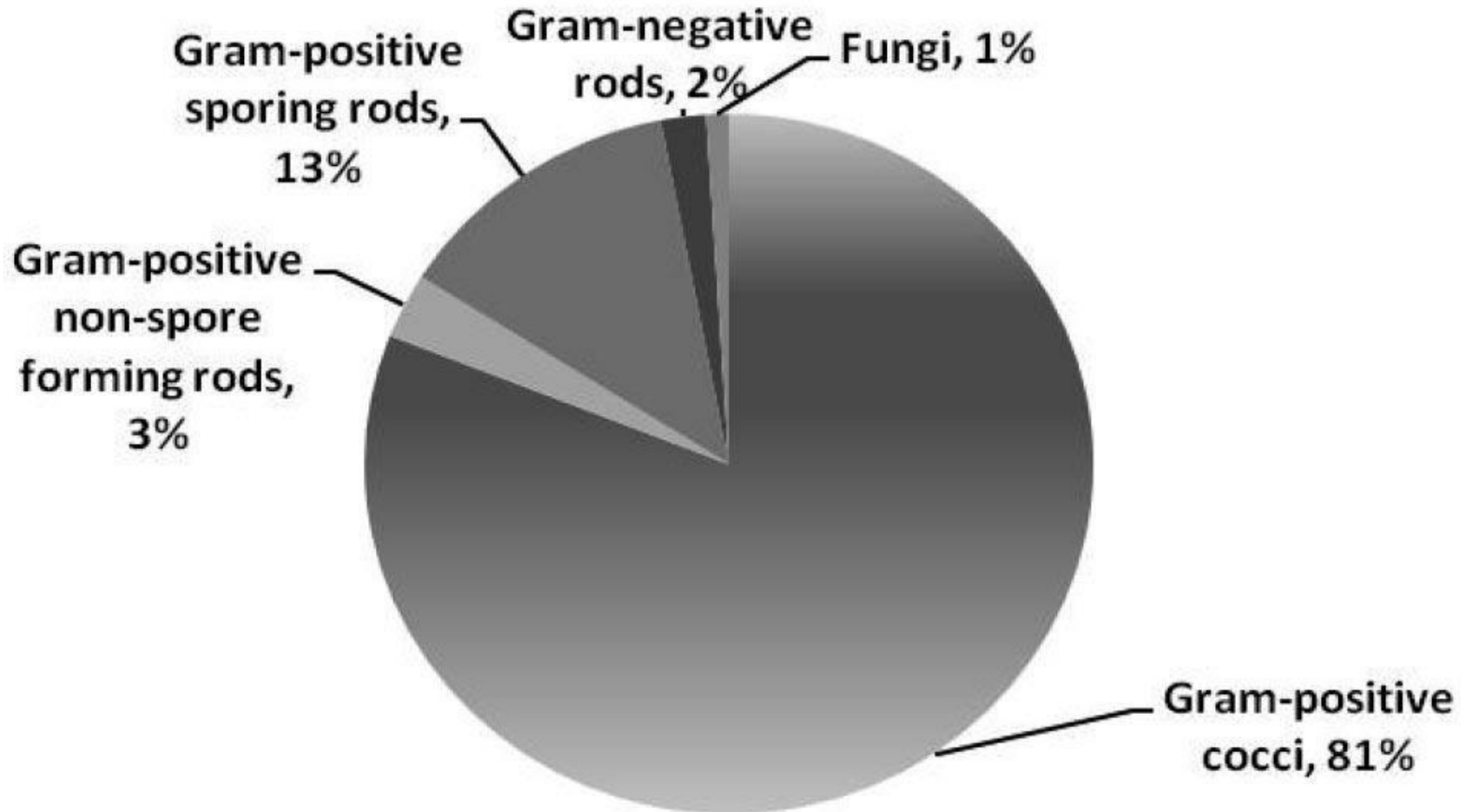
- Tim Sandle
- PDA J Pharm Sci and Tech 2011, 65:392-403
- **A Review of Cleanroom Microflora: Types, Trends, and Patterns**

- Examined isolates from 2000-2009 in U.K.
- Grade A/B and C/D



Review - Microflora in Cleanrooms (U.K.)

Grade A and Grade B microflora by group, 2001-2009





Review - Microflora in Cleanrooms (U.K.)

Genus	A/B (6729)	C/D (2500)
<i>Micrococci</i> (and related)	38%	40%
<i>Staphylococci</i>	21%	11%
<i>Bacillus</i> (and related)	13%	10%
<i>Pseudomonas</i> (and related)	<1%	8%
<i>Corynebacterium</i> (and related)	3%	5%
<i>Rhodococci</i>	<1%	N/A
Fungi	N/A	3%



Microorganism Resistance Hierarchy

	Microorganism	Examples
 <p>More Resistant</p>	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>
Less Resistant	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



Molds common to cleanrooms and cold rooms

- *Aspergillus* spp.
- *Penicillium* spp.
- *Stachybotrys* spp.
- *Cladosporium* spp.
- *Mucor* spp.
- *Scopulariopsis* spp.
- *Trychophyton* spp.
- *Chaetomium* spp.
- *Candida albicans* (yeast)



Bacterial Spores in Operations

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

B. cereus Group:

B. cereus, *B. anthracis*, *B. mycoides*, *B. thuringiensis*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. manliponensis*



Aspergillus niger

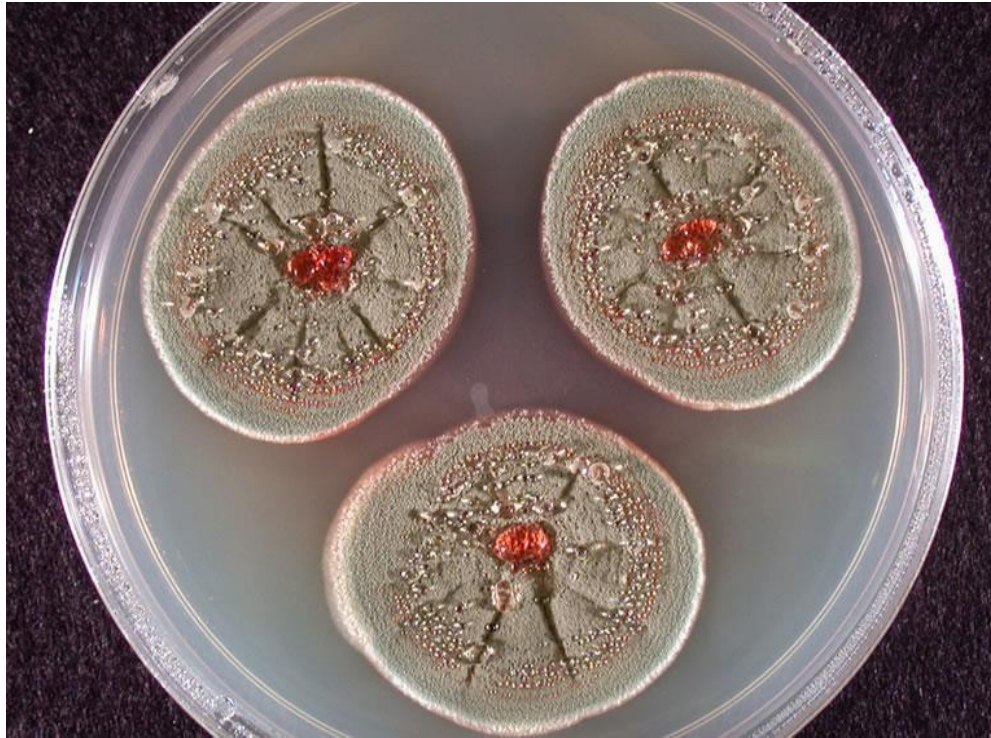
<http://nigergallery.blogspot.com/2012/10/aspergillus-niger.html>



<http://genome.jgi-psf.org/Aspni5/Aspni5.home.html>



Penicillium



Courtesy Ann Larson



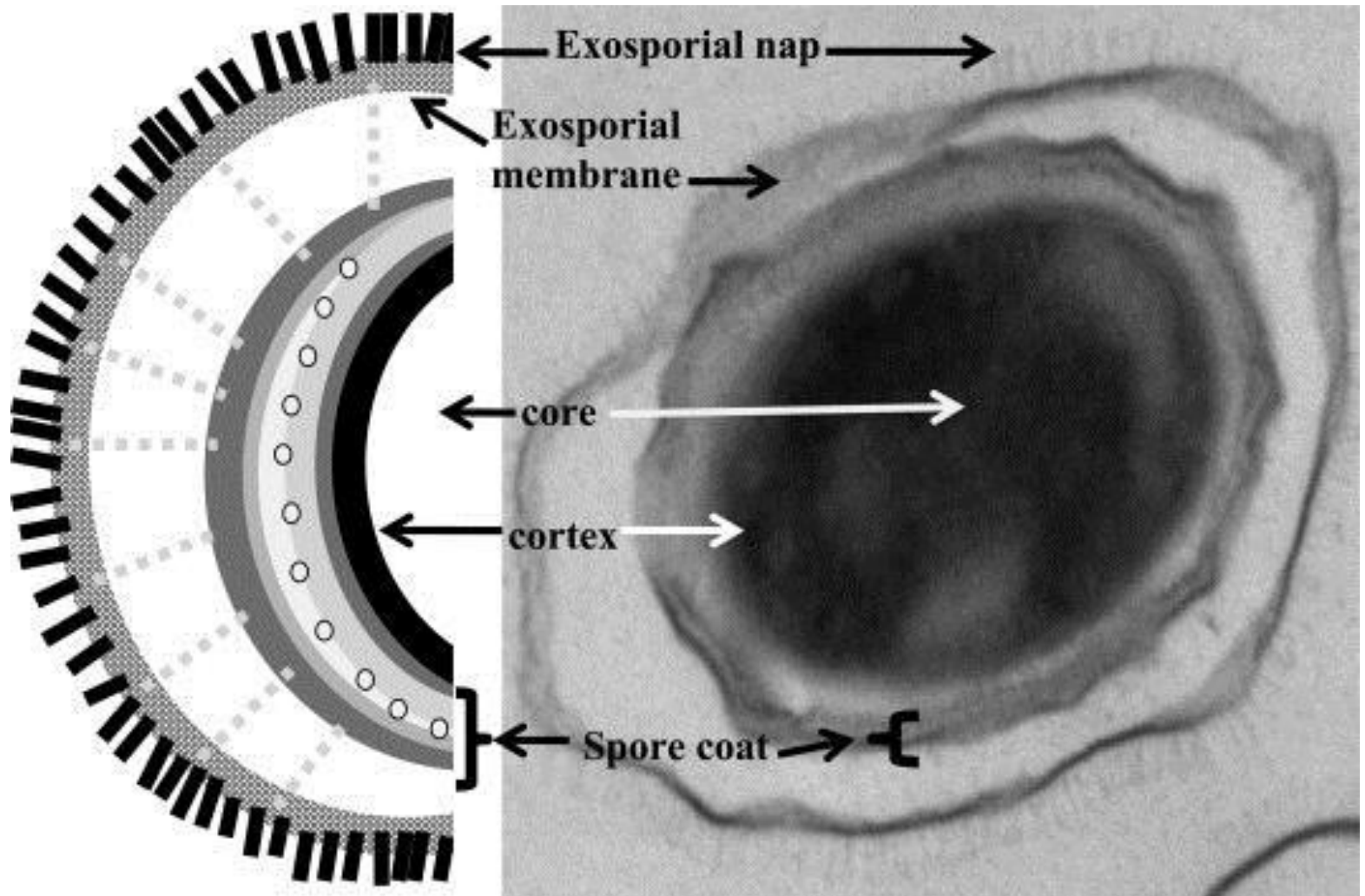
Cleanroom Fungi



Courtesy Dan Klein



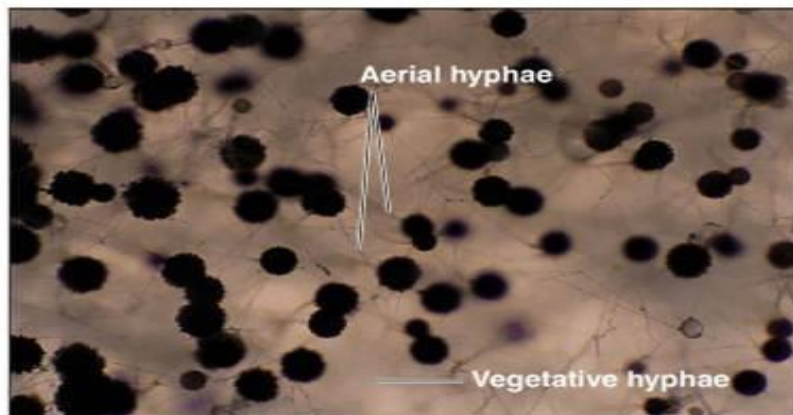
Exosporium – *B. anthracis*



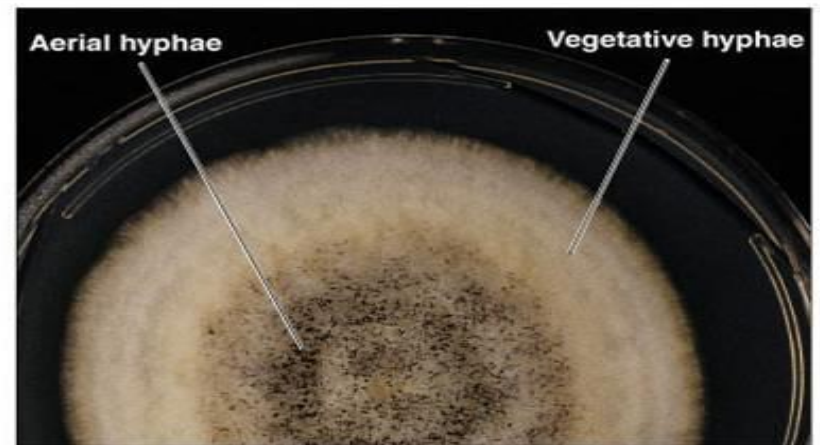
Inoculum Preparation

Fungal Spores

Cultures need to be incubated for a sufficient length of time before harvesting spores



(a) *Aspergillus niger*



(b) *A. niger* on agar



Agenda

➤ **Common sources of Bioburden**

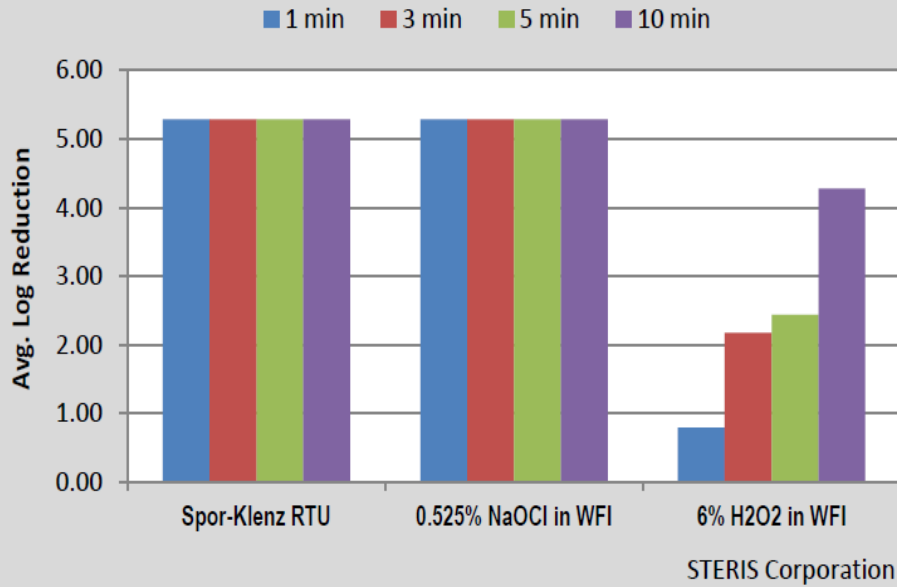
➤ **Disinfectant Qualification Studies**

• **Bringing the Cleanroom Online**

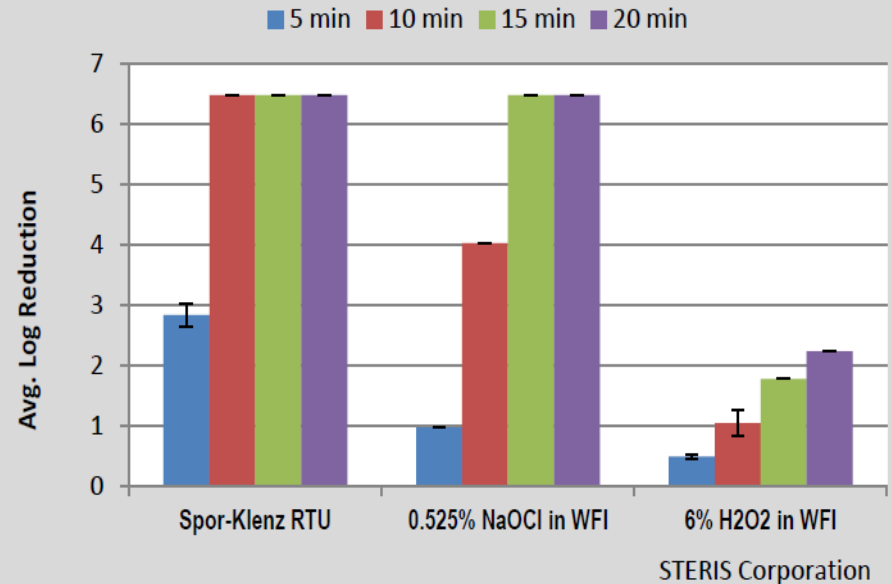


Efficacy of Sporicides

Efficacy of Aseptic Products in a Time Kill Study
A. brasiliensis ATCC 16404 Baseline 5.28 log₁₀

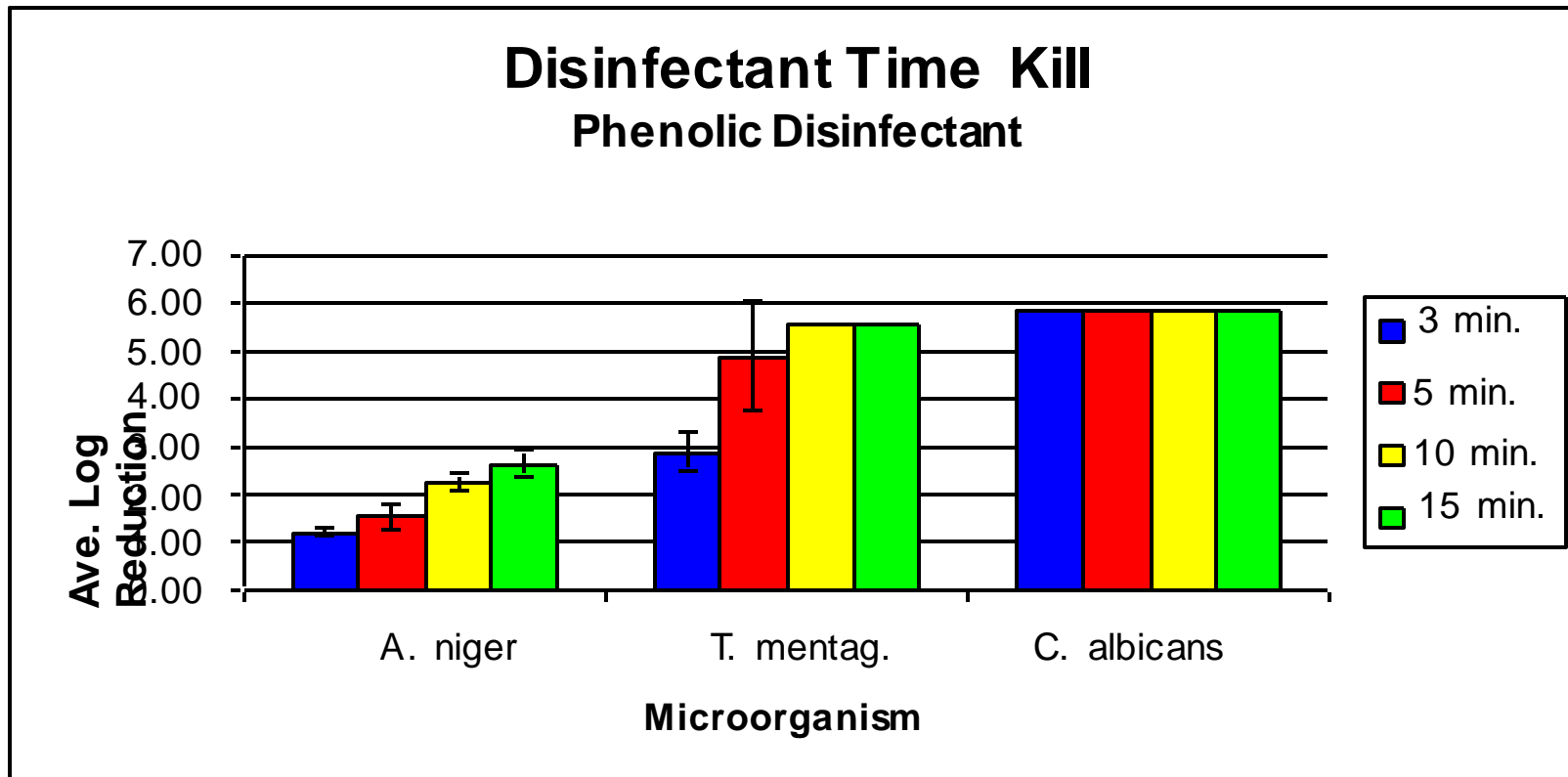


Efficacy of Aseptic Products in a Time Kill Study
B. subtilis ATCC 19659 Baseline = 6.48 log₁₀



Testing Against Fungal Spores

- *Trichophyton mentagrophytes* is US EPA standard (easily killed)
- Cleanroom users test *Aspergillus brasiliensis* (typically the most difficult to kill mold)



Case Study on Substrates

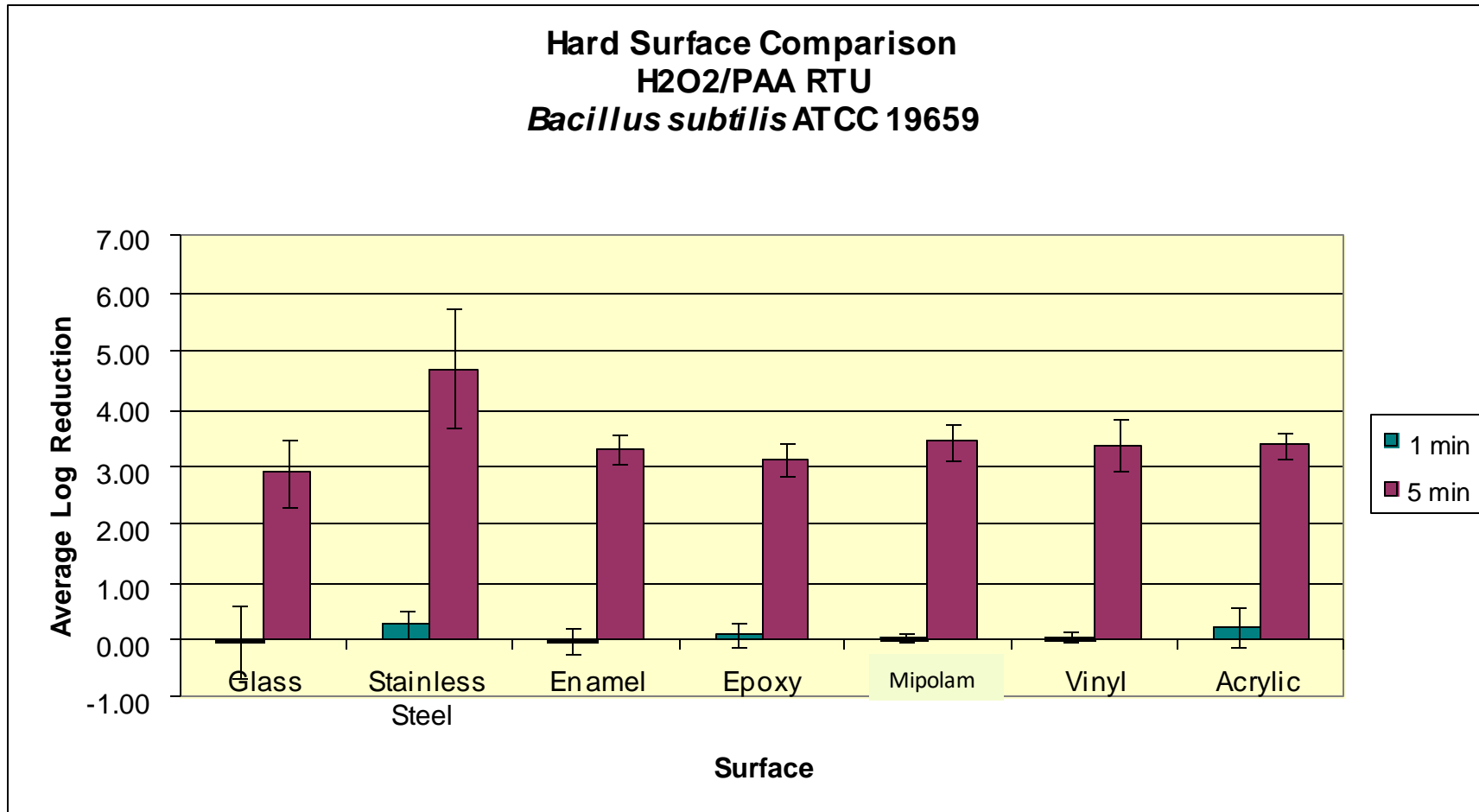
Efficacy (log reduction) of Low pH phenolic: (1:256) against test microorganisms on representative surfaces

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

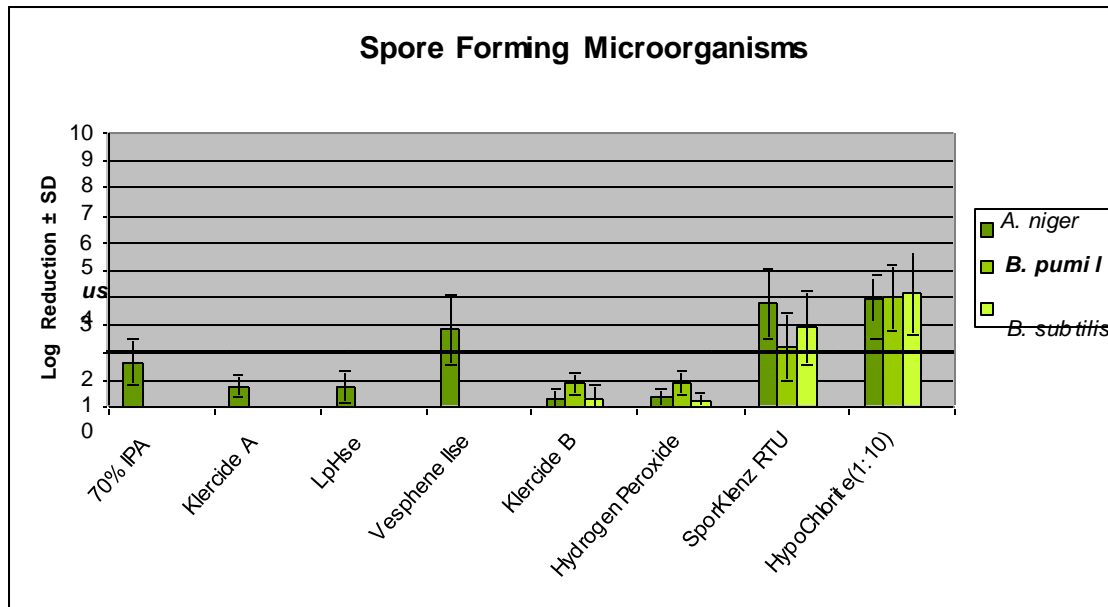
^a Disinfectant Efficacy = (Log MSP_(positive control) - Log MSP_(test coupons)), where MSP_(Positive Control) = Mean surviving population on positive control coupons; MSP_(test coupon) = Mean surviving population on test coupons after disinfectant treatment; ^b Each of triplicate coupons showed no growth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



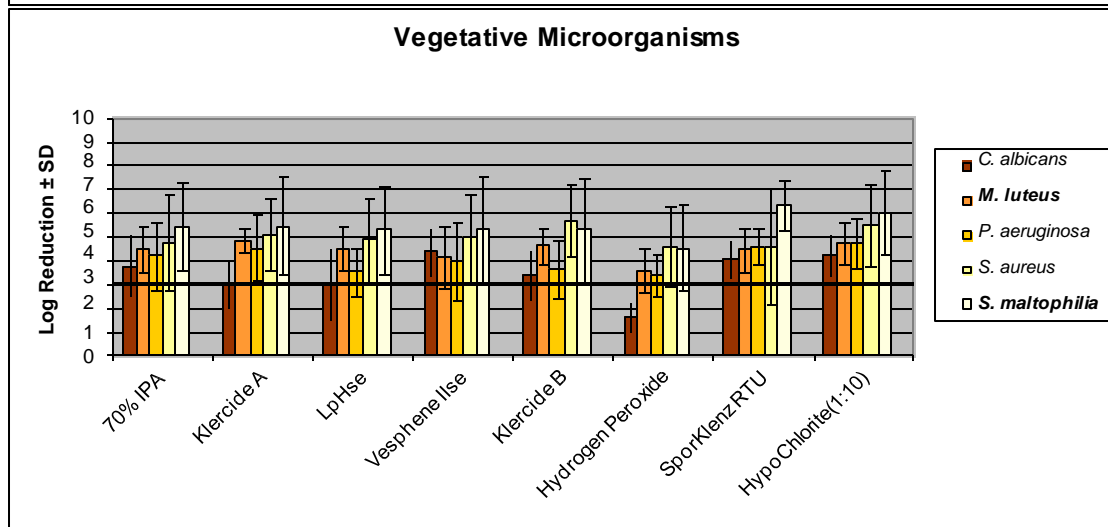
Hard Surface Test Results



Environmental Isolate Testing



2 Log Reduction Target

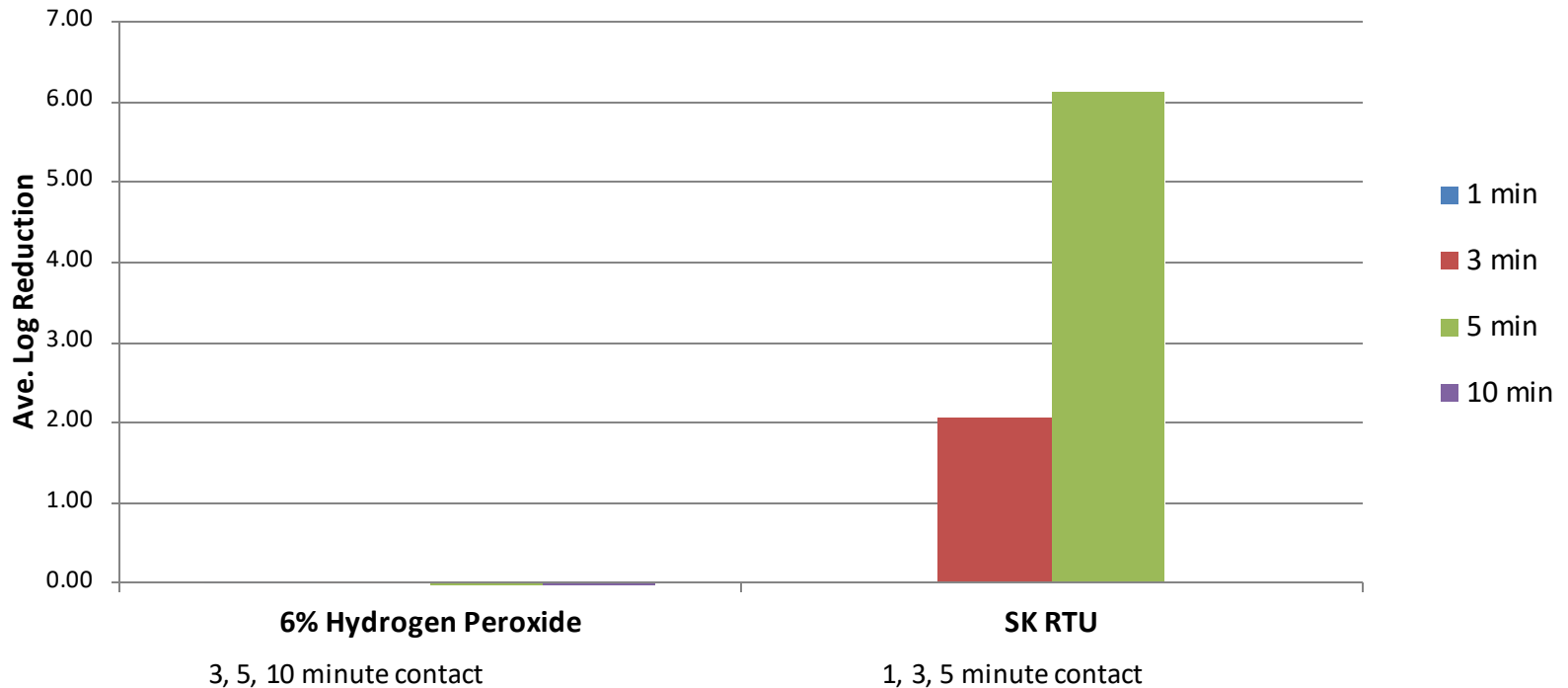


3 Log Reduction Target



Spore Testing

6% H₂O₂ vs. Spor-Klenz RTU
Standard Time Kill Study 13 Jun 2007
***B. subtilis* spores 19659 Baseline = 6.60 log₁₀**



STERIS Corporation

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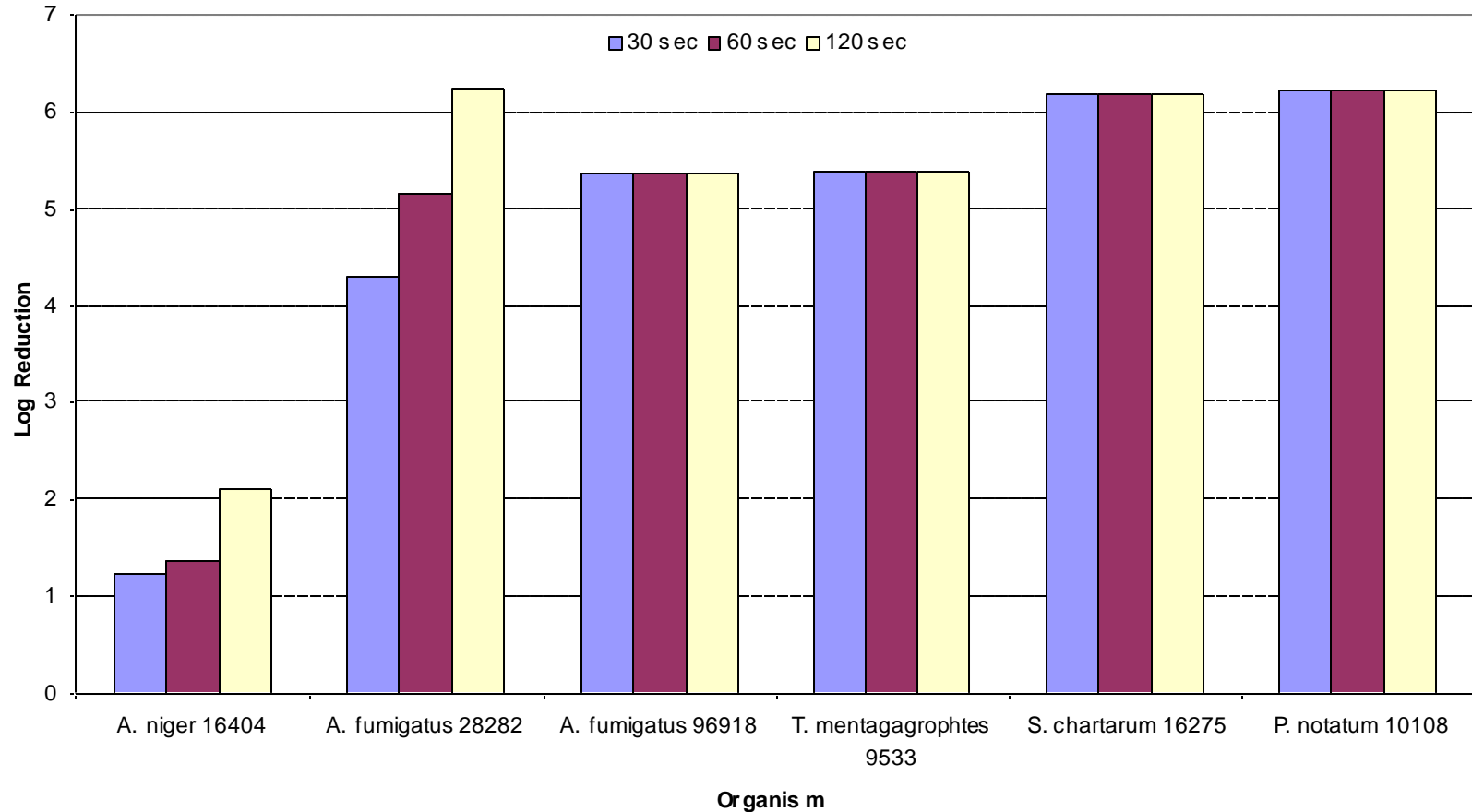


70% IPA Efficacy against Molds



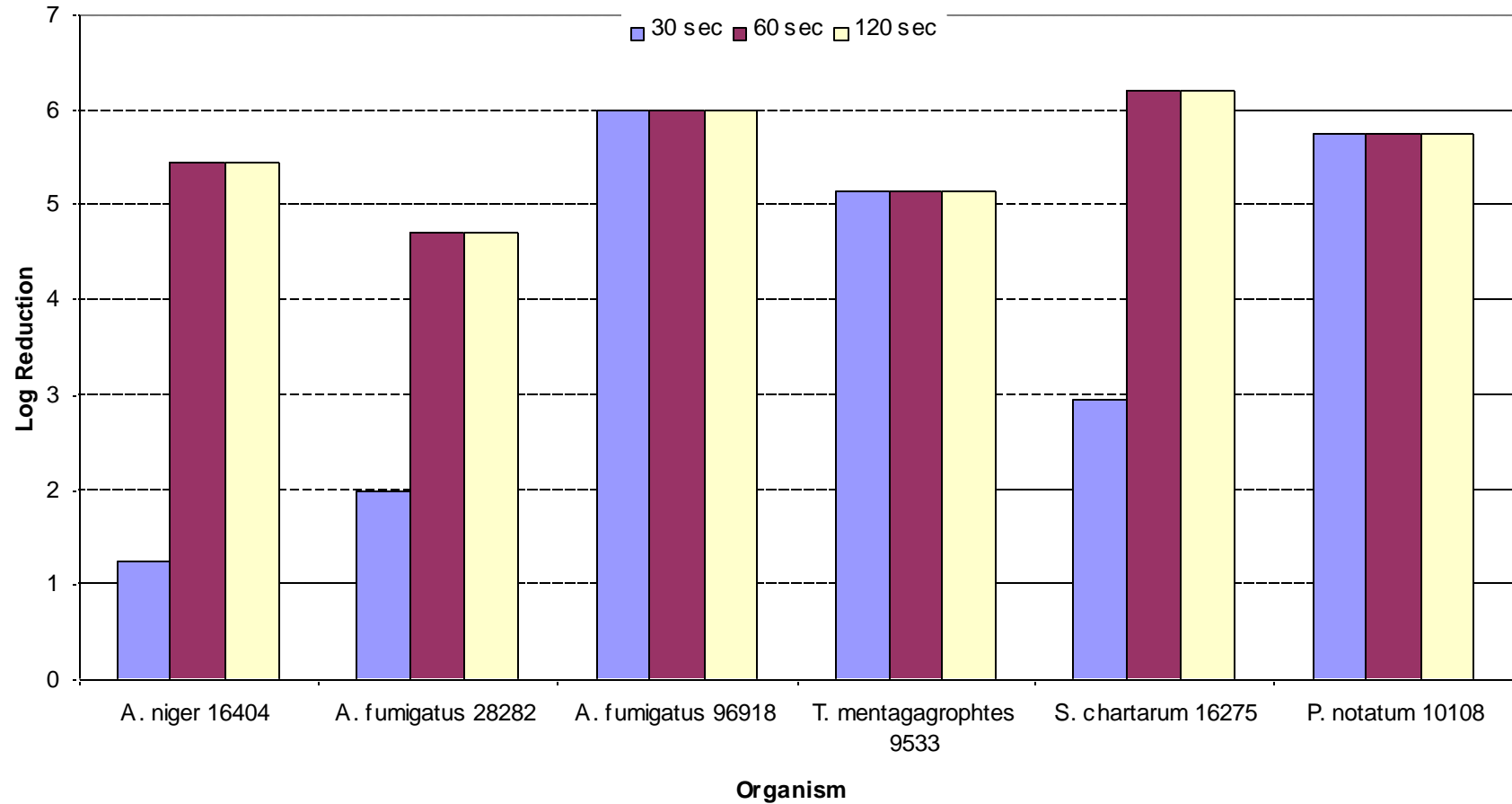
Life Sciences

Fungicidal Activity of 70% Isopropyl Alcohol using Time Kill Method



H2O2/PAA RTU against Molds

Fungicidal Activity of H2O2/PAA RTU using Time Kill Method

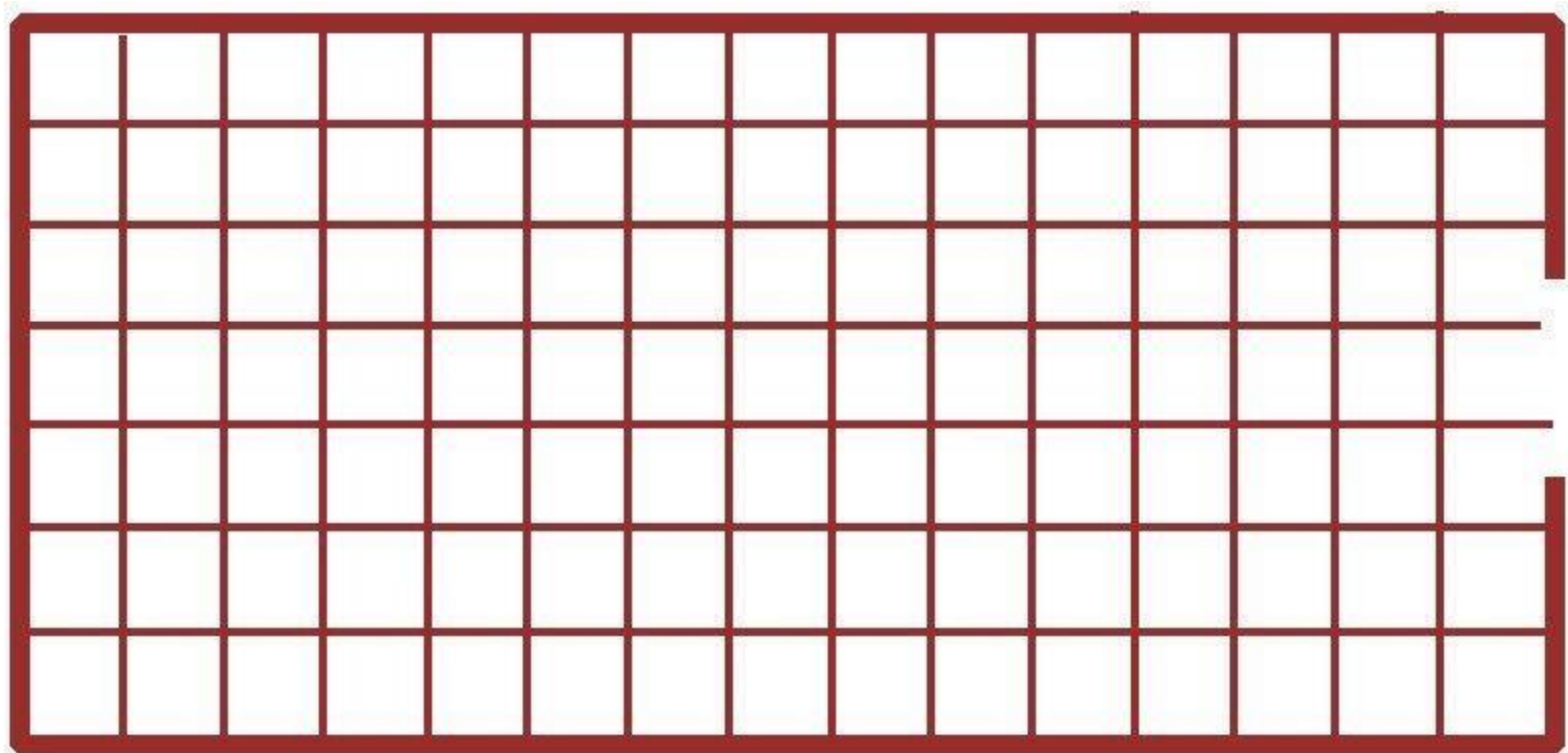


Case Study: Construction Event

- Worst Case Events
- 9X Clean [1X Sporicide + 2X Phenolic repeated on days 1,2,3]
- Fogging
- VHP®
- Triple Clean
 - Defined 3X Disinfectants and Sporicide (Different Definitions)
 - EM frequency (Static and Dynamic)
 - Release of the room



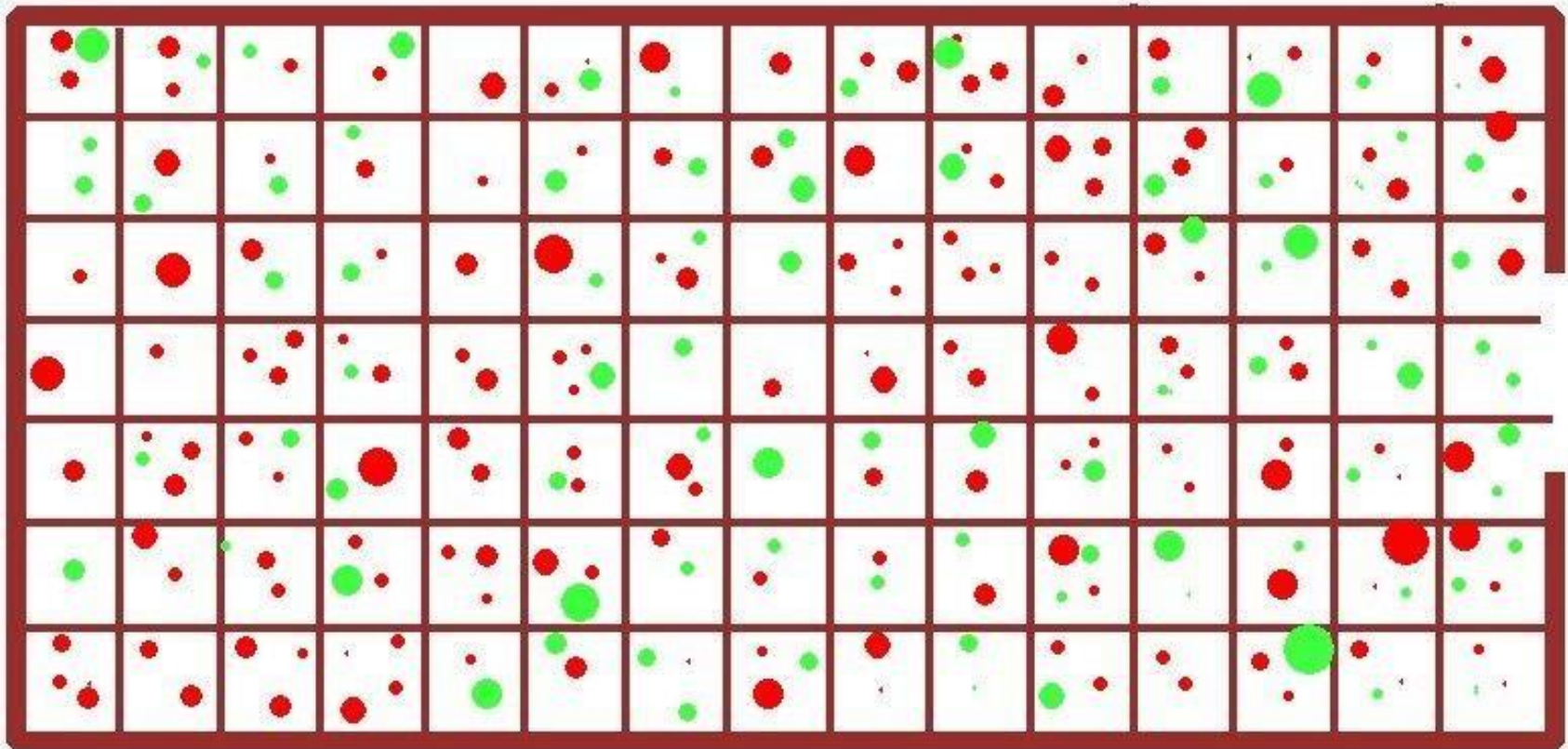
Cleaning and Disinfection Efficacy - *In situ* study



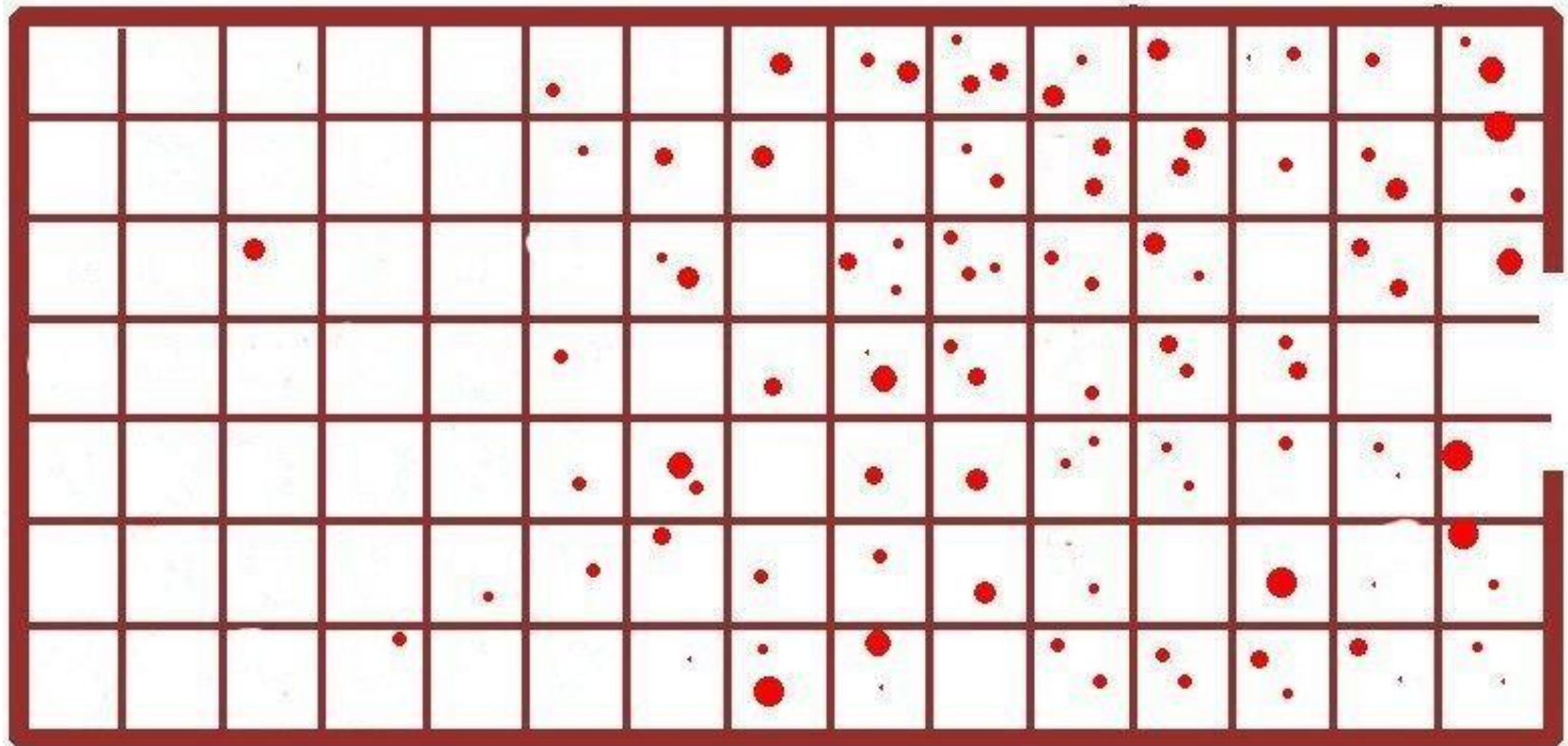
Time 0

Red = Spore formers

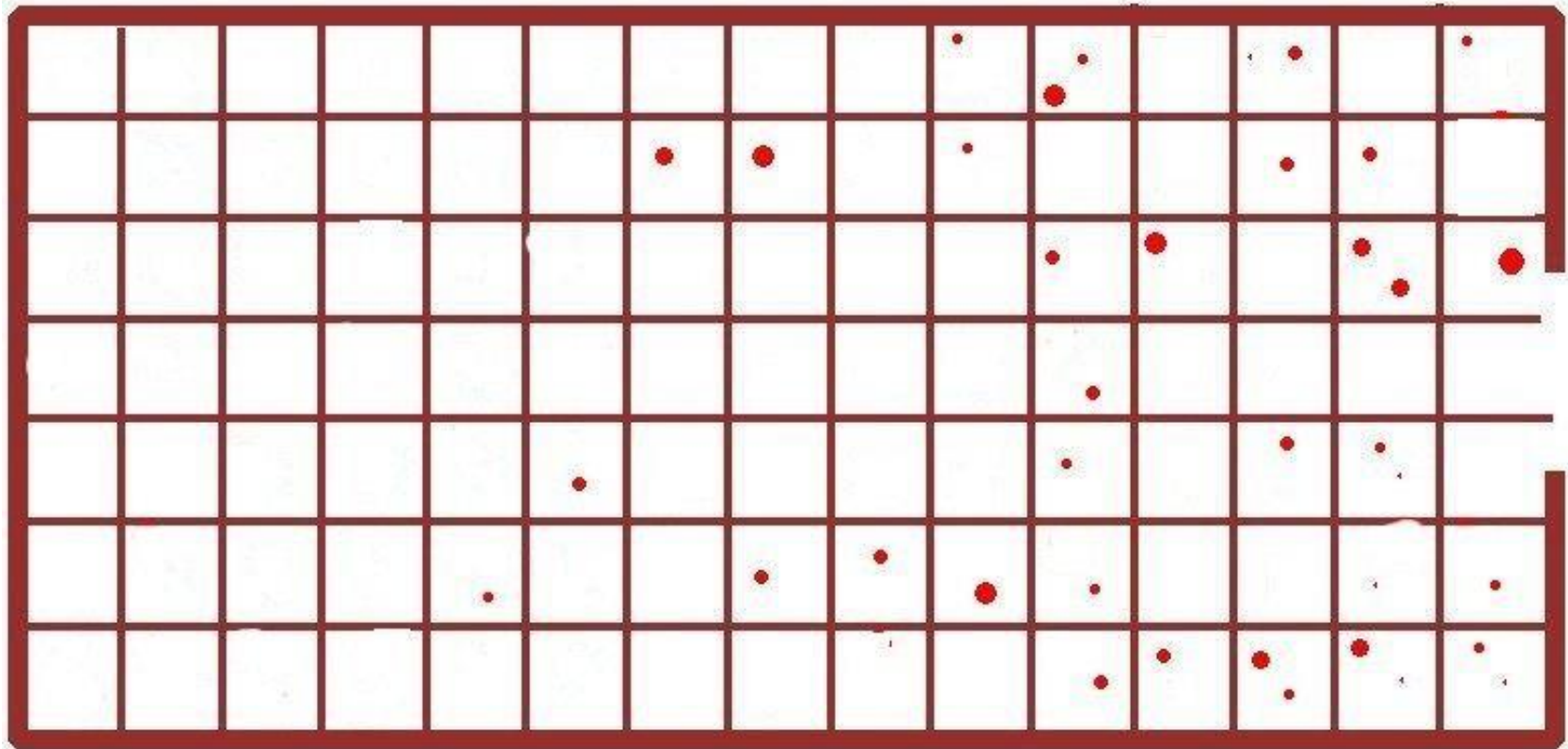
Green = Other



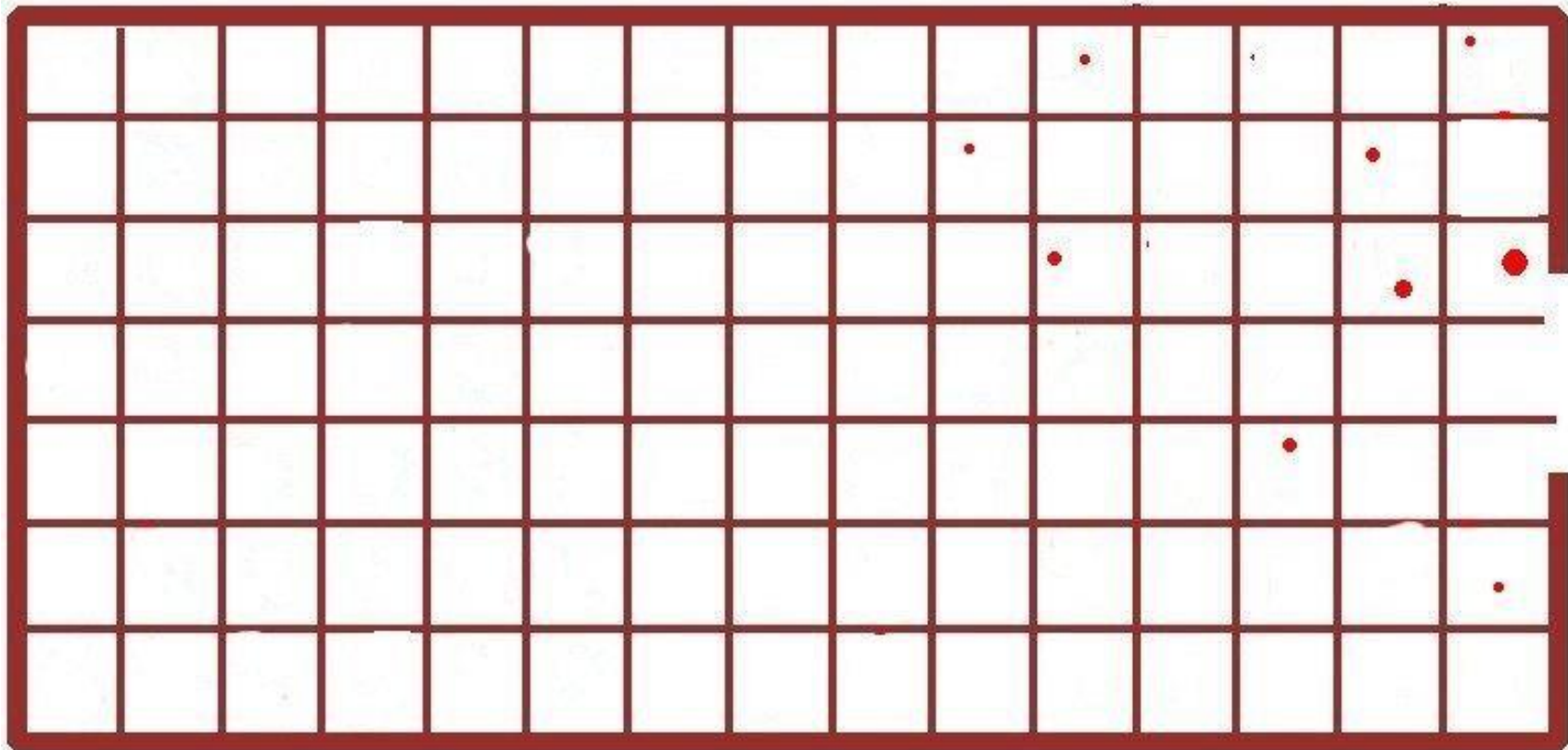
After 1X Cleaning - No Sporicide



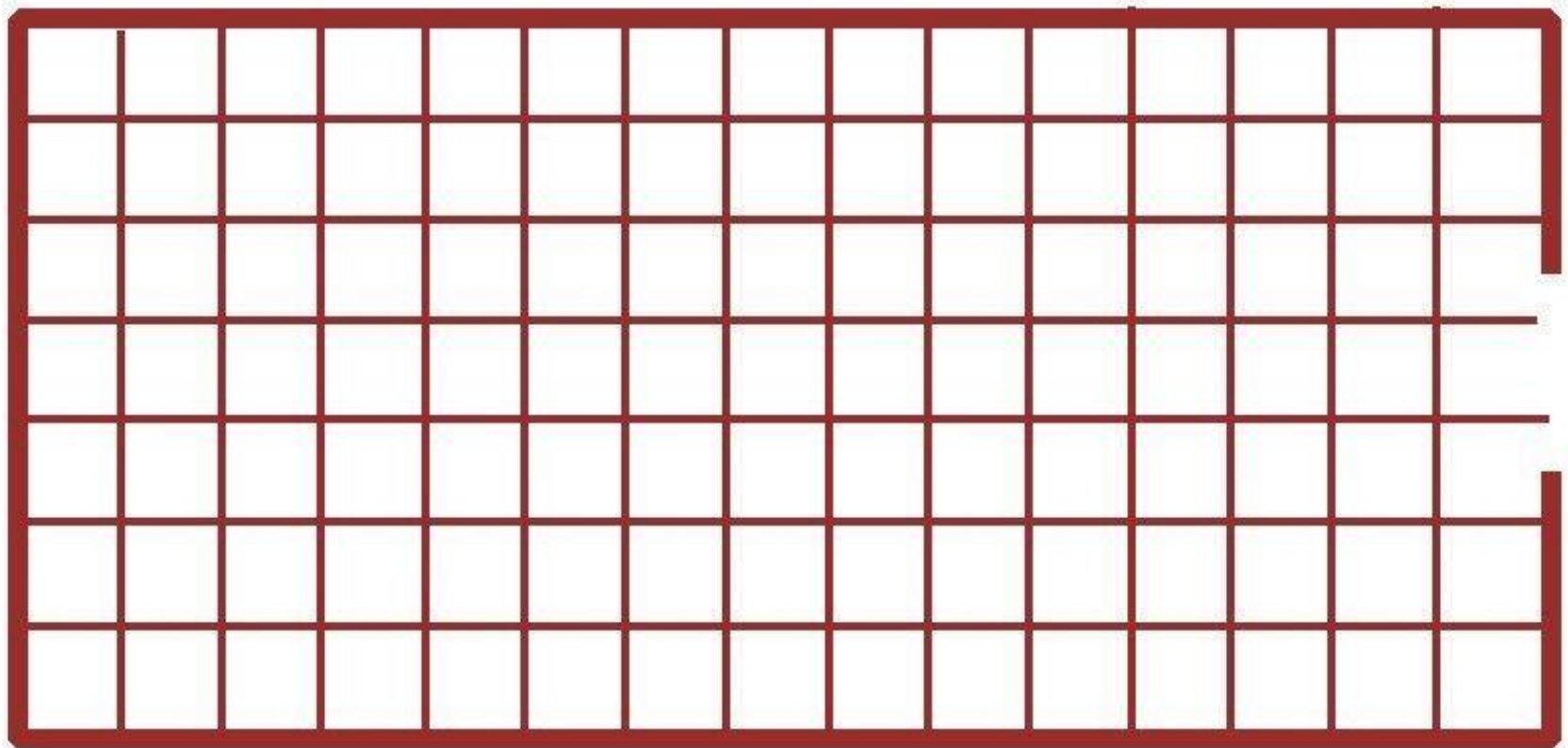
After 2X Cleaning – No Sporicide



After 3X Cleaning - No Sporicide

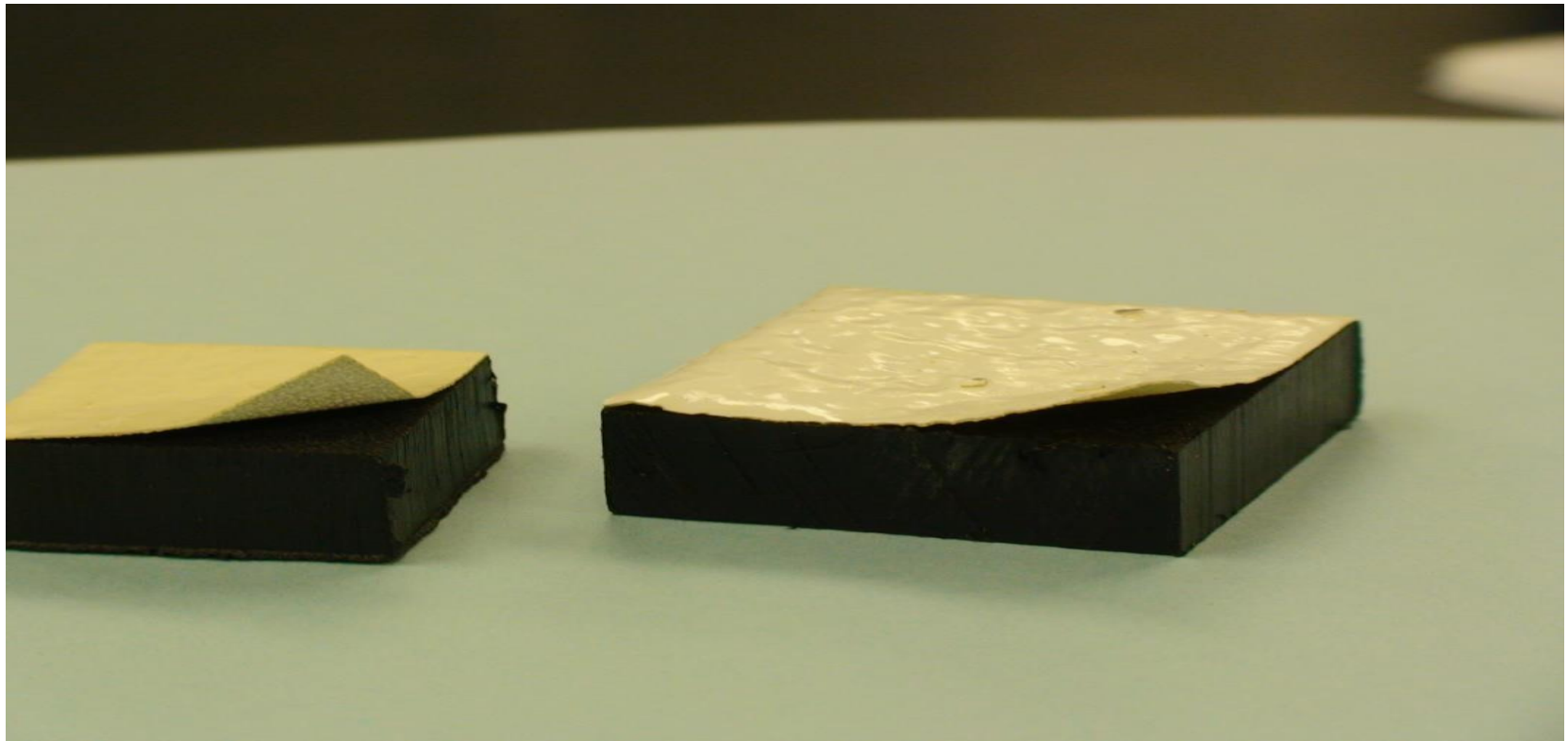


After Sporicide

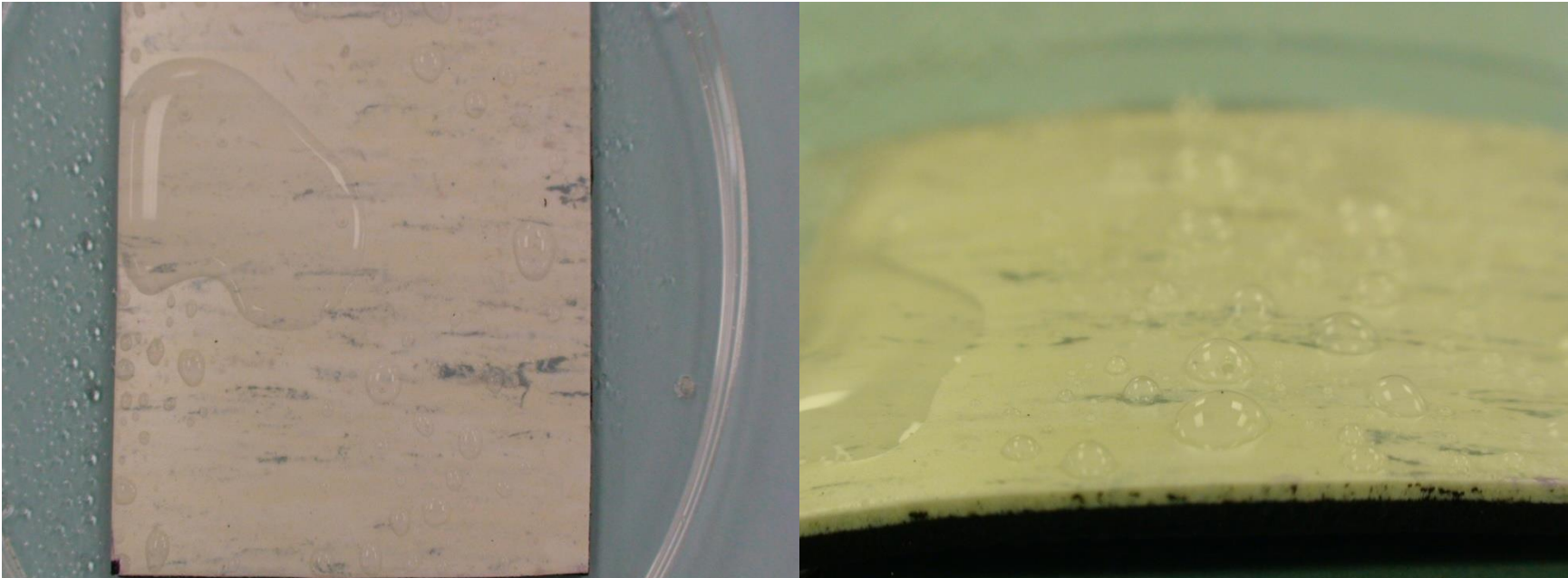


Surface Preparation

Autoclaving may not be acceptable for some surfaces (Saniflex)

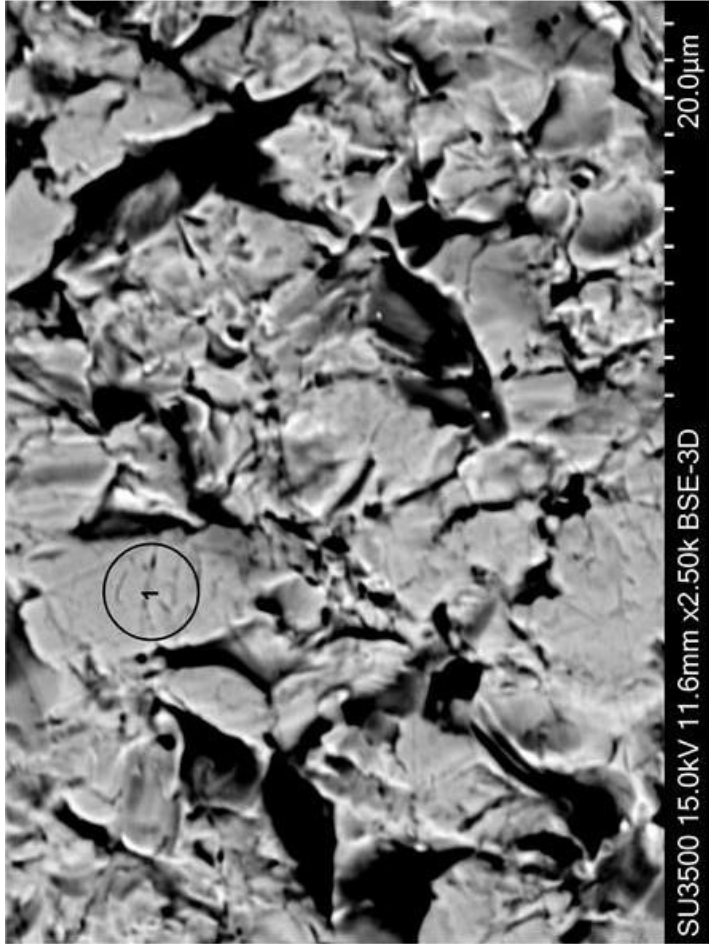
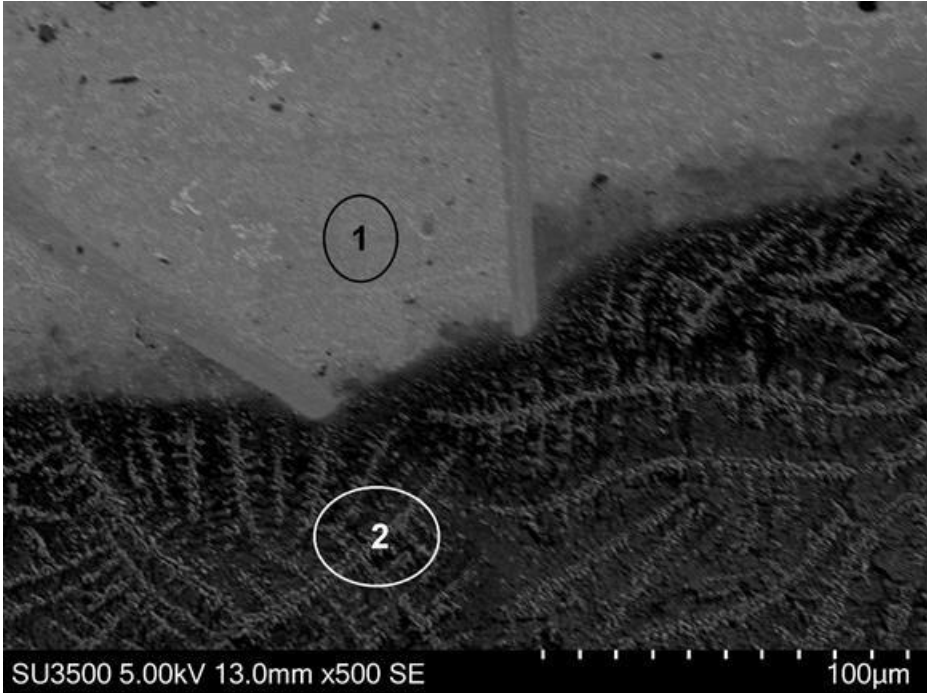


Surface Tension Issue



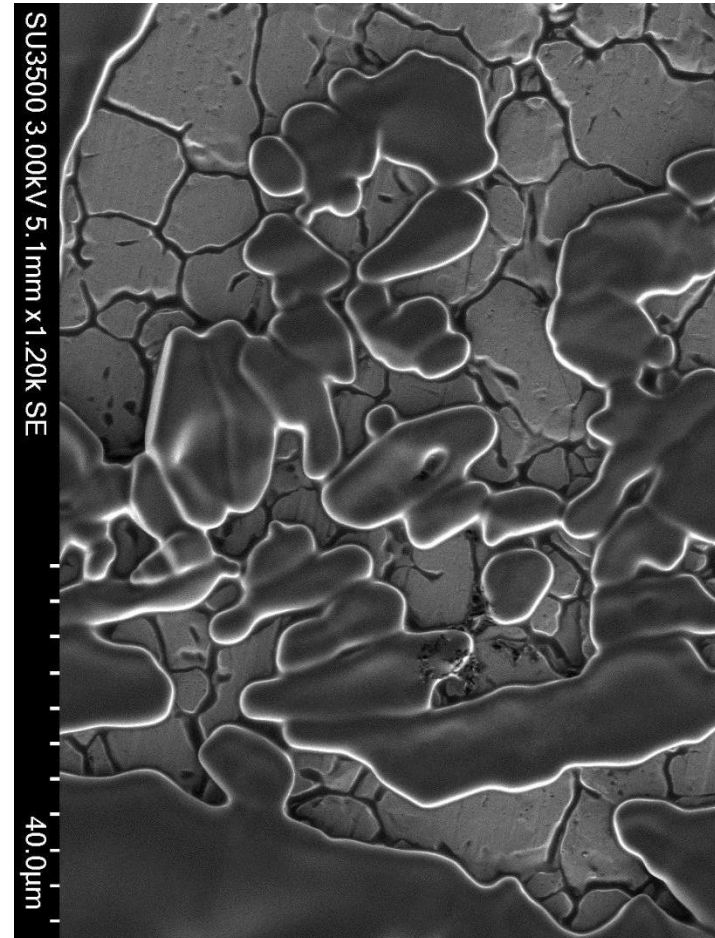
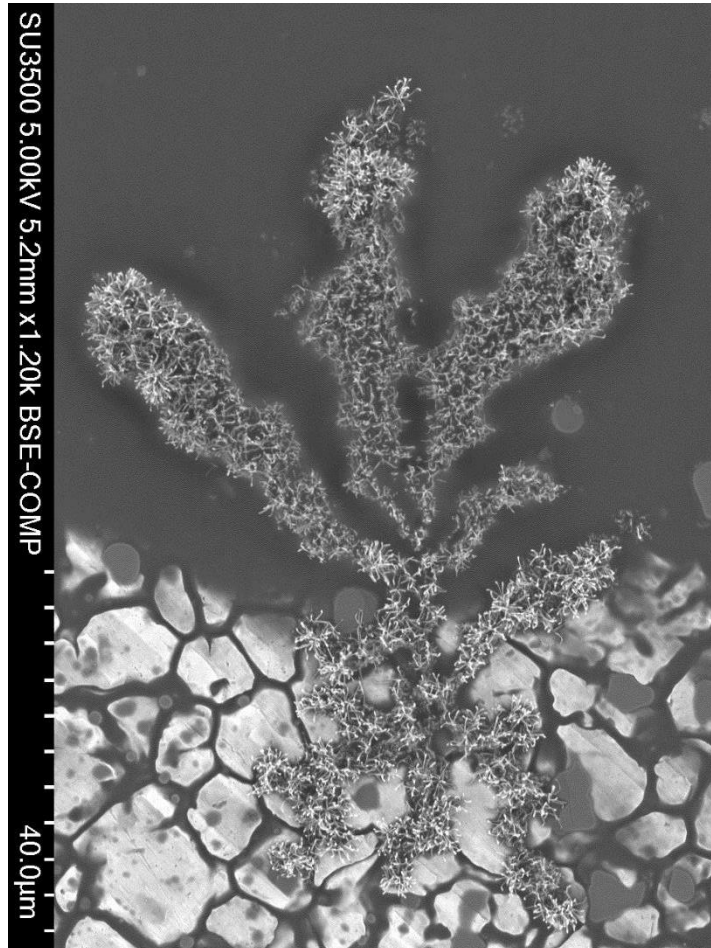
SEM Cleanroom Surfaces

Courtesy of Bruce Ritts



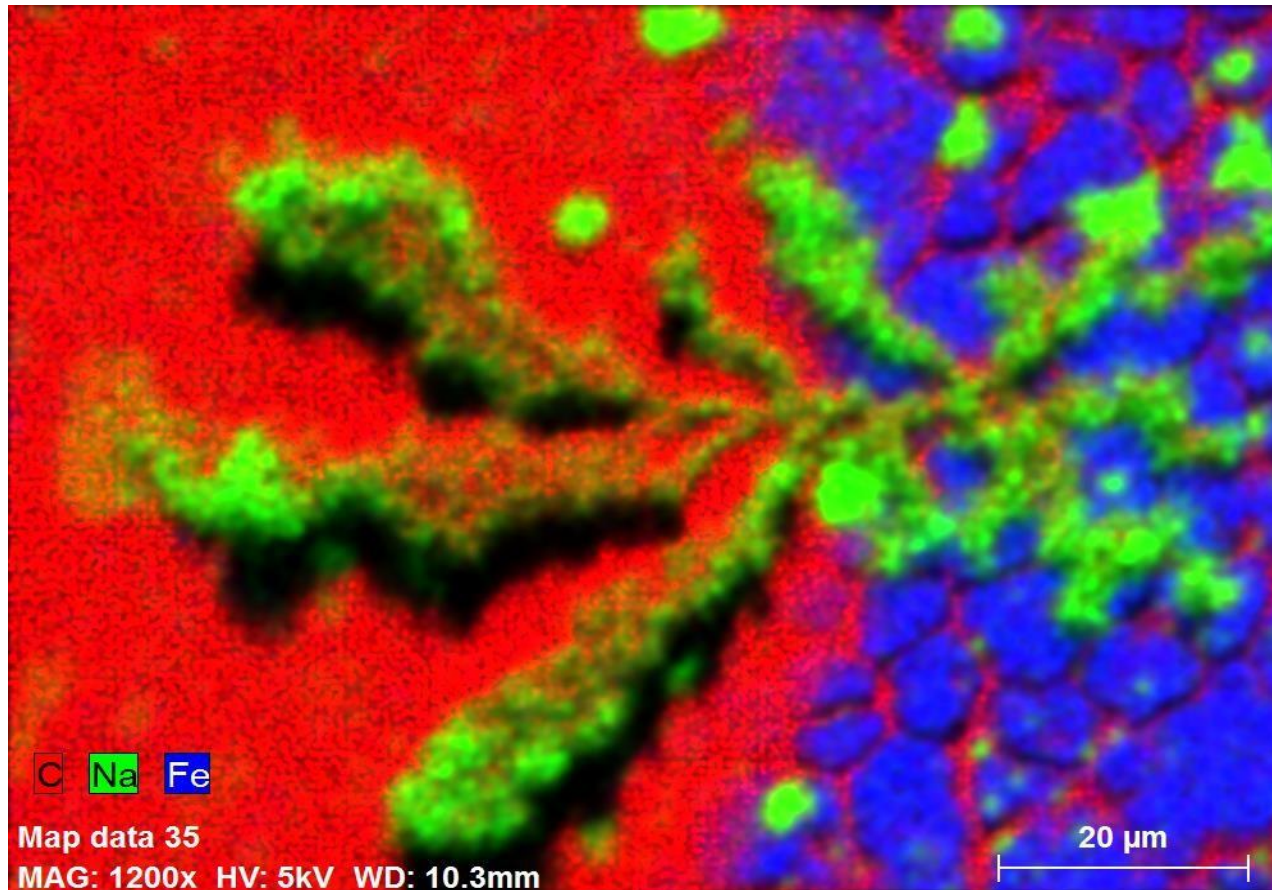
Residues: SEM

Courtesy of Bruce Ritts



Residue Analysis: SEM

Courtesy Bruce Ritts



Summary

- Common sources of bioburden when starting up the cleanroom
- Disinfectant Qualification Studies
- Bringing the cleanroom online



Control of Fungal and Bacterial Spores in Cleanrooms and Controlled Areas



Thank you
for
your
attention
jim_polarine@steris.com

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