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OMICS International is a pioneer and leading science event organizer, which publishes around 700+ open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 1000+ conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Case Studies of Human Flora and Spore Contamination in Cleanrooms

STERIS
Life Sciences

Jim Polarine MA.

August 17, 2015

Science & Solutions for Life

Agenda

✓Case Studies in Bioburden Control

✓Starting Up a New Cleanroom

Bioburden Agenda

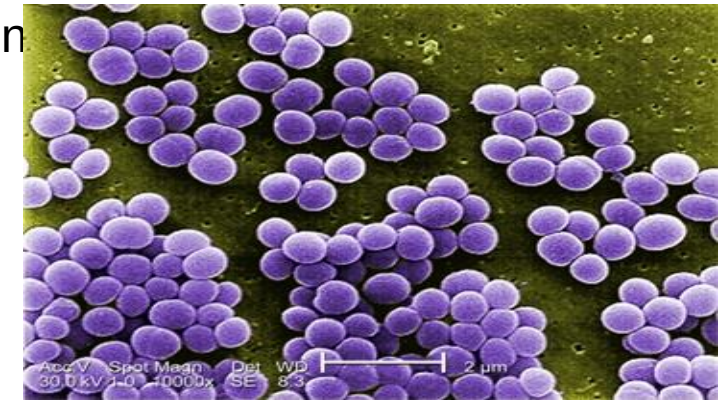
- ✓ Operator Contamination
- Fungal Spore Contamination
- Bacterial Spore Contamination

Operator contamination

- *Staphylococcus*
- *Propionibacterium acnes*

Human Skin Flora

- *Staphylococcus* cleanroom outbreak
  - Source
    - Gowning Material
    - Traced back to one operator
    - Skin infection
    - Another case was from a Ton
    - Non-sterile drug product



Courtesy Ann Larson

Propionibacterium acnes

- Gowning room
  - BSL Hood
  - Sources
    - Operator with acne
      - Solution
        - » Antimicrobial Handwash
        - » Antimicrobial Bodywash

Courtesy Ann Larson

Agenda

- ✓ Operator Contamination
- ✓ Fungal Spore Contamination
- Bacterial Spore Contamination

VALIDATION – Microorganism Selection

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

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

Fungal Spores

- *Penicillium*
- *Aspergillus*
- *Cladosporium*

Common sources of Spores

- Items brought into the Cleanroom
  - Bags, Boxes, Intervention Equipment, Pallets, Pallet Jacks, Scrubbers, Cart Wheels, Shoes, Shoe Covers
  - Raw Materials

Penicillium

- ISO-7 Cleanrooms
- Action Levels of 10 and picking up >100
  - Engineering Investigating
  - HVAC
  - Duct Work
  - HEPA Filters
  - Cooling Coils
  - Wall Coverings
  - Airflow Vents

Penicillium Investigation

- Entry and Exit Procedures
- Gowning Procedure
- Cart Wheels
- Construction
  - Further Investigation
  - Use of Sporicides
  - Containers in the Cleanroom
  - Coldroom Cleaning Procedures
  - Documentation
  - Assignable Cause

Penicillium

Courtesy Ann Larson

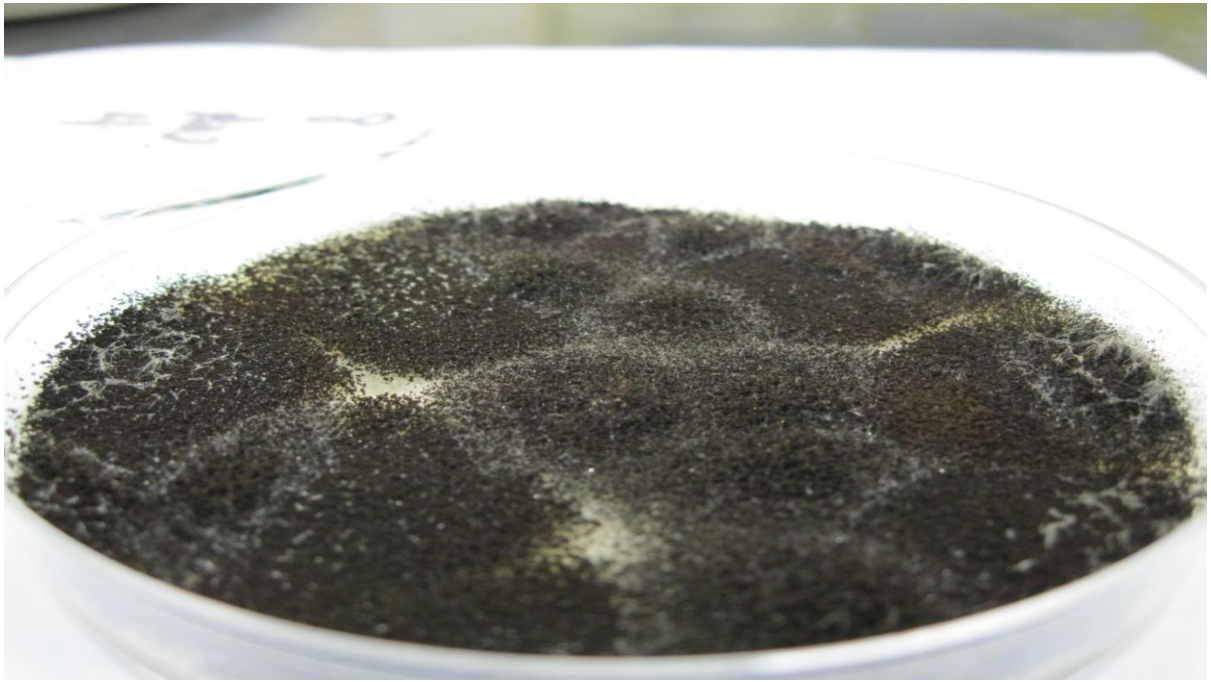
Aspergillus

- ISO-5 Cleanroom
  - Source
    - Door Kick Plate
    - High Impingement Spraying Device
- Exceeding Limits in ISO-7 areas
  - Dock Doors proximal to ISO-7 cleanroom
  - Storage room with limited control
  - No limits for mold spores
  - Limited control for incoming and outgoing items

Aspergillus Investigation

- Sporicidal usage in pass thru
- Clean and dirty area on the Dock
- Better control of gowning area
- Cart Wheel control
- Better gowning control

Fungi



Courtesy Dan Klein

Cleanroom Fungi

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Courtesy Dan Klein

Inoculum Preparation—Fungal Spores

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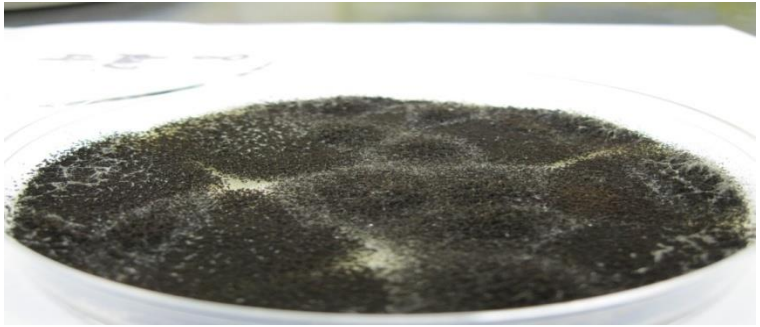
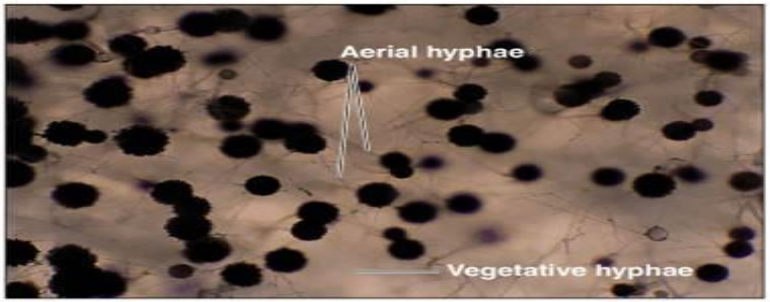
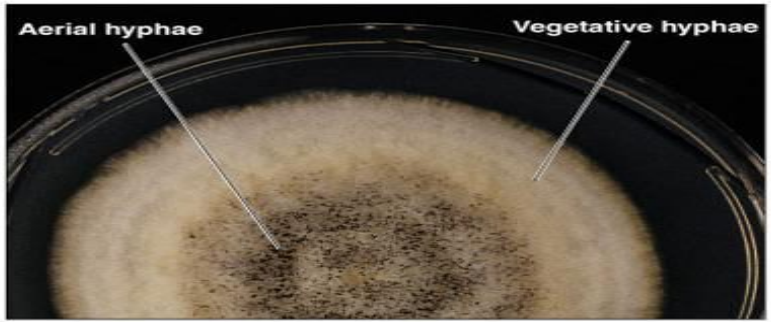


- Cultures need to be incubated for a sufficient length of time before harvesting spores



(a) *Aspergillus niger*

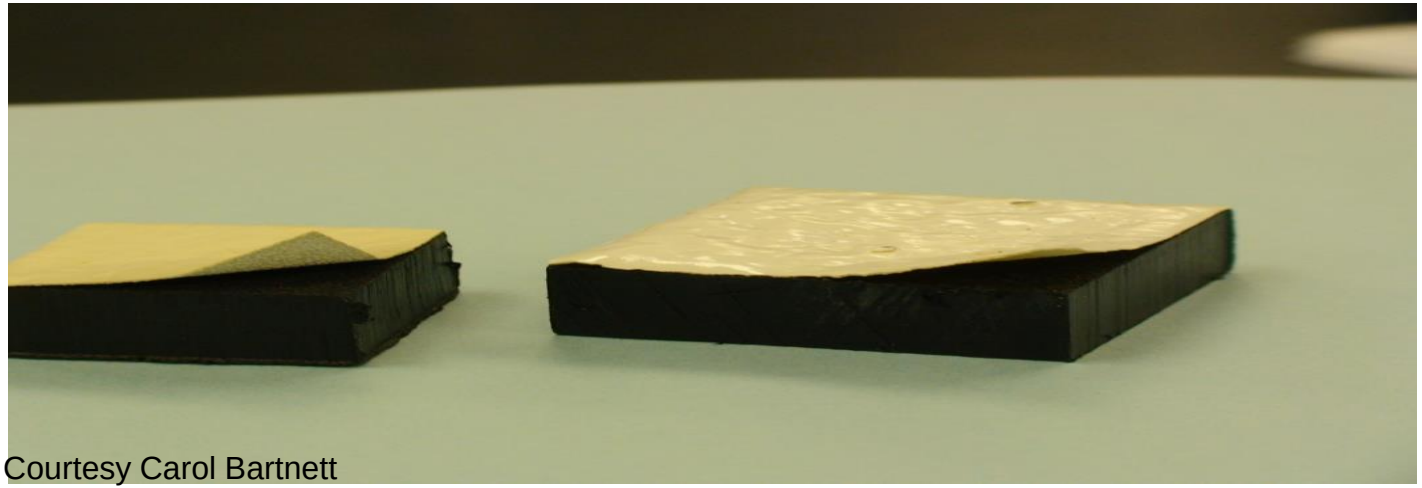


(b) *A. niger on agar*

Courtesy Dan Klein

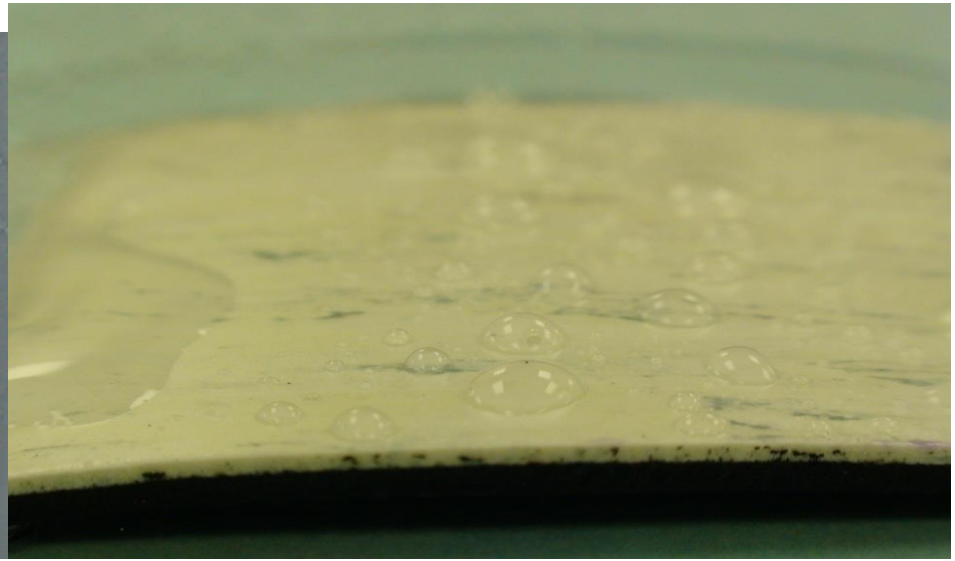
Surface Preparation

- Autoclaving may not be acceptable for some surfaces (Saniflex)



Courtesy Carol Bartnett

Surface Tension Issue



Cladosporium

- Elevated levels in Puerto Rico
- Common in Southern CA
  - Increase Sporicide Usage
  - Sources
    - Humidity and temperature
    - Burning of Sugar Cane fields

Case Study on Substrates

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

Surface	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*	*Corynebacterium glutamicum*	*Candida albicans*	*Aspergillus niger*	*Penicillium chrysogenum*
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
Print	5.85	5.86	5.74	4.51	<3.00[c]	3.38
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

70% IPA Efficacy Against Molds

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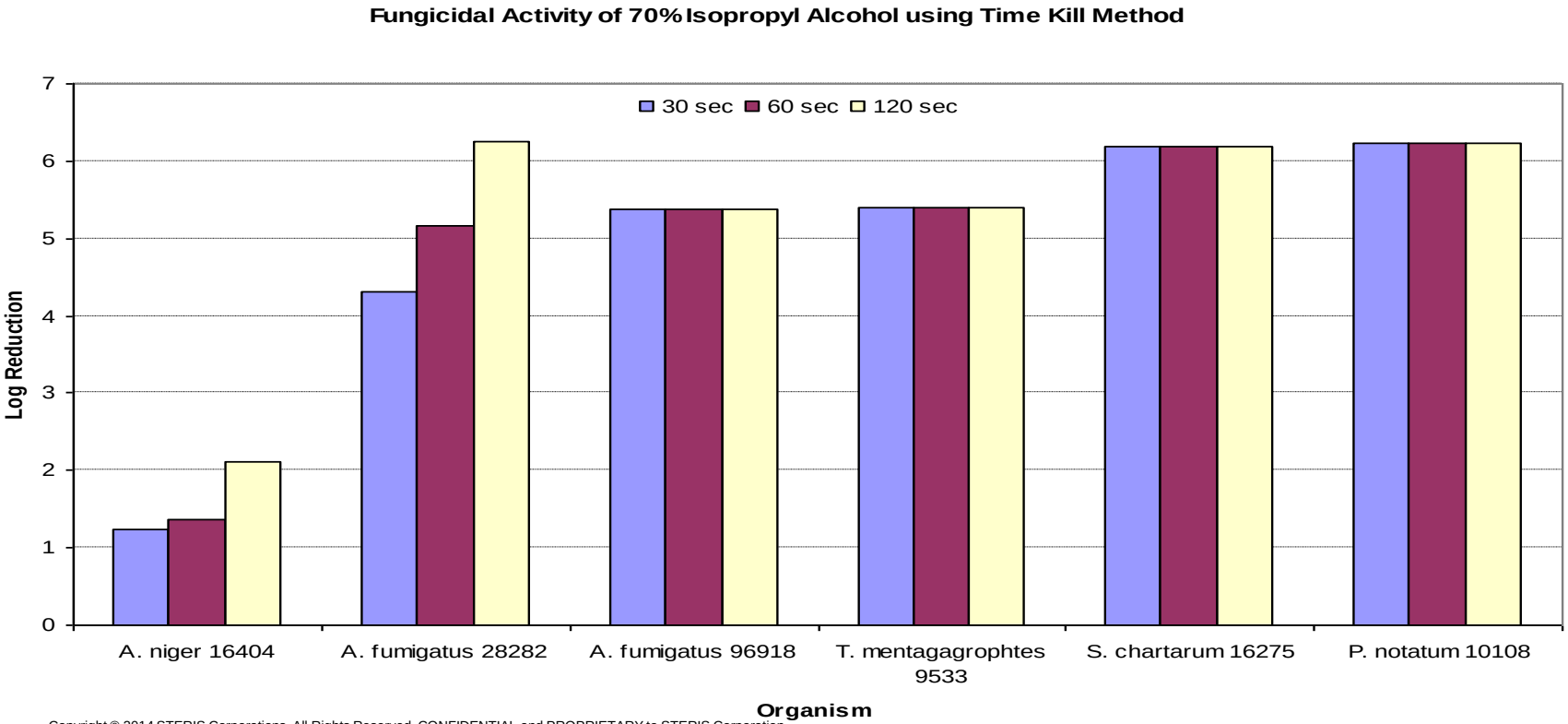
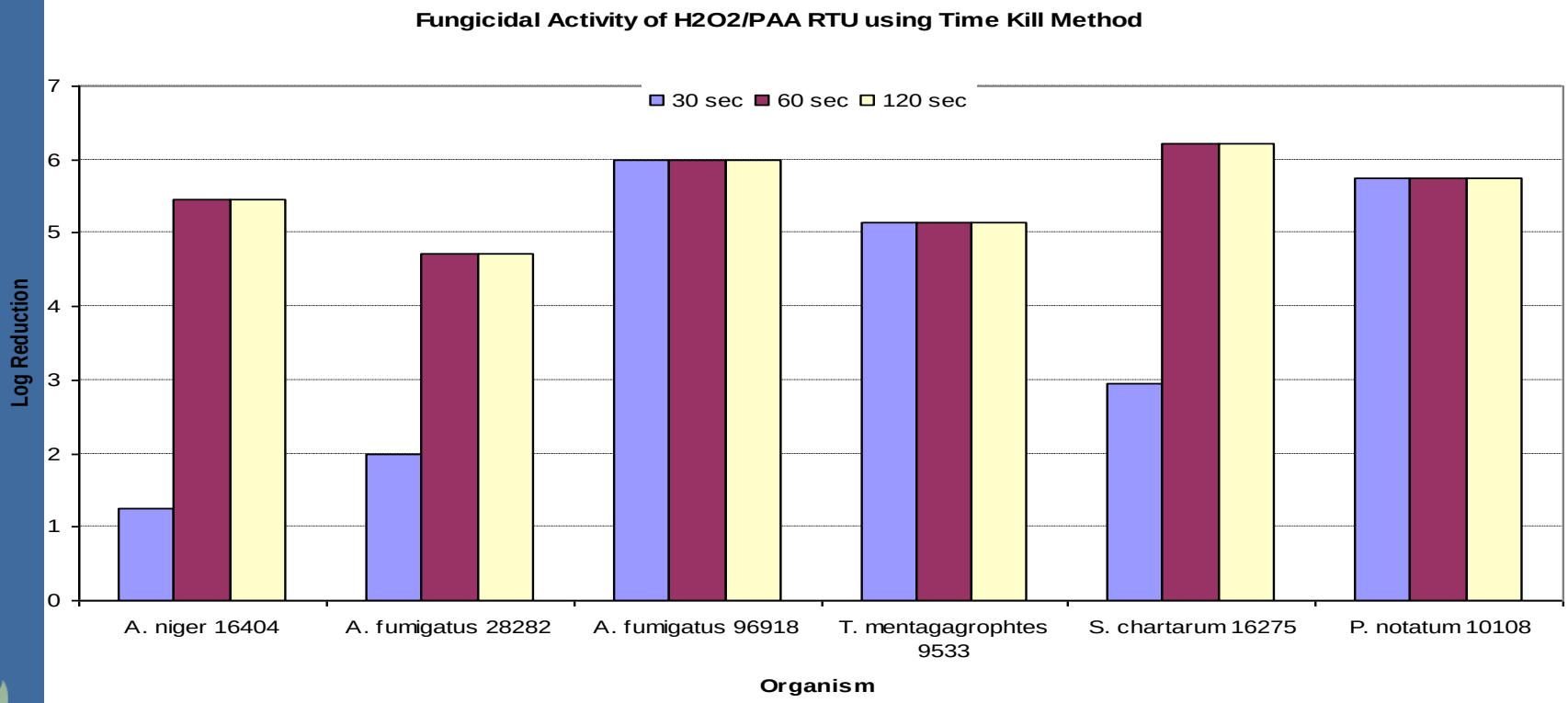


H2O2/PAA RTU Against Molds



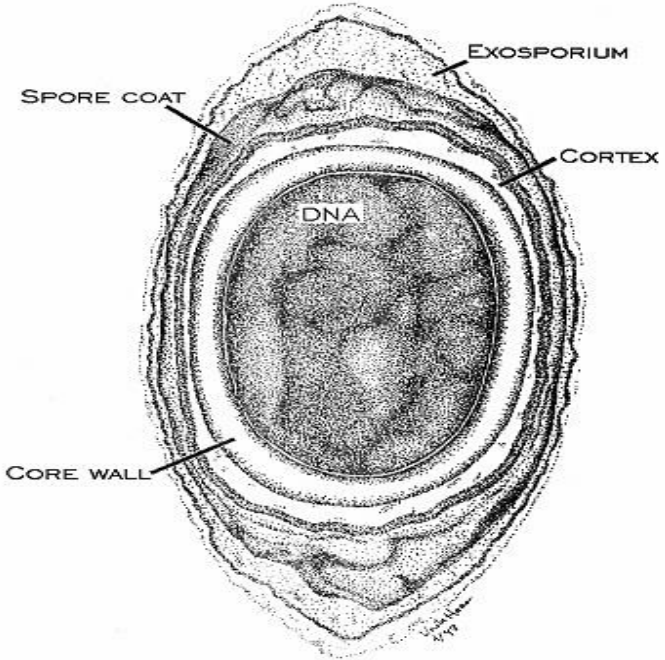
Agenda

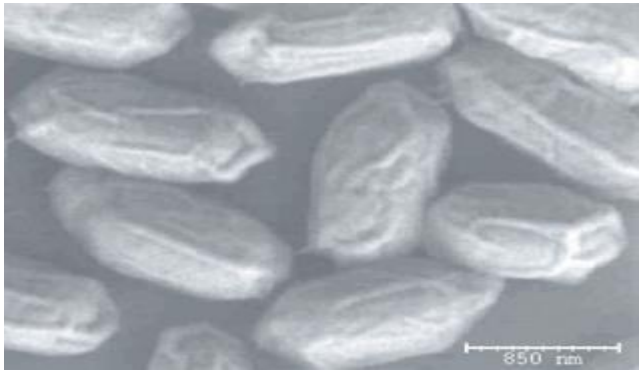
- ✓ Operator Contamination
- ✓ Fungal Spore Contamination
- ✓ Bacterial Spore Contamination

Bacterial Spores

- *Bacillus cereus*
- *Bacillus circulans*

Bacterial Endospore





Courtesy Dan Klein

Bacterial Spore Structure

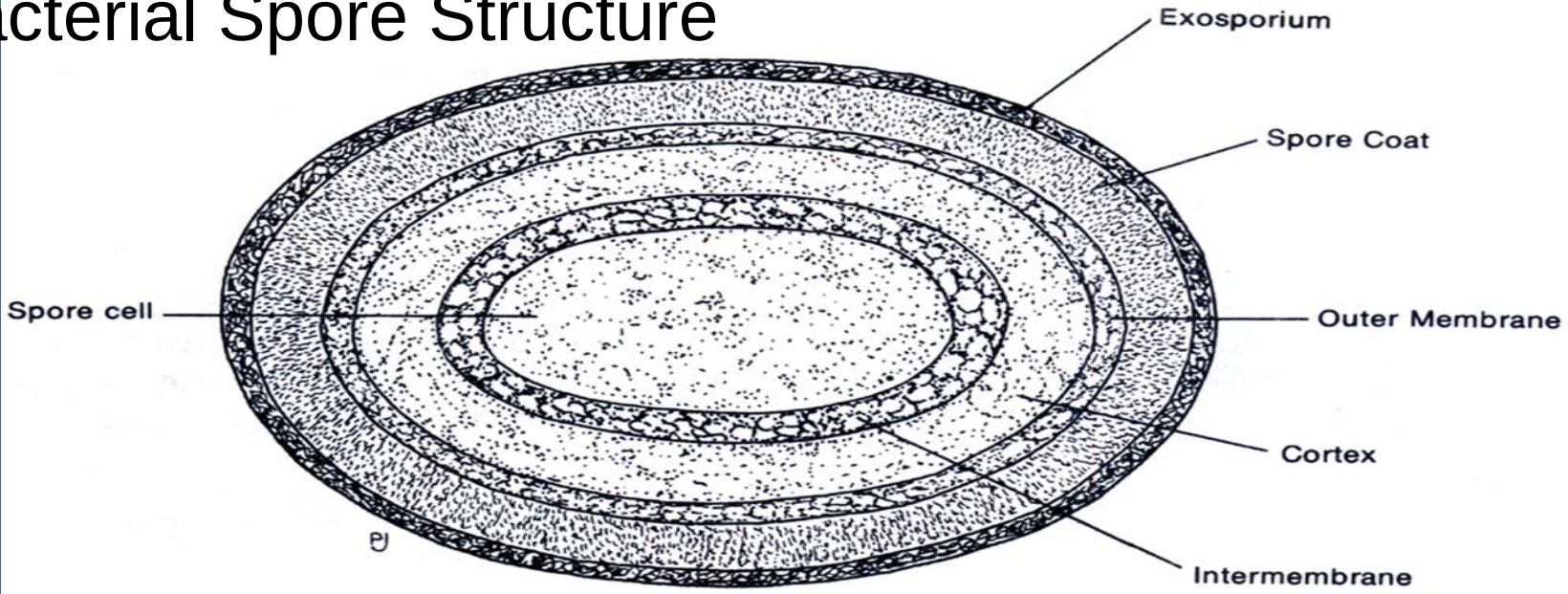


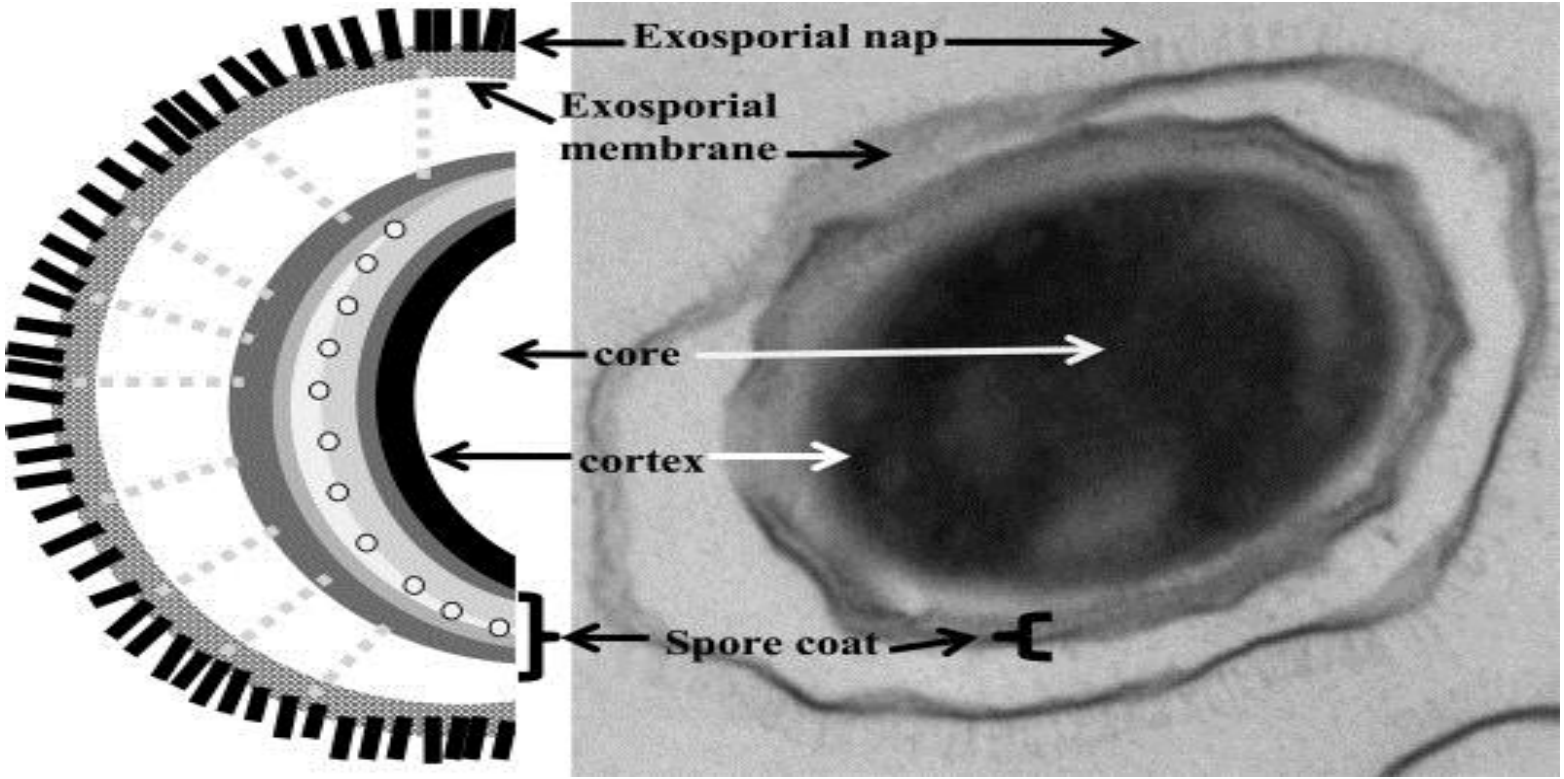
Fig. 8.1. Endospore

Bacillus cereus

- ISO-7 and ISO-8 cleanrooms
- Process Vessels
  - Source Locations
    - Cleanroom Shoe Cover
    - Fermentor
    - Process Vessels
    - ✓ The Source was a Raw Material

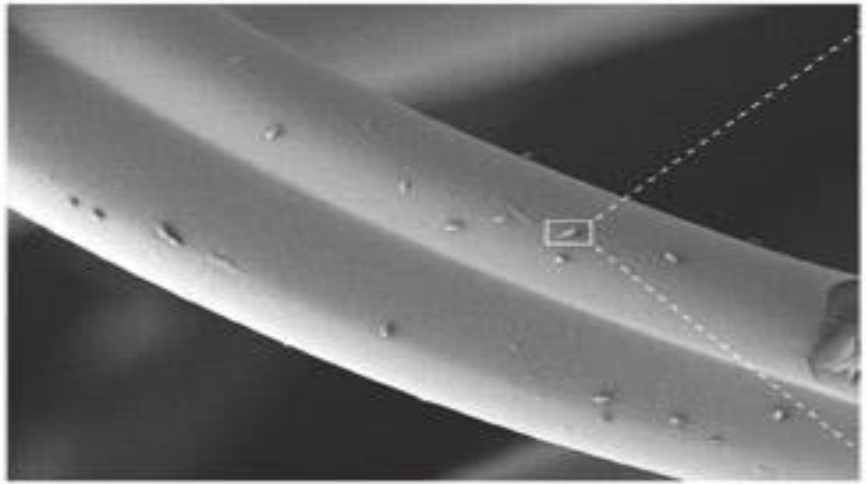
Exosporium – *B. anthracis*

Cote CK et al. 2011. Microbes and Infection 13(14-15):1146-55.

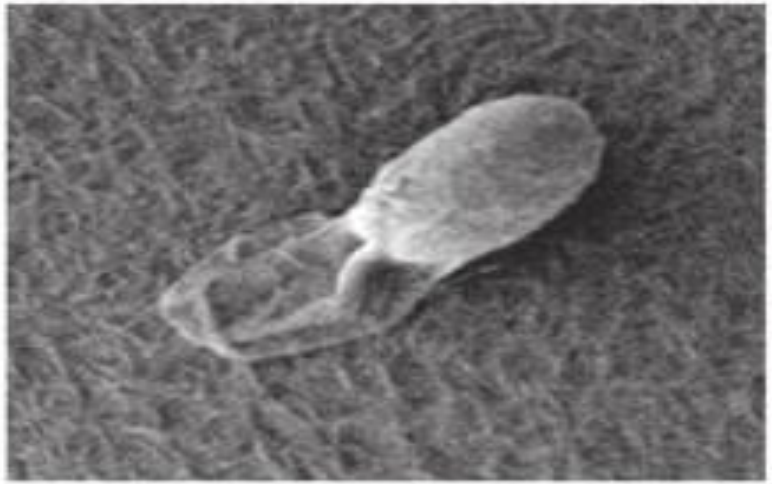


Exosporium – *B. anthracis*

Hydrophobicity helps adhere to fibers



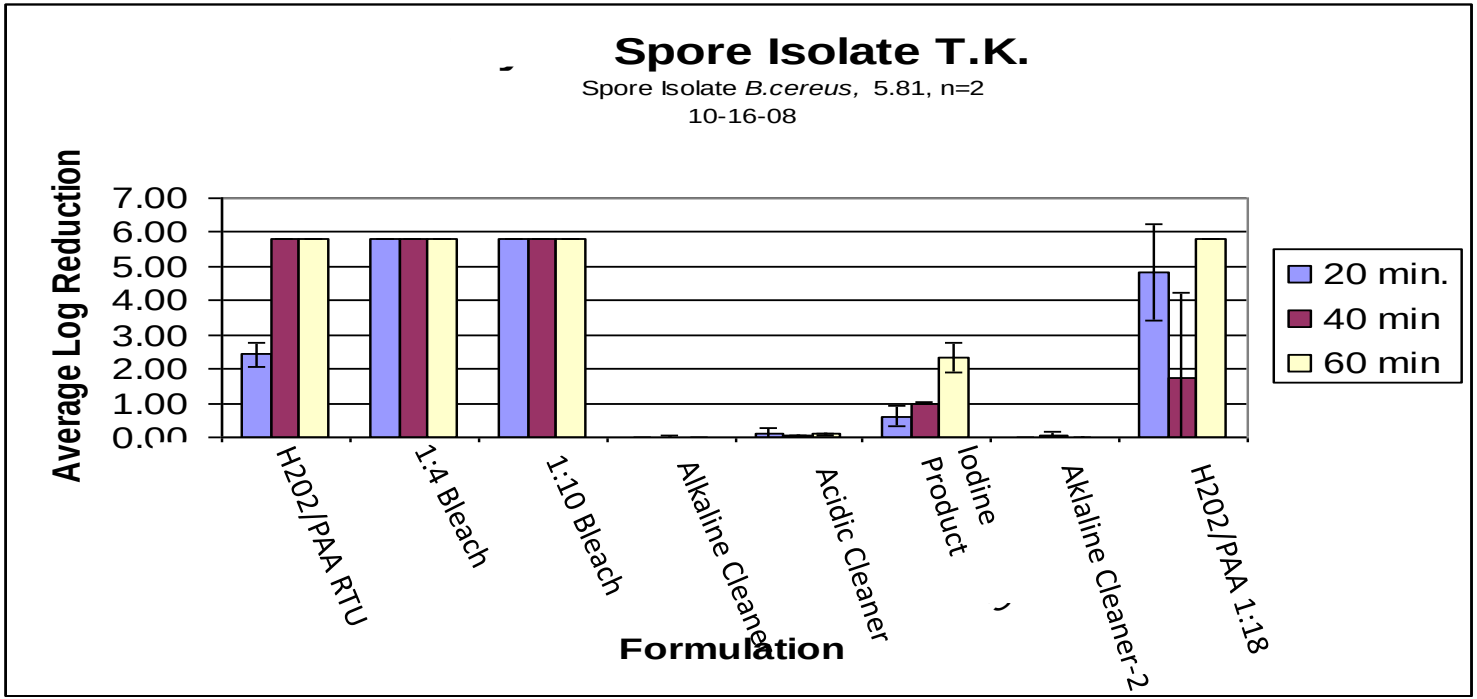
10 micrometers



1 micrometer

https://www.llnl.gov/str/Sep06/Velsko.html

Bacillus Testing



Bacillus circulans

- Efficacy Testing Failures (different test methods)
  - Vinyl surfaces
    - Sent out for ID
      - *Paenibacillus*

Case Study: Construction Event at Biotech Site

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

Cleaning and Disinfection Efficacy

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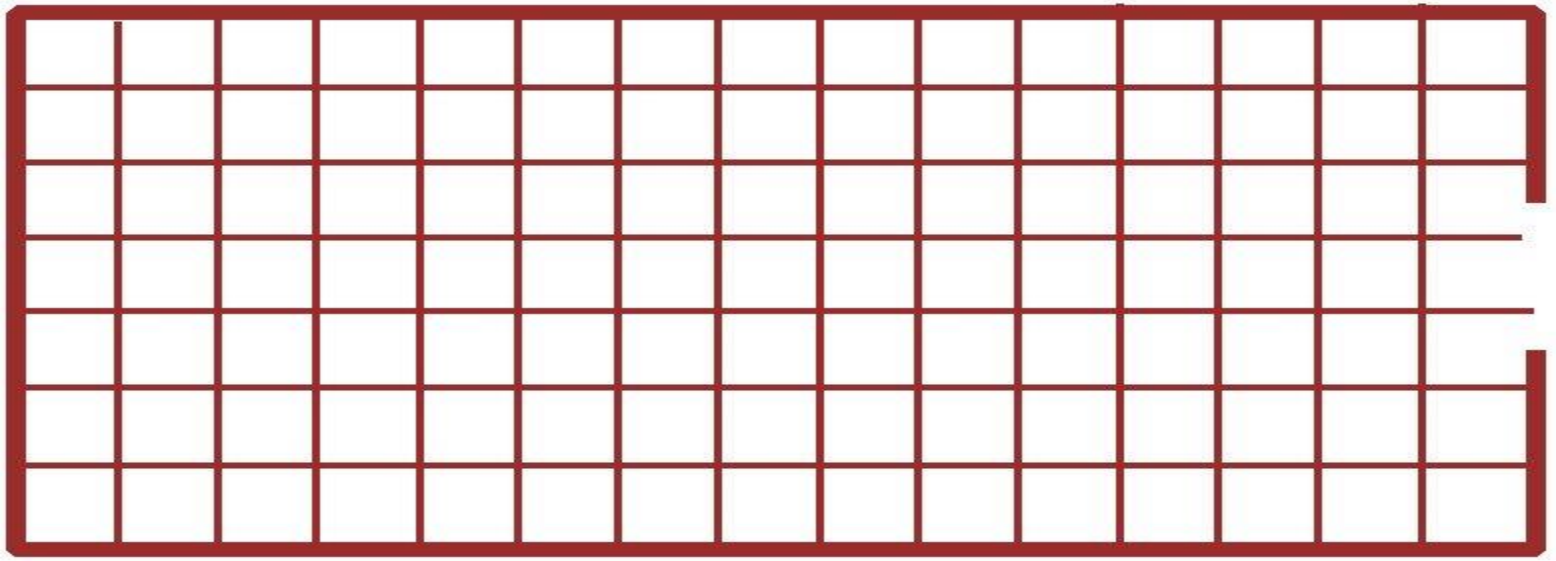

Time 0

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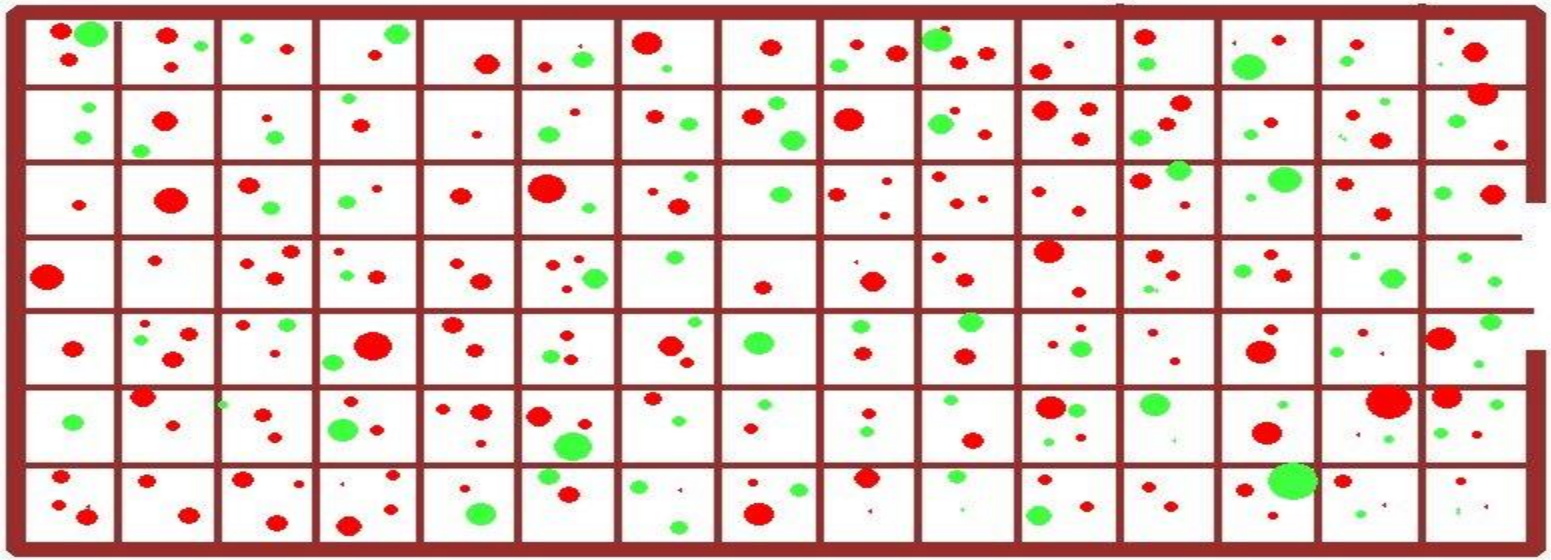
Red = Sporeformers Green = Other



After 1X Cleaning - NO Sporicide

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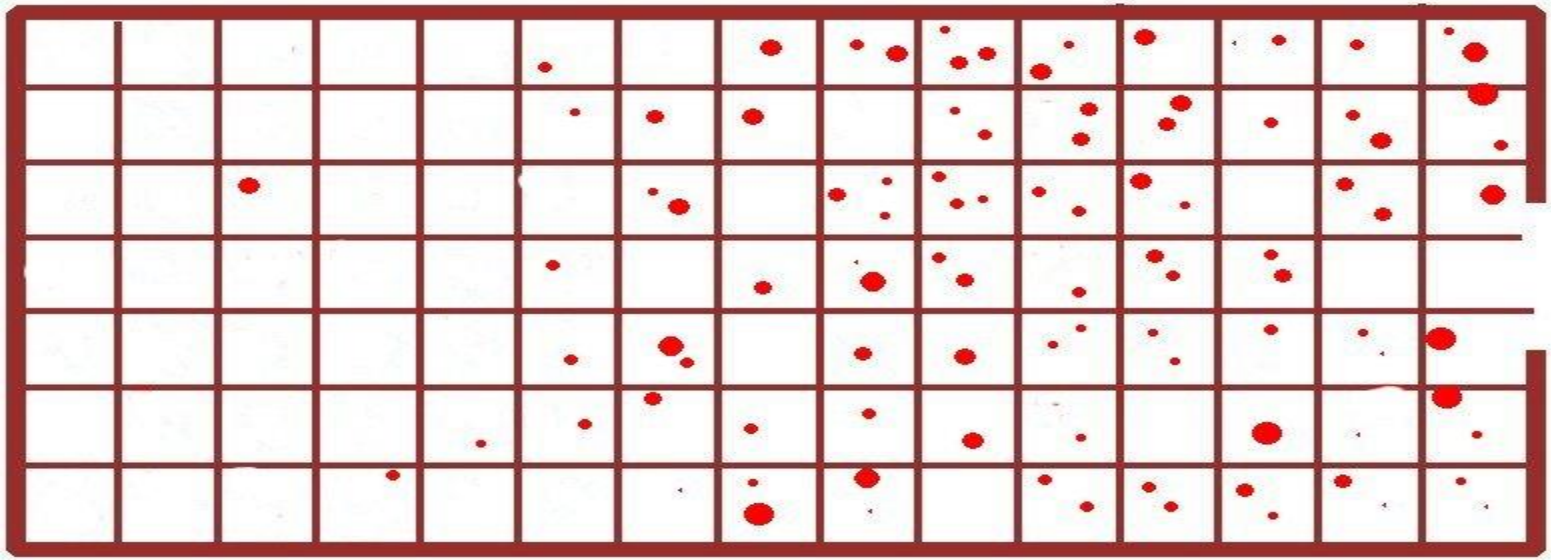

After 2X Cleaning – NO Sporicide

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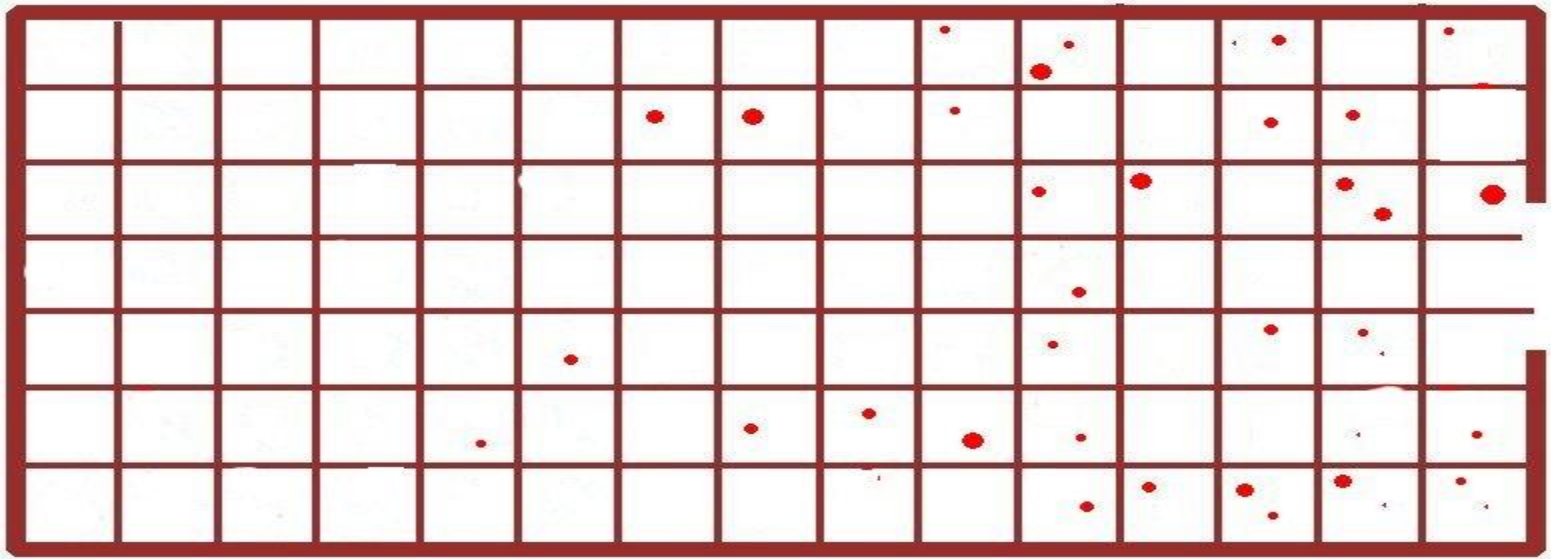
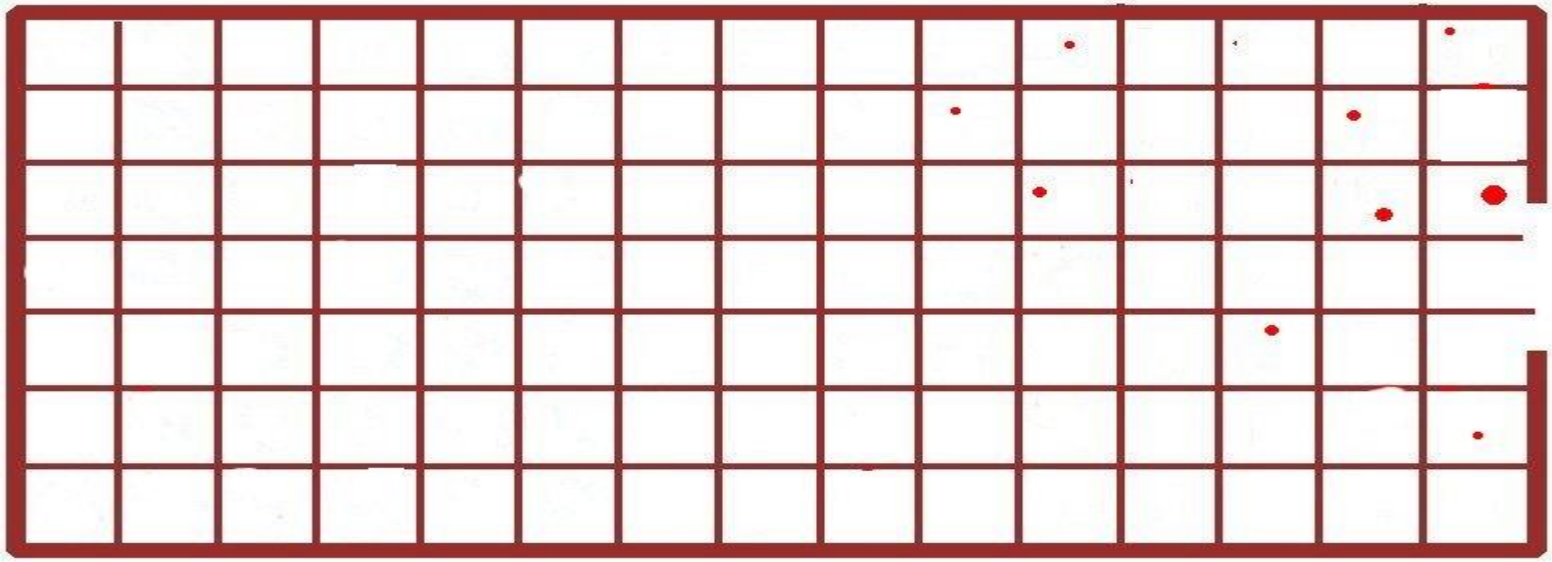
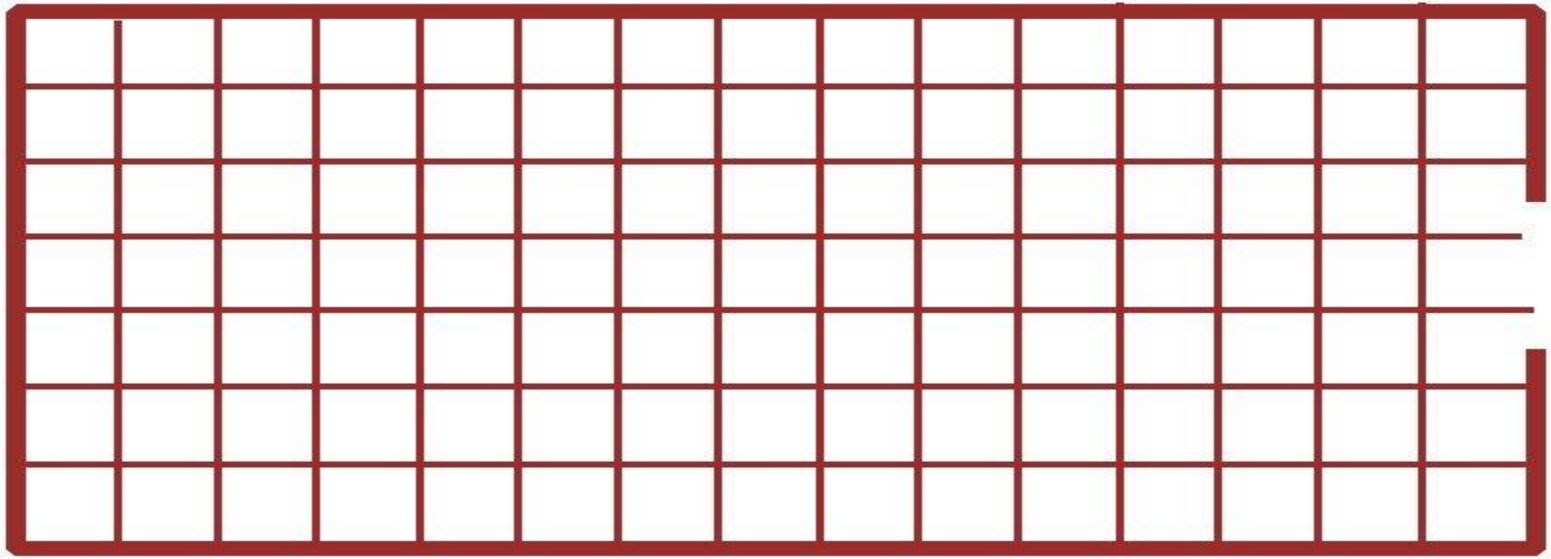


After 3X Cleaning - No Sporicide



After Sporicide



Bioburden Agenda

- Operator Contamination
- Fungal Spore Contamination
- Bacterial Spore Contamination

Summary

- Case Studies in Bioburden Control
- Starting up a New Cleanroom

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Thank You for Your Time

Jim Polarine Jr. MA.
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Let us meet again..

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