# Case Studies in Fungal and Bacterial Spore Excursions in Cleanrooms

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PDA/ISPE Australia
September 20, 2019
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# Factors Influencing Biocidal Performance

# Factors in Performance

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- pH
- Temperature
- Contact time
- Concentration
- Surface
- Presence of organic matter
- Water Quality (hardness)

# A Risk Based Approach to Choosing a Disinfectant

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- How to choose???
  - Performance – may need multiple products
  - Substrate compatibility
  - Cleaning ability
  - Change Control
  - Globally Available
  - Supply Chain
  - Disaster Response Plan
  - Ease of application
  - Validatability
    - SDS, COA available
    - Stability Studies (Opened Container, Closed Container, Use Dilution)
    - Rinsability, Compatibility, Toxicity Studies, Analytical Methods
  - Application and contact time requirements

# Disinfectants are a balance

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# A Risk Based Approach to Cleaning Frequency

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How often to clean???

- Environmental cleaning frequency determined by:
  - ISO Classification of area
  - The level of risk in the cleanroom
  - Activity level in area or use
  - Environmental monitoring data and feedback
  - Type of process being performed & equipment used

# Chemical types

- Disinfectants and sanitizers
  - Phenolics
  - Quats
  - Alcohols
  - Hydrogen Peroxide 3%

- Sterilants and sporicides (potentially)
  - Sodium hypochlorite
  - Chlorine dioxide
  - Hydrogen peroxide 6%
  - Peracetic acid
  - Peracetic acid/hydrogen peroxide blends
  - Glutaraldehyde/formaldehyde
  - Ozone
  - Nitrogen Dioxide
  - Vaporized Peracetic Acid and VHP®

# Contamination Sources

# Contamination Sources

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## Facility

- Poor design
- Aging facility
- Maintenance
- Cleaning and disinfection

## Materials

- Pass through sterilizers (autoclaves, dry heat ovens, depyrogenation tunnels, etc.)
- Decontamination chambers (EO, VHP, UV, etc.)
- Material handling airlocks

## People

- Gowning rooms
- Attire (clothing, shoe covers, hoods, face masks, goggles, etc)
- Conduct
- Standard Operating Procedures

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# Good Science: Risk Based Approach

# Warning Letter

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….revealed that numerous HEPA filters, HEPA filter supporting grid work, HEPA filter screens, and HEPA filter screen tracks contained varying amounts of discolored areas, chipping paint, multicolored coalescing droplets, and clumps of dark material that FDA testing later revealed was *mold.*

*FDA WARNING LETTER May 2013 (13-ATL-17)*

# Recalls due to Mold Contamination

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Reports of floating matter in IV bags manufactured. Foreign matter should not be present in a sterile injectable product. Subsequent microbiological analysis identified the matter as a *Cladosporium mold*.

*(FDA Public Health alert 2010)*

# Compounding Pharmacy Fungal Contamination

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There was no investigation by the firm when levels exceeded the action limits and no identification of isolates. No documented corrective actions were taken to remove *microbial contamination (bacterial and fungal)* from the facility.

*(FDA WL October 2, 2012)*

# Review - Microflora in Cleanrooms (U.K.)

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- 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.)

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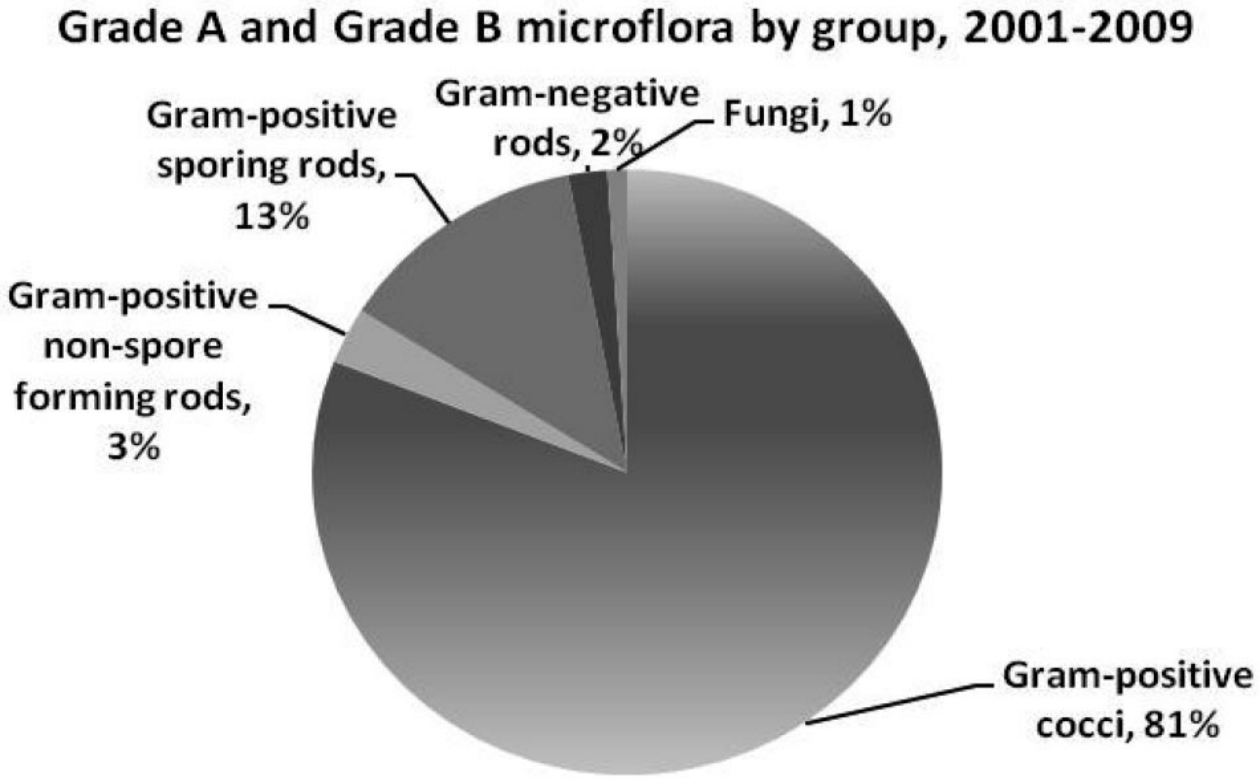

# Review - Microflora in Cleanrooms (U.K.)

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

# Risk Based Approach

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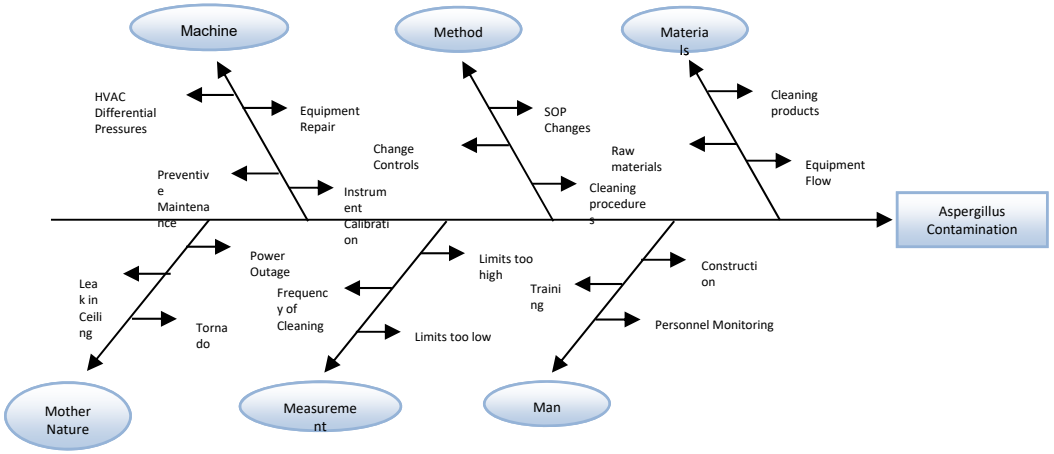
- Pathogenic or Toxic Molds
- Does it affect product contact surfaces
- Where is it picked up onsite
- Create a risk map or diagram
- What are the possible assignable causes
- Proactively prevent the mold from reoccurance
- How many mold spore require a CAPA?
- What do you do with one mold spore hit?

# Where does the Investigation team begin? Cause & Effect Diagram (Fishbone Diagram) example:



# Investigation Strategies: Critical Zones

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Trend history of organisms in the area versus the contaminant.

| Sample Type | Sample Frequency | Minimum Data Review Period |
|---|---|---|
| Water | Daily | ≥ 3 weeks prior to the excursion |
| | Weekly | ≥ 3 months prior to the excursion |
| Pure Steam | Weekly<br>Monthly<br>Quarterly | ≥ 3 months prior to the excursion<br>≥ 3 months prior to the excursion<br>≥ 6 months prior to the excursion |
| Compressed Air/Gas | Quarterly | ≥ 1 year prior to the excursion |
| Environmental Monitoring | Daily | ≥ 2 months prior to the excursion |
| Environmental Monitoring | Bi-Weekly or Monthly | ≥ 6 months prior to the excursion |
| Personnel | Applicable Facility SOP | ≥ 3 months prior to the excursion |

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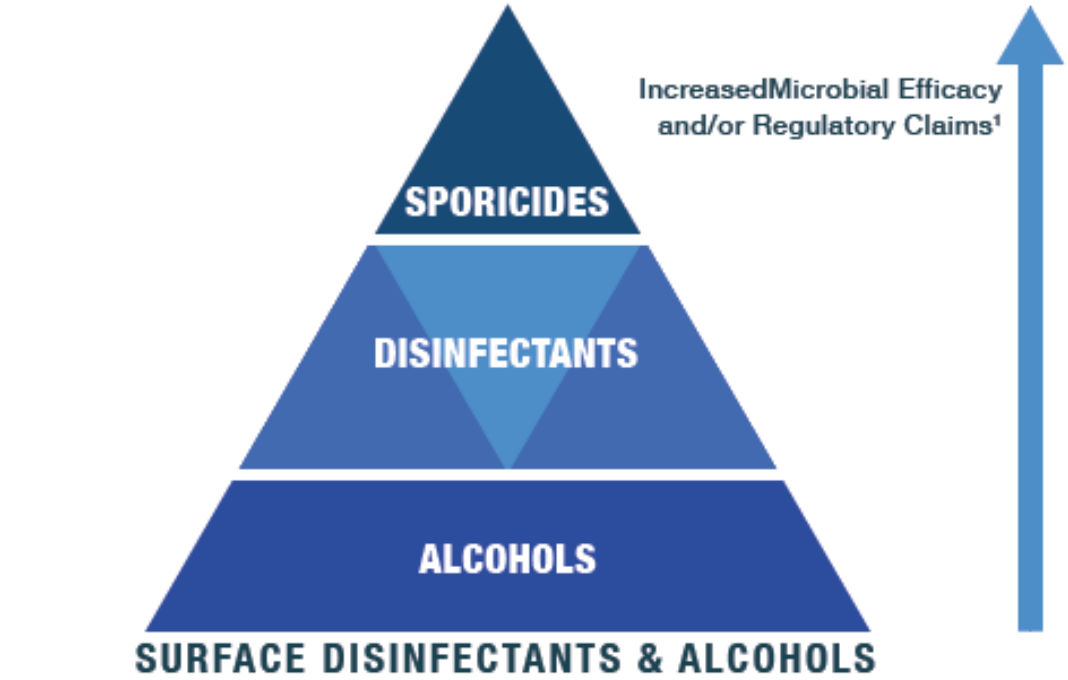


[1]Products that fall into the categories at the bottom of the pyramid are most frequently used and are generally not sporicidal. Progression up the pyramid indicates stronger performance overall and a broader spectrum of claims.

# Common Sources of Spores

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- Items brought into the Cleanroom
  - Bags, Boxes, Intervention Equipment, Pallets, Pallet Jacks, Scrubbers, Cartwheels, Carts, Shoes, Shoe Covers, Markers, Pens, Cellphones, Tools
  - Raw Materials and components

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# *Bacillus cereus* Contamination Case Study

# Microorganism Resistance Hierarchy

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| | 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 |

***Bacillus cereus / sphaericus***

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

***Clostridium* spp.**

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

# Bacterial Spores in Operations

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- *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*

# Bacterial Endospore

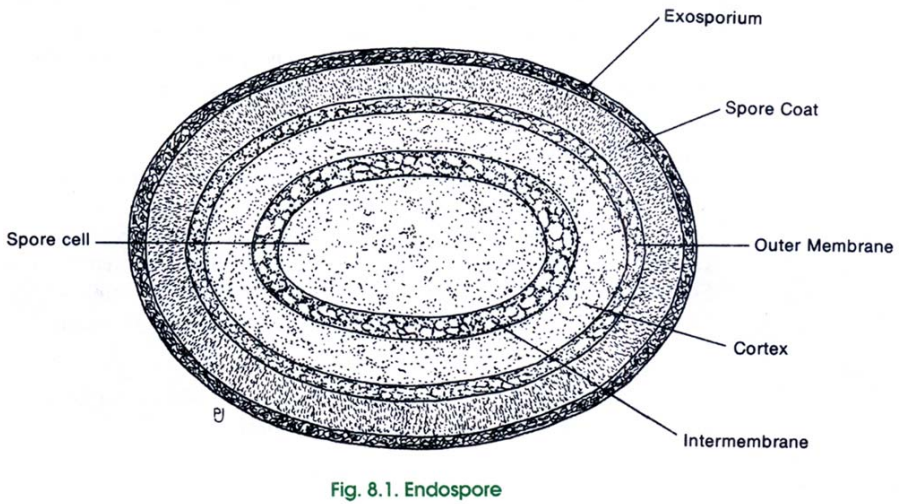
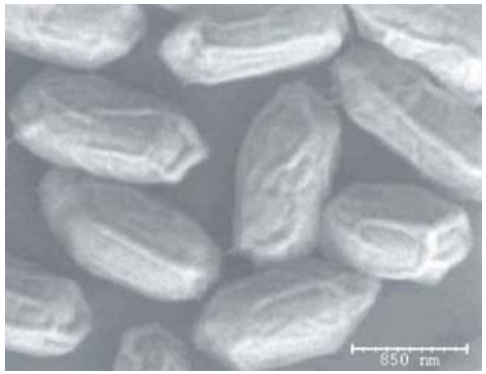



Fig. 8.1. Endospore

Courtesy Dan Klein



# Bacterial Spore Structure

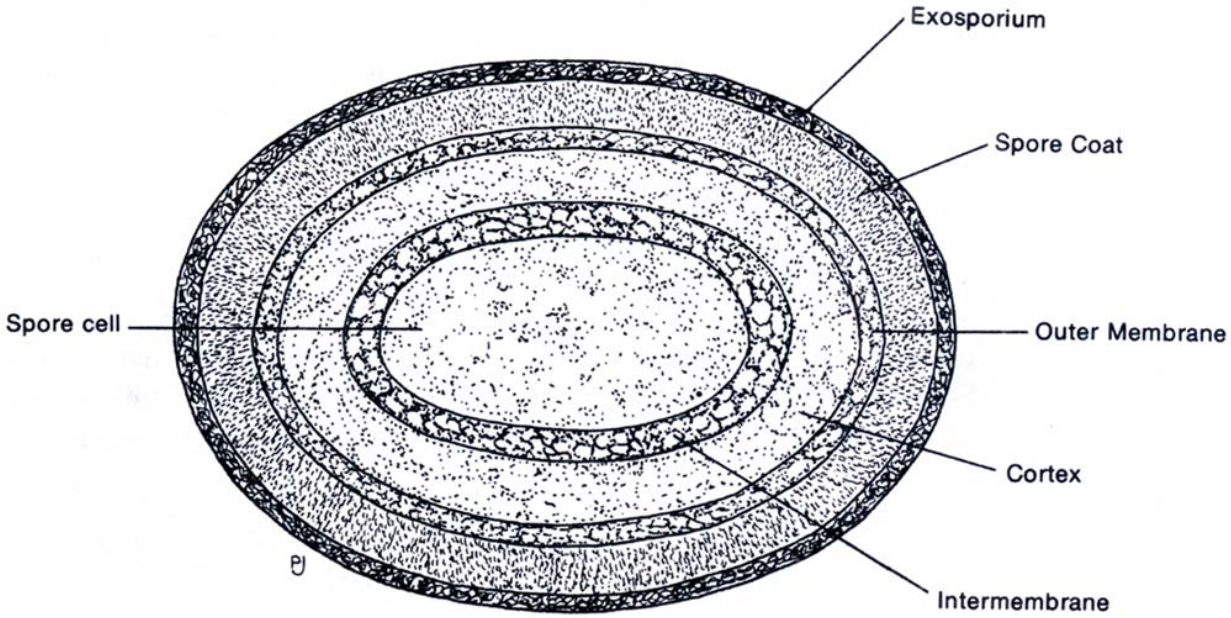



Fig. 8.1. Endospore

# Bacillus cereus Case Study

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- ISO-7 and ISO-8 cleanrooms
- Spores tracked throughout the facility
- Process Vessels
  - Source Locations
    - Cleanroom Shoe Cover
    - Fermenter
    - Process Vessels
    - ✓The Source was a Raw Material

# Exosporium – B. anthracis

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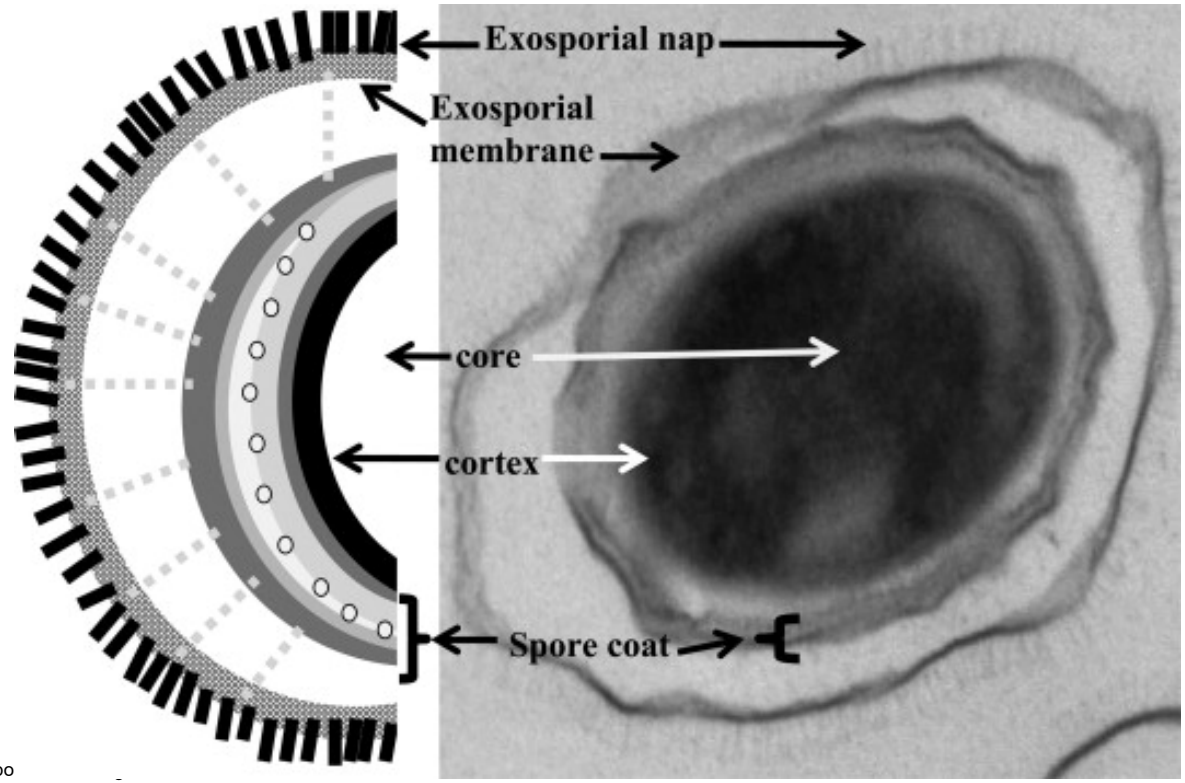
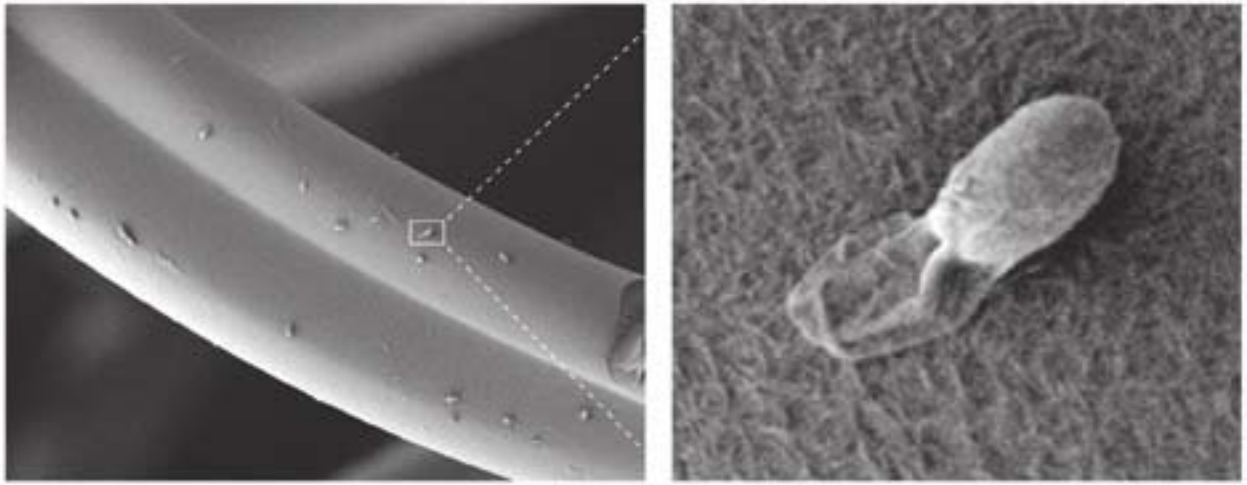
Cote CK et al. 2011. Microbes and Infection 13(14-15):1146-55.



# Exosporium – B. anthracis

Hydrophobicity helps adhere to fibers




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

# Model of *B. cereus* exosporium

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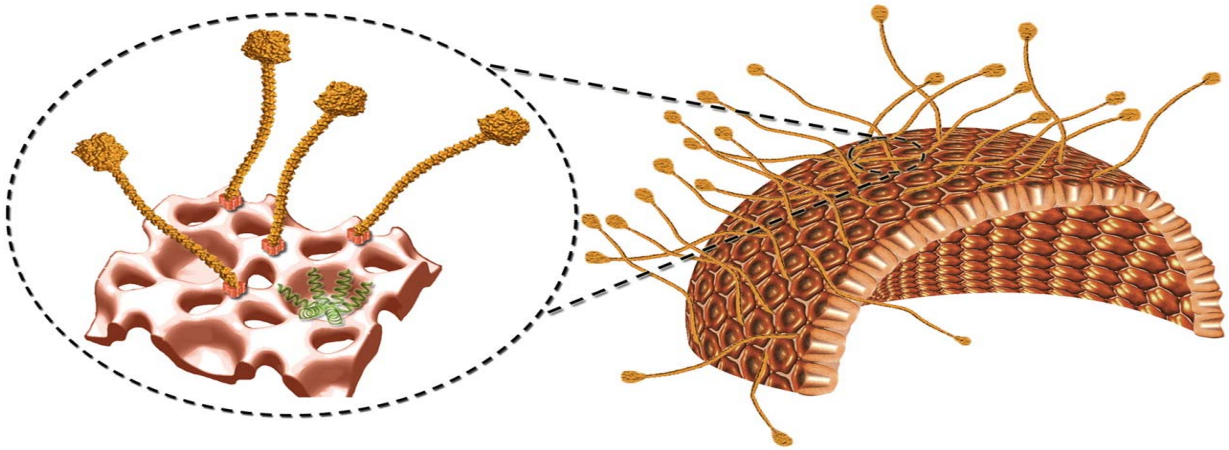
**A schematic diagram illustrating a possible model for the exosporium of the *B. cereus* family.**

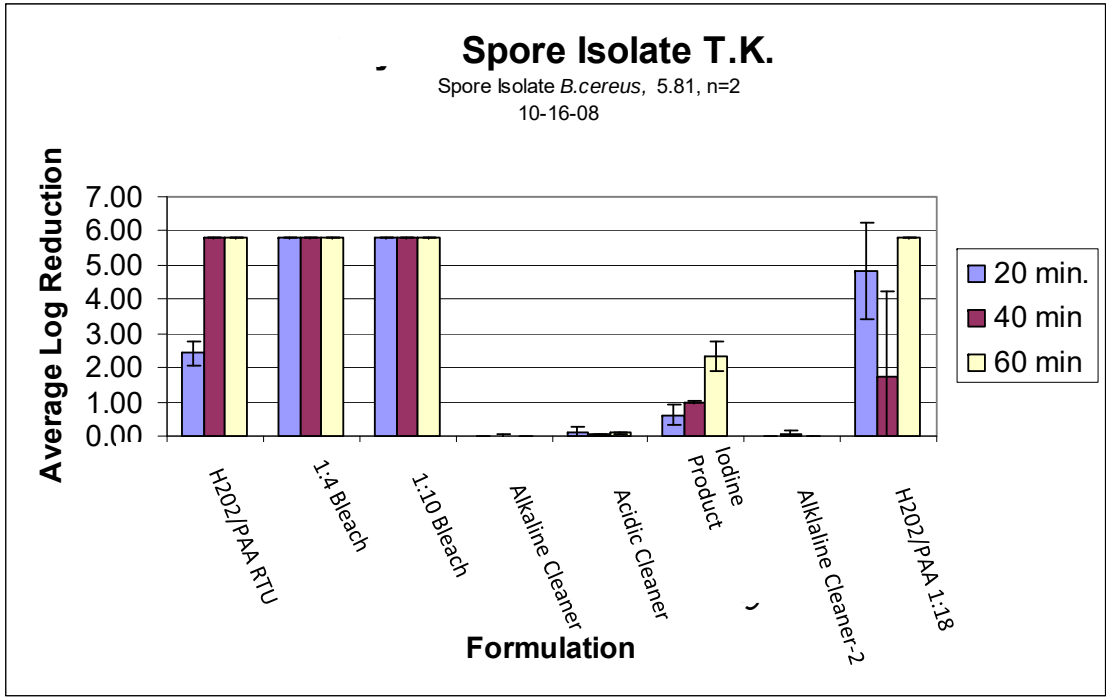


**Kailas L et al. PNAS 2011;108:16014-16019**



PNAS

# *Bacillus* Testing



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# Fungal Spore Contamination Case Studies

# Molds common to cleanrooms and cold rooms

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

# Acremonium Investigation

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- Establishing Realistic Limits
- Finding one mold spore in a filled product
- One hit found near a pump on ultrafiltration skid
- Using a proactive science-based approach
- Is Zero Mold possible?
- CAPA investigation
- Using Good Science

# *Aspergillus*

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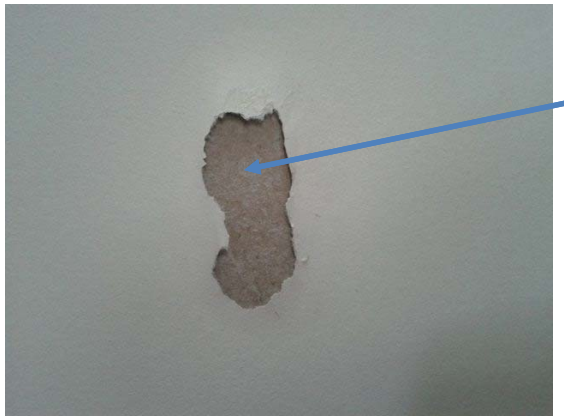
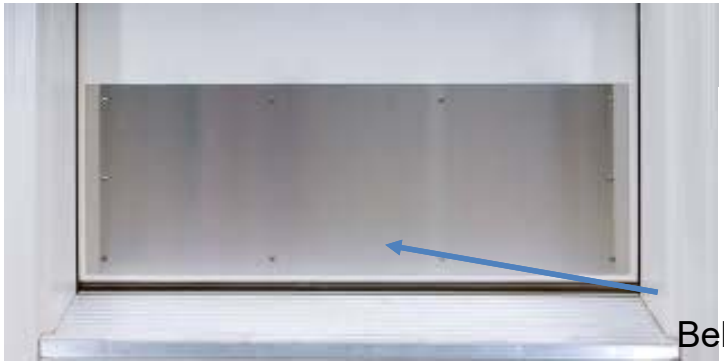
- ISO-5 Cleanroom
  - Source
    - High Impingement Spraying Device
    - Broken Pipes
    - HVAC Shut Down
- Exceeding Limits in ISO-7 areas
  - Dock Doors proximal to ISO-7 cleanroom
  - Storage room with limited control
  - No limits for mold spores (Establish Limits)
  - Limited control for incoming and outgoing items

# High Pressure Impingement Sprayers



Holes
in Walls

# Sources of *Aspergillus*



Behind Cleanroom Door Kickplate

Black Sharpie
Marker Tip

# *Aspergillus brasiliensis*

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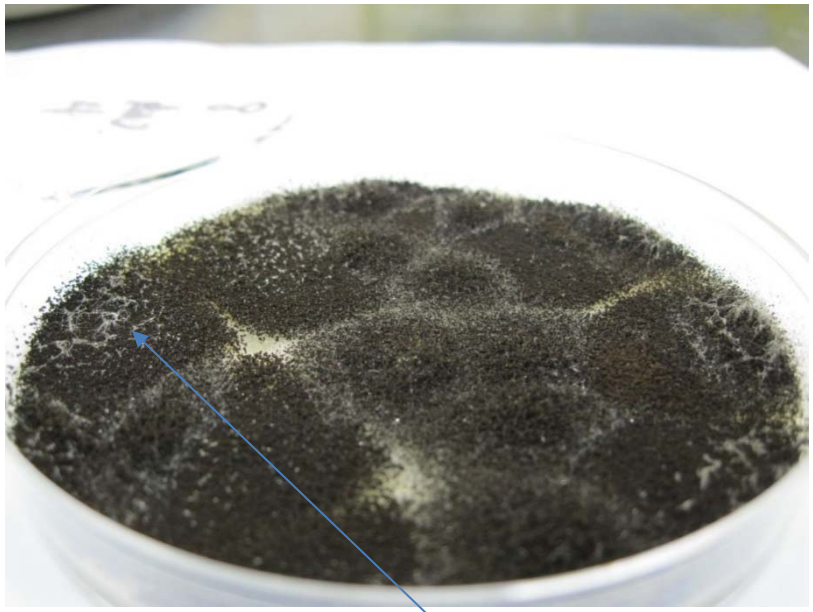

Courtesy Dan Klein

Conidiospores

# *Aspergillus* Investigation

- Sporicidal usage in pass-through
- Clean and dirty area on the dock
- Better control of gowning area
- Cart Wheel control methods
- Better gowning control

Photo: Terra Universal

# NECC Fungal Contamination

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- There was no investigation by the firm when levels exceeded the action limits and no identification of isolates. No documented corrective actions were taken to remove microbial contamination (bacterial and fungal) from the facility. (FDA WL October 2, 2012)
- 83 out of 321 vials of methylprednisolone acetate contaminated (fungal contamination found)
- 64 deaths and 750 Illnesses

# *Aspergillus brasiliensis*

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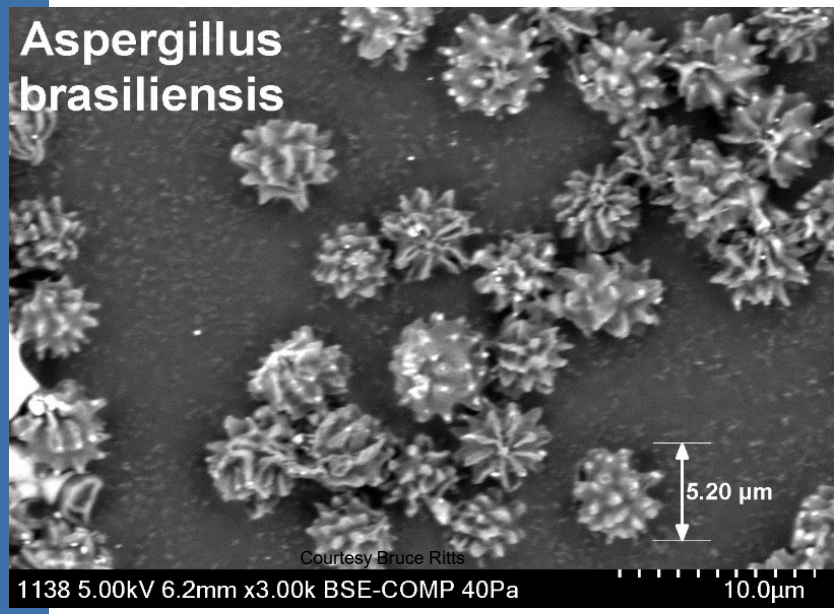
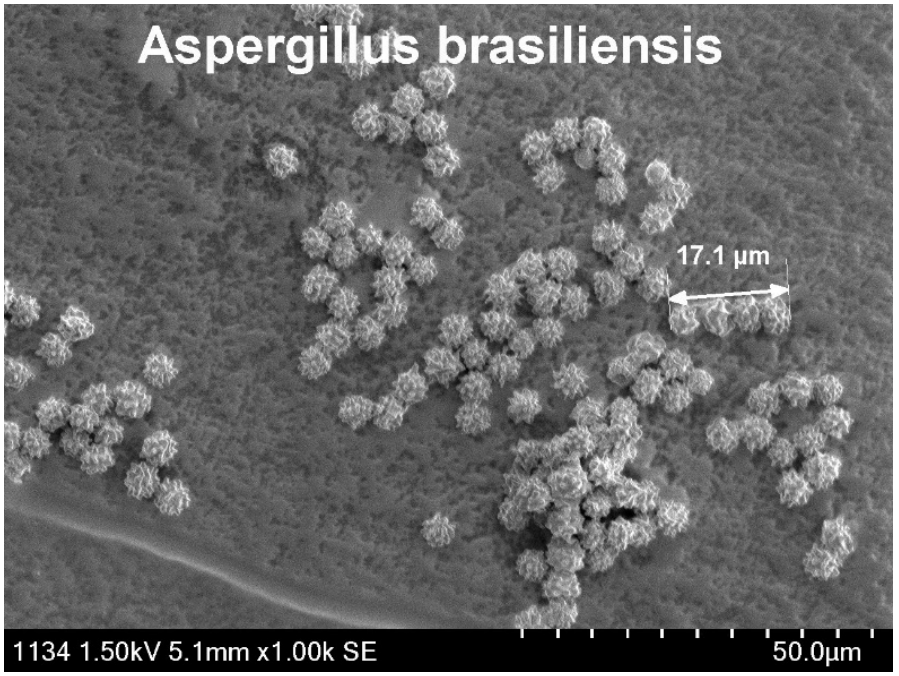
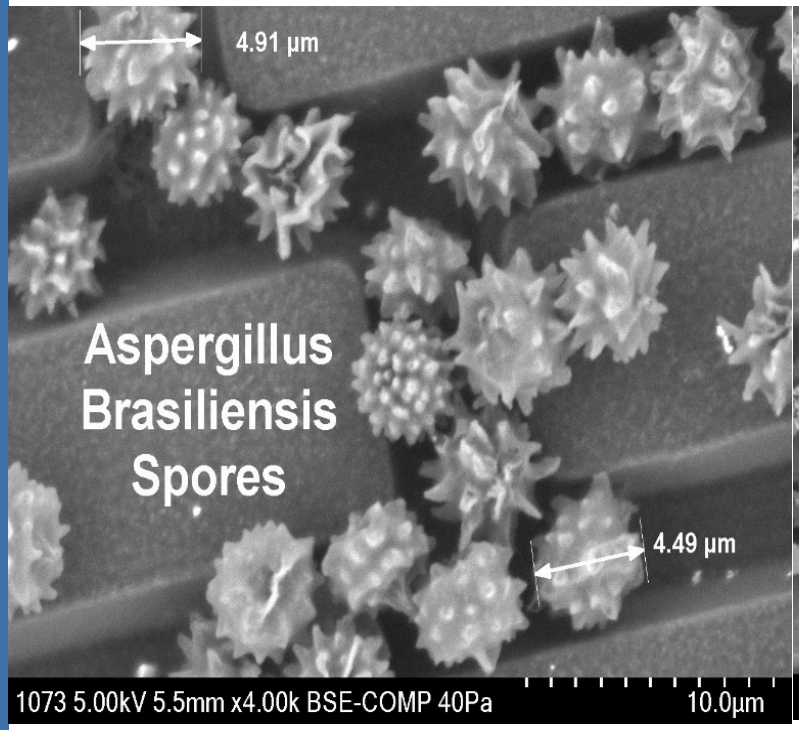
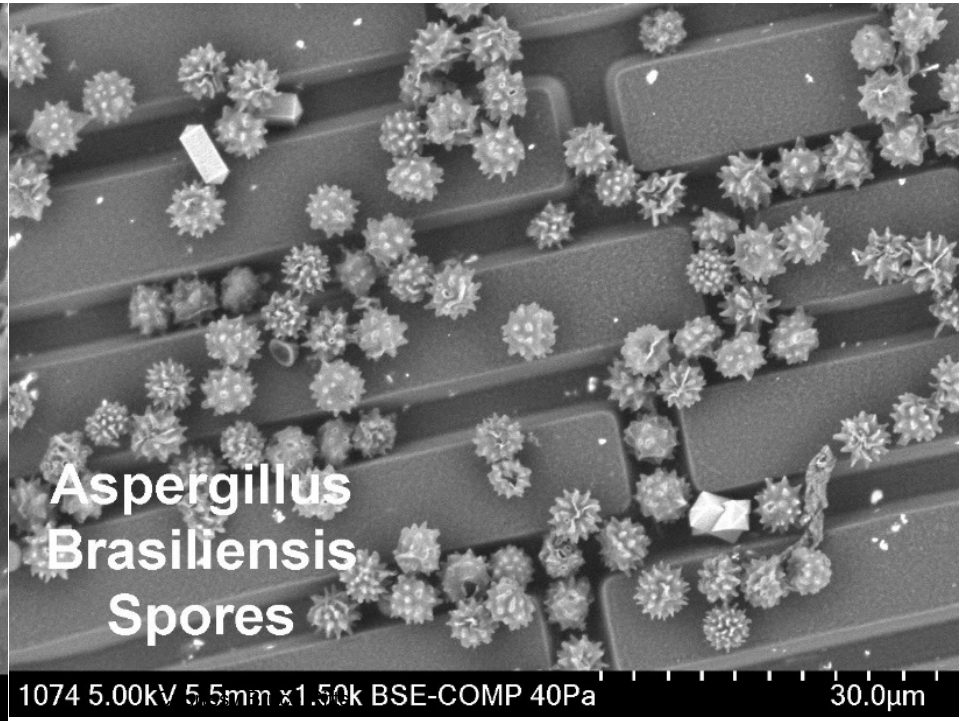


# *Aspergillus brasiliensis*



# *Spiny* Spores

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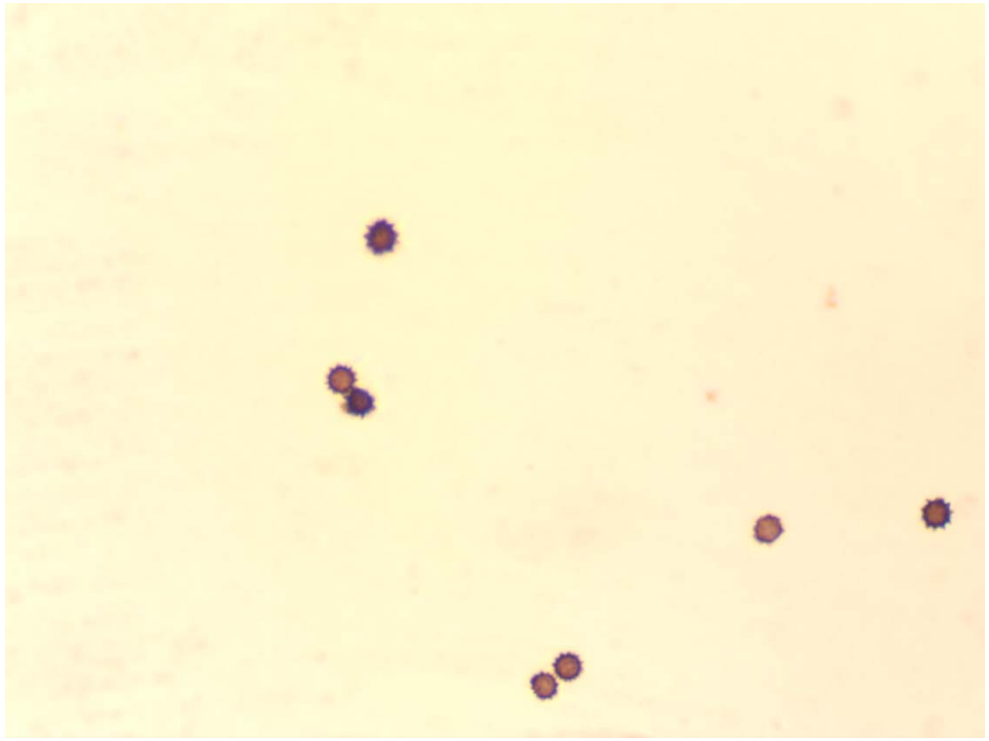

Courtesy Dave Shields

# Cleanroom Fungi

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Courtesy Dan Klein

# *Penicillium*

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- Two ISO-7 Cleanrooms
- Action Levels of 10 and picking up >100 Fungal Spores
  - Engineering Investigating
  - HVAC
  - Duct Work
  - HEPA Filters
  - Cooling Coils (two hits)
  - Wall Coverings
  - Airflow Vents

# *Penicillium* Investigation

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- Entry and Exit Procedures
- Gowning Procedures (Triple Gowning)
- Cart Wheels
- Construction (Current Maintenance Log)
  - Further Investigation
  - Use of Sporicides and Frequency
  - Plastic Containers in the Cleanroom
  - Coldroom Cleaning Procedures (0° Celsius to -5° Celsius)
  - Documentation of Cleaning Process
  - Designate Assignable Causes

# *Penicillium* Investigation

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Cleanroom Cooling Coils

Plywood made up the sliding door

# *Penicillium*

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Courtesy Ann Larson

# *Penicillium* Spores

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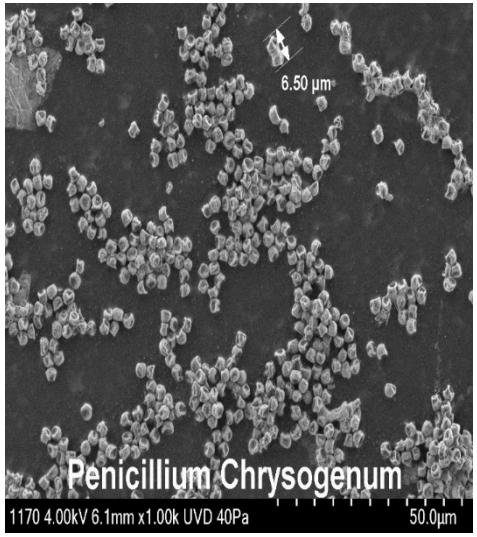
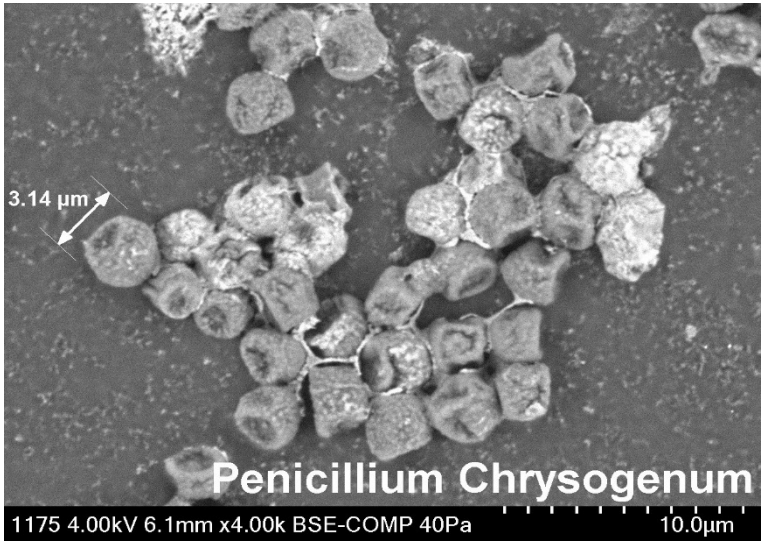
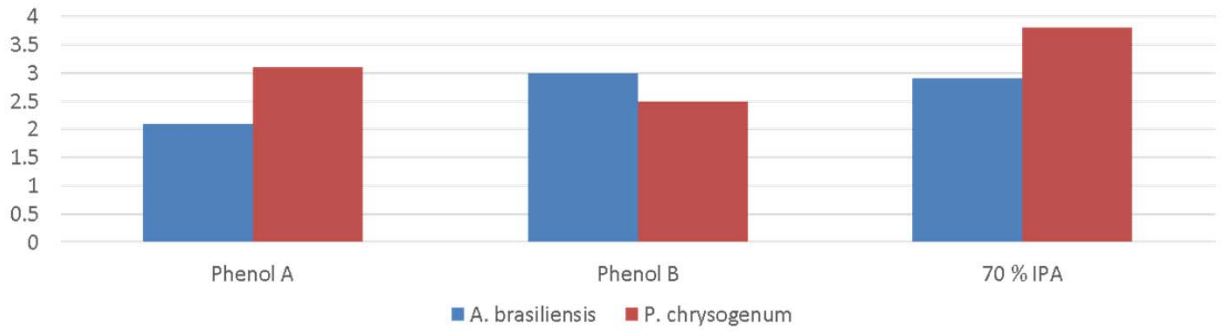
Courtesy Bruce Ritts

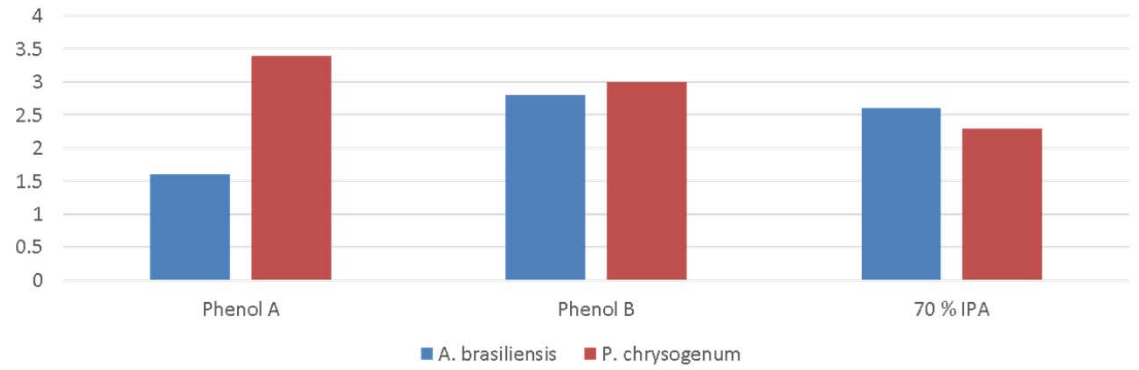


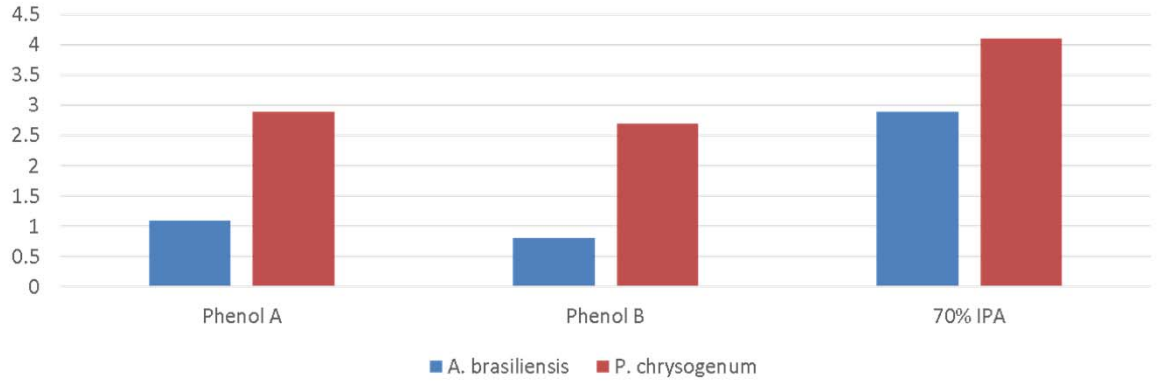
# Glass Evaluation



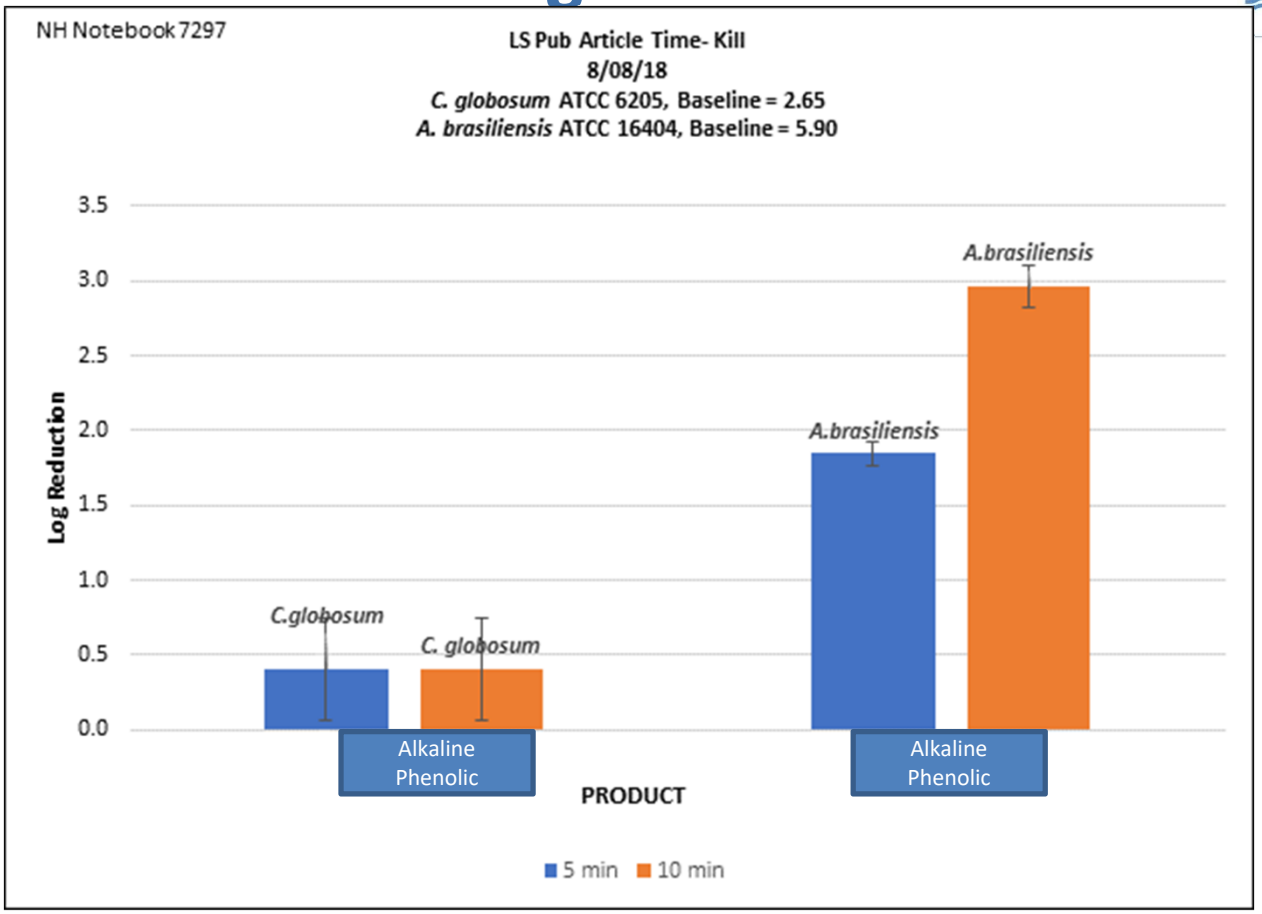
# Stainless Steel Evaluation



# Flooring Evaluation



# Chaetomium Testing



# Vesta Syde SQ vs. Competitive Quats

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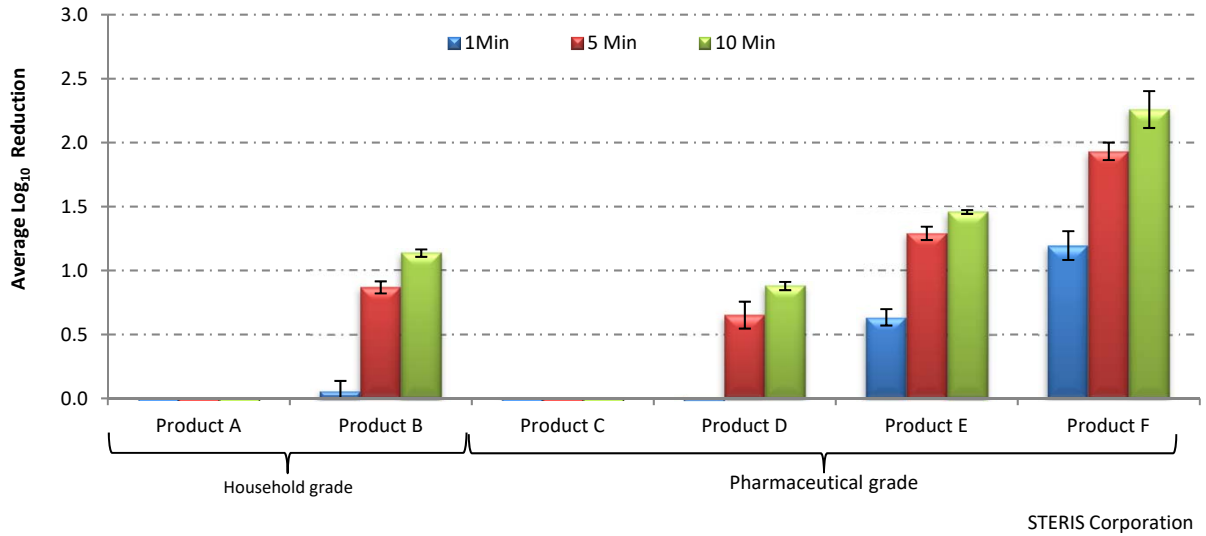
**Time Kill data showing Susceptibility of *Aspergillus brasiliensis* 16404 to products containing Quaternary Ammonium Compounds**

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# Graph

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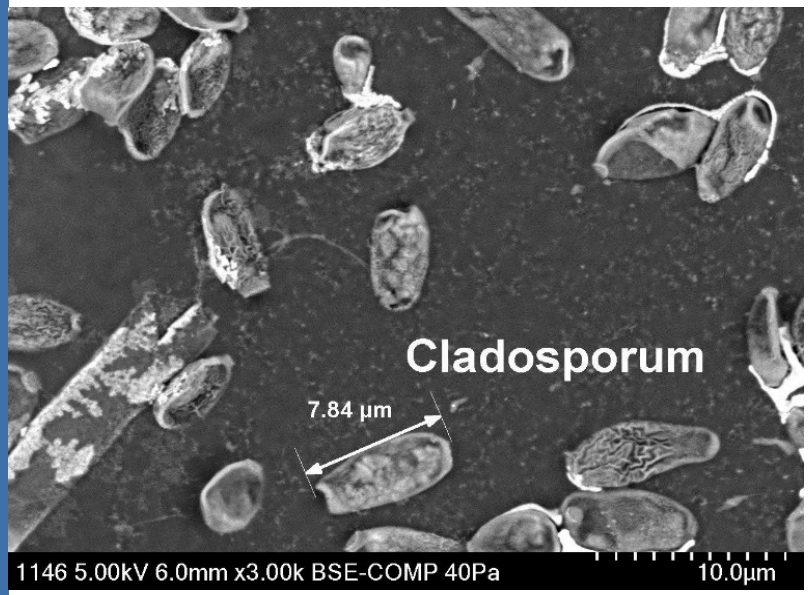
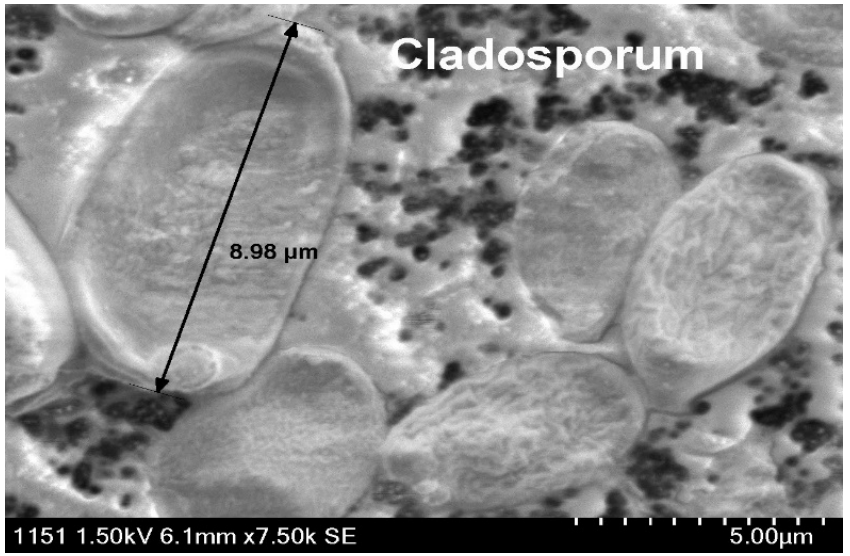
- Attached is the Vesta-Syde SQ graph that was in the JVT article. The product codes are as follows:
- Product A Tilex Bathroom Cleaner
- Product B 409 All Purpose Cleaner
- Product C Klercide CR Biocide A
- Product D Veltek Decon-Quat
- Product E Process NPD
- Product F Vesta-Syde SQ

# Cladosporium Spores

Courtesy Bruce Ritts



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# *Deinococcus* Contamination Case Study

# Medical Device Company

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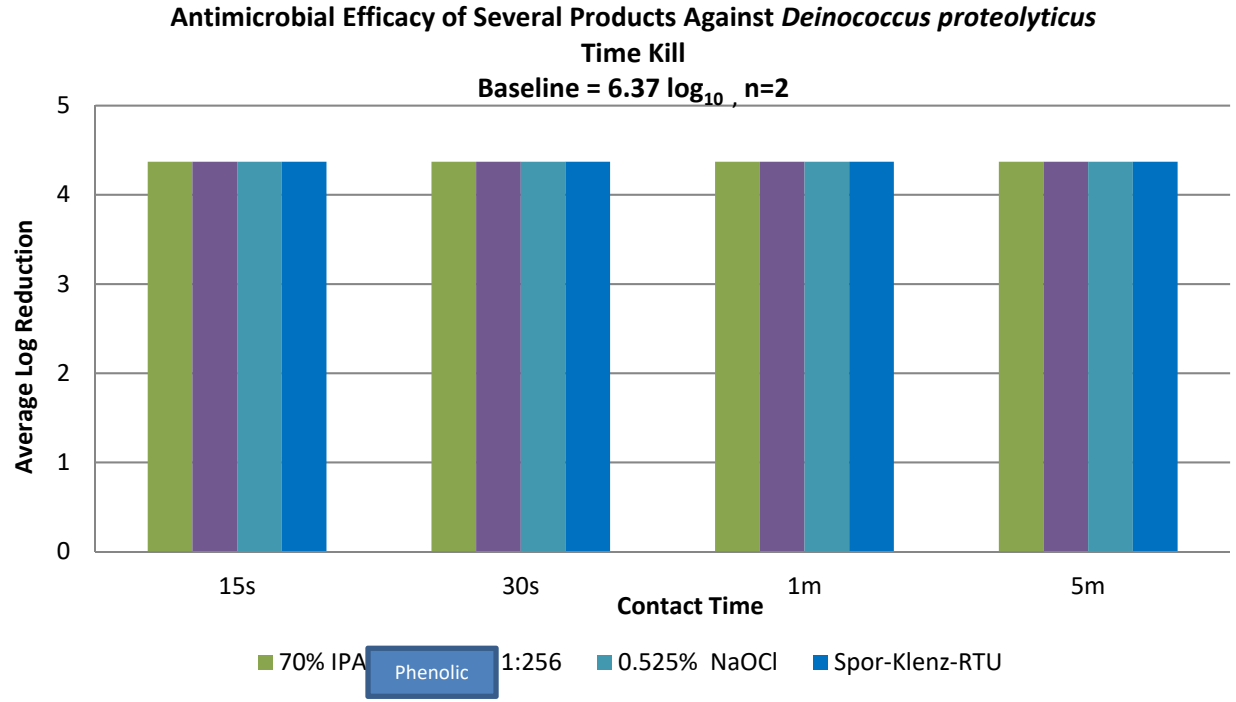
- Failing Bioburden Testing on Surgical Light Covers and Camera Cases
- Visited the site
  - Cardboard
  - Plywood
  - Poor Gowning Practices
  - Poor Cleanroom Behavior Practices
  - Performed EM and Site Audit
  - Solution
    - Move from Gamma Irradiation to ETO

# Performance against Deinococcus

**Figure 1. Disinfectant Evaluation—Time Kill Study**



# Covers for Surgical Lights and Camera Cases

# *Deinococcus*

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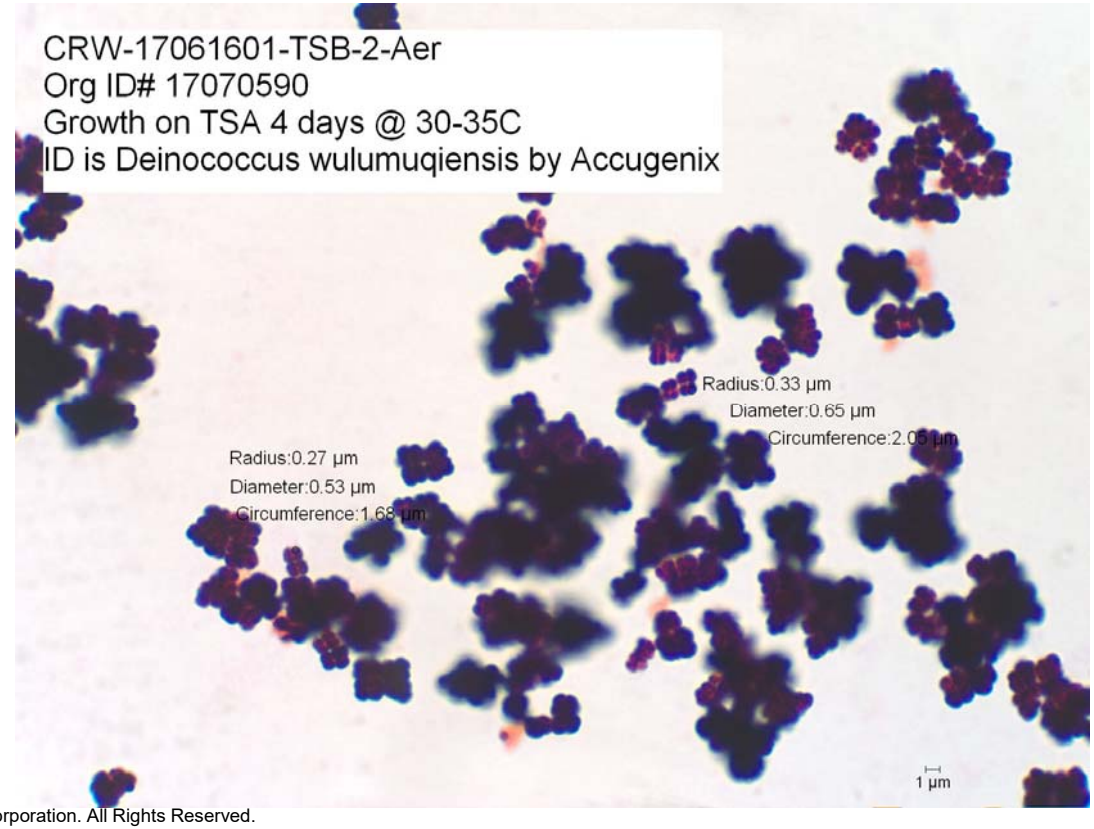

# *Deinococcus*

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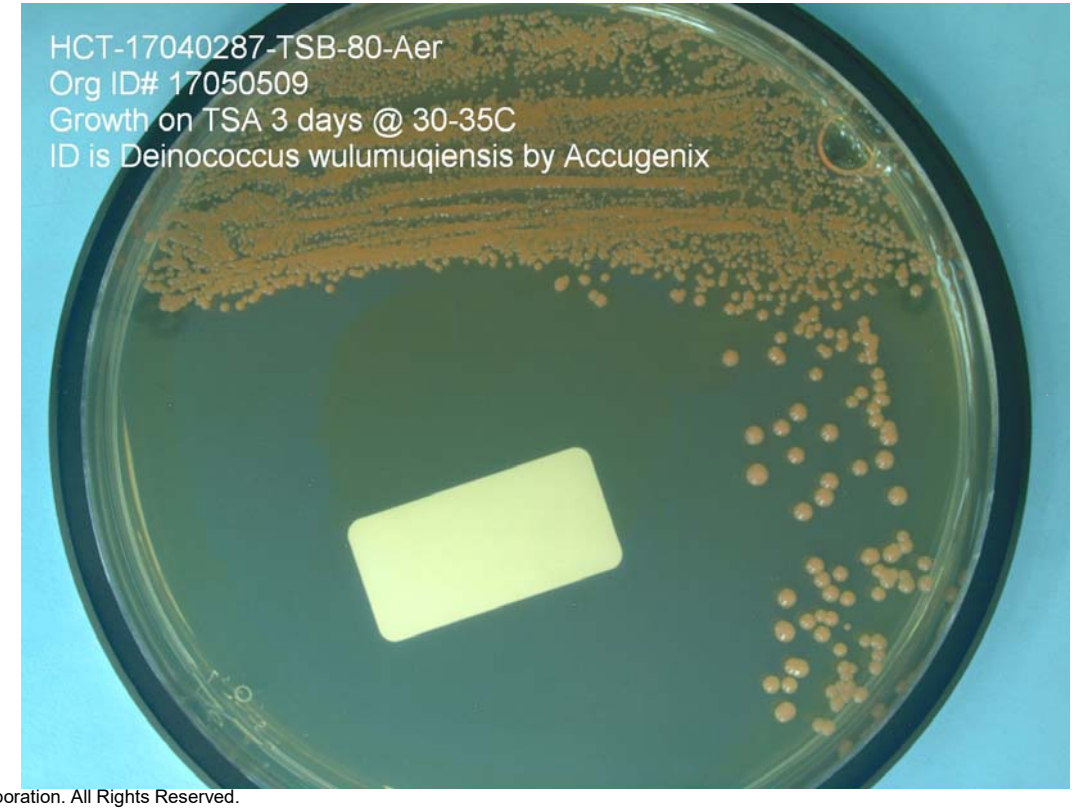

# Industry References

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- USP 42 <1072> Disinfectants and Antiseptics
- Draft Annex 1 (2018/2019) and MHRA Orange Guide (2015)
- FDA Aseptic Processing Guide (2004)
- FDA, MHRA, HPRA, CFDA, ANSM, ANVISA, FDAHA, Swissmedic, & EMA Expectations
- Industry Articles (Ex. Dr. Scott Sutton, Jose Martinez, Dr. Tim Sandle, Richard Prince, Rebecca Smith, Jeanne Moldenhauer, Crystal Booth)
- PDA Cleaning and Disinfection TR No. 70 (October, 2015)
- PDA TR No. 69 on Biofilms (2015)
- The CDC Handbook - A Guide to Cleaning & Disinfecting Cleanrooms (Dr. Tim Sandle 2016)
- A Guide to Disinfectants and their use in the Pharmaceutical Industry (Pharmig 2018)
- USP 42 <1116> Microbiological Control and Monitoring of Aseptic Processing Environments
- USP 42 <1115> Bioburden Control of Non-Sterile Drug Substances and Products
- PIC/S Guide to Good Practices for the Preparation of Medicinal Products in Healthcare Establishments (2014)
- WHO Annex 6
- PHSS Technical Monograph #20 “Bio-contamination characterization, control, monitoring and deviation management in controlled/GMP classified areas
- IEST-RP-CC018.4 Cleanroom Housekeeping: Operating & Monitoring Procedures (2019)

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