Case Studies in Fungal and Bacterial Spore Excursions in Cleanrooms

PDA/ISPE Australia
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Senior Technical Service Manager
Factors Influencing Biocidal Performance
Factors in Performance

- pH
- Temperature
- Contact time
- Concentration
- Surface
- Presence of organic matter
- Water Quality (hardness)
A Risk Based Approach to Choosing a Disinfectant

• 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

- Efficacy
- Compatibility
- Rinsability
- Stability
- Residue
- Efficacy
A Risk Based Approach to Cleaning Frequency

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

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

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

Materials
- Pass through sterilizers (autoclaves, dry heat ovens, depyrogenation tunnels, etc.)
- Decontamination chambers (EO, VHP, UV, etc.)
- Material handling airlocks
Good Science: Risk Based Approach
….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

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

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.)

• 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

- Gram-positive sporing rods, 13%
- Gram-negative rods, 2%
- Fungi, 1%
- Gram-positive non-spore forming rods, 3%
- Gram-positive cocci, 81%
### Review - Microflora in Cleanrooms (U.K.)

<table>
<thead>
<tr>
<th>Genus</th>
<th>A/B (6729)</th>
<th>C/D (2500)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micrococcic</em> (and related)</td>
<td>38%</td>
<td>40%</td>
</tr>
<tr>
<td><em>Staphylococcic</em></td>
<td>21%</td>
<td>11%</td>
</tr>
<tr>
<td><em>Bacillis</em> (and related)</td>
<td>13%</td>
<td>10%</td>
</tr>
<tr>
<td><em>Pseudomonas</em> (and related)</td>
<td>&lt;1%</td>
<td>8%</td>
</tr>
<tr>
<td><em>Corynebacterium</em> (and related)</td>
<td>3%</td>
<td>5%</td>
</tr>
<tr>
<td><em>Rhodococcic</em></td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>Fungi</td>
<td>N/A</td>
<td>3%</td>
</tr>
</tbody>
</table>
Risk Based Approach

• 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 reoccurrence
• 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:

- Machine
  - HVAC Differential Pressures
  - Preventive Maintenance
- Method
  - Equipment Repair
  - Change Controls
- Material
  - Cleaning products
  - Raw materials
- Measurement
  - HVAC Differential Pressures
  - Power Change
  - Frequent Cleaning
- Man
  - Training
  - Personnel Monitoring
- Nature
  - Aspergillus Contamination

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**Investigation Strategies: Critical Zones**

Trend history of organisms in the area versus the contaminant.

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

- 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
Bacillus cereus Contamination Case Study
## Microorganism Resistance Hierarchy

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

**More Resistant**

- **Bacillus cereus / sphaericus**
- **Bacillus subtilis / G. stearothermophilus**
- **Clostridium spp.**

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*
Bacterial Endospore

Fig. 8.1. Endospore

Courtesy Dan Klein
Bacterial Spore Structure

Fig. 8.1. Endospore
Bacillus cereus Case Study

• 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

Exosporium – B. anthracis

Hydrophobicity helps adhere to fibers

https://www.llnl.gov/str/Sep06/Velsko.html
Model of *B. cereus* exosporium

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

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

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Bacillus Testing

Spore Isolate T.K.
Spore Isolate B. cereus, 5.81, n=2
10-18-08

Formulation

Average Log Reduction

- 20 min.
- 40 min
- 60 min
Fungal Spore Contamination
Case Studies
Molds common to cleanrooms and cold rooms

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

- 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

• 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

Conidiospores

Courtesy Dan Klein
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

- 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

[Images of Aspergillus brasiliensis cells with measurements 5.20 μm and 17.1 μm]
Aspergillus brasiliensis

Aspergillus Brasiliensis Spores

Aspergillus Brasiliensis Spores

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**Spiny Spores**

Courtesy Dave Shields
Cleanroom Fungi

Courtesy Dan Klein
Penicillium

- 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**

- 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°C Celsius to -5°C Celsius)
  - Documentation of Cleaning Process
  - Designate Assignable Causes
Penicillium Investigation

Cleanroom Cooling Coils

Plywood made up the sliding door
Penicillium

Courtesy Ann Larson
Penicillium Spores

Courtesy Bruce Ritts

Penicillium Chrysogenum

Penicillium Chrysogenum
Glass Evaluation

![Bar chart showing the comparison of different phenolic compounds and their effects on two types of bacteria (A. brasiliensis and P. chrysogenum). The chart includes data for Phenol A, Phenol B, and 70% IPA.]
Stainless Steel Evaluation

![Bar chart showing the evaluation of Phenol A, Phenol B, and 70% IPA on A. brasiliensis and P. chrysogenum.](chart.png)
Flooring Evaluation

![Bar chart showing Flooring Evaluation results for Phenol A, Phenol B, and 70% IPA.]
Chaetomium Testing

NH Notebook 7297

LS Pub Article Time-Kill
8/08/18
*C. globosum* ATCC 6205, Baseline = 2.65
*A. brasiliensis* ATCC 16404, Baseline = 5.90

![Bar graph showing log reduction of *C. globosum* and *A. brasiliensis* with Alkaline Phenolic products at 5 and 10 minutes](graph.png)
Vesta Syde SQ vs. Competitive Quats

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

Steris Corporation

Household grade

Pharmaceutical grade

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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
Deinococcus Contamination
Case Study
Medical Device Company

• 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

Antimicrobial Efficacy of Several Products Against *Deinococcus proteolyticus*

Time Kill

Baseline = 6.37 log_{10}, n=2

<table>
<thead>
<tr>
<th>Contact Time</th>
<th>15s</th>
<th>30s</th>
<th>1m</th>
<th>5m</th>
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<tbody>
<tr>
<td>70% IPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LpHse 1:256</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.525% NaOCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spor-Klenz-RTU</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Average Log Reduction

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Covers for Surgical Lights and Camera Cases
Deinococcus

CRW-17061601-TSB-2-Aer
Org ID# 17070590
Growth on TSA 4 days @ 30-35C
ID is Deinococcus wulumuqiensis by Accugenix
**Deinococcus**

HCT-17040287-TSB-80-Aer
Org ID# 17050509
Growth on TSA 3 days @ 30-35C
ID is Deinococcus wulumugiensis by Accugenix
Industry References

- USP 42 <1072> Disinfectants and Antiseptics
- 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)
- USP 42 <1116> Microbiological Control and Monitoring of Aseptic Processing Environments
- USP 42 <1115> Bioburden Control of Non-Sterile Drug Substances and Products
- WHO Annex 6
- PHSS Technical Monograph #20 “Bio-contamination characterization, control, monitoring and deviation management in controlled/GMP classified areas
Acknowledgements

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• Dave Shields – Manager, STERIS Laboratories
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