Agenda

✓ Regulations
✓ Bioburden Control
✓ Cleaning and Disinfection
  o Why? FDA 483s
  o Safety
  o Application & Equipment
  o Frequency based on ISO 14644 classification
  o Rotation & Rinsing
  o Disinfectant Validation
European Regulations

- BPD-The effects on Biocides in Europe.
- REACH Directive
Biocidal Product Directive

- Approval Process for biocides: active substances and formulations
  - Annex I: positive list of approved substances
  - Listing on Annex I triggers review of Formulations
- Review Program for existing active substances
  - Range of Product Types
  - Driven by Review Regulations
  - Identified and Notified Substances
    - Identified – Active Substances that are stated to be on the market by suppliers
    - Notified – Active Substances that organisations commit to supporting through the review process
Biocidal Product Directive

• Active substance identified but not notified
  – For example, para tertiary amyl phenol (ptap)
  – Products containing this active must be off the market by 1 September 2006
    • No supply or use

• Active substance notified
  – National legislation, if in place, applies until review +/- December 2010
Biocidal Product Directive

• Next Steps for Suppliers of Active Substances in Disinfectants and General Biocidal Products
  – Submit active substance technical dossiers by 31 July 2007
  – Member States review deadline November 2008
  – EU discussion forum deadline November 2009
  – EU decision and active substance listing for that product type +/- December 2010
  – Formulated Product Dossiers due for these product types +/- December 2010 + 3 months
US Disinfectant Regulation

- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
- All germicidal cleaners fall under the FIFRA as amended (1988) and administered by EPA
- FDA regulation as medical device per Food Quality Protection Act of 1996 - if used to reprocess other medical devices or if used as a sterilant for medical devices
EPA requirements

- Environmental Protection Agency (EPA)
  - Safety, use, disposal
  - Efficacy
    - AOAC Official Methods of Analysis
EPA classifications

- Sanitizer
- Disinfectant
- Sterilant
Sanitizer

- Proper use results in bacteria reduction of
- > 99.9%
  - Used on precleaned surfaces
Disinfectant

• Proper use results in 100% kill of vegetative bacteria, target viruses and target fungi
• 4 Log reduction of bacteria
• 3 Log reduction of viruses
• 6 Log reduction of fungi
  • May or may not require precleaning
    ▪ Serum efficacy—5% BSA and EN methods differ
Sterilant

Proper use results in 100% kill of all microorganisms, including bacterial spores

\(B. \ subtilis, \ C. \ sporogenes\)

6-7 Log reduction

- Always requires pre-cleaning
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Review - Microflora in Cleanrooms (U.K.)

- Tim Sandle
- 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-positive non-spore forming rods, 3%
- Gram-negative rods, 2%
- Fungi, 1%
- 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>Micrococi (and related)</td>
<td>38%</td>
<td>40%</td>
</tr>
<tr>
<td>Staphylococi</td>
<td>21%</td>
<td>11%</td>
</tr>
<tr>
<td>Bacillus (and related)</td>
<td>13%</td>
<td>10%</td>
</tr>
<tr>
<td>Pseudomonas (and related)</td>
<td>&lt;1%</td>
<td>8%</td>
</tr>
<tr>
<td>Corynebacterium (and related)</td>
<td>3%</td>
<td>5%</td>
</tr>
<tr>
<td>Rhodococci</td>
<td>&lt;1%</td>
<td>N/A</td>
</tr>
<tr>
<td>Fungi</td>
<td>N/A</td>
<td>3%</td>
</tr>
</tbody>
</table>
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 Tongue Stud
    - 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
Fungal Spores

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

*Penicillium*, photos: Ann Larson
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
  - Cold room Cleaning Procedures
  - Documentation
  - Assignable Cause
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-through
- Clean and dirty area on the dock
- Better control of gownsing area
- Cart Wheel control
- Better gowning control

Photo: Terra Universal
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

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

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

<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
Bacterial Spores

- *Bacillus cereus* group (7 species*)
- *Bacillus circulans*
- *Paenibacillus glucanolyticus*

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

Exosporium – *B. anthracis*

Hydrophobicity helps adhere to surfaces (tubing, 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
Bacillus cereus

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

EM B. cereus isolate
5.81 log inoculum

Average Log Reduction

Formulation

- H2O2/PAA RTU
- 1:4 Bleach
- 1:10 Bleach
- Alkaline Cleaner
- Acidic Cleaner
- Iodine
- Product
- Alkaline Cleaner-2
- H2O2/PAA 1:18

20 min.
40 min
60 min
**Bacillus circulans**

- Efficacy Testing Failures
  - Vinyl surfaces
    - Sent out for ID
      - *Paenibacillus*
Efficacy Testing on Vinyl Coupons

<table>
<thead>
<tr>
<th>Time</th>
<th>B. subtilis</th>
<th>B. cereus</th>
<th>Paenibacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min.</td>
<td>6.64 log</td>
<td>6.51 log</td>
<td>5.39 log inoculum</td>
</tr>
<tr>
<td>15 min.</td>
<td>6.64 log</td>
<td>6.51 log</td>
<td>5.39 log inoculum</td>
</tr>
</tbody>
</table>
Efficacy Testing on SS Coupons

B. subtilis 19659 - 6.64, B. cereus 14576 - 6.51, Paenibacillus - 5.39
H2O2/PAA RTU SS coupons

Ave. Log Reduction

- B. subtilis
- B. cereus
- Paenibacillus

10 min. vs. 15 min.
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“There was no investigation by the firm when levels exceeded their action limits and there was no identification of the isolates. No documented corrective actions were taken to remove microbial contamination (bacteria and mold) from the facility.”

FDA Warning Letter 10-26-12
“The spaces between the machine surfaces are not subject to direct cleaning and sanitization. This area was previously identified during special environmental monitoring studies to be a location where *Stenotrophomonas maltophilia* was present at levels that were recorded as being too numerous to count.”

GMP Trends November 1, 2013
Warning Letter – Application equipment in sterile areas

“General Cleaning, Sanitization and Disinfection of Aseptic and Controlled Areas,” also lacks adequate details on how many times mops and wipes can be used. Your response is inadequate because you did not provide scientific data that the corrective actions implemented in SOP 101.74, "General Cleaning, Sanitization and Disinfection of Aseptic and Controlled Area" are adequate. While this SOP instructs staff to replace mops, wipes, and other supplies when visually soiled, it is unclear whether this revision will provide for acceptable standards of sanitization and disinfection in the controlled area.”

FDA Warning Letter November 27, 2013
“Your surface disinfectant study (stainless steel and plastic curtain surfaces) does not address the impact of the disinfectant use on the subject surface. Bleach is used for weekly wall cleaning and monthly on the ceilings and light fixtures in the filling rooms, staging area and airlocks.”

GMP Trends May 1, 2014
Inadequate surfaces tested

“a. The coupons used in the "Disinfectant Efficacy Verification for Hard Surfaces" VP-2008-065-PV approved: 04/26/2010, were not representative of the surfaces found in the Tissue Processing Laboratories (TPL) and BioAdhesive laboratories. For example, (b)(4) was used in the study to represent the biological safety cabinets, laminar flow hoods, and tables in the processing and manufacturing areas. However, the equipment is comprised of (b)(4). All surfaces that are used in critical processing and manufacturing areas were not evaluated in the "Disinfectant Efficacy Verification for Hard Surfaces" VP-2008-065-PV approved: 04/26/2010.

Your response to this observation is not adequate. The (b)(4) work-top surface is an area that is monitored and is located in a classified area. Therefore, the effectiveness of its cleaning should be evaluated just as the other surfaces.”
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Safety: Data sources

- Sources of information
  - Safety Data Sheet (SDS)
  - Product label
  - Your safety/EHS department (industrial hygienist)
  - Supplemental toxicity studies from supplier
- Involves product AND process
Overexposure issues (sporicides)

- Inhalation
  - Sore throat
  - Sneezing
  - Headache
- Skin contact
  - Bleaching or whitening of skin
  - Minor irritation (tingling)
- Eye contact
  - Watering eyes
  - Corrosive to eye tissues
Potential Toxicity Testing Summary

- Acute Oral Toxicity
- Acute Inhalation Toxicity
- Primary Dermal Irritation
- Acute Dermal Toxicity
- Primary Eye Irritation
- Skin Sensitivity
- Acute Intravenous Toxicity
- Cytotoxicity
General Safety Guidance

Proper Use

- Avoid splashing
- Use in a well ventilated area
- Avoid manual fine mist spraying
- Keep solution covered or contained
- Rubber or nitrile gloves
- Ocular cavity fit goggles
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Application: Two and Three Bucket Systems

Courtesy Perfex Corp.

Courtesy Micronova
Bucket Systems

- Sterilant (Disinfectant) in front bucket and rinse bucket;
- Optional to put some sterilant (Disinfectant) in waste bucket (beneath 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, MicronSwep System (Vileda/ Micronclean/Aramark)
Newer Mopping Systems

Mop King Jr.

http://www.am-king.com/mopkingjr.htm

- Stainless steel
- Battery operated and electronically monitored
- Holds 15 Rayon or Microfiber flat mops
- Holds 1.5 gal solution
- Dispensed with the precise amount of solution
- Fits on housekeeping cart
- Flat mops guided along rail to a wetting tray
- Pump activates, dispenses solution to mop head

AmKing Technologies, Bedford, NH
Newer Mopping Systems

MicronSweep system by Vileda Professional and Micronclean

(www.micronsweep.com)
Cleanroom Curtain Devices

• Surfaces
  - Floors
  - Walls
  - Isolators
  - Lyophilizers
  - Cabinets
  - Tanks
  - Curtains/Softwalls

Courtesy Micronova
Commonly Used Equipment

Courtesy Micronova
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-5,6 (Grade A & B)
  - 1,000 square feet in (93 square meters) ISO-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
Application Technique

Figure 1. Proper Surface Cleaning

Illustration of Pull-lift Technique

1st Stroke: Lift the sponge mop and place it on the surface at a manageable distance. Pull it toward you.

2nd Stroke: Lift the mop again, place it down at the start of the first stroke, overlapping the first stroke by around 10% to 20%. Pull it toward you.

3rd Stroke: Repeat.

Links to Mopping Resources

- http://www.am-king.com/mopkingjr.htm
- http://www.youtube.com/watch?v=Q_56ut2UQkk
- http://www.youtube.com/watch?v=pF5iPQMXKdU
- www.micronswep.com
- http://www.micronova-mfg.com/
- http://www.perfexonline.com/001.htm
- http://www.youtube.com/watch?v=qTWaYQIX2IY
Alternative Technique

- Foaming
- VHP®
- Spraying (also known as fogging)
  - Aerosolizes disinfectant
- Fumigation
  - Vaporizes disinfectant
- Full immersion
  - Disinfectant soak
Fogger / DynaFogger

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

Courtesy of Curtis Dyna-Fog
Room Decontamination

Walk-In Refrigerator Type Construction

Oklahoma Medical Research Foundation, Oklahoma City, OK
Application Conditions

✓ Concentration
✓ Contact Times
✓ Temperature
✓ Surface
✓ Bioburden
✓ Soil levels
✓ Water hardness
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
## Grade D (ISO 8 at rest)

<table>
<thead>
<tr>
<th>Surface</th>
<th>Method</th>
<th>Cleaning Agent</th>
<th>Frequency</th>
<th>Rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floors • Around Drains • Foot Traffic Paths • Spill Areas • Access Ports</td>
<td>Mop</td>
<td>Disinfectant with surfactant</td>
<td>Daily at shutdown, between process changeover</td>
<td>Not necessary after each application†</td>
</tr>
<tr>
<td>Walls, Ceilings • General</td>
<td>Wipe or Mop</td>
<td>Disinfectant with surfactant</td>
<td>Monthly</td>
<td>Not necessary after each application†</td>
</tr>
<tr>
<td>• Doors, Handles, High-Traffic Areas</td>
<td>Wipe or Mop</td>
<td>Disinfectant with surfactant</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>Equipment • Adjacent to Access Port</td>
<td>Spray or Wipe</td>
<td>Disinfectant with surfactant</td>
<td>Daily during processing</td>
<td>As needed to remove residue buildup</td>
</tr>
<tr>
<td>• Surface Upstream Airflow Path to Process Opening</td>
<td>Wipe</td>
<td>Disinfectant with surfactant</td>
<td>Weekly</td>
<td></td>
</tr>
<tr>
<td>Other Surfaces • Sinks • Benches • Trash Containers</td>
<td>Wipe</td>
<td>Disinfectant with surfactant</td>
<td>Daily</td>
<td>Not necessary after each application†</td>
</tr>
</tbody>
</table>

A sporicidal agent must be used quarterly, semi-annually or as needed in response to microbial monitoring.  
† Any contamination control program should incorporate a residue removal component. See the Residue Removal Section for details.
Grade C (ISO 7 at rest, ISO 8 in operation)

<table>
<thead>
<tr>
<th>Surface</th>
<th>Method</th>
<th>Cleaning Agent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floors • Normal Traffic Paths</td>
<td>Mop</td>
<td>Disinfectant with surfactant</td>
<td>Daily after transfers</td>
</tr>
<tr>
<td>• Proximity to Open Process or Transfer Areas</td>
<td>Wipe or Mop</td>
<td>Disinfectant with surfactant followed by a sporicide</td>
<td>Weekly or monthly, if necessary</td>
</tr>
<tr>
<td>Walls • General</td>
<td>Wipe or Mop</td>
<td>Disinfectant with surfactant followed by a sporicide</td>
<td>Weekly or monthly</td>
</tr>
<tr>
<td>• Door Plate</td>
<td></td>
<td>Disinfectant with surfactant</td>
<td>Daily</td>
</tr>
<tr>
<td>Equipment • Shelving • Portable Tanks • Processing Items</td>
<td>Spray or Wipe</td>
<td>Disinfectant with surfactant</td>
<td>Before and after use</td>
</tr>
<tr>
<td>• Carts (wheels)</td>
<td></td>
<td>Sporicide</td>
<td></td>
</tr>
<tr>
<td>Other Surfaces • Furniture</td>
<td>Spray or Wipe</td>
<td>Disinfectant with surfactant</td>
<td>Daily</td>
</tr>
<tr>
<td>• Chair (wheels)</td>
<td></td>
<td>Sporicide</td>
<td></td>
</tr>
</tbody>
</table>
Grade A (ISO 4.8) or B (ISO 5 at rest, ISO 7 in operation)

<table>
<thead>
<tr>
<th>Surface</th>
<th>Method</th>
<th>Cleaning Agent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>External Hoods</td>
<td>Wipe</td>
<td>Sterile disinfectant with surfactant</td>
<td>Daily</td>
</tr>
<tr>
<td>• Back, Sides, Top</td>
<td></td>
<td>Sterile disinfectant with surfactant</td>
<td>Daily</td>
</tr>
<tr>
<td>• Door, Sliding Panel</td>
<td>Wipe</td>
<td>Sterile Sporicide</td>
<td>Weekly or in response to microbial monitoring</td>
</tr>
<tr>
<td>Inside Hood or Curtain</td>
<td>Wipe</td>
<td>Sterile disinfectant with surfactant</td>
<td>Daily, preuse and postuse</td>
</tr>
<tr>
<td>• Work Surface</td>
<td></td>
<td>Sterile Sporicide</td>
<td>Weekly or in response to microbial monitoring</td>
</tr>
<tr>
<td>• Sidewalls</td>
<td></td>
<td>Sterile Sporicide</td>
<td>Sterile WFI or 70% IPA as needed to remove residue buildup</td>
</tr>
<tr>
<td>• Apparatus/Critical Surfaces</td>
<td></td>
<td>Sterile Sporicide</td>
<td>Weekly or in response to microbial monitoring</td>
</tr>
<tr>
<td>Curtains</td>
<td>Wipe or Mop</td>
<td>Sterile Sporicide</td>
<td>Weekly or in response to microbial monitoring</td>
</tr>
<tr>
<td>Adjacent Flooring and Walls</td>
<td>Mop</td>
<td>Sterile disinfectant with surfactant followed by a sterile sporicide, as necessary</td>
<td>Weekly or in response to microbial monitoring</td>
</tr>
</tbody>
</table>

Sterile WFI or 70% IPA as needed to remove residue buildup
Agenda

✓ Regulations
✓ Bioburden Control
✓ Cleaning and Disinfection
  o Why? FDA 483s
  o Safety
  o Application & Equipment
  o Frequency based on ISO 14644 classification
  o Rotation & Rinsing
  o Disinfectant Validation
Surface Types and Topography

- Sticky mats
- Drains
- Edges and corners
When To Rinse

Rinse as needed to control residue

– Appearance (visible at ~ 4 µg/cm² on stainless steel)
– Functionality
– Product risk
– Interaction/interference with other chemical agents being used
– Safety issue (stickiness, tackiness, slippery)
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
Current Rotation Practices

“Rotation of a common disinfectant and a sporicidal helps ensure that bacterial spores do not take hold in manufacturing and aseptic areas. But the rotation of common disinfectants such as those based on phenol-derivatives, aldehydes, and oxidizing agents has no scientific basis.”

Current Rotation Practices

“The need for the rotation of disinfectants in a pharmaceutical cleanroom sanitization program is not supportable from a scientific basis. The assumptions that proponents of the practice assert as facts, e.g. generation of resistant organisms, greater efficacy of alternating agents, are not supported by the literature. However, even when using a validated disinfectant as part of a well-managed cleanroom sanitization program, periodic use of a sporicide is a prudent—even an essential—component of the sanitization program. It is needed to address the occasional appearance of spore-forming organisms in the environmental monitoring program and therefore ensure the cleanest possible environment for manufacturing.”

Current Rotation Practices

“It is clear that using a sporicide is highly important, but agents that have sporicidal activity tend to be harsh and unacceptable for everyday use. For this reason it is recommended that a sporicide is used in rotation with another effective disinfectant that is more suitable for regular use.”

“Given this knowledge, the pharmaceutical and biotechnology industries have moved away from the rotation of two disinfecting agents. This formerly common practice led to high residue levels and subordinate efficacy performance. Today most firms use a system whereby a disinfectant is rotated with a sporicide to more effectively reduce the bioburden levels. The rotation of a disinfectant with a sporicide is superior to the use of rotations of multiple disinfectants.”

PDA TR 2014
Agenda

✓ Regulations
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Vendor - Common AOAC International Tests

- Use-Dilution Method Tests for Liquids
  - 955.14 S. enterica
  - 955.15 S. aureus
  - 964.02 P. aeruginosa
- Germicidal Spray Products Test
- Confirmatory Tuberculocidal Activity Test
- Fungicidal Activity of Test Substances
- Sporicidal Activity of Disinfectants (966.04)
Testing / Validation Protocols

Regulatory

United States
Methods typically taken from AOAC INT’L. Primarily qualitative
Primarily use ring carriers
Pass/Fail Criteria differ for bacteria, TB, fungi and spores

Europe
Methods divided into 3 tiers
Phase 1
   Basic suspension tests
Phase 2
   Simulation studies
   Use hard surfaces
Phase 3
   Tests under practical conditions
3 Disinfectant Validation Components

• *In vitro* testing (Disinfectant Qualification)
  – Suspension testing (also called Time-Kill Study)
  – Carrier Testing (also called Coupon Testing)

• *In situ* testing

• Environmental monitoring
  – Data trending (over a long duration of time, typically 6-12 months)
  – Identification of organisms (fungi, yeasts, and bacteria)
End User Disinfectant Qualification

- USP 37 <1072> Disinfectants and Antiseptics
  - Use-dilution tests
  - Surface Challenge tests

- ASTM E2614-08 Guide for evaluation of Cleanroom Disinfectants

- ISO 14698 (parts 1-3)
  - Surface evaluation, focus on cleaning

- PDA Technical Report on Cleaning and Disinfection (Draft Document)
Considerations for *In vitro* Testing

- Use-dilution
- Temperature (hot WFI drops, use in cold room?)
- Technique
  - Suspension vs. carrier
  - Substrates
  - Neutralization/dilution
  - Subculture techniques
- Microorganisms
- Efficacy requirements
Microorganism Selection

- Environmental isolates **must** be considered
  - Broad spectrum
  - Most frequently occurring
  - High levels in the Environment
  - Demonstrated decontamination difficulty at the facility
  - “Worst Case”
- USP (ATCC or USDA) challenge organisms may also be considered but environmental isolates are the most critical
### Hierarchy of Resistance

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

In vitro Options for Testing

• AOAC
  – Use-dilution Test
  – Sporicidal Activity of Disinfectants
  – Germicidal Spray Products as Disinfectants

• ASTM
  – Time Kill Method
  – Spray Slide
  – Sanitizer method (E1153)
  – Wipe method
  – **Quantitative Carrier Method (E2111 & E2197, QCT)**
  – Biofilm Method (E1427)
  – Viral Testing (Suspension E1052)
  – Viral Testing (Carrier E1053)
  – **Standard Guide for Evaluation of Cleanroom Disinfectants (E2614-08)**

• Variations of all of the above
More Options for *In vitro* Testing

- **EN**
  - 1276 (bacterial suspension test)
  - 1040 (bacterial suspension test)
  - 1650 (fungal suspension test)
  - 13704 (sporicidal suspension test)
  - 13697 (Carrier test)
  - 14476 (Viral Testing)
  - 13610 (Viral Testing Hard Surface)
  - 14348 (TB Testing)
- **AFNOR (France)**
  - NFT 72-150 Suspension
  - NFT 72-190 Carrier Test
- **DGHM/VAH (GER; Carrier & Suspension Tests)**
- **TGA (Australia)**
Neutralization Methods

• Elimination of inhibitory residual disinfectant activity
  • Chemical neutralization of the active
  • Dilution - generally not effective alone (alcohols)
  • Filtration + Rinsing – separating the active from the organism

• Issues
  • Antimicrobial activity of neutralizer (toxicity)
    ▪ Thioglycollate and sodium sulfite can be toxic
  • Mechanical separation causing damage to cells

• Validation of neutralization is required
### USP <1227> Common Neutralizers

<table>
<thead>
<tr>
<th>Neutralizer</th>
<th>Biocide Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisulfate</td>
<td>Glutaraldehyde, Mercurials</td>
</tr>
<tr>
<td>Dilution</td>
<td>Phenolics, Alcohol, Aldehydes, Sorbate</td>
</tr>
<tr>
<td>Glycine</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>Lecithin</td>
<td>Quaternary Ammonium Compounds (QACs), Parabens, Bis-biguanides</td>
</tr>
<tr>
<td>Mg(^{+2}) or Ca(^{+2}) ions</td>
<td>EDTA</td>
</tr>
<tr>
<td>Polysorbate (Tween)*</td>
<td>QACS, Iodine, Parabens</td>
</tr>
<tr>
<td>Thioglycollate</td>
<td>Mercurials</td>
</tr>
<tr>
<td>Sodium thiosulfate</td>
<td>Mercurials, Halogens, Aldehydes</td>
</tr>
</tbody>
</table>

*Tween 20 or 80, & Lubrol (Brij 58) are nonionic detergents

**Catalase for H\(_2\)O\(_2\)**
Surface/Coupon Issues

• Surface type and condition can have a huge impact on efficacy
• Preparation of surfaces prior to testing
  – Autoclaving may not be acceptable for some surfaces
  – Residues must be removed
• Some surfaces pose a challenge during qualification studies:
  – Peeling after sterilization
  – Surface tension
Surface Type and Condition

- Visually smooth surfaces can be irregular
- Older or damaged surfaces can be more challenging
- Glass and stainless steel typically the least challenging
Recovery Method Issues

- Typical surface recovery methods
  - Contact plates (rarely used)
  - Swabs
  - Direct inoculation of coupons into neutralizing media
    - Requires sterile coupons
    - May include manual or automated dislodging
      - Stomacher bags
- Recovery method must be validated
- Final plates must be countable to calculate log reduction
Surface Preparation

• Autoclaving may not be acceptable for some surfaces (Saniflex)
Surface Tension Issue
Disinfectant Qualification Study Tips

- AOAC methods are inappropriate for this testing (but some procedures such as inoculum prep, etc. can be of value)

- EN-13697 offers valuable insight into quantitative surface testing
- Up-front planning is extremely important
- Combining physical removal and chemical kill in one study is not recommended
- Consistency is crucial to a positive outcome
- Reading the product labels to understand product claims and limitations is necessary
- Incorporate expiry dating specified in internal SOPs into the study
- Using a contract lab to perform testing sounds easy but still requires time, effort, and vigilance
In situ Testing

• “…a statistical comparison of the frequency of isolation and the numbers of microorganisms isolated prior to and after the implementation of a new disinfectant.” USP General Informational Chapter <1072>

• “The effectiveness of these sanitization procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces (i.e., via obtaining samples before and after sanitization).” Sterile Drug Products Produced by Aseptic Processing – September, 2004 FDA
Case Study: Construction Event at Biotech Site

- Worst Case Events
- 9X Clean [1X Sporicide + 2X Phenolic repeated on days 1,2,3]
- Fogging
- VHP®
- Triple Clean
  - Defined 3X Disinfectants and Sporicide
  - EM frequency (Static and Dynamic)
  - Release of the room
Cleaning and Disinfection Efficacy
Time 0

Red = Spore formers
Green = Other
After 1X Cleaning - NO Sporicide
After 2X Cleaning – NO Sporicide
After 3X Cleaning - No Sporicide
After Sporicide
Summary

- Case Studies in Bioburden Control – read 483s
- Current best practices in Cleaning and Disinfection
  - Safety (PPE, Toxicity, SDS)
  - New mopping systems
  - Rotation = Disinfectant + Sporicide
  - Incorporate Rinsing
- Disinfectant Validation
  - Vendor (AOAC for EPA registration)
  - End-user (USP 1072, ASTM, or EN methods)
  - Use of in-house isolates + surfaces crucial