Disinfectant Efficacy Testing for Critical Environments

Marc Rogers Ph.D.
Technical Service Specialist
Life Sciences Formulated Chemistries

PDA SE Chapter
Greenville, NC
April 18, 2013
AGENDA

- Disinfectant Regulation & EPA Registration
- Vendor Label Claims and Testing
- Disinfectant Validation Study
  - Why run?
  - 3 components
- Test Types & Methods
- In vitro Testing Considerations
  - Examples/Data

Copyright © 2013 STERIS Corporations. All Rights Reserved. CONFIDENTIAL and PROPRIETARY to STERIS Corporation.
Disinfectant Regulation

- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (Title 7 of the US Code) - Pesticides
- All germicidal cleaners fall under FIFRA as amended (1977), administered by EPA (OPPTS)
- FDA regulation as medical device if used to reprocess other medical devices or used as a sterilant for medical devices
  - Per Food Quality Protection Act (1996)
  - 21 CFR 880.6890 General Purpose Disinfectants – noncritical
  - 21 CFR 880.6885 – critical and semicritical HLD & sterilants
Disinfectant Regulation

- EPA approves and registers all label claims for antimicrobial pesticides
  - Safety
  - Directions for Use
  - Directions for Disposal
  - Efficacy (AOAC INTERNATIONAL)

- Intended to meet test requirements of FIFRA (Title 7 U.S.C. 136)
- Sporicides/sterilants
- Germicidal Spray Products
- Disinfectants
  - Limited efficacy
  - General efficacy (broad spectrum)
  - Hospital or med environment efficacy
- Fungicide, Tuberculocide
- Virucide (modified AOAC)
EPA Requirements - Vendors

- **Sterilant** (60 carriers each on two surfaces); spores of *B. subtilis* ATCC 19659 and *C. sporogenes* ATCC 3584; 3 lots (720 carriers)
- **Disinfectant** (60 carriers representing 3 lots) against 3 bacteria; *S. enterica* ATCC 10708, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442
- **Fungicide** (10 carriers rep. 2 lots killing all spores of *T. mentagrophytes* ATCC 9533)
- **Tuberculocide** (2 lots killing all *M. tuberculosis var. bovis* (BCG) on all carriers) or 4 LRV in quantitative test
- **Virucide** (2 lots at 4 replicates per each dilution showing inactivation at all dilutions if no cytotoxicity) – 4 LRV (3 LRV if cytotoxicity)
- **Sanitizer-N-FC** (3 LRV on surfaces within 5 min against *S. aureus* ATCC 6538 and *K. pneumoniae* ATCC 4352 or *E. aerogenes* ATCC 13048
Disinfectant Effectiveness Tests

• AOAC International analyses include carrier tests & use-dilution tests for bactericidal, mycobactericidal, sporicidal, fungicidal, and virucidal activity

• In EU, efficacy can be demonstrated:
  – Kelsey-Sykes Capacity test
  – European Committee for Normalization (CEN)
  – TC 216 work program “Chemical Disinfectants and Antiseptics”
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
30 min contact as a sporicide on hard non-porous surfaces
AGENDA

• Disinfectant Regulation & EPA Registration
• Vendor Label Claims and Testing
• Disinfectant Validation Study
  – Why run?
  – 3 components
• Test Types & Methods
• In vitro Testing Considerations
  – Examples/Data
Why Do End-Users Validate Disinfectants/ Biocides?

• Context is non-product contact cleanroom surfaces
• FDA Aseptic Processing Guide (Sep, 2004)
  – Each manufacturer must have a formal program governing the qualification, use and disposal of disinfectants (p 34)
• USP <1072> Disinfectants and Antiseptics
• FDA Form 483’s and Warning Letters
FDA 483/ WL Categories

• Inadequate Sanitizer and Disinfectant Study:
  – No data to support the appropriateness of the disinfectants used
  – Did not include efficacy studies for solutions currently used
  – Did not use materials found in the aseptic processing area (APA) floors, walls, work surfaces
  – No data to support the expiration date
  – No data to support the contact time
Recent Warning Letter

“The **coupons used** in the “Disinfectant Efficacy Verification for Hard Surfaces….” were not **representative** of the surfaces found in the tissue processing laboratories (TPL) and BioAdhesive laboratories. For example, ___ was used in the study to represent biological safety cabinets, laminar flow hoods, and tables in the processing and manufacturing areas. However, the equipment is comprised of ___”

“All surfaces that are used in critical processing and manufacturing areas were not evaluated…”

Warning Letter Jan 29, 2013
“Your Disinfectant qualification for ___and___ bi-spore disinfectants documented that the log reduction criteria (Bacteria >4, Fungi >3) was not met when challenged with multiple organisms in variety of surfaces. After disinfection you recovered Micrococcus luteus on vinyl,____, stainless steel, glass and wall laminate and Enterobacter cloacae, Rhodococcus sp, Burkholderia cepacia, Pseudomonas aeruginosa on glass. However your procedures for routine cleaning of the aseptic manufacturing area continue to require the use of unqualified disinfectants ….”

Warning Letter October 7, 2011
Recent Warning Letter

“The materials that were tested in the Disinfectant Efficacy study were not representative of all the surfaces present in the Aseptic Processing Area.” “The stainless steel coupon tested did not represent these damaged surfaces” May 25, 2011
“There is no assurance that the disinfectant _____ is effective against mold, since it did not meet your established recovery rate acceptance criterion in the December 2001 “Disinfectant Validation and Efficacy Study of ____ by the Surface Test Method” study.”

May 24, 2007
What do you know?

What the Vendor tells you

- Chemical makeup
  
  Label lists actives/concentration, MSDS lists only hazardous ingredients

- Recommended prep method (use-dilution)

- Efficacy using AOAC
  
  Tested against ATCC organisms

- Usually 10 minute contact time
What you need to know

How the disinfectant performs:

• in YOUR facility
• prepped by YOUR procedures
• applied by YOUR methods
• with YOUR contact time
• on YOUR surfaces
• against YOUR resident microbes
Qualification and Validation

• Qualification could involve assessing vendor data against ATCC microbes, i.e. disinfectant is “qualified for use”
• May be verified with a suspension test against ATCC recommended microbes
• Validation typically involves coupon studies with in-house environmental isolates from the facility
• To FDA, “validation” typically refers to a process
• In-house isolates should include yeast, bacteria, spore forming bacteria and mold, and possibly viruses
End-User Disinfectant Validation Components

- In vitro testing
  - Suspension testing (also called Time Kill Study)
  - Carrier Testing (also called Coupon Testing)
- In situ testing
- Environmental monitoring
  - Data trending (6-12 months, reviewed monthly*)
  - Identification of organisms (mold, yeast, and bacteria); i.d. to species level and bank them (recommended)

See USP <1116> for incident rate review/recalculation
Disinfectant Validation Procedure

Recommendations

- USP <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)
In Vitro Options for Testing

- **AOAC**
  - Use-dilution Test Methods (955.14, 955.15, 964.02)
  - Sporicidal Activity of Disinfectants (966.04)
  - Germicidal Spray Products as Disinfectants

- **ASTM**
  - Time Kill Method
  - Spray Slide
  - Sanitizer method (E1153)
  - Wipe method
  - Quantitative Carrier Method (E2111 & E2197)
  - Biofilm Method (E1427)
  - Viral Testing (Suspension E1052)
  - Viral Testing (Carrier E1053)

- Variations of all of the above
More In Vitro Options for Testing

- EN
  - 1276  (bacterial suspension test)
  - 1040  (bacterial suspension test)
  - 1650  (fungal suspension test)
  - 13704 (sporicidal suspension test)
  - 13697 (Carrier test)
  - 14476 (Viral Testing)
  - 14348 (TB Testing)
- AFNOR (France)
  - NFT 72-150 Suspension
  - NFT 72-190 Carrier Test
- DGHM (GER; Carrier & Suspension Tests)
- TGA (Australia)
Disinfectant Validation Study

Variables

• The cost of a study can vary widely depending on:
  – Who is conducting the study (In-house or outside microbiology lab)
  – Number/type of organisms tested
  – Number of Contact times tested (many companies test more than one time point)
  – Number of substrates tested
  – Number of disinfectants or sporicides tested
  – Age of product tested (i.e., 7 day use-dilution)
  – The different test methods (i.e., suspension and coupon?)
  – Any other variables they may want to consider in their testing (soiled vs. clean conditions, water quality, etc.)
  – Range from $50K –1M +
Key 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
Substrates for Carrier Testing

- Traditional methods (AOAC and ASTM)
  - Stainless steel disks, penicylinders or coupons
  - Watch glasses or glass slides
  - Porcelain penicylinders and silk suture loops

- Cleanroom disinfectant validations – representative materials, large surface areas
  - Stainless steel (416, 316, 316L, 306, 304)
  - Various plastics and elastomers
  - Lexan curtains
  - Kydex (thermoplastic alloy used for ceilings and walls)
  - Bodycote aluminum wall
  - Epoxy-coated flooring
  - Polymeric flooring
  - MMA Flooring
  - Vinyl Flooring
  - Terrazo Flooring
  - Acrylic and Grout
  - Paints & Sealants
  - Gaskets (EPDM, Teflon)
  - Rubber or Nitrile gloves
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
## 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\(_2\)
### Neutralizing Broths

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>AOAC</th>
<th>DEB</th>
<th>LET</th>
<th>NIH</th>
<th>TAT</th>
<th>TPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casitone</td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine</td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>10.0</td>
<td></td>
<td>5.5</td>
<td></td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>7.0</td>
<td>0.7</td>
<td></td>
<td>5.0</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Peptamin</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43.2</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td></td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Sodium bisulfite</td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
<td>5.0</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>1.0</td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium thiosulfate</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soytone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Tryptone</td>
<td>5.0</td>
<td></td>
<td></td>
<td>20.0</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.5</td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Microorganisms

• 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
### Microorganism Selection

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


---

**B. cereus / B. sphaericus**

**B. subtilis / G. stearothermophilus**

**Clostridium spp.**
Virus Efficacy Testing Trends

• Most requests still from Vendors seeking EPA registration of virucidal claims; occasional requests from end-users for validation on cleanroom surfaces

• Rate of requests from end-users is increasing

  – Kelleen Gutzmann, ATS Laboratories, Eagan, MN
  – S. Steve Zhou, Dir. Virology & Molecular Biology, Microbiotest Laboratories, Sterling VA
Virus Efficacy Testing Trends

• Efficacy requirements for EPA (3 LRV beyond cytotox level), FDA no requirements
• EPA requires GLP studies (40 CFR Part 160); FDA will accept GLP or cGMP
• Most influenza tests now done with TC-adapted strains, almost never use eggs*
• Neutralization typically E1482 or gel-filtration columns (i.e. Sephadex); toxicity of neutralizer has occasionally been an issue and requires higher titer

*Unless higher titers needed, then use eggs
Virus Efficacy Testing Trends

• Most fastidious/difficult viruses for testing include porcine circovirus (PCV) and bovine polyomavirus (BPyV)

• Most common cause of failure is surface roughness/porosity

• Expense driven by virus type and host cell feeding schedules/technician time e.g. duck hepatitis B virus (DHBV) primary cells

• Pricing generally fixed per solution per contact time per surface per virus
General Industry Efficacy Recommendations (non-virus)

• Suspension acceptance criteria
  • 4-5 log reduction

• Carrier acceptance criteria $<10^{72}$
  • 2 log reduction bacterial spores
  • 3 log reduction vegetative bacteria, yeast, mold spores
• “...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
Cleaning Efficacy In Situ
Time 0

- Red = Spore formers
- Green = Other
After 1X cleaning - NO Sporicide
After 2X Cleaning – NO Sporicide
After 3X cleaning-No Sporicide
After Sporicide
In Situ Protocols

- Use actual cleaning procedure SOPs (update prior to Validation study)
- “Worst case” conditions
- Compare environmental data before and after procedures
  - Should include data from more than one cleaning event
- Preparation and storage of disinfectants
  - Dilution accuracy is critical
    - SOP development before validation
  - Monitor and control storage of dilution
    - Expiry dating
  - Filter to remove microorganisms if necessary (ISO Class 5)
    - Filter validation (Compatibility and Bubble Point Testing)
In Situ Testing Frequency

- New Cleanroom
- At Shut Down
- After Construction
- After a Power Failure
- After a Worst Case Event (Natural Disaster)

Part 3: Environmental Monitoring & Data Trending (recalculate monthly)
AGENDA

• Disinfectant Regulation & EPA Registration
• Vendor Label Claims and Testing
• Disinfectant Validation Study
  – Why run?
  – 3 components
• Test Types & Methods
• In vitro Testing Considerations
  – Examples/Data
In Vitro Testing Considerations
Contributors to Test Failures

- Recovery issues post-drying (*P. aeruginosa*)
- Inoculum prep (e.g. fungal spores)
- Coupon prep (autoclaving – peeling Saniflex)
- Improper dilution of Concentrate
- Inappropriate biocide for spores
- Insufficient contact time – should match SOP
- US vs. EU requirements
Hard Surface Test Results

Hard Surface Comparison
H2O2/PAA RTU
Bacillus subtilis ATCC 19659

Average Log Reduction

Glass Stainless Steel Enamel Epoxy Miplan Vinyl Acrylic

Surface

1 min 5 min

\( \bar{I} \) = error bar

Copyright © 2013 STERIS Corporations. All Rights Reserved. CONFIDENTIAL and PROPRIETARY to STERIS Corporation.
## Case Study on Substrates

Efficacy (log reduction) of Low pH phenolic: (1:256 ) 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 brasiliensis</th>
<th>Penicillium chrysogenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless Steel</td>
<td>6.62</td>
<td>&gt;6.10 (^b)</td>
<td>4.18</td>
<td>&gt;4.31 (^b)</td>
<td>&lt;3.00 (^c)</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 (^b)</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 (^b)</td>
<td>&lt;3.00 (^c)</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 (^c)</td>
<td>&lt;3.00 (^c)</td>
</tr>
<tr>
<td>Enamel</td>
<td>&gt;6.05 (^b)</td>
<td>&gt;5.72 (^b)</td>
<td>5.45</td>
<td>&gt;3.92 (^b)</td>
<td>&lt;3.00 (^c)</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 (^c)</td>
<td>&lt;3.0 (^c)</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 (^c)</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 (^c)</td>
<td>2.1</td>
</tr>
<tr>
<td>Hardwood</td>
<td>5.18</td>
<td>&gt;4.54 (^b)</td>
<td>5.26</td>
<td>3.2</td>
<td>&lt;3.00 (^c)</td>
<td>2.59</td>
</tr>
<tr>
<td>Melamine Covered Wood</td>
<td>&gt;5.38 (^b)</td>
<td>&gt;5.64 (^b)</td>
<td>&gt;5.09 (^b)</td>
<td>&gt;5.12 (^b)</td>
<td>3.65</td>
<td>3.95</td>
</tr>
<tr>
<td>Plastic</td>
<td>&gt;5.73 (^b)</td>
<td>&gt;5.32 (^b)</td>
<td>&gt;5.05 (^b)</td>
<td>&gt;4.04 (^b)</td>
<td>&lt;3.00 (^c)</td>
<td>2.44</td>
</tr>
<tr>
<td>Plexiglas</td>
<td>&gt;5.90 (^b)</td>
<td>5.62</td>
<td>4.83</td>
<td>&gt;4.40 (^b)</td>
<td>&lt;3.00 (^c)</td>
<td>3.85</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 (^c)</td>
<td>2.61</td>
</tr>
</tbody>
</table>

\(^a\) Disinfectant Efficacy = (Log MSP\(_{\text{positive control}}\) - Log MSP\(_{\text{test coupons}}\)) , where MSP\(_{\text{Positive Control}}\) = Mean surviving population on positive control coupons; MSP\(_{\text{test coupon}}\) = 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
**Inoculum Preparation - Viability**

- Prepare inoculum suspensions from 18-24 hr cultures
- Titer (cfu/mL) and viability must be verified at the end of every test day
### Inoculum Preparation - Fungal Spores

- Use fungal **spore** suspensions for testing
- Hyphae/mycelia can prevent disinfectant from contacting and penetrating spore

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>More Resistant</td>
<td></td>
</tr>
<tr>
<td>Prions</td>
<td>Scrapie, Creutzfeld-Jacob disease, Chronic wasting disease</td>
</tr>
<tr>
<td>Bacterial Spores</td>
<td>Bacillus, Geobacillus, Clostridium</td>
</tr>
<tr>
<td>Protozoal Occysts</td>
<td>Cryptosporidium</td>
</tr>
<tr>
<td>Helminth Eggs</td>
<td>Ascaris, Enterobius</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Mycobacterium tuberculosis, M. tenea, M. chelonae</td>
</tr>
<tr>
<td>Small, Non-Enveloped Viruses</td>
<td>Poliovirus, Paroviruses, 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, Escherichia</td>
</tr>
<tr>
<td>Vegetative Fungi and Algae</td>
<td>Aspergillus, Tychophyton, Candida, Chlamydomonas</td>
</tr>
<tr>
<td>Vegetative Helminths and Protozoa</td>
<td>Ascaris, Cryptosporidium, Giardia</td>
</tr>
<tr>
<td>Large, non-enveloped viruses</td>
<td>Adenoviruses, Rotaviruses</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td>Staphylococcus, Streptococcus, Enterococcus</td>
</tr>
<tr>
<td>Enveloped viruses</td>
<td>HIV, Hepatitis B virus, Herpes Simplex virus</td>
</tr>
<tr>
<td>Less Resistant</td>
<td></td>
</tr>
</tbody>
</table>
Inoculum Preparation—Fungal Spores

Cultures need to be incubated for a sufficient length of time before harvesting spores.
Testing Against Fungal Spores

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

![Disinfectant Time Kill](chart.png)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Ave. Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td></td>
</tr>
<tr>
<td>T. mentag.</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td></td>
</tr>
</tbody>
</table>

Disinfectant: Phenolic Disinfectant

- **3 min.**
- **5 min.**
- **10 min.**
- **15 min.**
Inoculum Preparation—Bacterial Endospores

- May need to use media additives to enhance sporulation (MnSO$_4$)
- Use a microscope and/or staining technique to verify spore concentration; FDA may ask for confirmation, especially when testing a sporicide
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
Surface Preparation

- Autoclaving may not be acceptable for some surfaces (Saniflex)
Surface Tension Issue
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
Disinfectant Validation 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
Working with Contract Labs

- Percentage of business related to disinfectant effectiveness testing for pharma/med device customers
- Audit the lab. Are SOPs in place regarding culture storage and maintenance, basic laboratory procedures? Employee training documents?
- Ask to observe testing
- Make sure all acceptance criteria is spelled out in the protocol (inoculum concentrations, spore concentrations, preparation of diluents, neutralization and recovery, final log reduction, etc.)
- Policy for repeating studies if acceptance criteria is not met due to lab error
- Understand that not all contract labs have the expertise and some may use temporary employees with little experience to perform the testing
Keys to a successful validation

- Antimicrobial agent
  - Choosing the proper disinfectant for the job

- Testing protocol (practical, achievable & verifiable)
  - Choose the method that best fits your situation

- Sanitization procedures
  - Set up the proper rotation of disinfectants to control all organisms

- Change control
  - Have all processes organized
THANK YOU!

Questions & Answers

marc_rogers@steris.com