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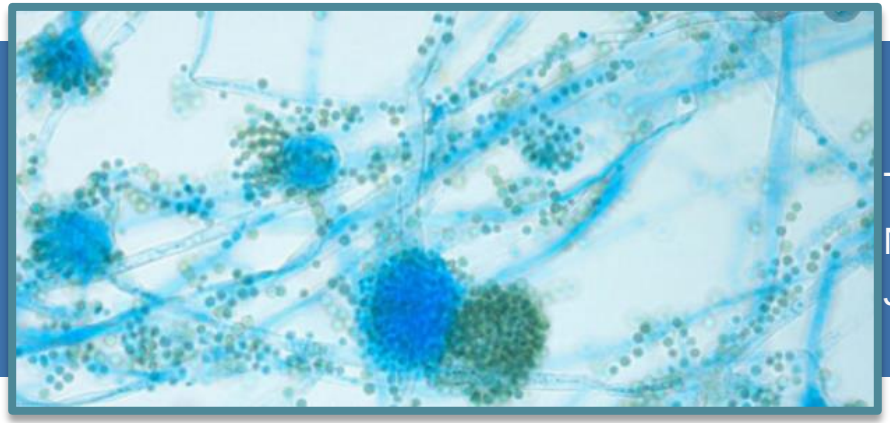
Debates and Challenges Concerning Disinfectant Validation and Methods to Circumvent Them



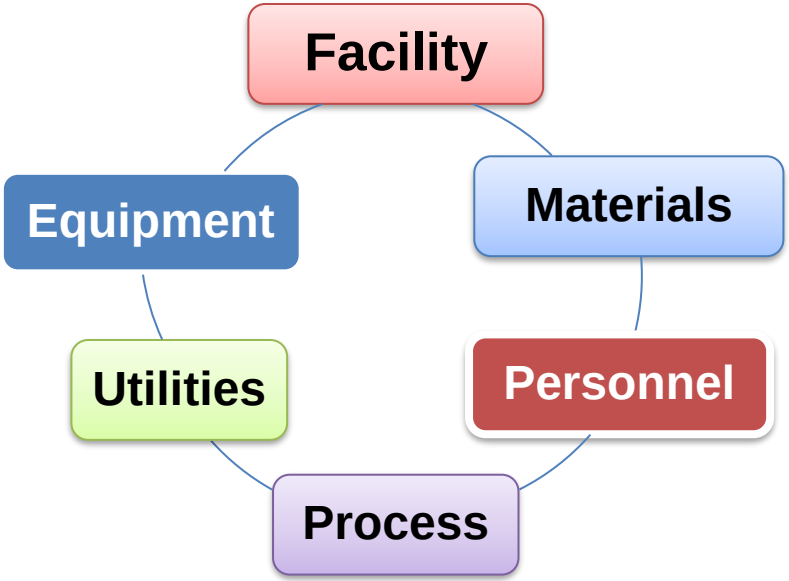
Cleanroom Technology
November 10-11, 2020
Jim Polarine MA.

Biography

Mr. Polarine is a senior technical service manager at STERIS Corporation. He has been with STERIS Corporation for twenty years. His current technical focus is microbial control in cleanrooms and other critical environments. Mr. Polarine is a 2019 PDA Michael S. Korczynski Award recipient. He has lectured in North America, Europe, Middle East, Asia, and Latin America on issues related to cleaning and disinfection, microbial control in cleanrooms and validation of disinfectants. Mr. Polarine is a frequent industry speaker and published several PDA book chapters and articles related to cleaning and disinfection and contamination control. He is active on the PDA's COVID-19 Task Force and the PDA's Microbial Diviations Task Force. He was a co-author on PDA's Technical Report #70 on Cleaning and Disinfection. Mr. Polarine teaches industry regulators as well as the pharmaceutical, biotech, and medical device industries at the PDA and the University of Tennessee. Mr. Polarine currently teaches the cleaning and disinfection course as part of the PDA Aseptic Processing Course and at the University of Tennessee Parenteral Medications Course. Mr. Polarine is current President for the PDA Missouri Valley Chapter and Technical Coordinator for the IEST.

Jim Polarine has a Master's of Arts in Biology from the University of Illinois in Champaign, IL..

Contamination Sources



Disinfectants are a balance

End-User Disinfectant Validation Components

1. *In vitro* testing
   - Suspension testing (also called Time Kill Study)
   - Carrier Testing (also called Coupon Testing)
2. *In situ* testing
3. Environmental monitoring
   - Data trending (6-12 months, reviewed monthly)
   - Identification of organisms (mold, yeast, and bacteria)

Disinfectant Qualification Procedure Recommendations

- USP 43 <1072> Disinfectants and Antiseptics
  - Use-dilution tests
  - Surface Challenge tests
- ASTM E2614-15 Guide for evaluation of Cleanroom Disinfectants
- ISO 14698 (parts1-3)
  - Surface evaluation, focus on cleaning
- PDA TR No. 70 on Cleaning and Disinfection (October, 2015)
- IEST RP-CC-018.5: Cleanroom Cleaning and Sanitization: Operating and Monitoring Procedures

PIC/S & Aseptic Guide References

- PIC / S PI 007-6 - Recommendation on the Validation of Aseptic Processes: *“The effectiveness of disinfectants and the minimum contact time on different surfaces should be validated”*

- FDA Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing, Current Good Manufacturing Practice: *“The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces.”*

In Vitro Options for Testing

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- AOAC
  - Use-dilution Test Methods (955.14, 955.15, 964.02)
  - Sporicidal Activity of Disinfectants (966.04)
  - Germicidal Spray Products as Disinfectants (961.02)
- ASTM
  - Time Kill Method (E2783)
  - Sanitizer method (E1153)
  - Wipe method (E2362)
  - Quantitative Carrier Method (E2111-11 & E2197-17)
  - Biofilm Method (E2871, E2799)
  - Viral Testing (Suspension E1052)
  - Viral Testing (Carrier E1053)
  - Standard Guide for Evaluation of Cleanroom Disinfectants (E2614-15)
- Variations of all of the above

More *In Vitro* Options

- European Norms (EN)
  - 1276 (bacterial suspension test)
  - 1040 (bacterial suspension test)
  - 1650 (fungal suspension test)
  - 13704 (sporicidal suspension test)
  - 13697 (2019 Updated, Carrier test)
  - 14476 (Viral Testing)
  - 14348 (TB Testing)
  - 14885 (2015, Guidance)
  - 16777 (Viral Hard Surface test)
  - 16615 (Wipe Method)

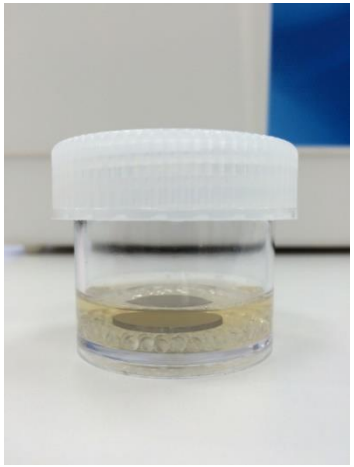
- Association Francaise de Normalisation (AFNOR) (France)
  - NFT 72-150 Suspension
  - NFT 72-190 Carrier Test
- Association for Applied Hygiene (VAH) (Germany, Carrier & Suspension Tests)
- Therapeutic Goods Administration (TGA) (Australia)

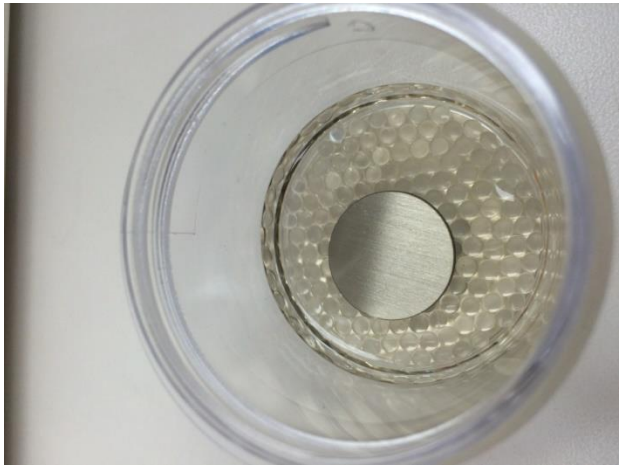
Wipe Methods

- Risk Analysis
  - EN-16615
    - Water Controls showed log reductions
    - Is the reduction from the wiping or the chemistry?
    - Different operators could get different wiping efficacy results.

EN 13697

- Being a prescriptive test method allows for consistency across European facilities





In Vitro Carrier Comparison

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EN 13697

Inoculum

Test Product

In Vitro Carrier Comparison

ASTM E 2197

Inoculum

Test Product

USP 43 <1072> 2”x2” Coupons?

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- USP 43 <1072> does not provide specific guidance on recovery methods
- Established reference methods that specify recovery methods, utilize smaller coupons
- Using larger coupons can negatively impact some recovery methods
- The volume of inoculum and test product used in prescriptive reference methods obviates the need for larger coupons
- USP 43 <1072> is meant to be a “wipe method” Tony Cundell

USP 43 <1072> 2”x2”

- Necessary?

Coupon Size Debate

- USP 43 <1072> Calls for 2” x 2” (5.08 cm x 5.08 cm) coupons-no other operatic details specified
- PDA TR # 70 Calls for 3.8 cm X 3.8 cm
- ASTM E2197-17 Calls for 1 cm disc
- EN 13697 (2019) Calls for 2 cm disc
- Some End Users 28 X 28cm and 5 X 5cm
- Larger coupons can limit possible recovery methods
- Having scientifically sound method, more important than arbitrary size

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 qualifications – representative materials
  - 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
  - Acyrlic and Grout
  - Saniflex
  - Paints (Epoxy and Water Based) & 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, thiosulfate, and sodium sulfite can be toxic
  - If ineffective, contact time is inaccurate

- Validation of neutralization is required

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

Microorganism Selection

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

Bacillus cereus / sphaericus

Bacillus subtilis / G. stearothermophilus

***Clostridium* spp.**

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

Debate Regarding Coupon Testing

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- Pros for not testing
  - Reduce testing and resources costs significantly
  - Have one centralized coupon study as a reference
  - BPOG and PQRI

- Cons for not testing
  - There are in fact more resistant strains of bacterial spores such as *Bacillus cereus* that do not conform

General Efficacy Recommendations

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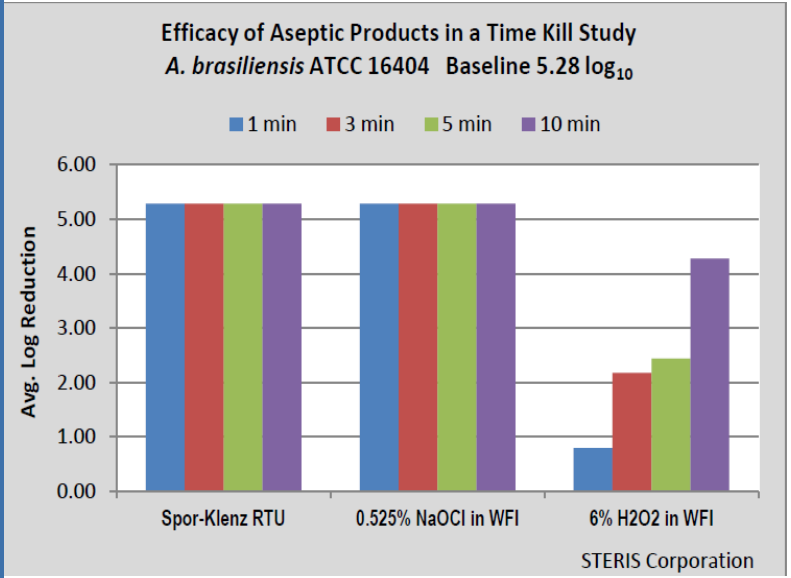
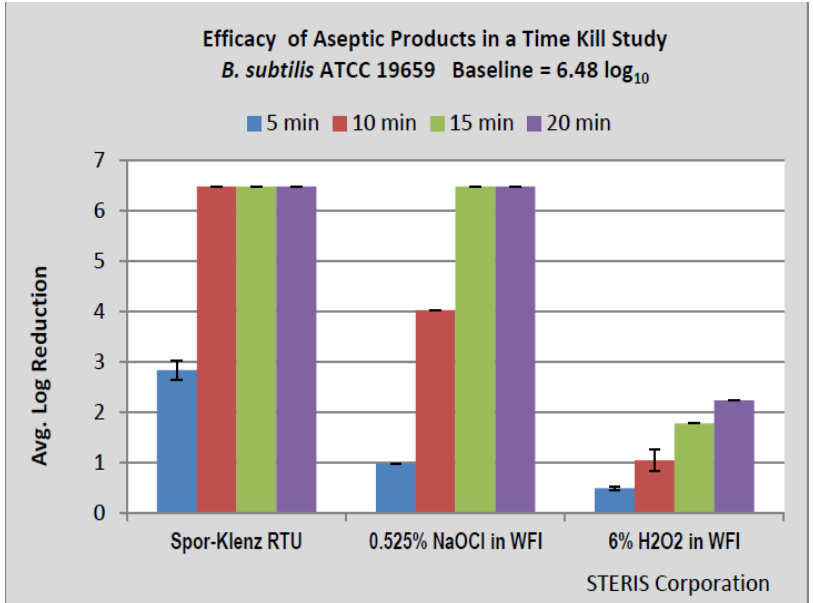
- Suspension acceptance criteria
  - 4-5 log reduction
- Carrier acceptance criteria USP 43 <1072>
  - 2 log reduction bacterial spores
  - 3 log reduction vegetative bacteria
  - PDA TR #70
    - 1-5 min disinfectant and sporicide >1 log reduction
    - 90 sec sanitizer >1 log reduction

PDA TR # 70: Table 5.2.2-1

Antimicrobial Chemical Agent	Organism Type	Suggested Contact Time	Suggested Minimum Reduction
Sanitizer	Non-spore formers	max. 90 sec	>1 Log
Disinfectant/Sporicide	Non-spore formers	1 - 5 min	>1 Log
Disinfectant/Sporicide	Mycoplasma	1 - 5 min	>1 Log
Sporicide	Mold Spores	1 - 5 min	>1 Log
Sporicide	Bacterial Spores	1 - 5 min	>1 Log

Efficacy of Sporicides





Pigeonhole for asking and ranking questions

Most Common Causes for Failures in Efficacy Testing

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General	• Testing biocide against inappropriate microbes • Using inappropriate methods • Inadequate planning • Insufficient contact time
Neutralization	• Inadequate neutralization • Neutralizer toxicity
Inoculum	• Poor viability of inoculum suspensions • Fungal and bacterial spore suspensions prepared incorrectly
Surfaces	• Porous surfaces • Coupons not amenable to steam sterilization • Uneven inoculation or product coverage due to curvature or surface tension
Recovery	• Lethality after drying (e.g. *P. aeruginosa)* • Setting artificially high log reduction targets • Final plates are not countable • Recovery method not validated

In Vitro Testing Considerations Contributors to Test Failures

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- 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

Case Study on Substrates

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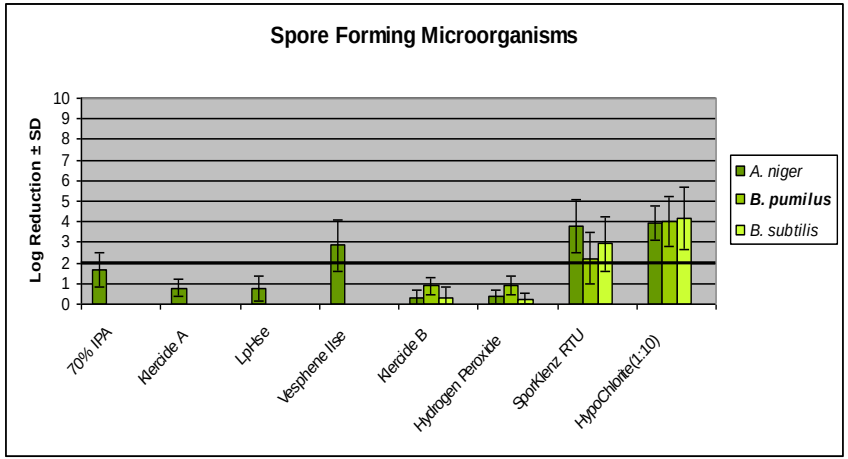
Efficacy (log reduction) of Low pH phenolic: (1:256) against test microorganisms on representative surfaces

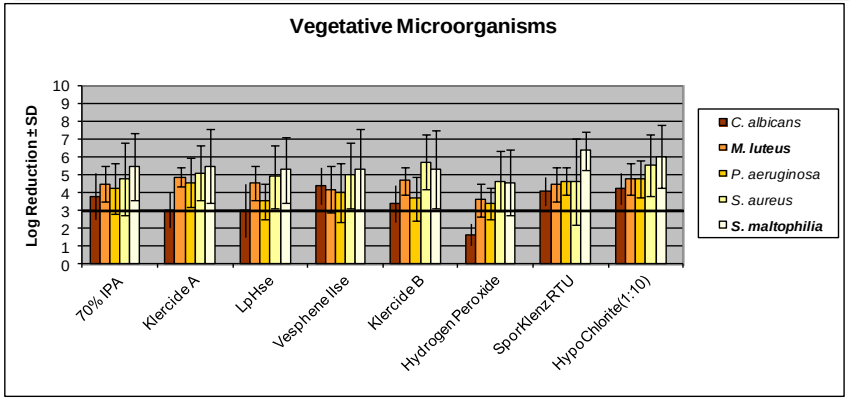
Surface	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*	*Corynebacterium glutamicum*	*Candida albicans*	*Aspergillus brasiliensis*	*Penicillium chrysogenum*
Stainless Steel	6.62	>6.10 [b]	4.18	>4.31 [b]	<3.00 [c]	4.95
Glass	6.85	6.42	5.26	>5.80 [b]	2.98	5.11
Aluminum	6.35	5.69	5.14	>3.93 [b]	<3.00 [c]	3.48
Epoxy	4.36	4.45	4.48	3.19	<3.00 [c]	<3.00 [c]
Enamel	>6.05 [b]	>5.72 [b]	5.45	>3.92 [b]	<3.00 [c]	2.83
Acrylic	4.53	6.06	4.49	2.92	<3.00 [c]	<3.0 [c]
Mipolam	4.36	3.87	4.29	4.37	<3.00 [c]	3.25
Vinyl	4.08	3.68	3.93	2.61	<3.00 [c]	2.1
Hardwood	5.18	>4.54 [b]	5.26	3.2	<3.00 [c]	2.59
Melamine Covered Wood	>5.38 [b]	>5.64 [b]	>5.09 [b]	>5.12 [b]	3.65	3.95
Plastic	>5.73 [b]	>5.32 [b]	>5.05 [b]	>4.04 [b]	<3.00 [c]	2.44
Plexiglas	>5.90 [b]	5.62	4.83	>4.40 [b]	<3.00 [c]	3.85
Chromium	6.55	5.95	6.63	4.08	<3.00 [c]	2.61

[a] Disinfectant Efficacy = (Log $MSP_{(positive\ control)}$ - Log $MSP_{(test\ coupons)}$), where $MSP_{(Positive\ Control)}$= Mean surviving population on positive control coupons; $MSP_{(test\ coupon)}$ = Mean surviving population on test coupons after disinfectant treatment; [b] Each of triplicate coupons showed no growth after disinfectant treatment; [c] Each of triplicate coupons showed TNTC growth

Environmental Isolate Testing



2 Log Reduction Target



3 Log Reduction Target

Neutralizers

PDA TR # 70: Table 5.2.1-1

Antimicrobial Chemical Agent	Neutralizing Agent
Alcohols	Dilution or Polysorbate 80
Sodium Hypochlorite	Sodium Thiosulfate
Quaternary Ammonium Compounds	Polysorbate 80 and Lecithin
Phenolic Compounds	Dilution or Polysorbate 80 and Lecithin
Hydrogen Peroxide/Peracetic Acid and Hydrogen Peroxide	Catalase

Common Chemical Neutralizers

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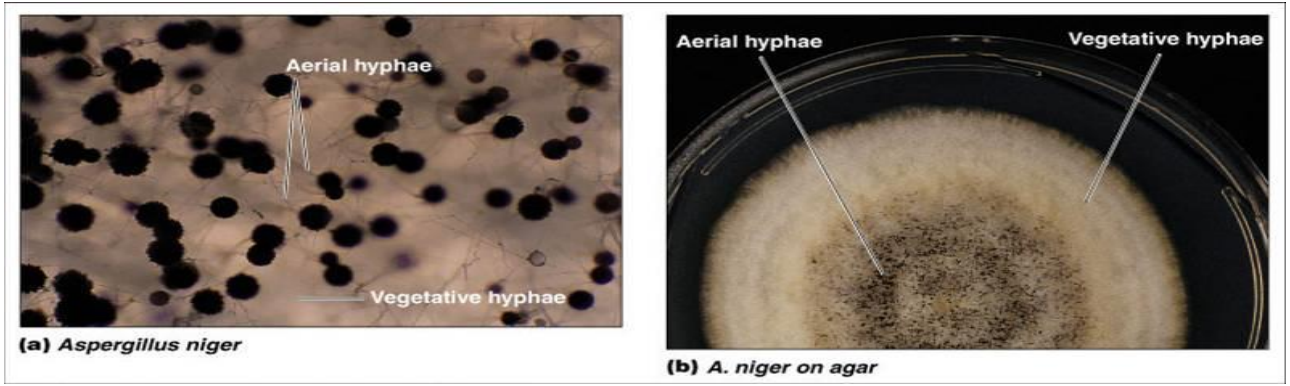
Neutralizer	Biocide Class
Bisulfate	Gluteraldehyde
Catalase	Hydrogen Peroxide
Glycine	Aldehydes
Lecithin	Quats, Phenolics, Bis-biguanides
Letheen	Quats
Mg+2 or Ca+2 ions	EDTA
Polysorbate (Tween)	Quats, Phenolics, Iodine
Sodium Thiosulfate	Sodium Hypochlorite, Iodine

Viability of Inoculum

- Making sure the bacteria at the right phase of growth
- Making sure to isolate the fungal spores with a glass gauze fritted filter or glass wool (testing spores and not mycelia or mycelial mat)
- Checking the viability of the culture and making sure no cross contamination is present

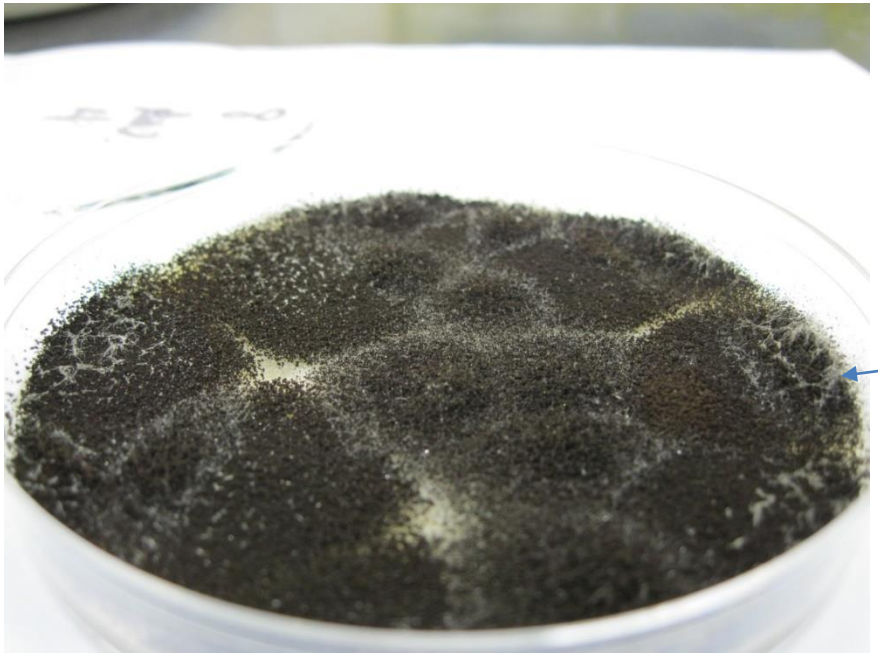
Inoculum Preparation—Fungal Spores

Incubate cultures for a sufficient length of time before harvesting spores



Courtesy Dan Klein

Aspergillus Spores



Cleanroom Fungi

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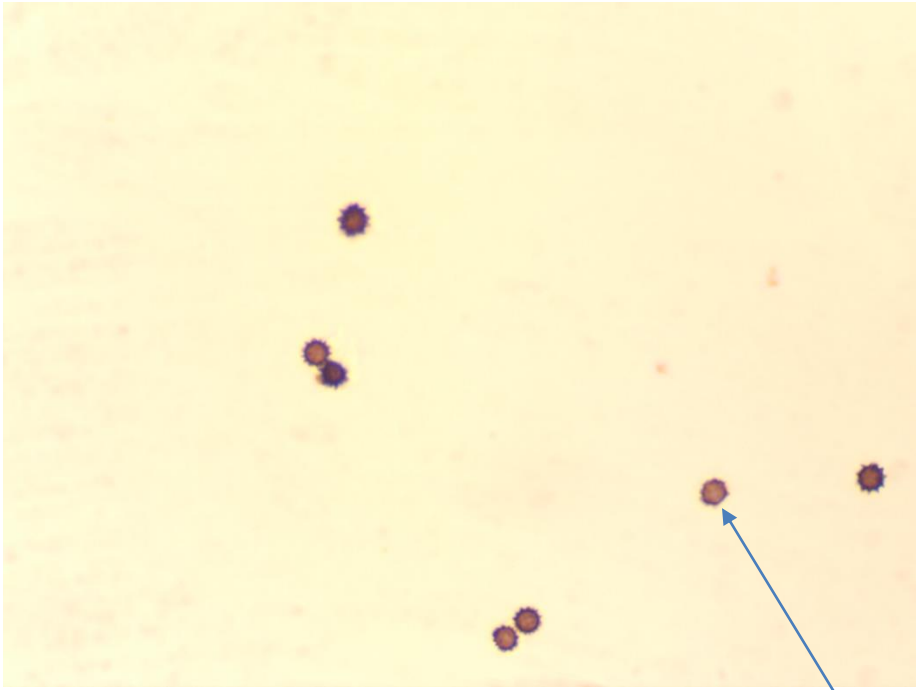
Courtesy Dan Klein

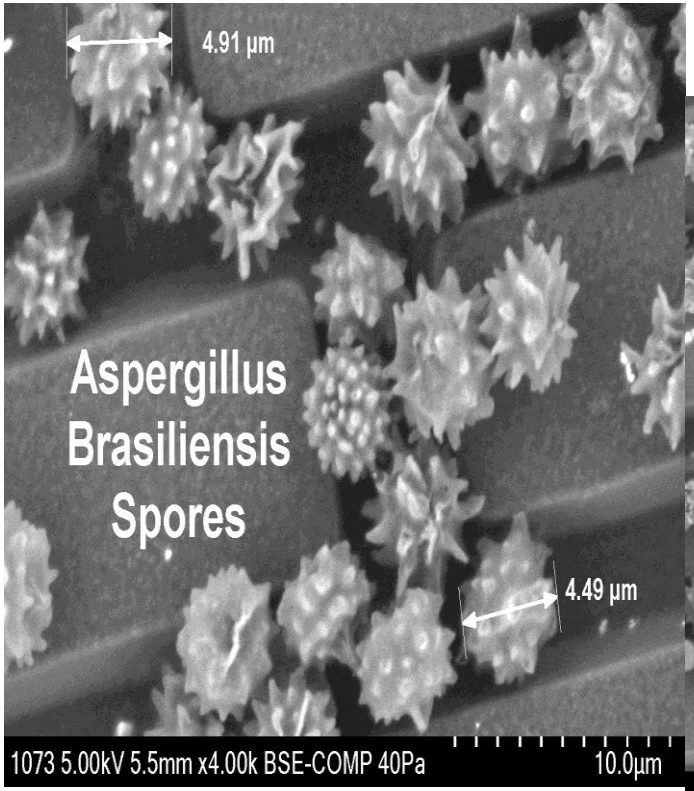
Spiny Spores

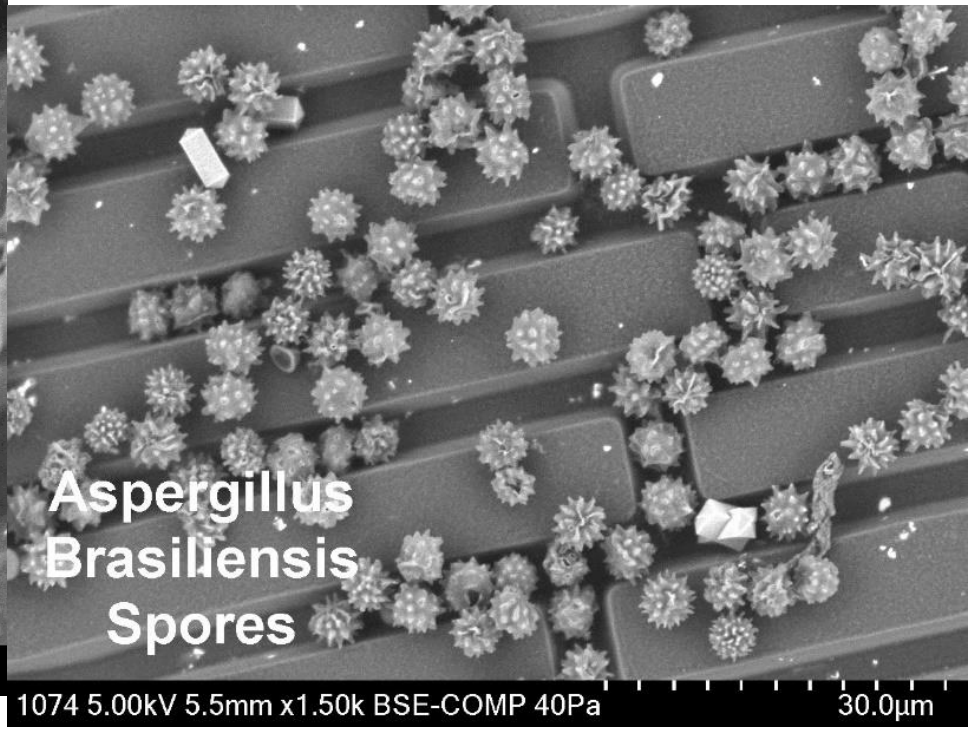


Courtesy Dave Shields

Spiny Spores

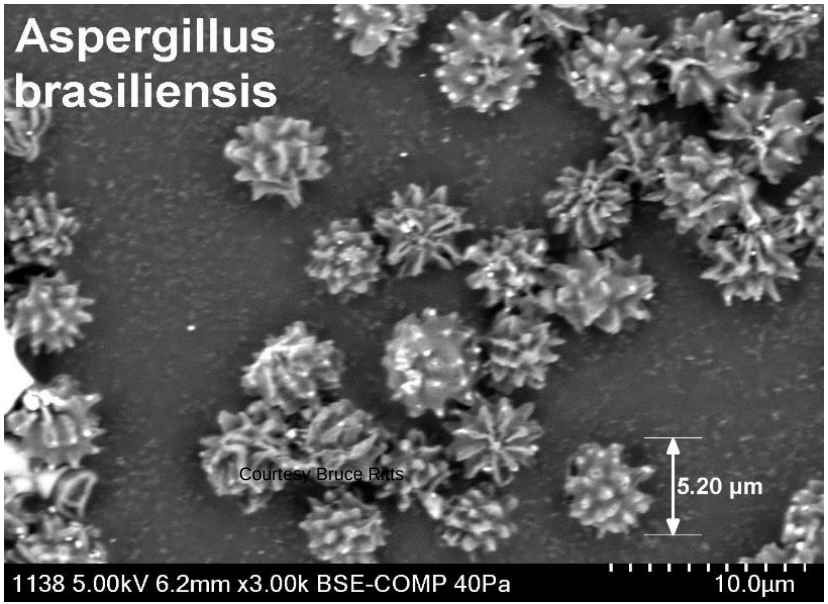
Aspergillus Spores

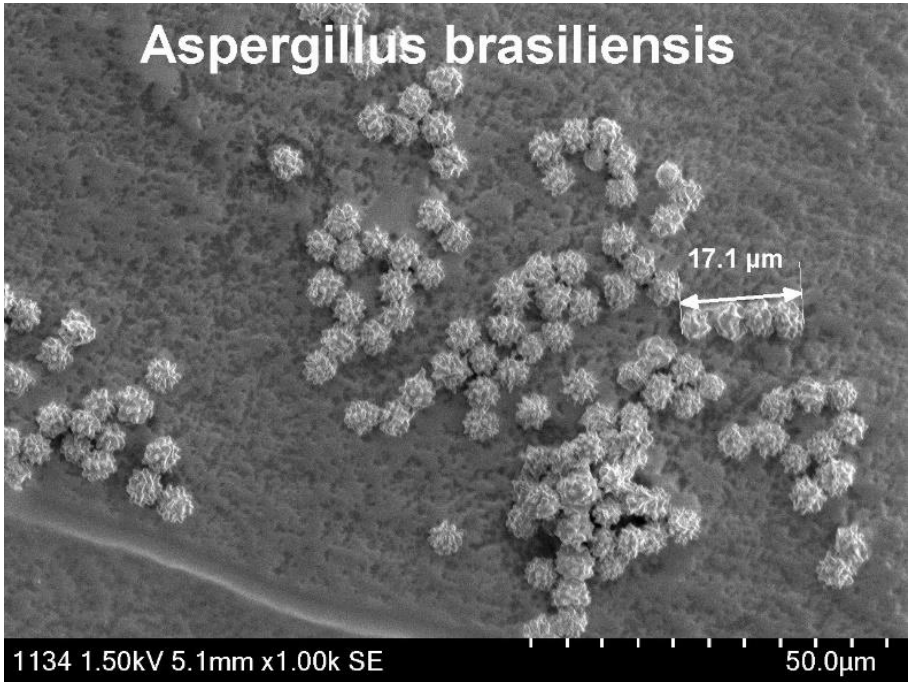




Courtesy Bruce Ritts

Aspergillus brasiliensis





Surface/Coupon Issues

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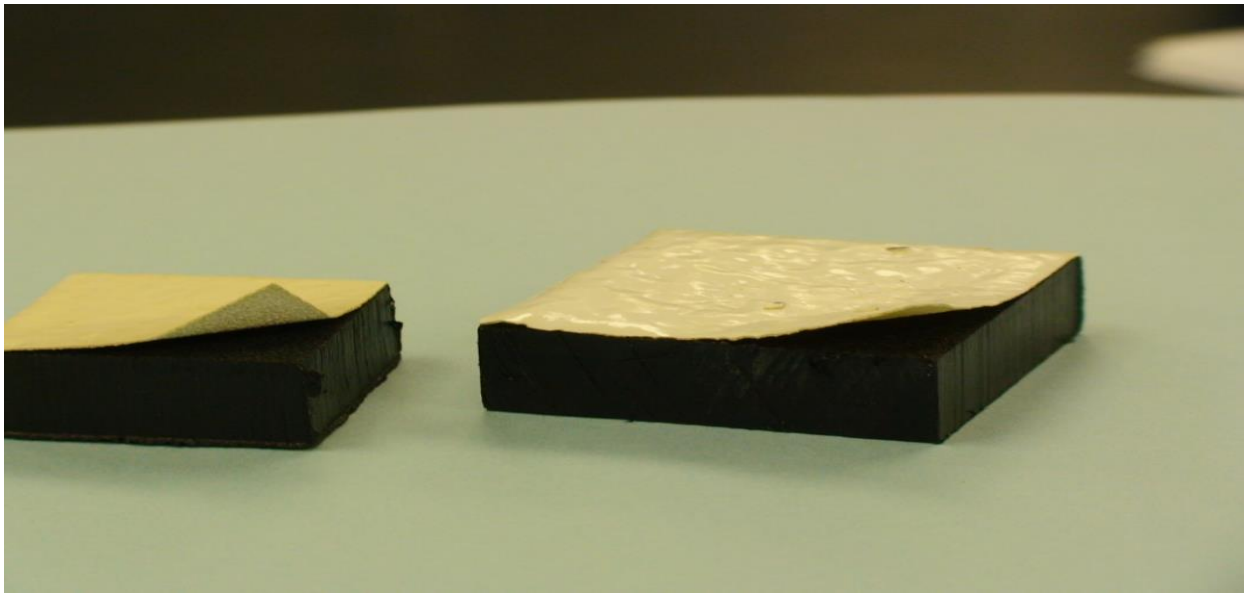
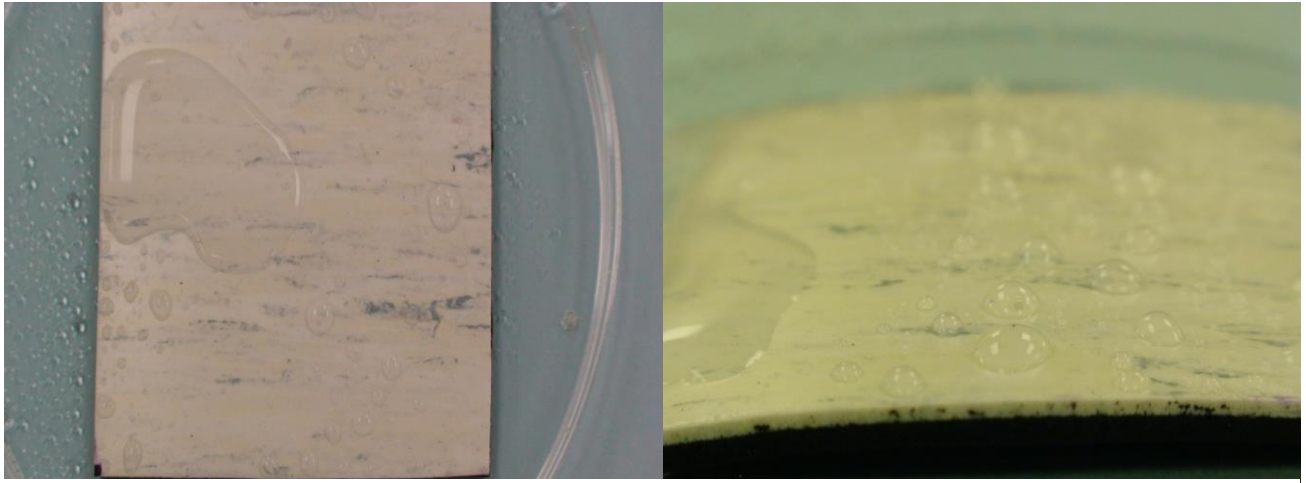
- Surface type and condition can have a huge impact on efficacy
- Preparation of surfaces prior to testing
  - Autoclaving may not be acceptable for some surfaces
  - Residues must be removed
  - No rusting or pitting of surfaces
- Some surfaces pose a challenge during qualification studies:
  - Peeling after sterilization
  - Surface tension (issue on Epoxy, Vinyl, and Terrazzo)
  - Paints and Glove Materials

Surface Preparation

Autoclaving may not be acceptable for some surfaces, gypsum board with paint (Saniflex)

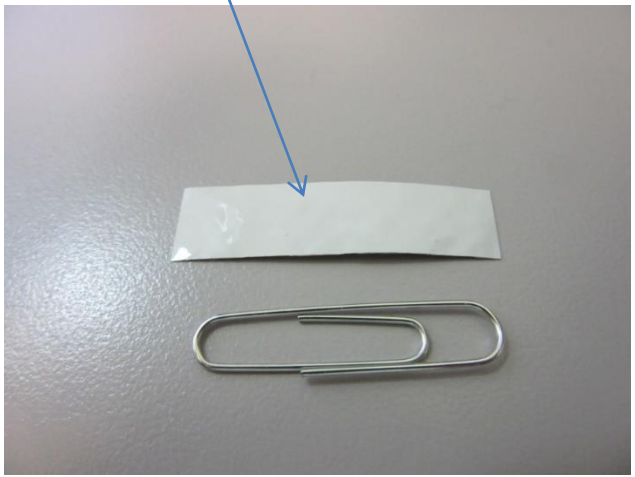


Surface Tension Issue

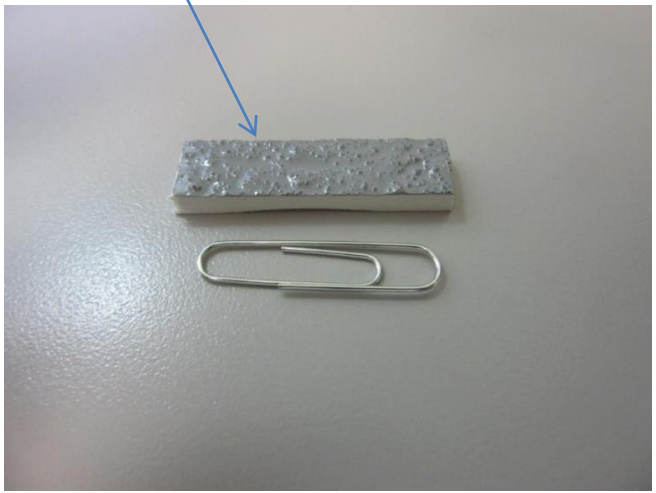


Coupon Issues

Surface Sterilization Issues



Surface Roughness



Surface Creation Issue

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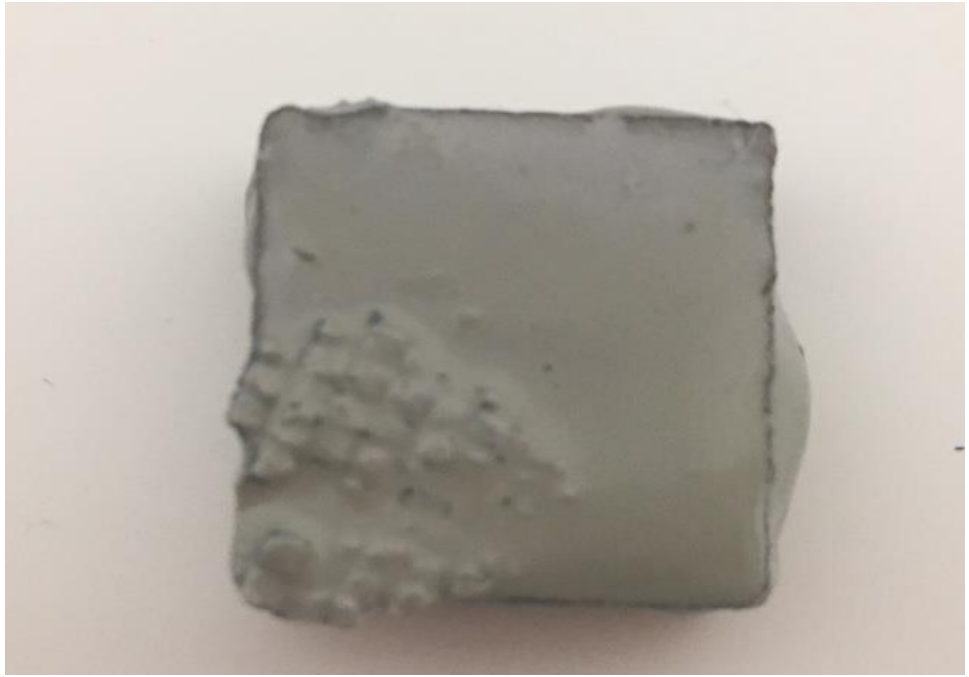
Coupon creation led to unrepresentative texture



Courtesy of Erin Kruesi, STERIS Laboratories

Surface Degradation Issue

Courtesy of Erin Kruesi, STERIS Laboratories

Coupon Epoxy Flooring

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Challenging Due to Surface Grit and Surface Finish

Courtesy of Erin Kruesi, AST Laboratories

Recovery Method Issues

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- 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 (Food Industry)
- Recovery method must be validated/verified
- Sonication, vortexing, and glass beads.
- Final plates must be countable to calculate log reduction

Disinfectant Qualification Study Tips

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- AOAC methods are inappropriate for this testing (but some procedures such as inoculum prep, spore prep etc. can be of value)
- EN-13697 (2019) and ASTM E2197-17 offer valuable insight into quantitative surface testing
- PDA TR #70 (2015) is useful in determining log reductions
- Up-front proactive 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
- Auditing the contract lab is very useful

Keys to a Successful Qualification

- Effective Antimicrobial agents
- Effective and repeatable testing protocol
- Effective sanitization procedures
- Effective change control procedures

Requalification

- Review annually to assess risk/ whether changes have occurred
- If new bioburden appears at high levels or inherently resistant organisms
- Re-evaluate every five to seven years to determine if any repeat testing is needed due to testing deficiencies

Summary Slide

- Current Industry Best Practice in Disinfectant Validation

Industry References

- **USP 43 <1072> Disinfectants and Antiseptics**
- **Draft Annex 1 v. 12 (Draft 2020) and MHRA Orange Guide (2017)**
- **FDA Aseptic Processing Guide (2004)**
- **FDA, MHRA, HPRA, CFDA, ANSM, ANVISA, FDAHA, ANMAT, 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 2017)**
- **USP 43 <1116> Microbiological Control and Monitoring of Aseptic Processing Environments**
- **USP 43 <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**
- **PIC/S**
- **Japanese and Chinese Pharmacopoeias**
- **PHSS Technical Monograph #20 "Bio-contamination characterization, control, monitoring and deviation management in controlled/GMP classified areas**
- **IEST-RP-CC018.5 Cleanroom Housekeeping: Operating & Monitoring Procedures (2020)**

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STERIS Technical Services

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