Debates and Challenges Concerning Disinfectant Validation and Methods to Circumvent Them

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PDA/ISPE Australia
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Agenda

- Disinfectant Validation Practices

End-User Disinfectant Validation Components

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- *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)

Disinfectant Qualification Procedure Recommendations

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- USP 42 <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)

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
- ASTM
  - Time Kill Method
  - Spray Slide
  - Sanitizer method (E1153)
  - Wipe method
  - **Quantitative Carrier Method (E2111-11 & E2197-11)**
  - Biofilm Method (E1427)
  - Viral Testing (Suspension E1052-11)
  - Viral Testing (Carrier E1053-11)
  - Standard Guide for Evaluation of Cleanroom Disinfectants (E2614-15)
- Variations of all of the above

More In Vitro Options

- EN
  - 1276 (bacterial suspension test)
  - 1040 (bacterial suspension test)
  - 1650 (fungal suspension test)
  - 13704 (sporicidal suspension test)
  - **13697 (Carrier test)-Revised 2015**
  - 14476 (Viral Testing)
  - 14348 (TB Testing)
  - 14885:2015
  - 16777 (Viral Hard Surface test)

- AFNOR (France)
  - NFT 72-150 Suspension
  - NFT 72-190 Carrier Test
- VAH (DGHM) (Germany, Carrier & Suspension Tests)
- TGA (Australia)

EN 13697: 2015 Revisions

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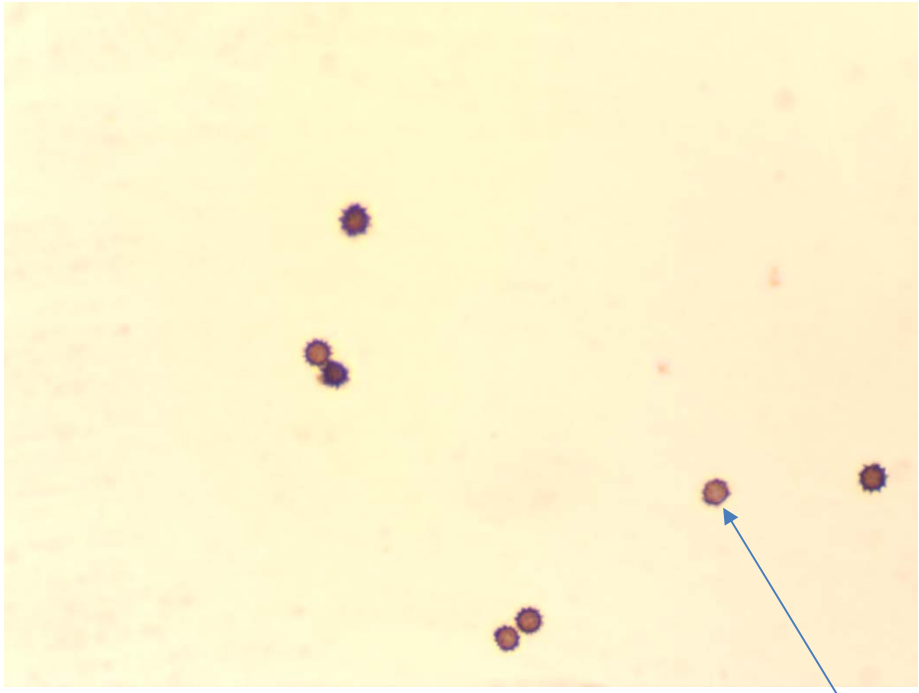
- Some of the key revisions include:
  - Obligatory soiling conditions for *P. aeruginosa* changed from bovine albumin to skimmed milk
  - An evaluation for 75% mature (spiny) *A. brasiliensis* spores prior to testing
  - Nearly all method verification acceptance criteria modified (now including a neutralizer toxicity evaluation)
  - Calculation of weighted mean for counts

Spiny Spores

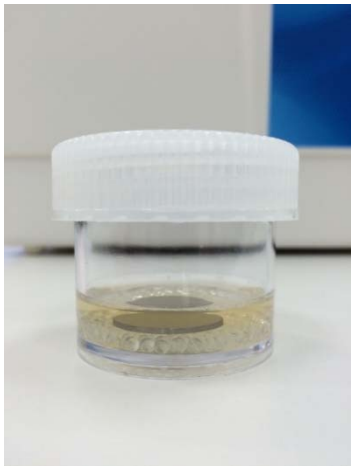


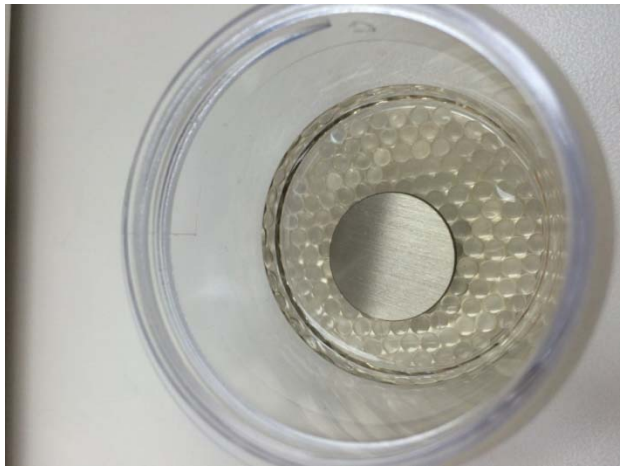
Courtesy Dave Shields

Spiny Spores

EN 13697

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





In Vitro Carrier Comparison

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EN 13697

Inoculum

Test Product

In Vitro Carrier Comparison

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ASTM E 2197

Inoculum

Test Product

USP 42 <1072> 2”x2” Coupons?

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- USP 42 <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 <1072> was really meant to be a “wipe method” Tony Cundell

USP 42 <1072> 2”x2”

- Necessary?

Coupon Size Debate

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- USP 42 <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 E2197-11 Calls for 1 cm disc
- EN 13697 (2015) 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

Key Considerations for In Vitro Testing

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- 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

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- 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

Suspension Testing

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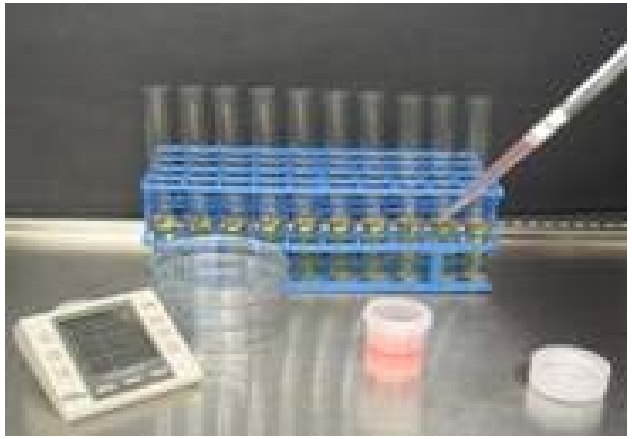
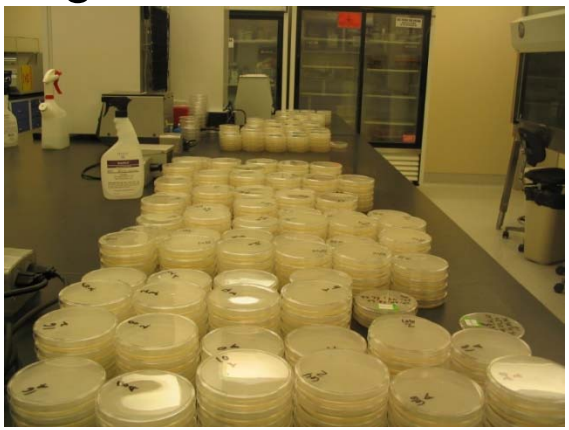
- Often called “Time Kill” study
- Estimates the *in vitro* activity of the biocide
- Often used for preliminary evaluation of several different biocides
- Not required, but useful screening tool





Carrier Testing

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- Simulates practical conditions of disinfectant use and application
- Test organisms are dried on coupons made of varied substrates
- End-user required to perform carrier tests to qualify disinfectants

Neutralization Methods

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- 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

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- 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 42 <1072>
  - 2 log reduction bacterial spores
  - 3 log reduction vegetative bacteria
  - PDA TR #70
    - 1-5min disinfectant and sporicide >1 log reduction
    - 90sec sanitizer >1 log reduction

PDA TR # 70: Table 5.2.2-1

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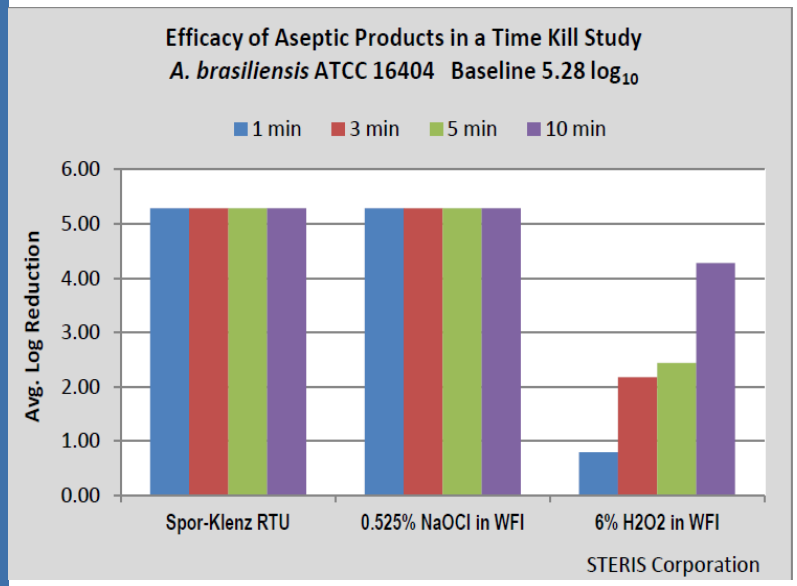
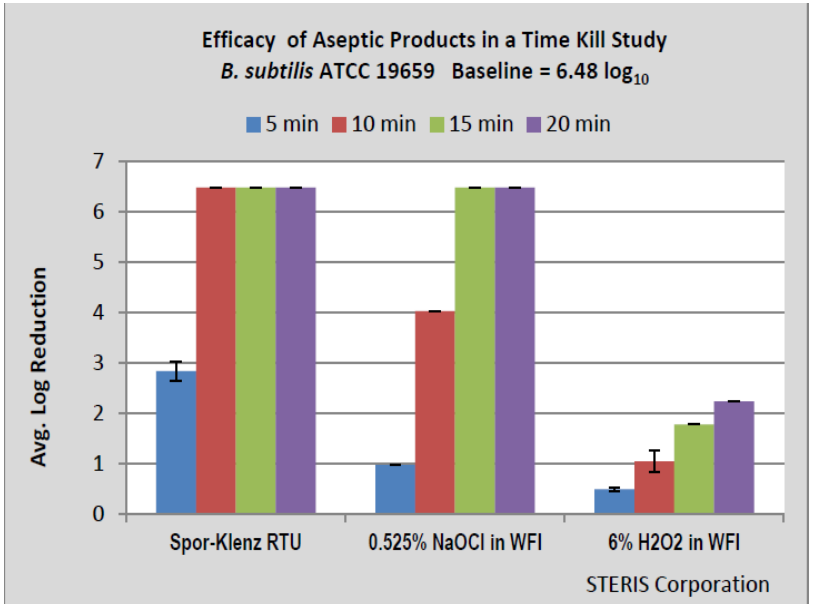
Antimicrobial Chemical Agent	Organism Type	Suggested Contact Time	Suggested Minimum Reduction
Sanitizer	Non-spore formers	max. 90 sec	>1 Log
Disinfectant/Sporicide	Non-spore formers	1 - 5 min	>1 Log
Disinfectant/Sporicide	Mycoplasma	1 - 5 min	>1 Log
Sporicide	Mold Spores	1 - 5 min	>1 Log
Sporicide	Bacterial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

Efficacy of Sporicides





In Vitro Testing Considerations Contributors to Test Failures

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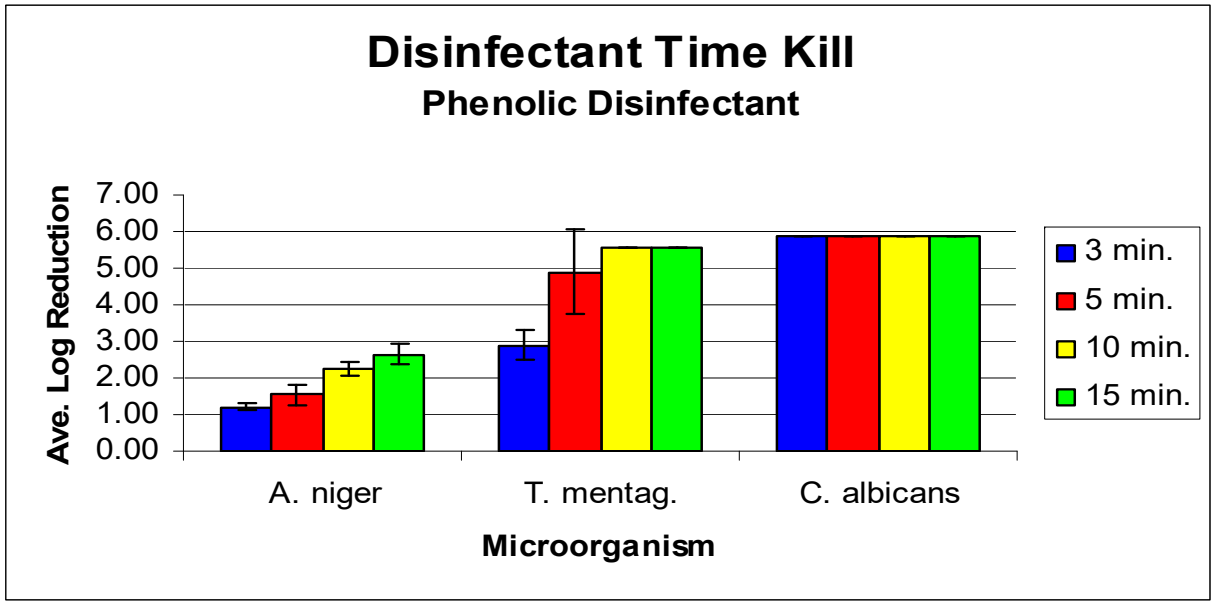
- Recovery issues post-drying (*P. aeruginosa*)
- Inoculum prep (e.g. fungal spores)
- Coupon prep (autoclaving – peeling Saniflex)
- Improper dilution of Concentrate
- Inappropriate biocide for spores
- Insufficient contact time – should match SOP
- US vs. EU requirements

Testing Against Fungal Spores

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



Case Study on Substrates

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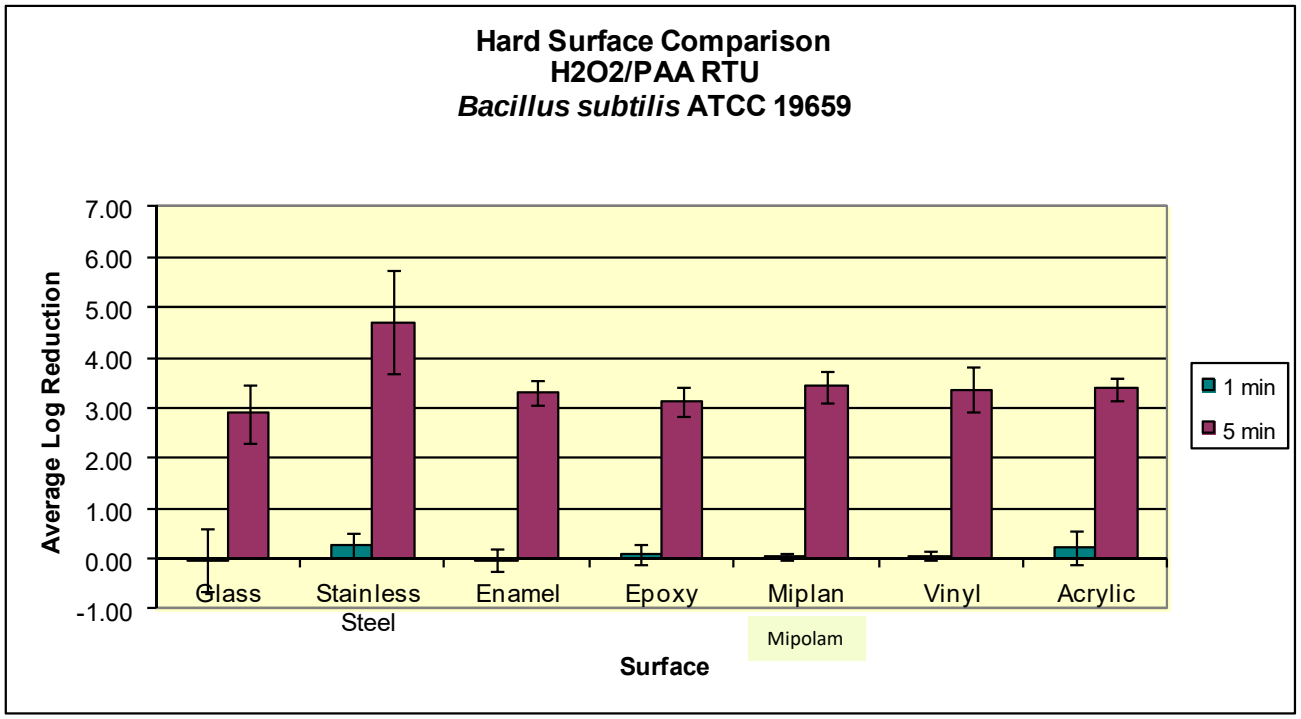
Efficacy (log reduction) of Low pH phenolic: (1:256) against test microorganisms on representative surfaces

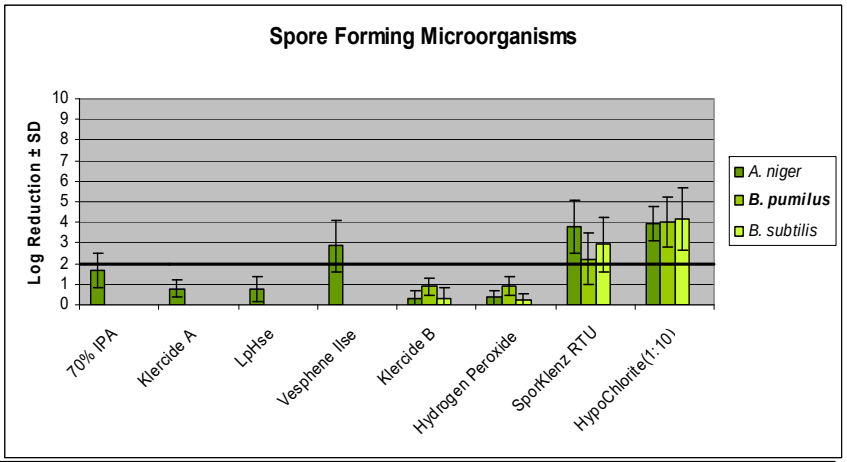
Surface	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*	*Corynebacterium glutamicum*	*Candida albicans*	*Aspergillus brasiliensis*	*Penicillium chrysogenum*
Stainless Steel	6.62	>6.10 [b]	4.18	>4.31 [b]	<3.00 [c]	4.95
Glass	6.85	6.42	5.26	>5.80 [b]	2.98	5.11
Aluminum	6.35	5.69	5.14	>3.93 [b]	<3.00 [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

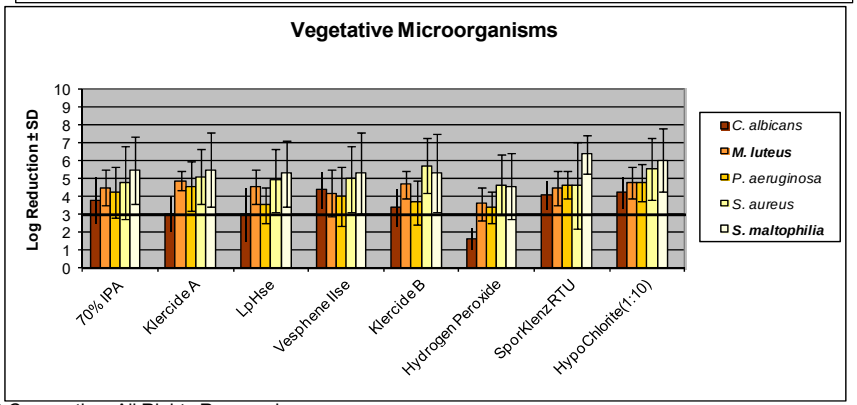
Hard Surface Test Results



Environmental Isolate Testing



2 Log Reduction Target



3 Log Reduction Target

Most Common Causes for Failures in Efficacy Testing

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

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

Neutralizing Broths

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

Sutton, SW et al. 2002. Validation of Microbial Recovery From Disinfectants. PDA J Pharma. Sci. Technol. 56(5):255-266.

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

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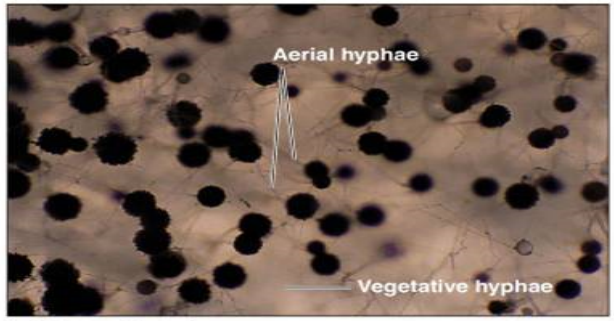
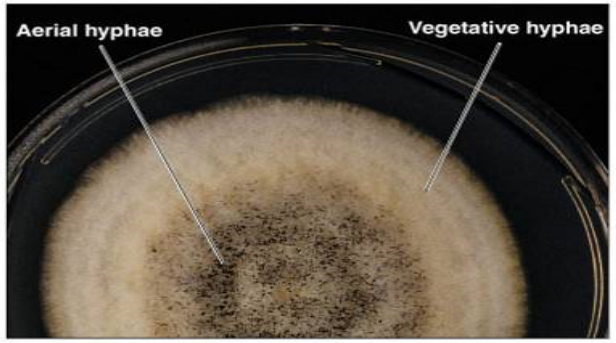
Incubate cultures for a sufficient length of time before harvesting spores



(a) *Aspergillus niger*



(b) *A. niger on agar*

Courtesy Dan Klein

Aspergillus Spores

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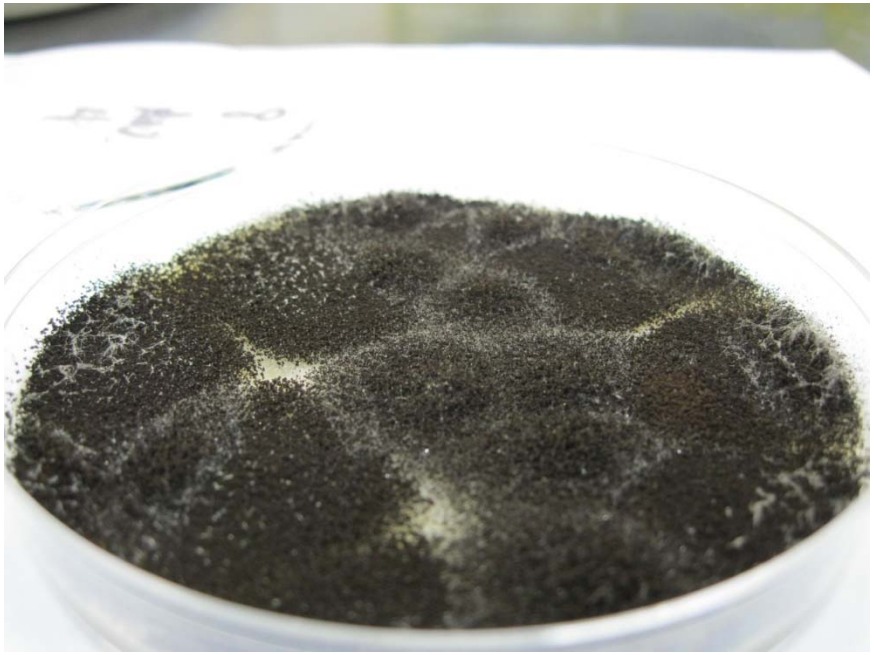

Cleanroom Fungi

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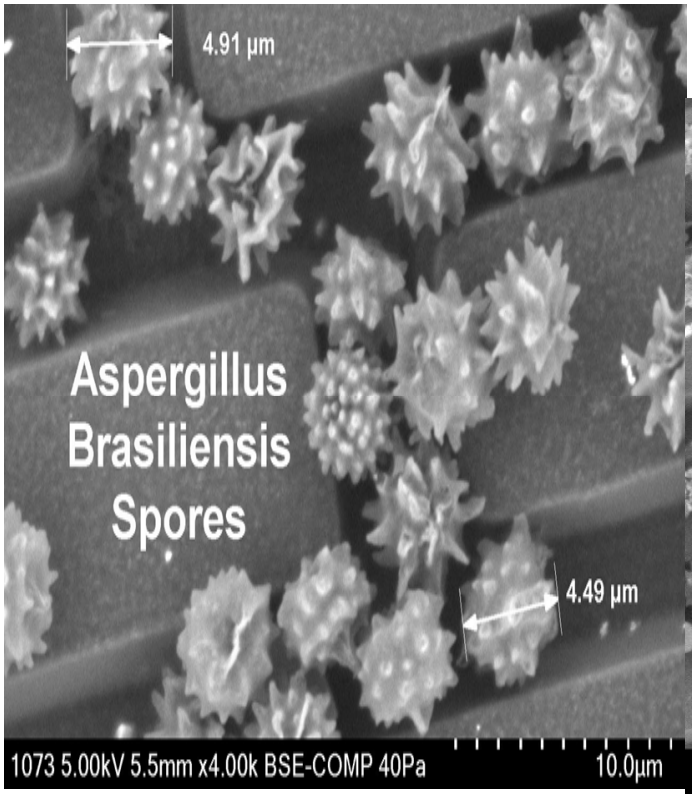
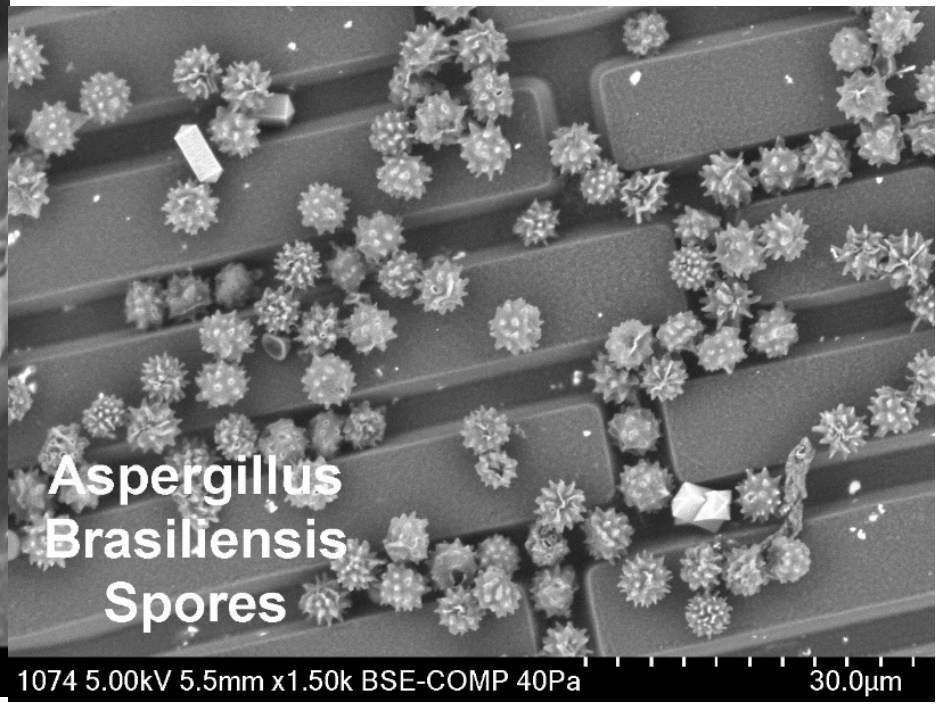
Courtesy Dan Klein

Aspergillus Spores





Courtesy Bruce Ritts

Aspergillus brasiliensis

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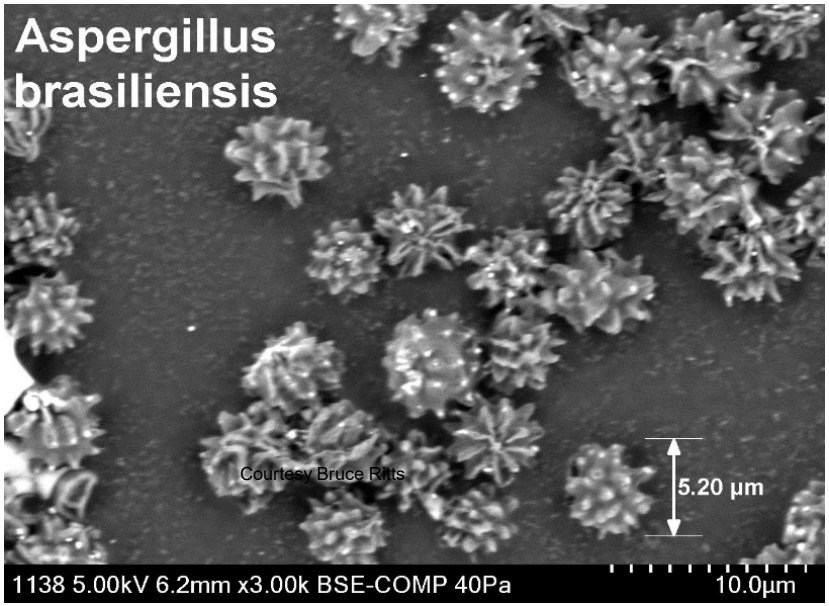

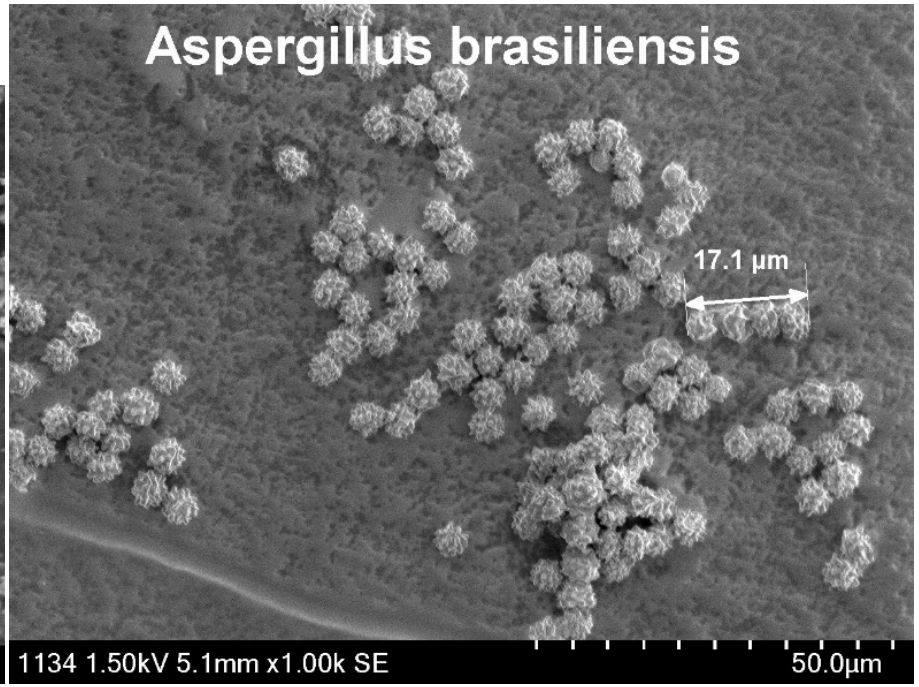

Penicillium Spores

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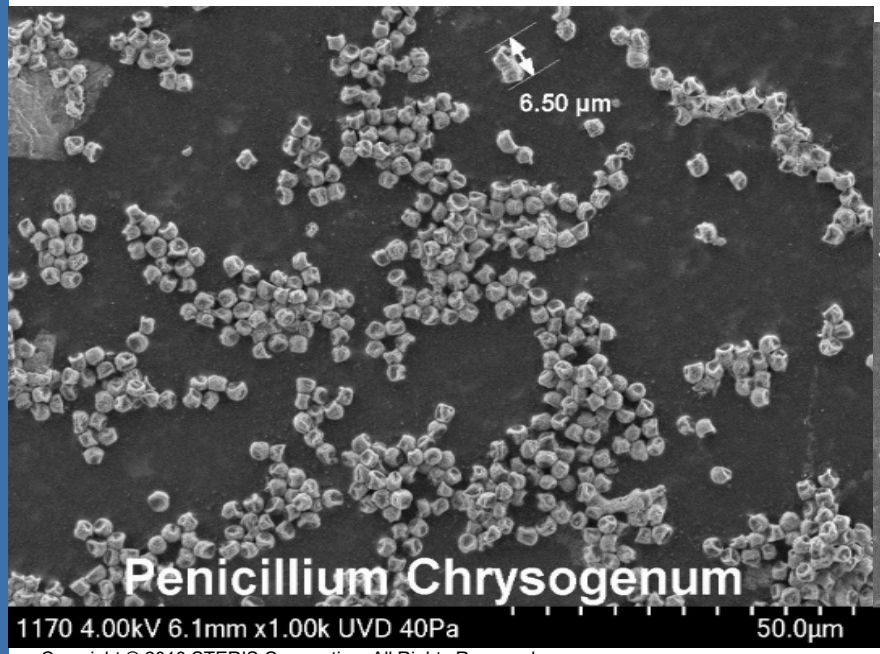
Courtesy Bruce Ritts



Cladosporium Spores

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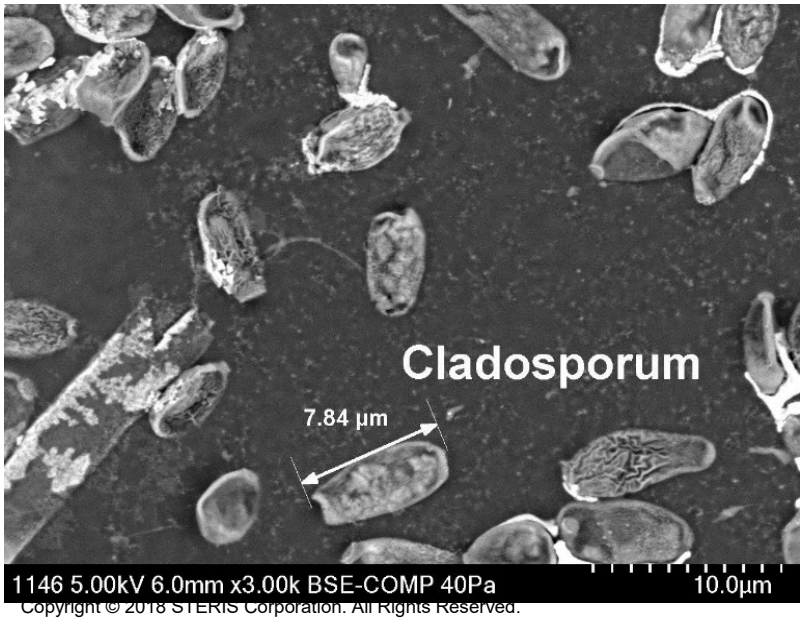
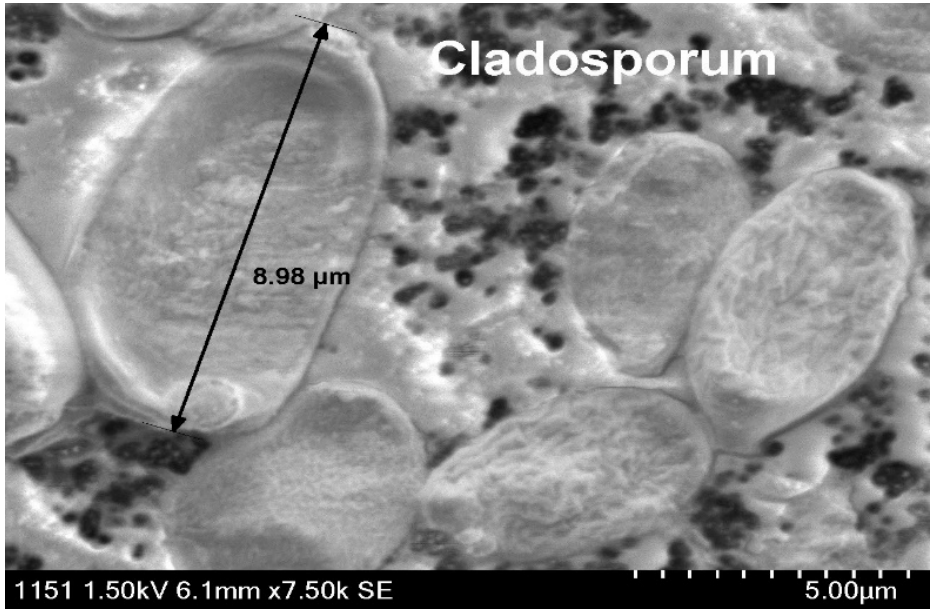
Courtesy Bruce Ritts





Paecilomyces Spores

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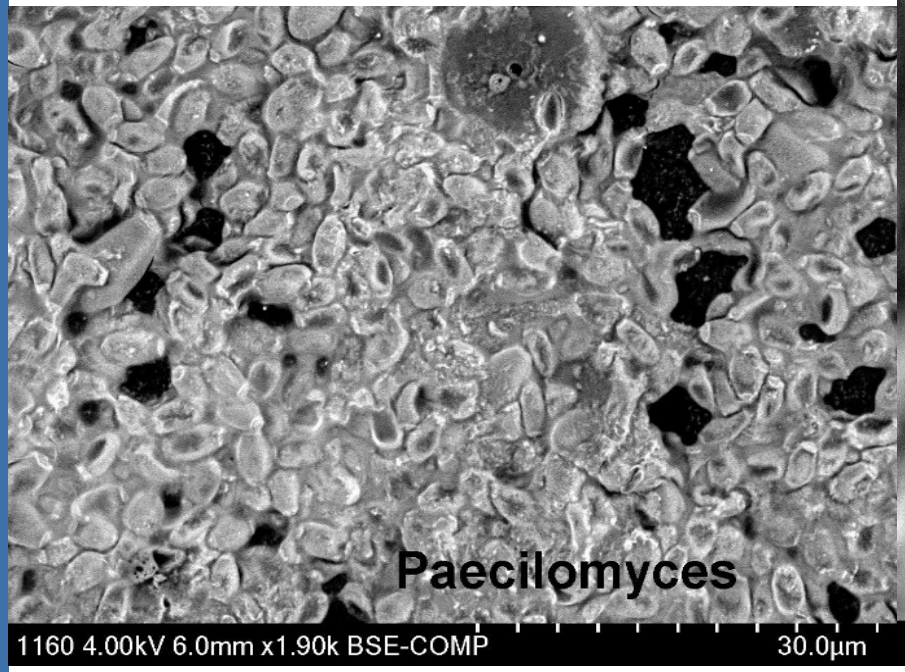
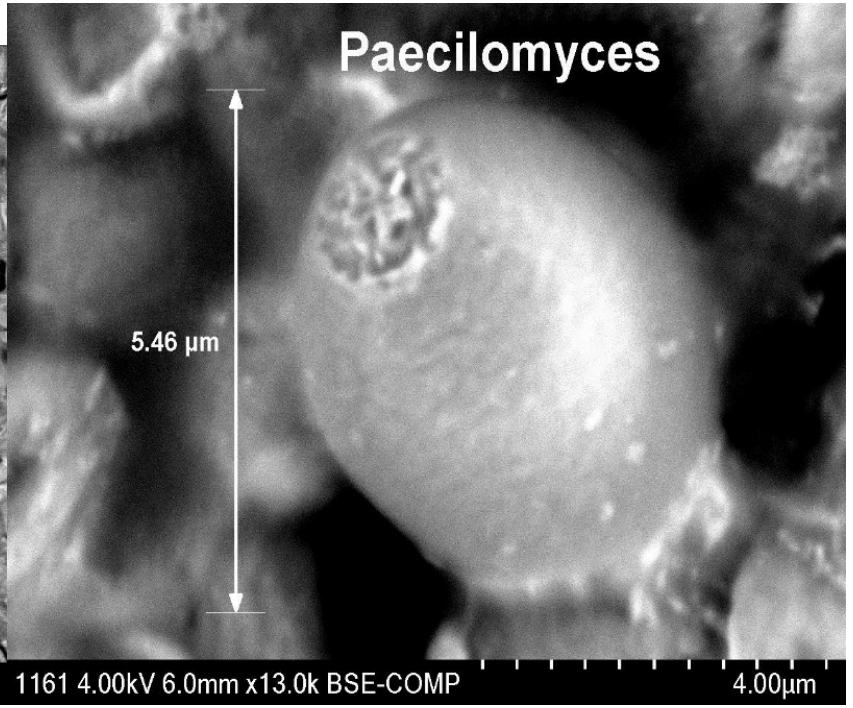
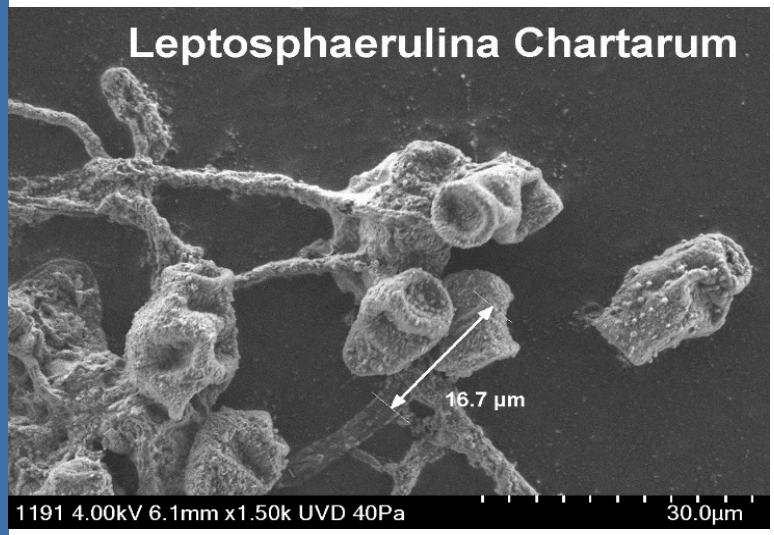
Courtesy Bruce Ritts

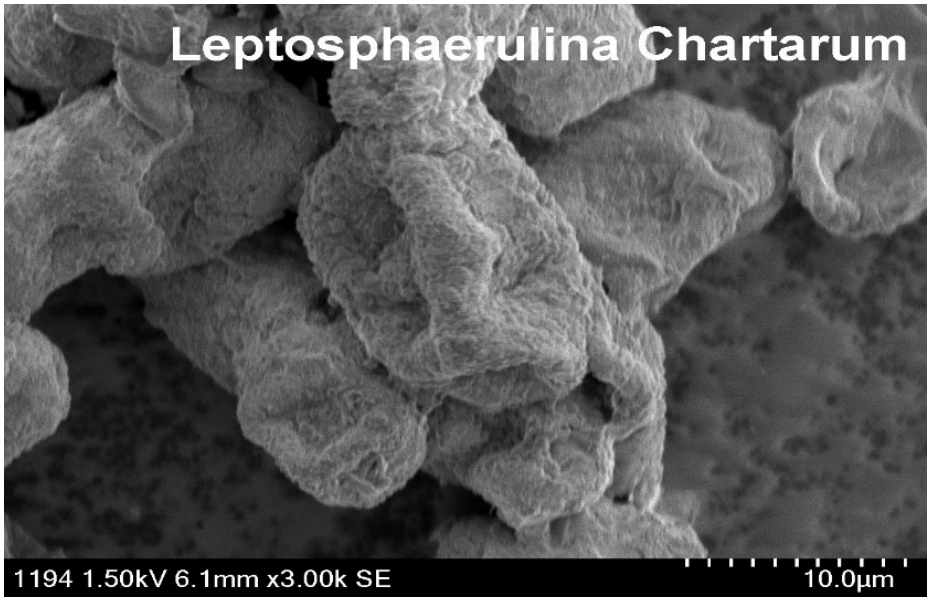




Leptosphaerulina Spores

Courtesy Bruce Ritts





SEM: Pseudomonas 5,000X magnification

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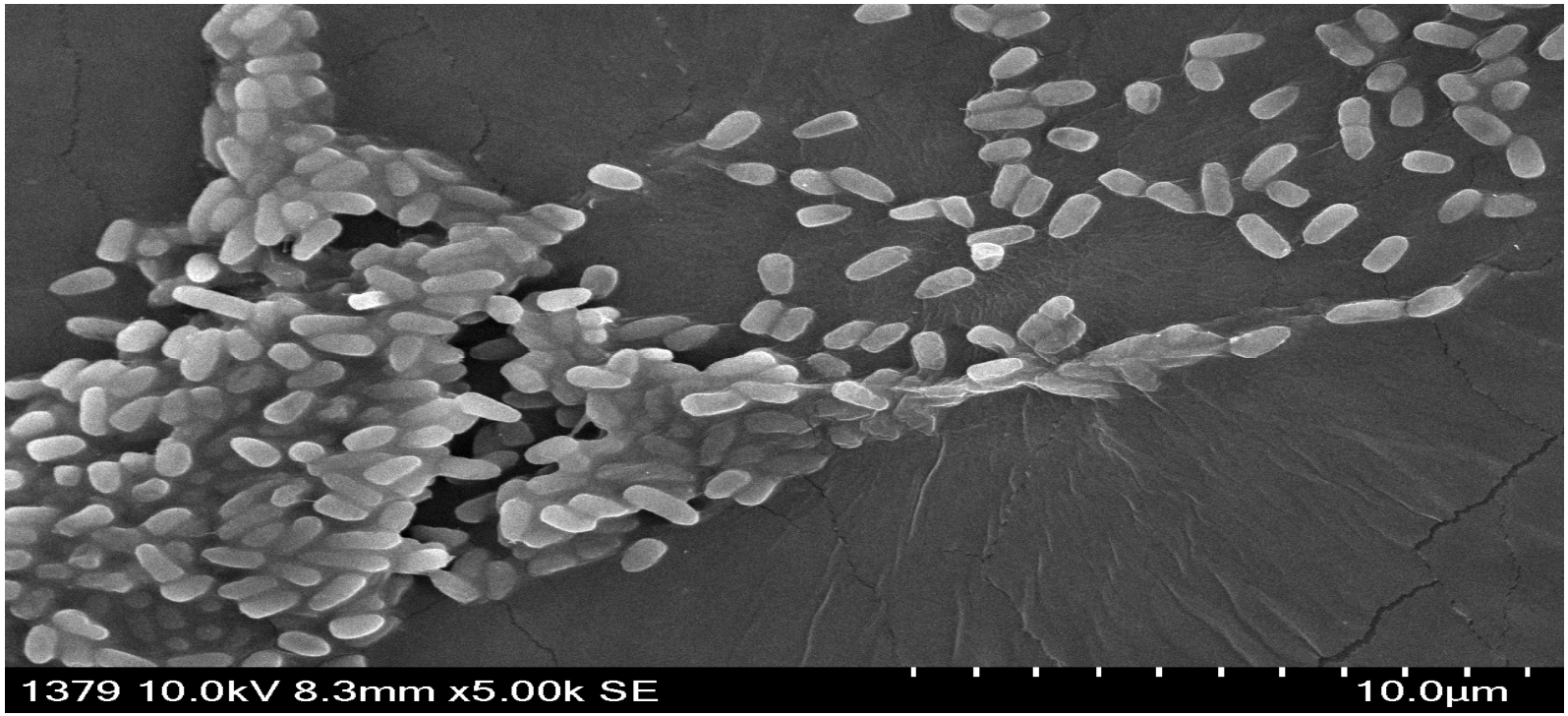
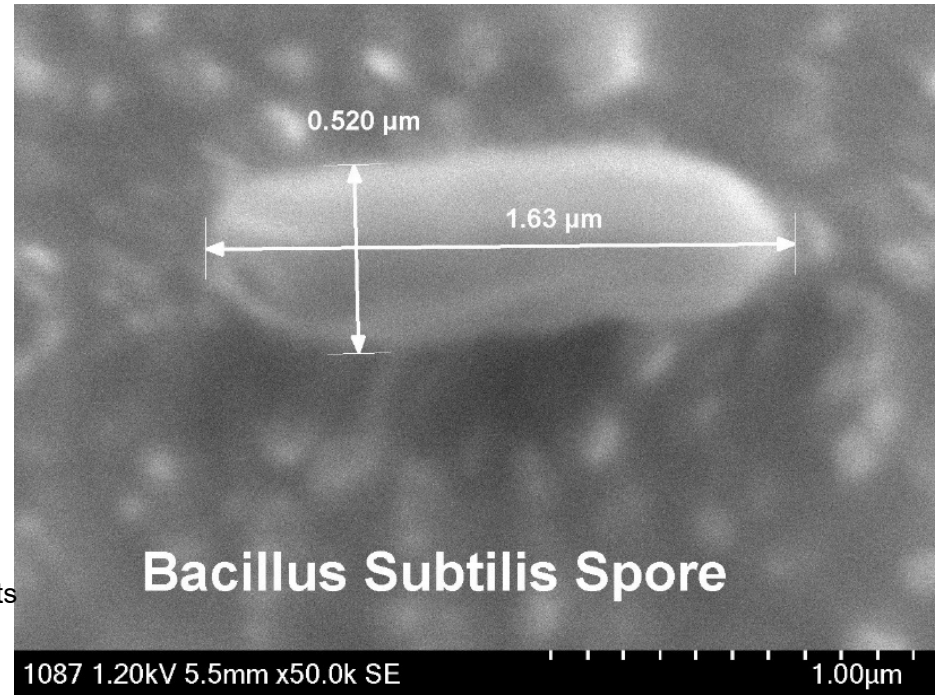


Bacillus Subtilis

Courtesy Bruce Ritts



Surface/Coupon Issues

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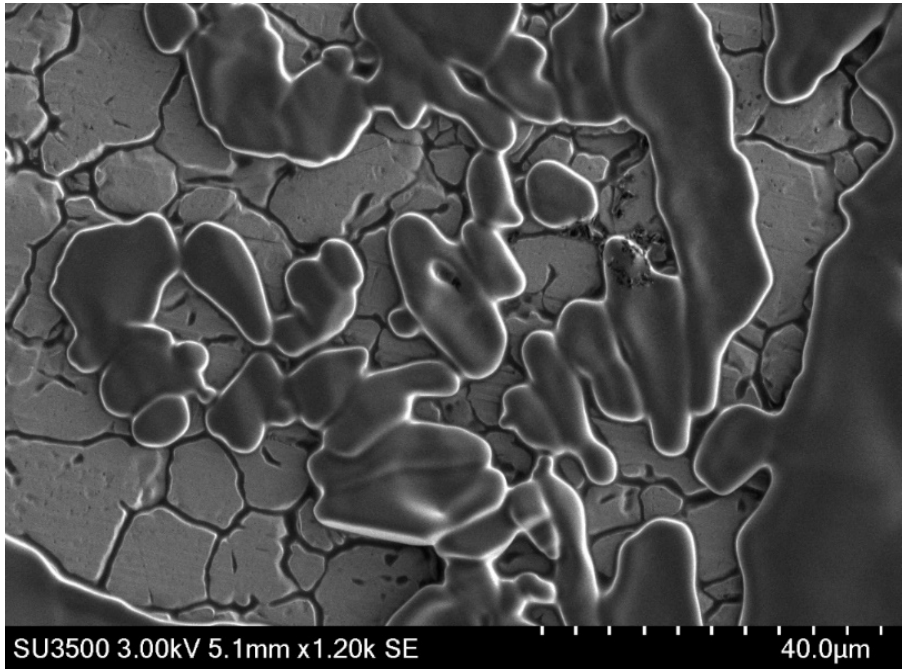
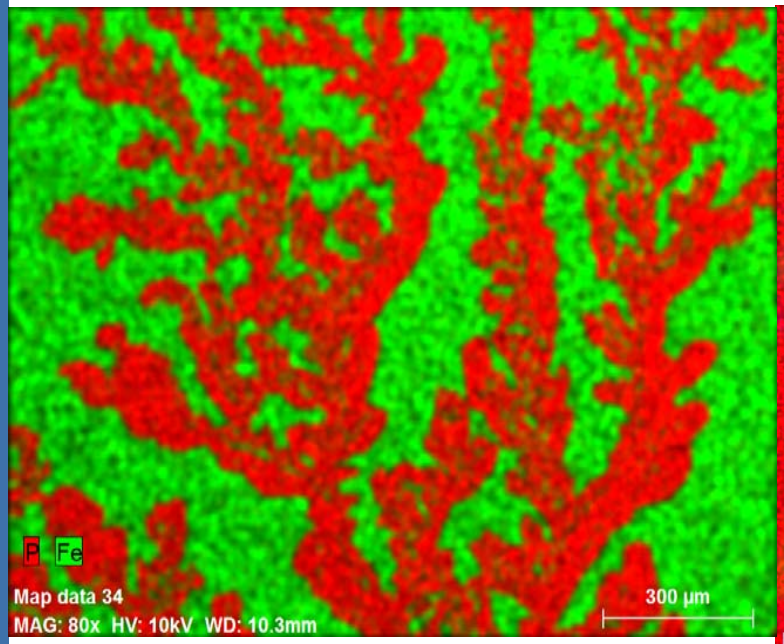
- Surface type and condition can have a huge impact on efficacy
- Preparation of surfaces prior to testing
  - Autoclaving may not be acceptable for some surfaces
  - Residues must be removed
  - No rusting or pitting of surfaces
- Some surfaces pose a challenge during qualification studies:
  - Peeling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

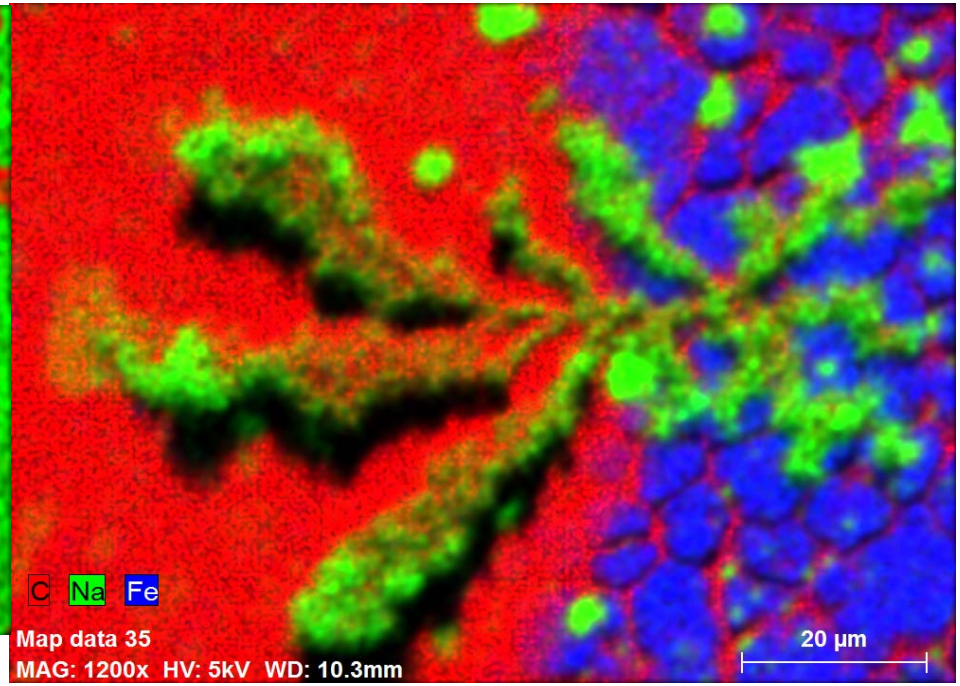
Surface Type and Condition

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



Residue Analysis: SEM





Courtesy Bruce Ritts

Surface Conditions Effect Performance

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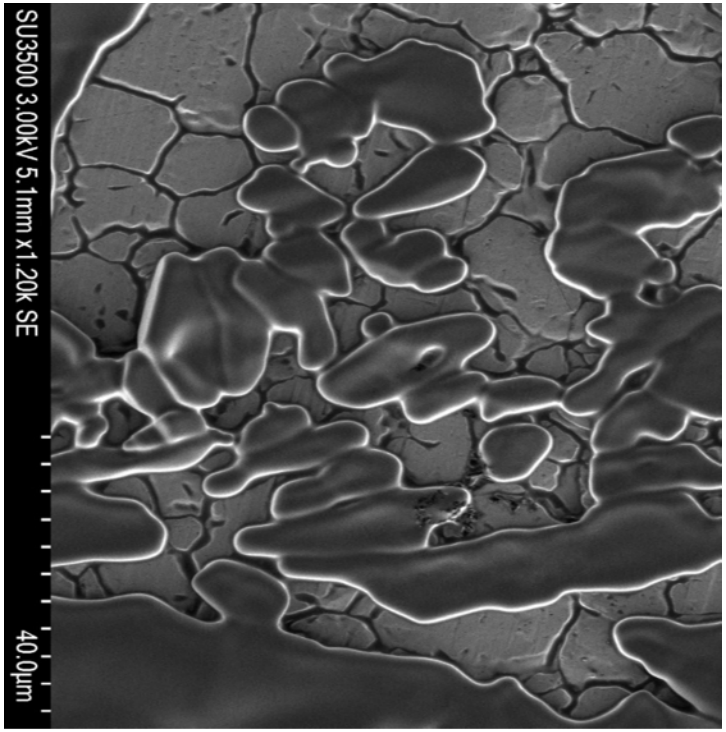
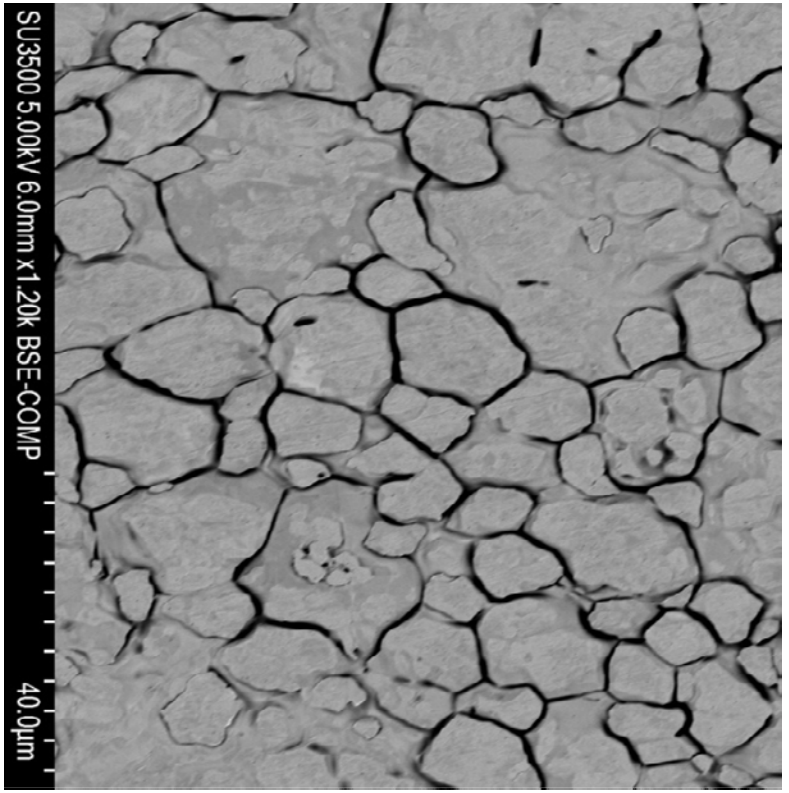


Courtesy Bruce Ritts



Surface Preparation

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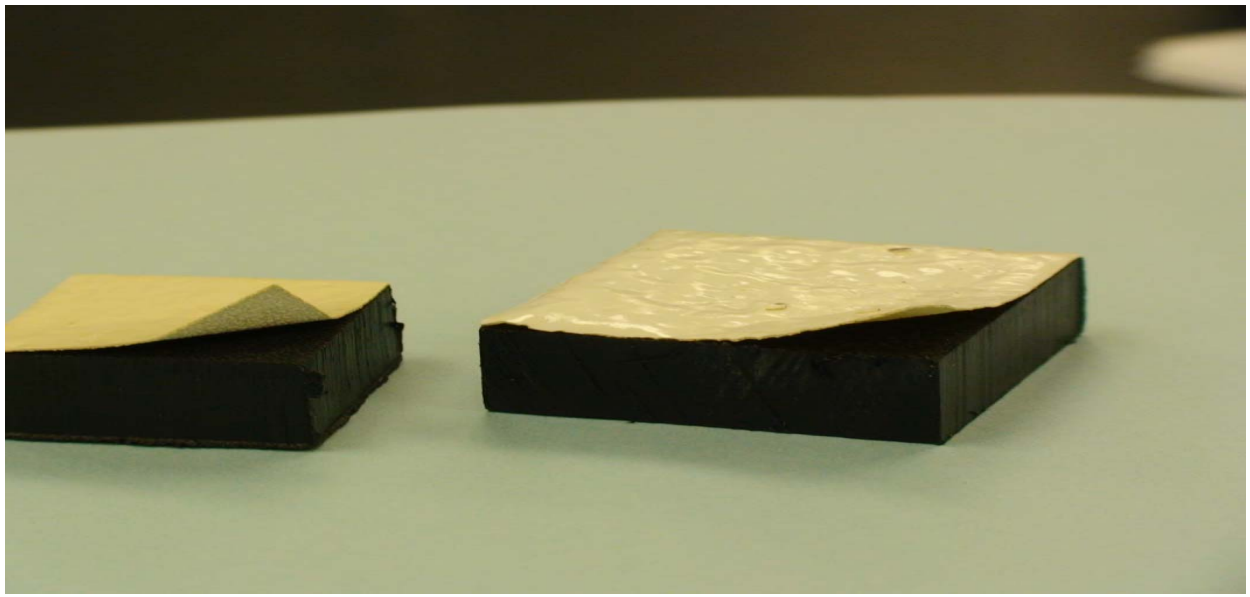
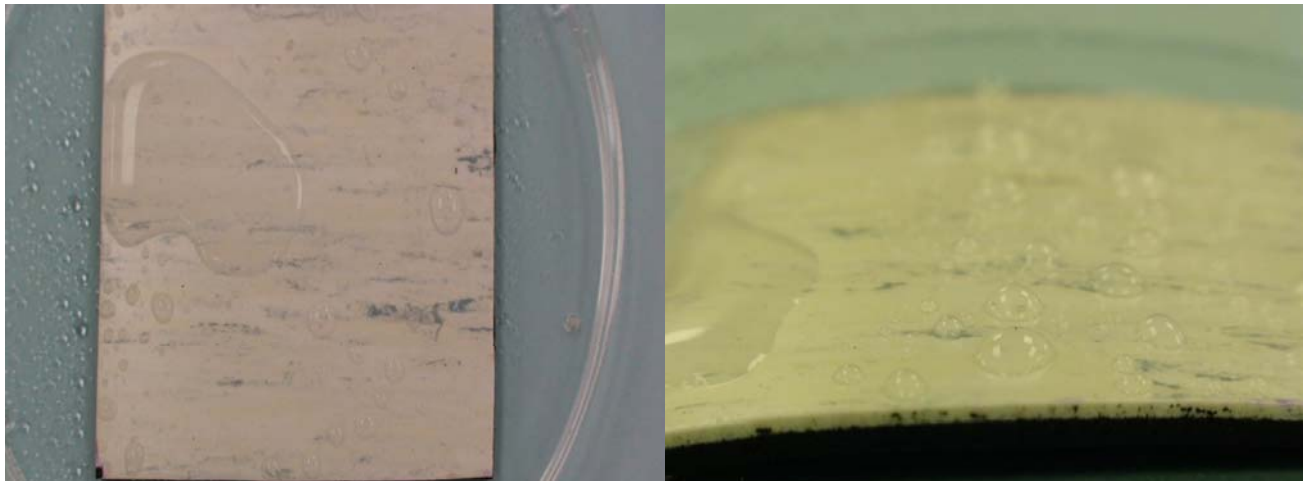
Autoclaving may not be acceptable for some surfaces, gypsum board with paint (Saniflex)



Surface Tension Issue



Coupon Issues

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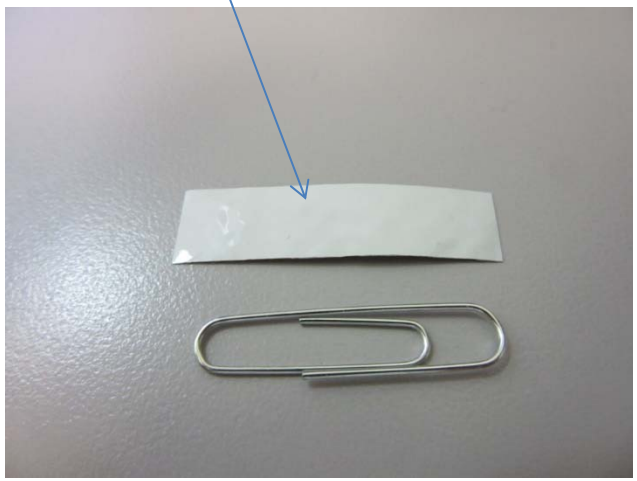
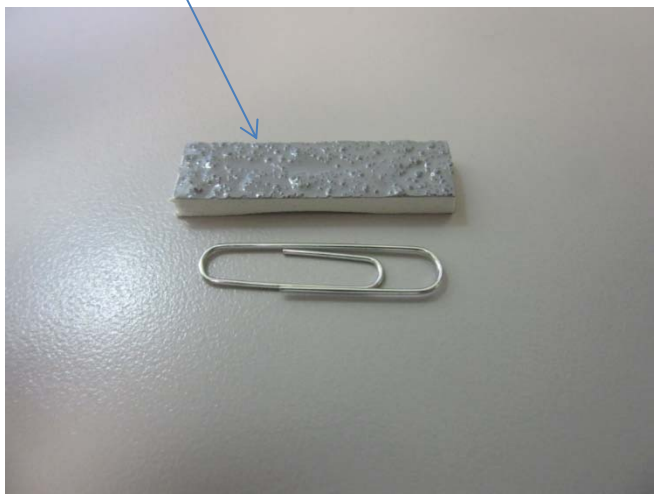
Surface Sterilization Issues



Surface Roughness



Surface Creation Issue

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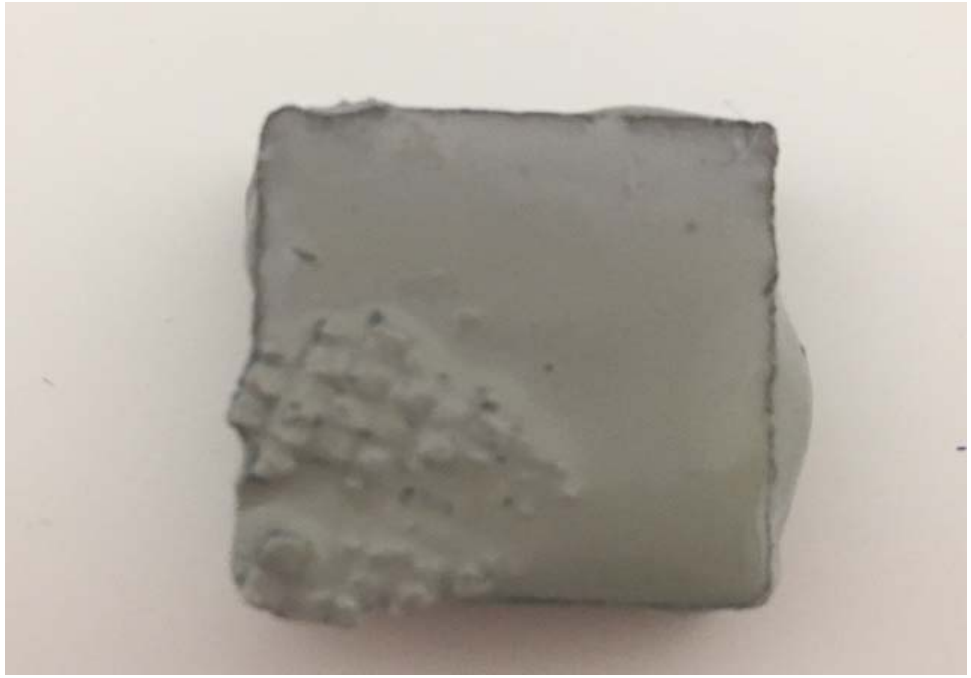
Coupon creation led to unrepresentative texture



Courtesy of
Erin Kruesi,
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Surface Degradation Issue

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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

Testing Bacterial Spore Formers

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- Bacterial spores are significantly more challenging to inactivate than vegetative cells (worst case for a strain)
- Bacterial spores generally require sporicidal agent to inactivate
- Spore form is most likely form of *Bacillus*, *Paenibacillus,* etc. to be encountered in classified area

Testing Bacterial Spore Formers

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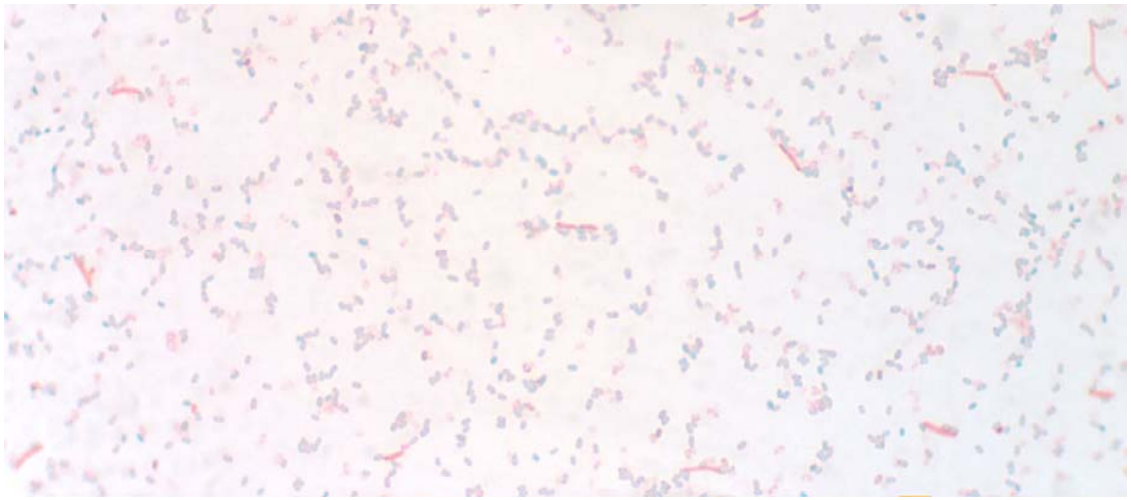
Can you consistently test against “vegetative” *Bacillus*?



Testing Bacterial Spore Formers in DET

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- Bacterial spore formation is a stochastic event
- Sporulation occurs within sub-populations (i.e. the entire suspension does not sporulate at the same time)
- Sporulation kinetics is complicated, depending on a multitude of environmental factors-NOT just nutrient scarcity

Paenibacillus 24 Hour Culture

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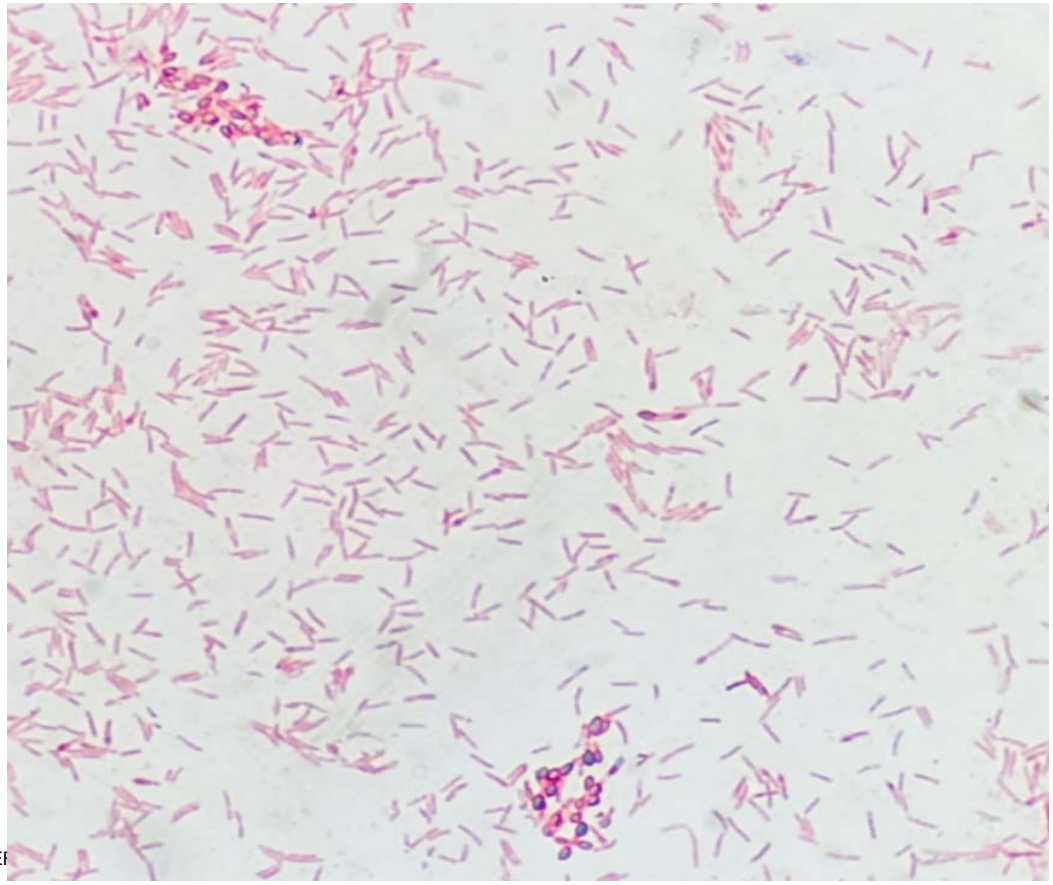

Courtesy of Jamie Knutson, STERIS Laboratories

Paenibacillus 18 Hour Culture

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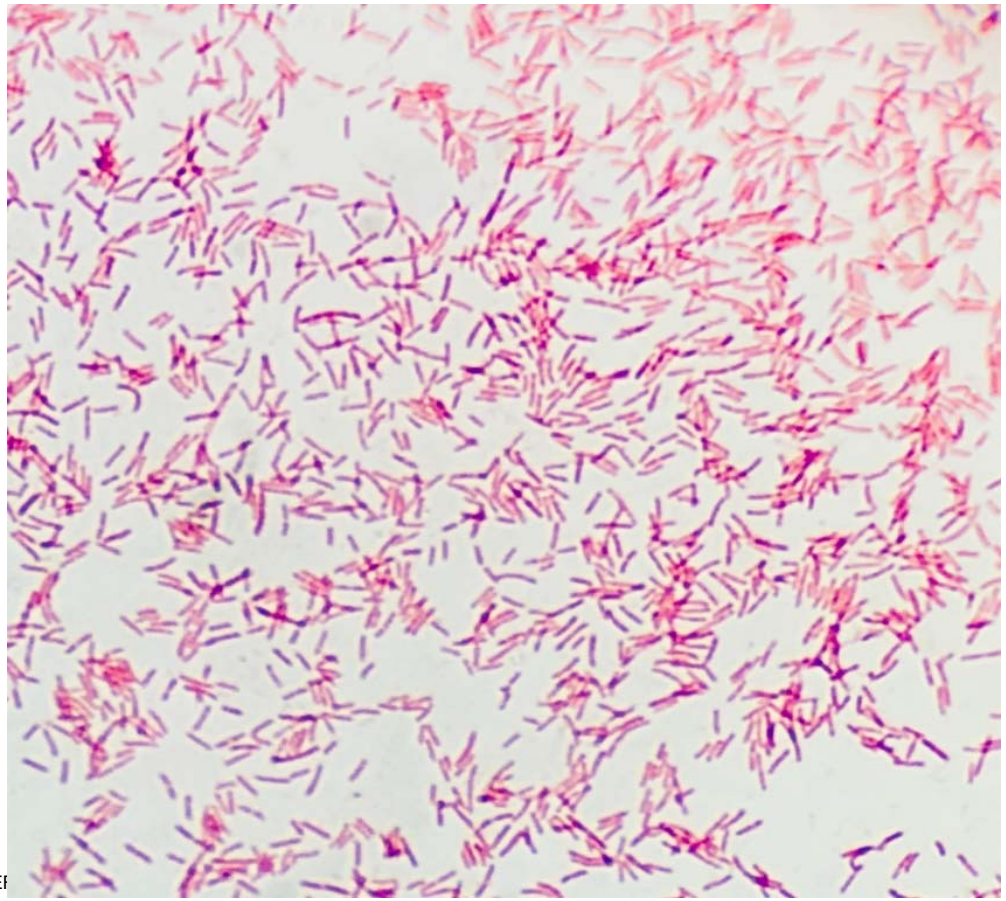

Courtesy of Jamie Knutson, STERIS Laboratories

18 Hour *Paenibacillus* Culture

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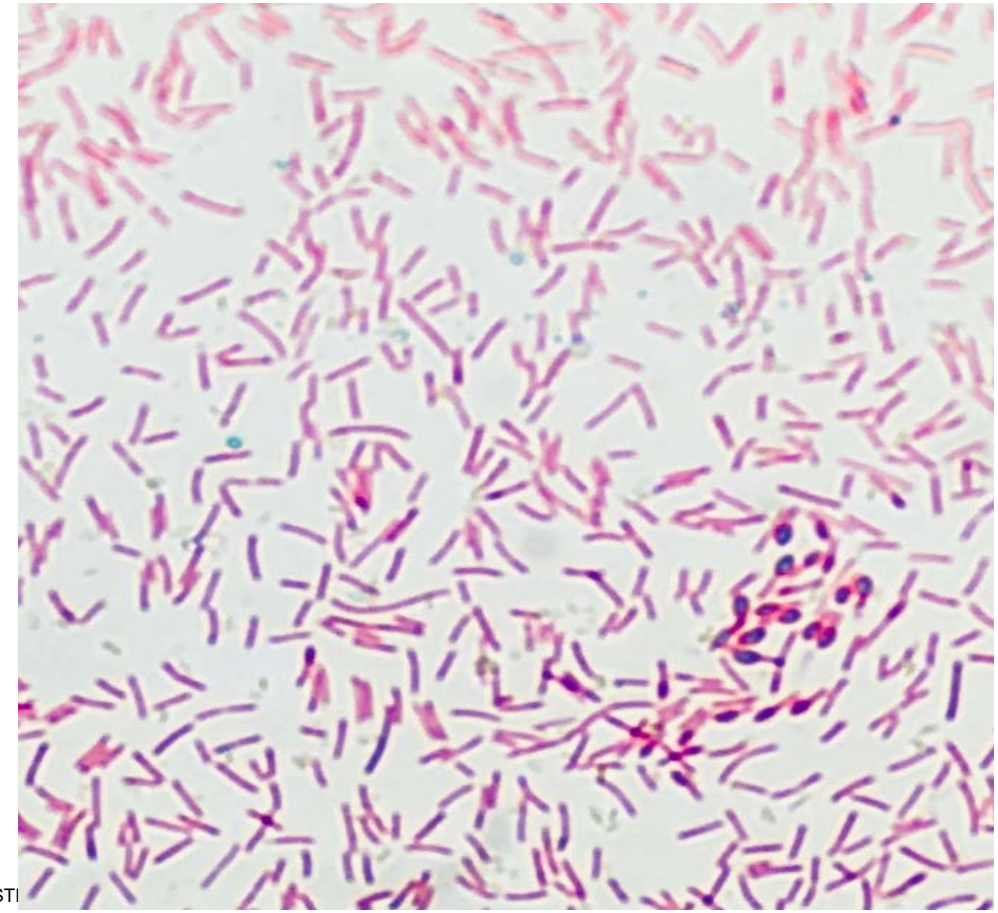

Courtesy of Jamie Knutson, STERIS Laboratories

Disinfectant Qualification Study Tips

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- AOAC methods are inappropriate for this testing (but some procedures such as inoculum prep, etc. can be of value)
- EN-13697 (2015) and ASTM E2197-11 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

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- 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 years to determine if any repeat testing is needed due to testing deficiencies

Summary Slide

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- Current Industry Best Practice in Disinfectant Validation

Acknowledgements

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- Special Thanks to Dan Klein and David Shields.