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Disinfectant Efficacy Testing for Critical Environments

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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

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

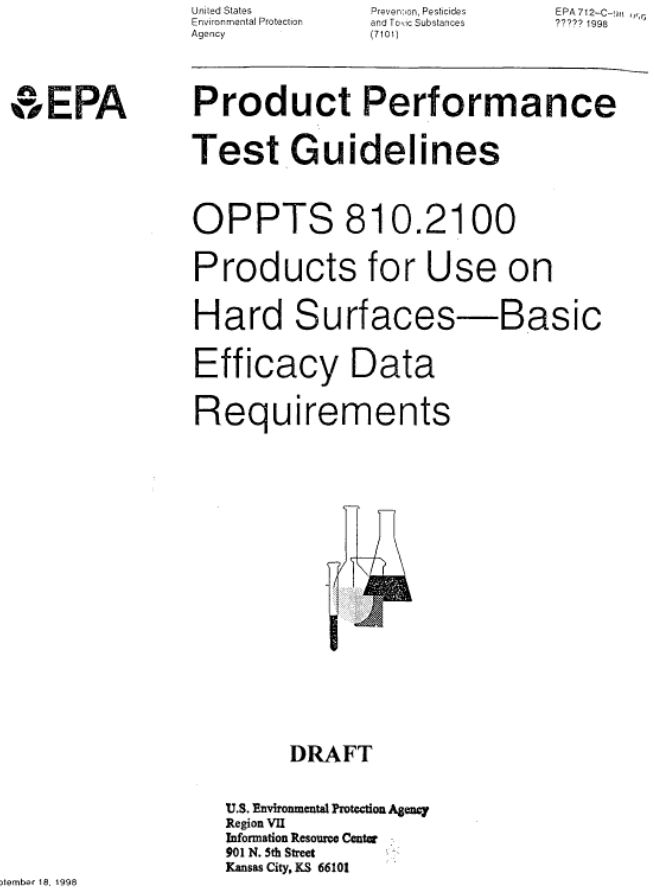
Disinfectant Regulation

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

EPA Guidance Document (1998) – for Vendors

- 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

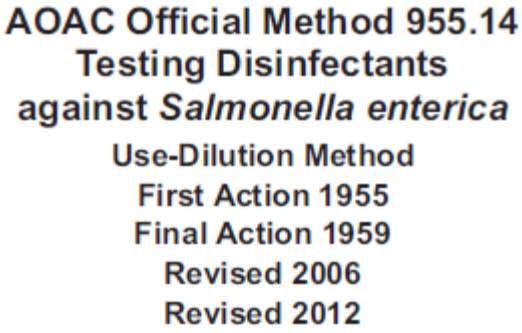
- Sterilant (60 carriers each on two surfaces); spores of *B. subtilis* ATCC 19659 and *C. sporogenes* ATCC 3584; 3 lots (720 carriers)
- Disinfectant (60 carriers representing 3 lots) against 3 bacteria; *S. enterica* ATCC 10708, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15442
- Fungicide (10 carriers 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”

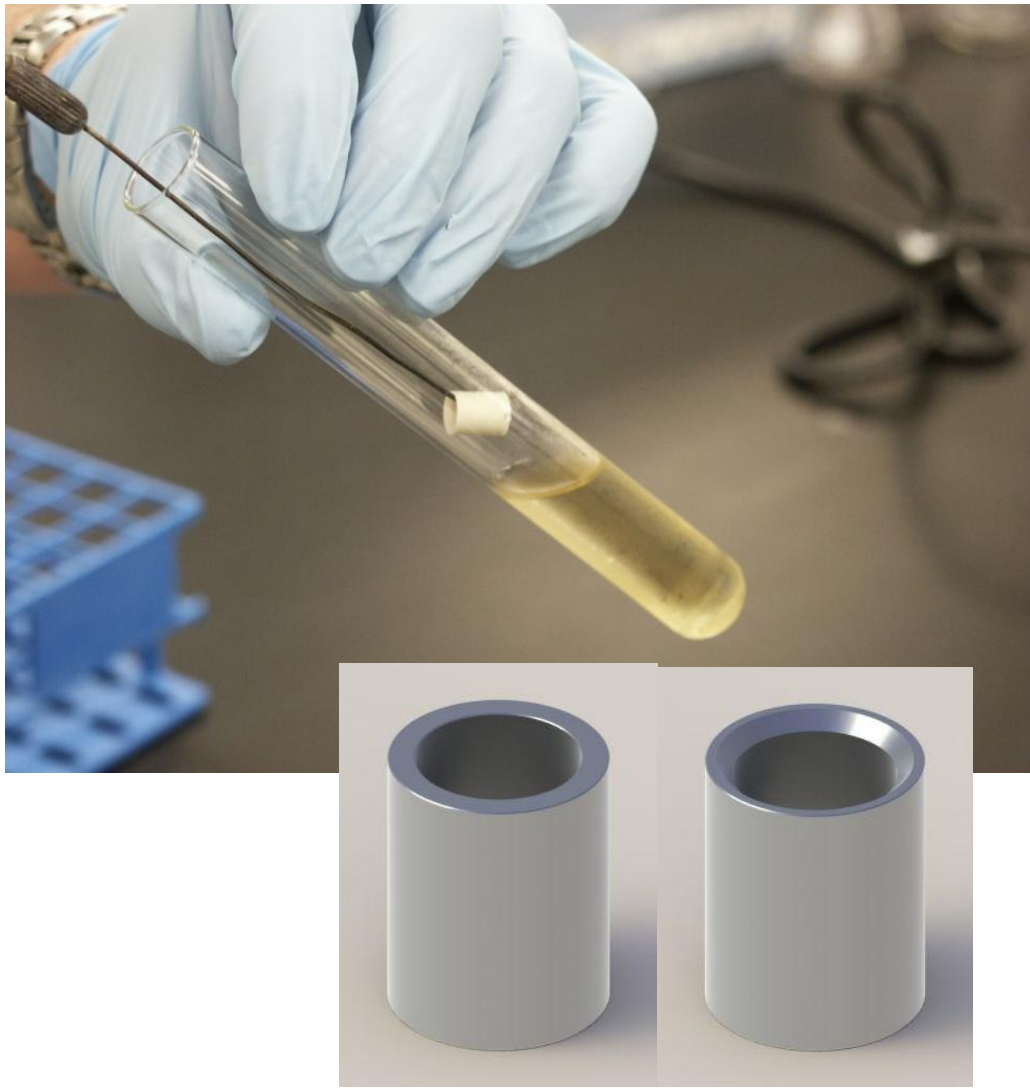
Common AOAC INTERNATIONAL Tests

- 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



Vendor Label Example

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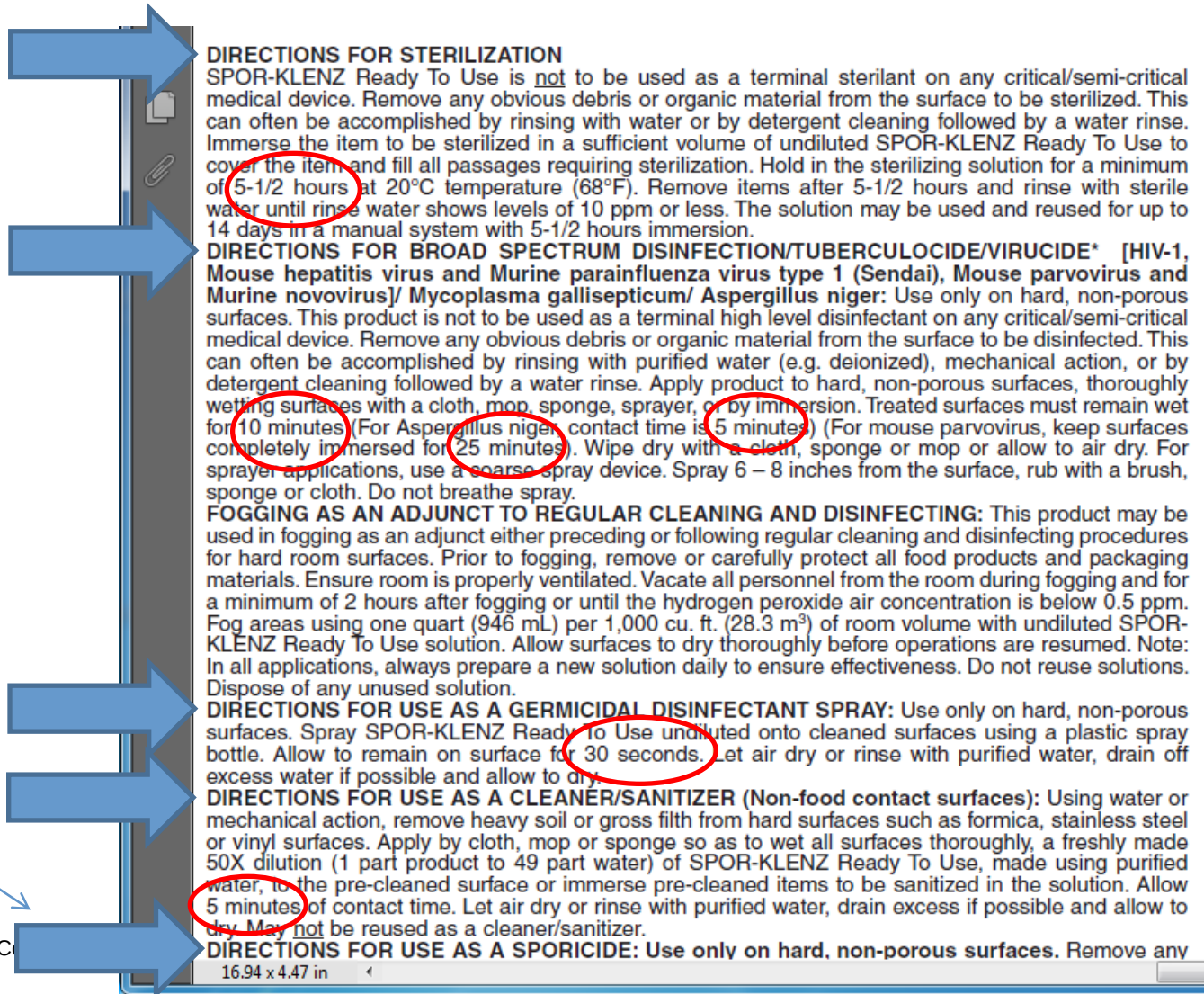

DIRECTIONS FOR STERILIZATION
SPOR-KLENZ Ready To Use is not to be used as a terminal sterilant on any critical/semi-critical medical device. Remove any obvious debris or organic material from the surface to be sterilized. This can often be accomplished by rinsing with water or by detergent cleaning followed by a water rinse. Immerse the item to be sterilized in a sufficient volume of undiluted SPOR-KLENZ Ready To Use to cover the item and fill all passages requiring sterilization. Hold in the sterilizing solution for a minimum of 5-1/2 hours at 20°C temperature (68°F). Remove items after 5-1/2 hours and rinse with sterile water until rinse water shows levels of 10 ppm or less. The solution may be used and reused for up to 14 days in a manual system with 5-1/2 hours immersion.

DIRECTIONS FOR BROAD SPECTRUM DISINFECTION/TUBERCULOCIDE/VIRUCIDE* [HIV-1, Mouse hepatitis virus and Murine parainfluenza virus type 1 (Sendai), Mouse parvovirus and Murine novovirus]/ Mycoplasma gallisepticum/ Aspergillus niger: Use only on hard, non-porous surfaces. This product is not to be used as a terminal high level disinfectant on any critical/semi-critical medical device. Remove any obvious debris or organic material from the surface to be disinfected. This can often be accomplished by rinsing with purified water (e.g. deionized), mechanical action, or by detergent cleaning followed by a water rinse. Apply product to hard, non-porous surfaces, thoroughly wetting surfaces with a cloth, mop, sponge, sprayer, or by immersion. Treated surfaces must remain wet for 10 minutes (For Aspergillus niger contact time is 5 minutes) (For mouse parvovirus, keep surfaces completely immersed for 25 minutes). Wipe dry with a cloth, sponge or mop or allow to air dry. For sprayer applications, use a coarse spray device. Spray 6 – 8 inches from the surface, rub with a brush, sponge or cloth. Do not breathe spray.

FOGGING AS AN ADJUNCT TO REGULAR CLEANING AND DISINFECTING: This product may be used in fogging as an adjunct either preceding or following regular cleaning and disinfecting procedures for hard room surfaces. Prior to fogging, remove or carefully protect all food products and packaging materials. Ensure room is properly ventilated. Vacate all personnel from the room during fogging and for a minimum of 2 hours after fogging or until the hydrogen peroxide air concentration is below 0.5 ppm. Fog areas using one quart (946 mL) per 1,000 cu. ft. (28.3 m^3) of room volume with undiluted SPOR-KLENZ Ready To Use solution. Allow surfaces to dry thoroughly before operations are resumed. Note: In all applications, always prepare a new solution daily to ensure effectiveness. Do not reuse solutions. Dispose of any unused solution.

DIRECTIONS FOR USE AS A GERMICIDAL DISINFECTANT SPRAY: Use only on hard, non-porous surfaces. Spray SPOR-KLENZ Ready To Use undiluted onto cleaned surfaces using a plastic spray bottle. Allow to remain on surface for 30 seconds. Let air dry or rinse with purified water, drain off excess water if possible and allow to dry.

DIRECTIONS FOR USE AS A CLEANER/SANITIZER (Non-food contact surfaces): Using water or mechanical action, remove heavy soil or gross filth from hard surfaces such as formica, stainless steel or vinyl surfaces. Apply by cloth, mop or sponge so as to wet all surfaces thoroughly, a freshly made 50X dilution (1 part product to 49 part water) of SPOR-KLENZ Ready To Use, made using purified water, to the pre-cleaned surface or immerse pre-cleaned items to be sanitized in the solution. Allow 5 minutes of contact time. Let air dry or rinse with purified water, drain excess if possible and allow to dry. May not be reused as a cleaner/sanitizer.

DIRECTIONS FOR USE AS A SPORICIDE: Use only on hard, non-porous surfaces. Remove any

16.94 x 4.47 in

30 min contact as a sporicide on hard non-porous surfaces

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Why Do End-Users Validate Disinfectants/ Biocides?

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- 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

Recent Warning Letter

"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

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"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

Older Warning Letter

"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

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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); i.d. to species level and bank them (recommended)

See USP <1116> for incident rate review/recalculation

Disinfectant Validation Procedure Recommendations

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- USP <1072> Disinfectants and Antiseptics
  - Use-dilution tests
  - Surface Challenge tests
- ASTM E2614-08 Guide for evaluation of Cleanroom Disinfectants
- ISO 14698 (parts1-3)
  - Surface evaluation, focus on cleaning
- PDA Technical Report on Cleaning and Disinfection (Draft Document)

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 & E2197)
  - Biofilm Method (E1427)
  - Viral Testing (Suspension E1052)
  - Viral Testing (Carrier E1053)

- Variations of all of the above

More In Vitro Options for Testing

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- 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

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- 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

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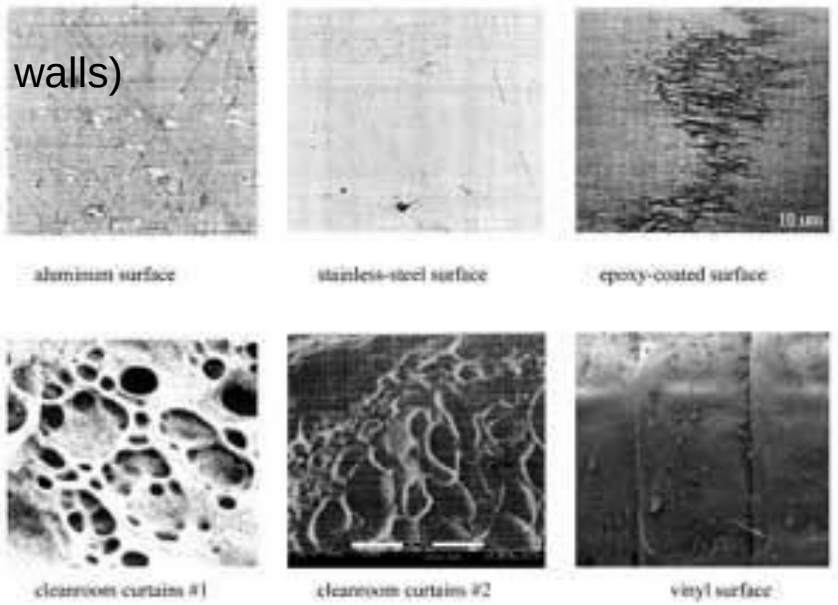
- Traditional methods (AOAC and ASTM)
  - Stainless steel disks, penicylinders or coupons
  - Watch glasses or glass slides
  - Porcelain penicylinders and silk suture loops

- Cleanroom disinfectant 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
  - Acyrlic and Grout
  - Paints & Sealants
  - Gaskets (EPDM, Teflon)
  - Rubber or Nitrile gloves



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 and sodium sulfite can be toxic
  - Mechanical separation causing damage to cells

- Validation of neutralization is required

<1227> Common Neutralizers

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

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

Catalase for H_2O_2

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

Microorganisms

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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

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	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

B. cereus / B. sphaericus

B. subtilis / G. stearothermophilus

***Clostridium* spp.**

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

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

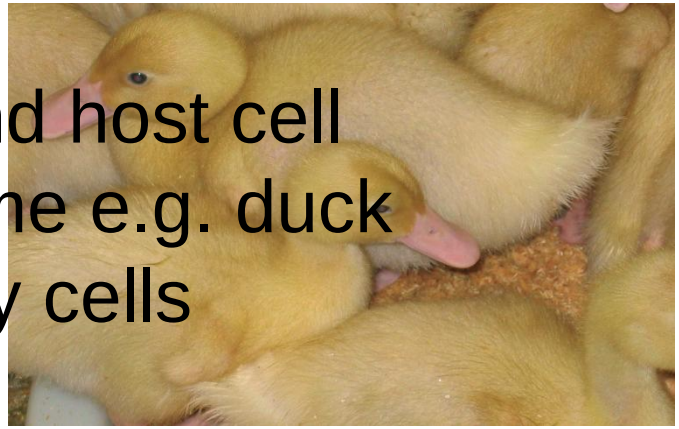
Virus Efficacy Testing Trends

- 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 <1072>
  - 2 log reduction bacterial spores
  - 3 log reduction vegetative bacteria, yeast, mold spores

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

Cleaning Efficacy In Situ Time 0

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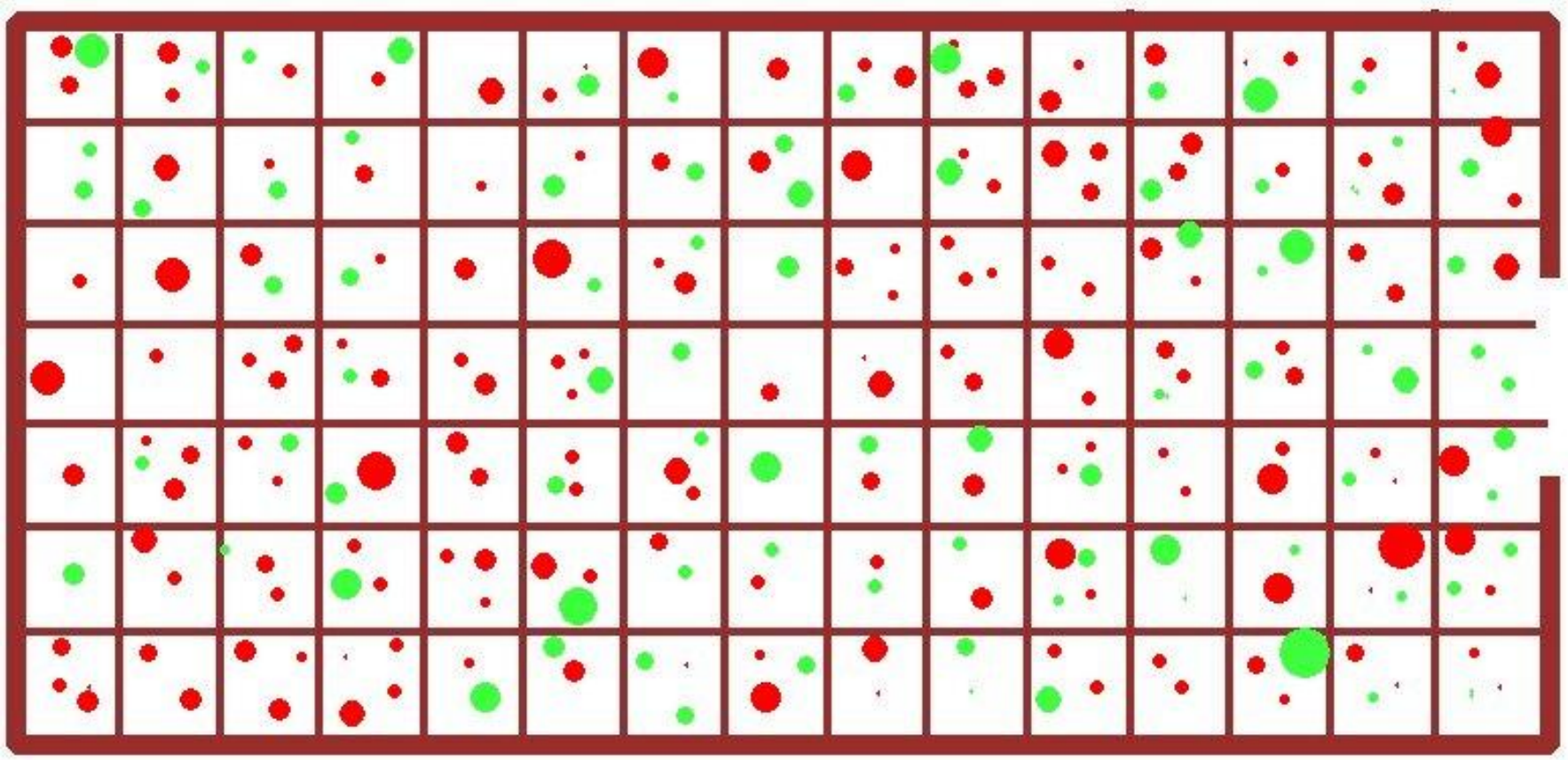
- Red = Spore formers Green = Other



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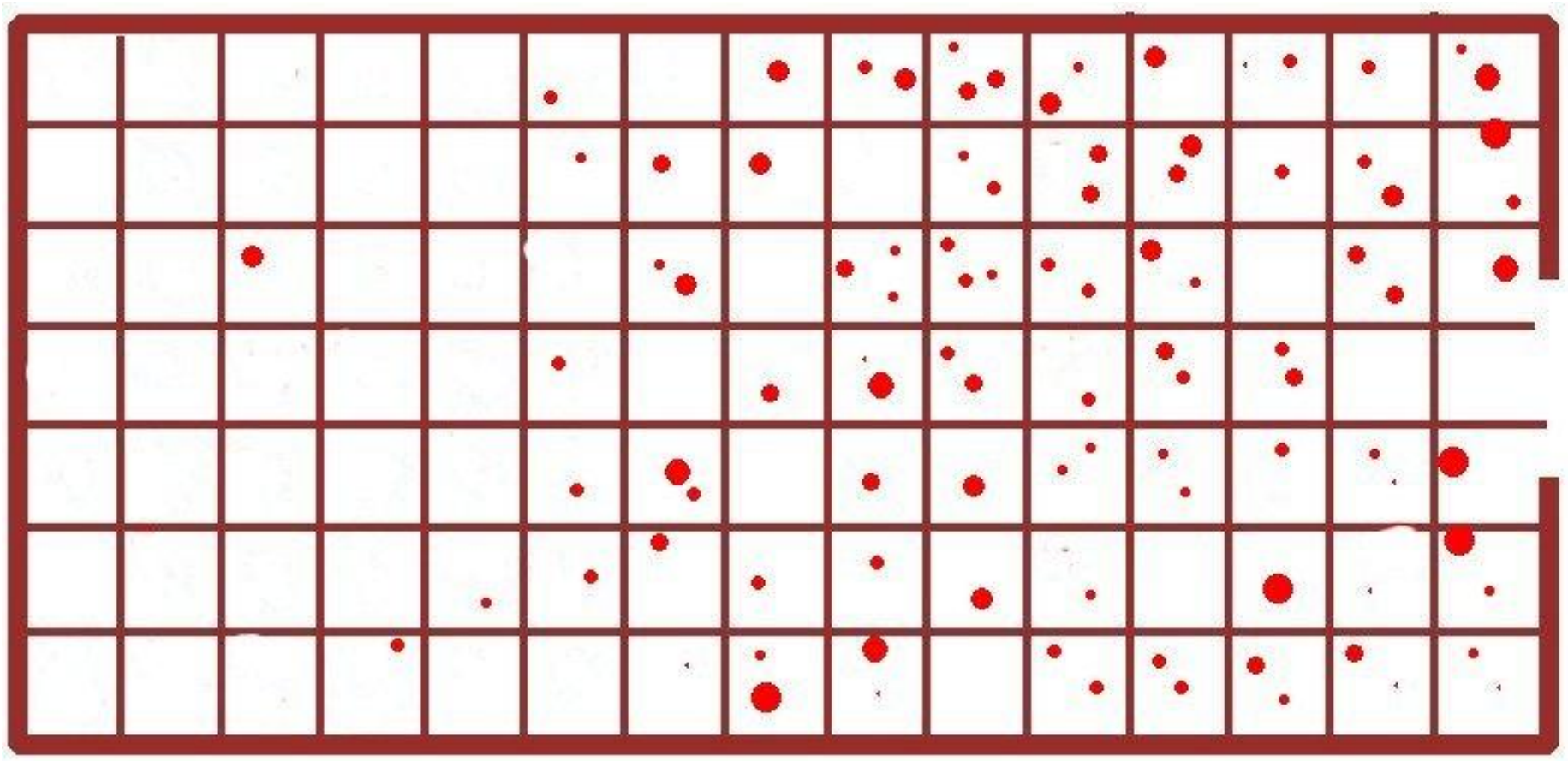
After 1X cleaning-NO Sporicide



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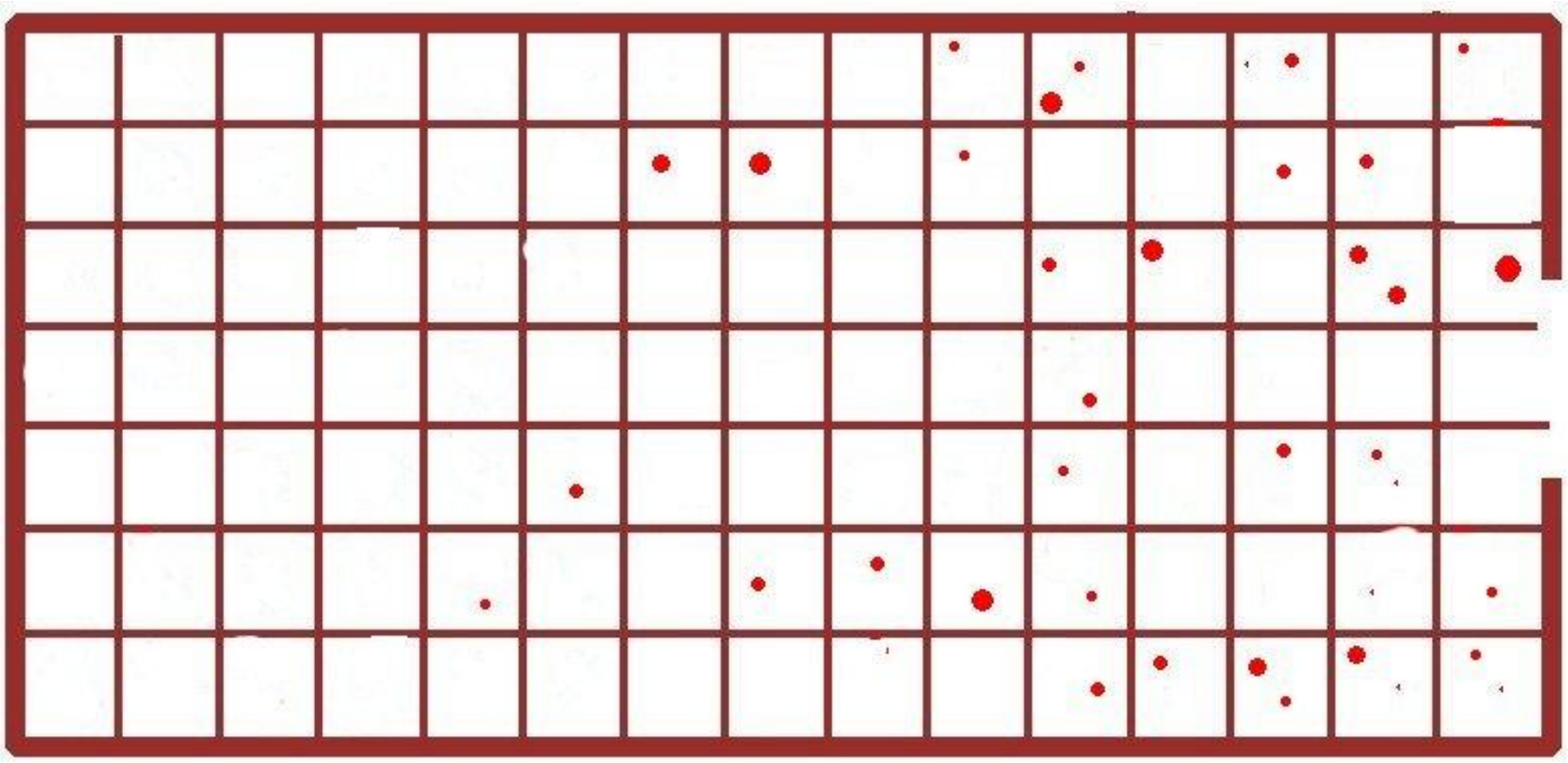
After 2X Cleaning – NO Sporicide



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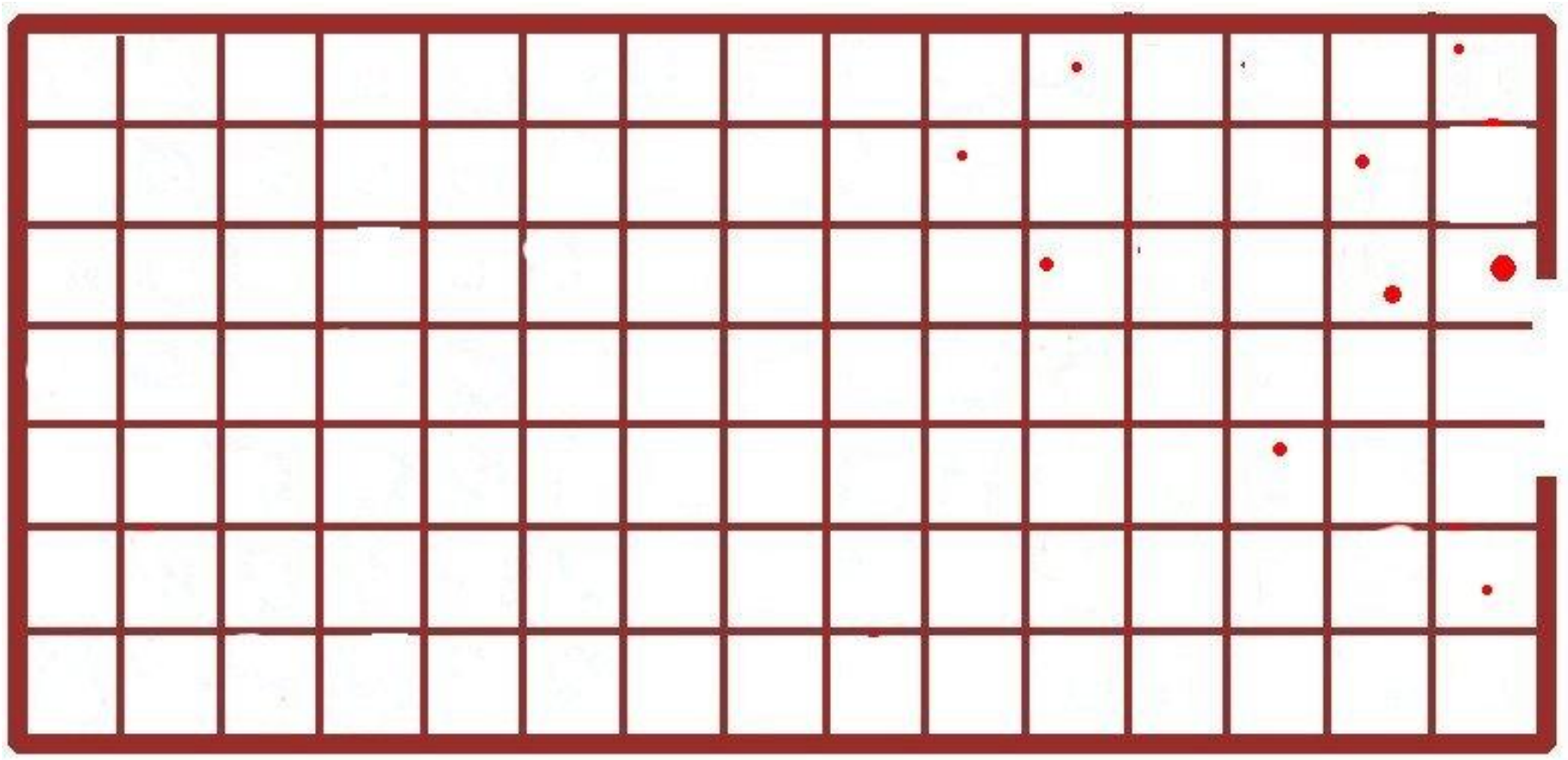
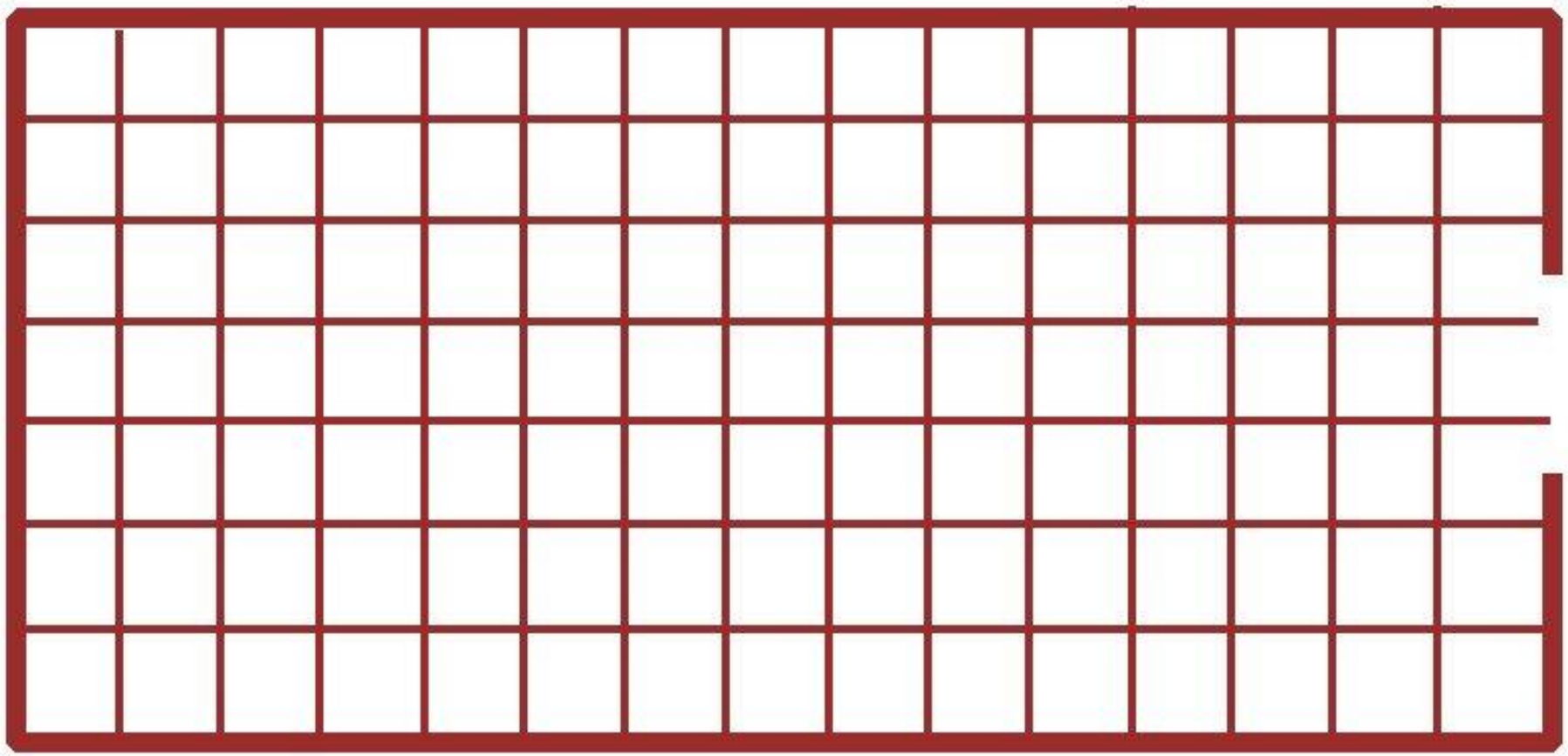
After 3X cleaning-No Sporicide



After Sporicide



In Situ Protocols

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- 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

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- 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)

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In Vitro Testing Considerations Contributors to Test Failures

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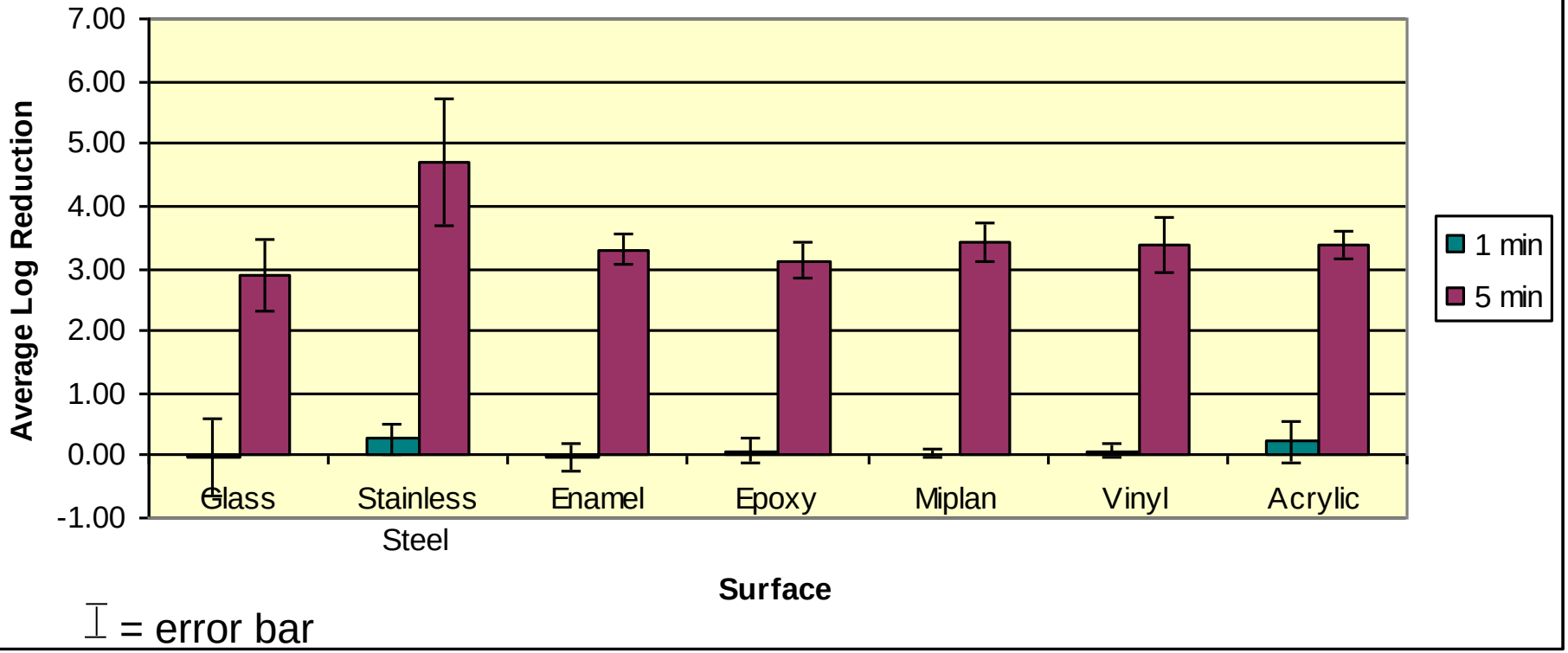
- Recovery issues post-drying (*P. aeruginosa*)
- Inoculum prep (e.g. fungal spores)
- Coupon prep (autoclaving – peeling Saniflex)
- Improper dilution of Concentrate
- Inappropriate biocide for spores
- Insufficient contact time – should match SOP
- US vs. EU requirements

Hard Surface Test Results



Case Study on Substrates

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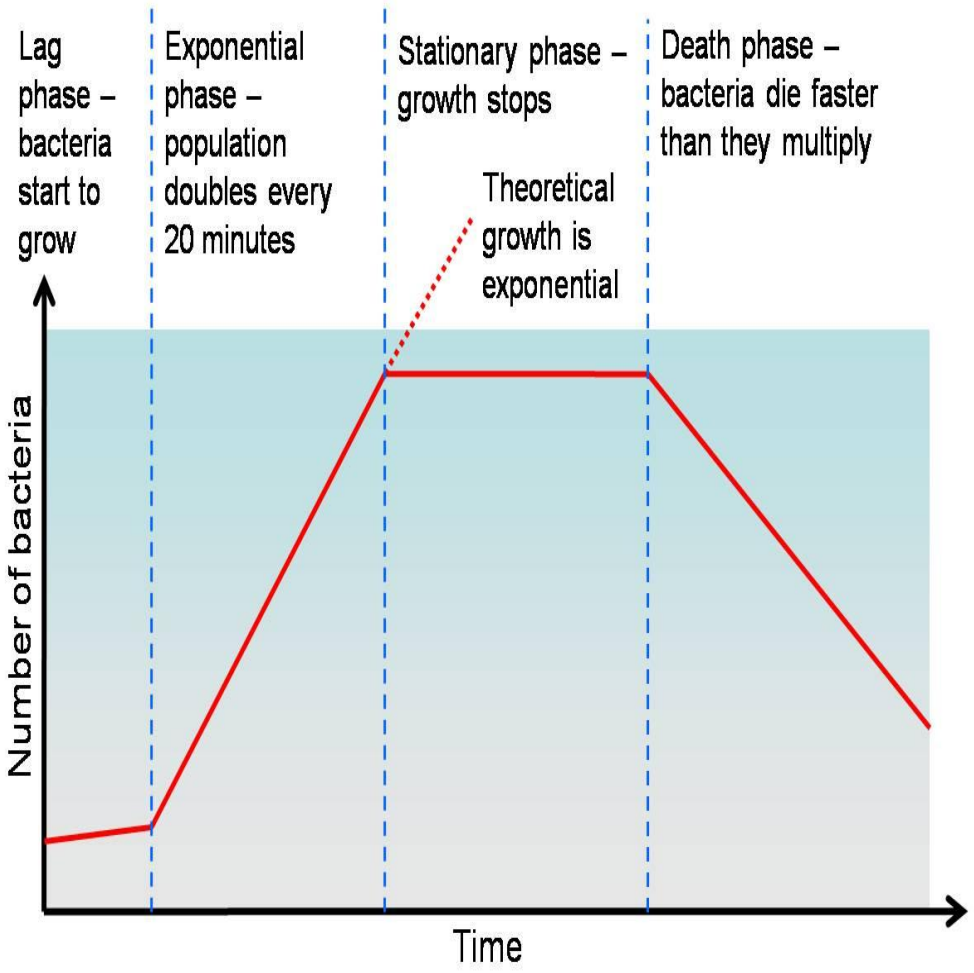
Efficacy (log reduction) of Low pH phenolic: (1:256) against test microorganisms on representative surfaces

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

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

	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

Inoculum Preparation—Fungal Spores

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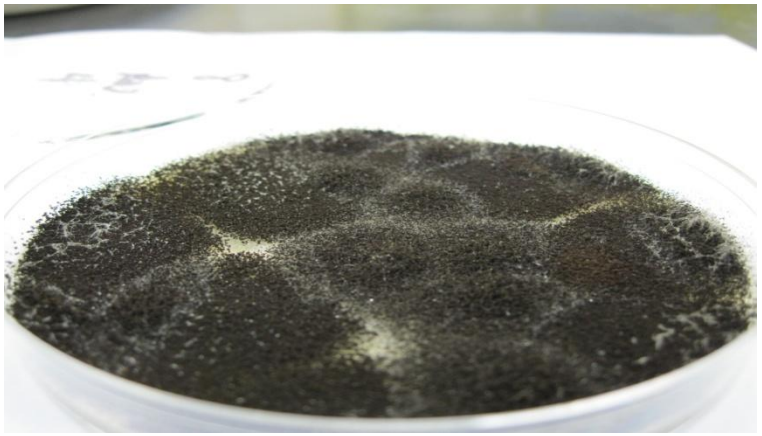
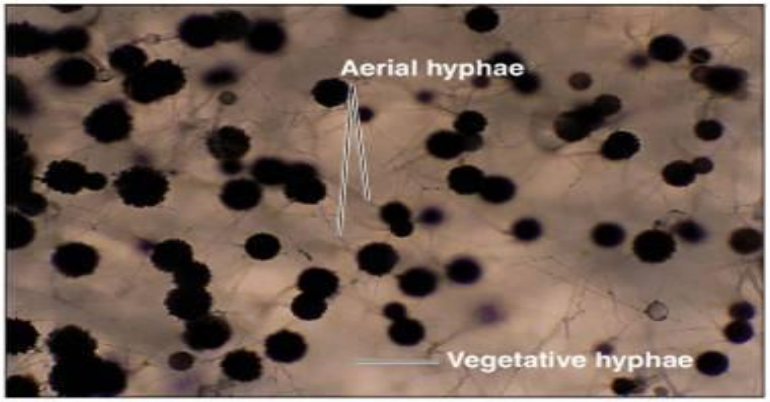
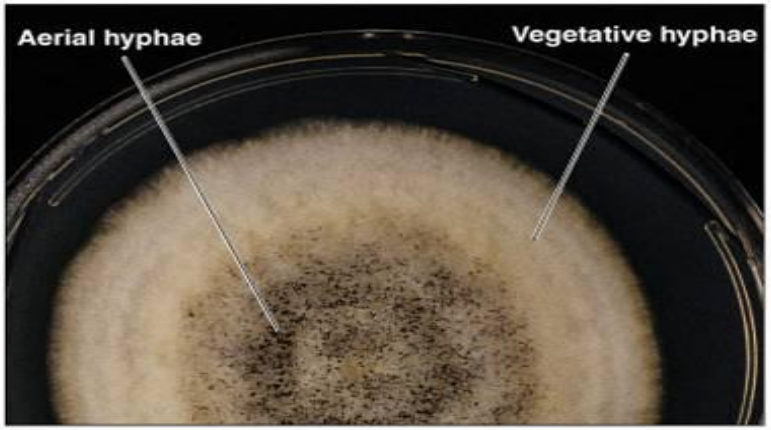
Cultures need to be incubated for a sufficient length of time before harvesting spores





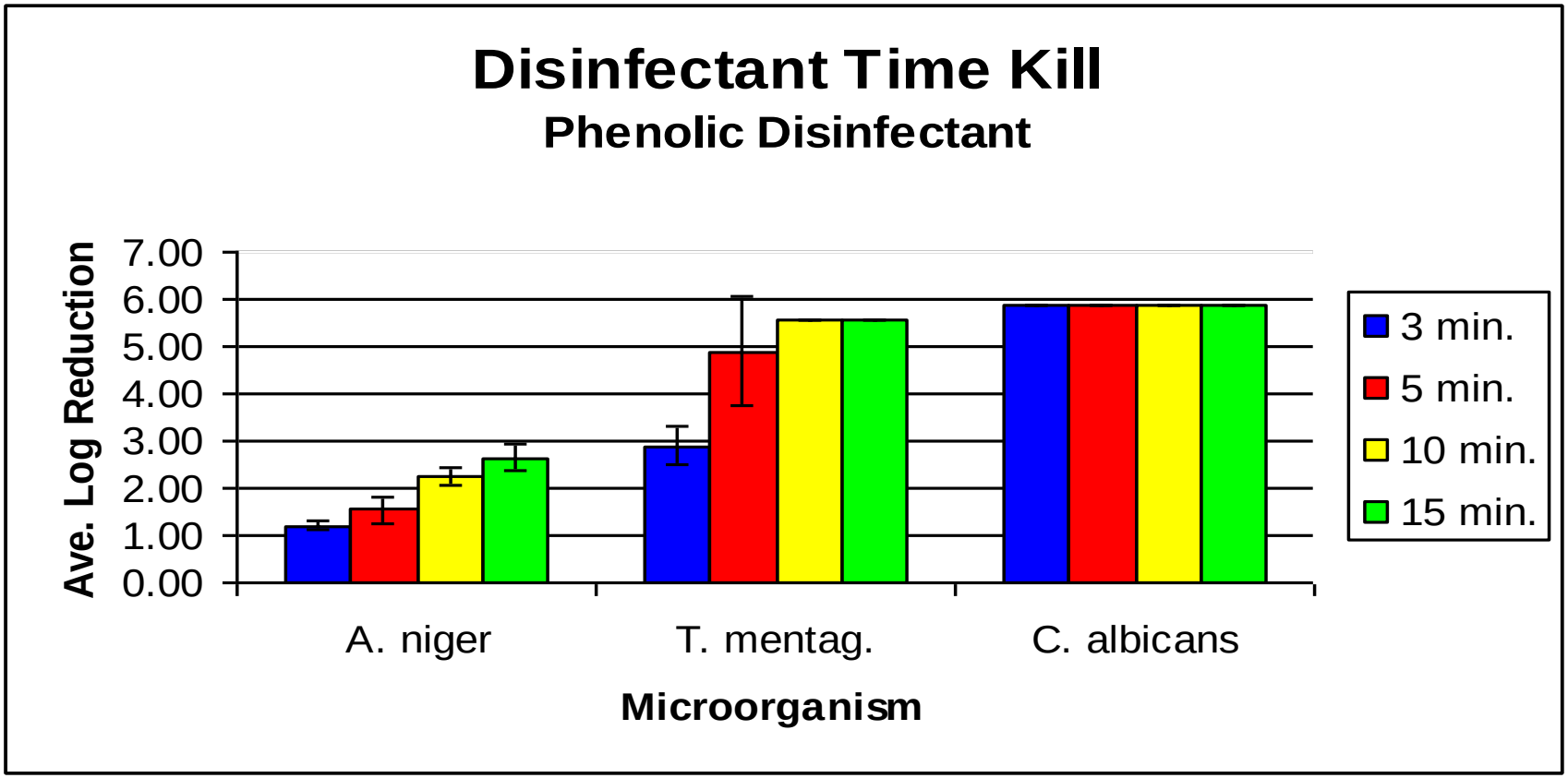
(a) *Aspergillus niger*



(b) *A. niger on agar*

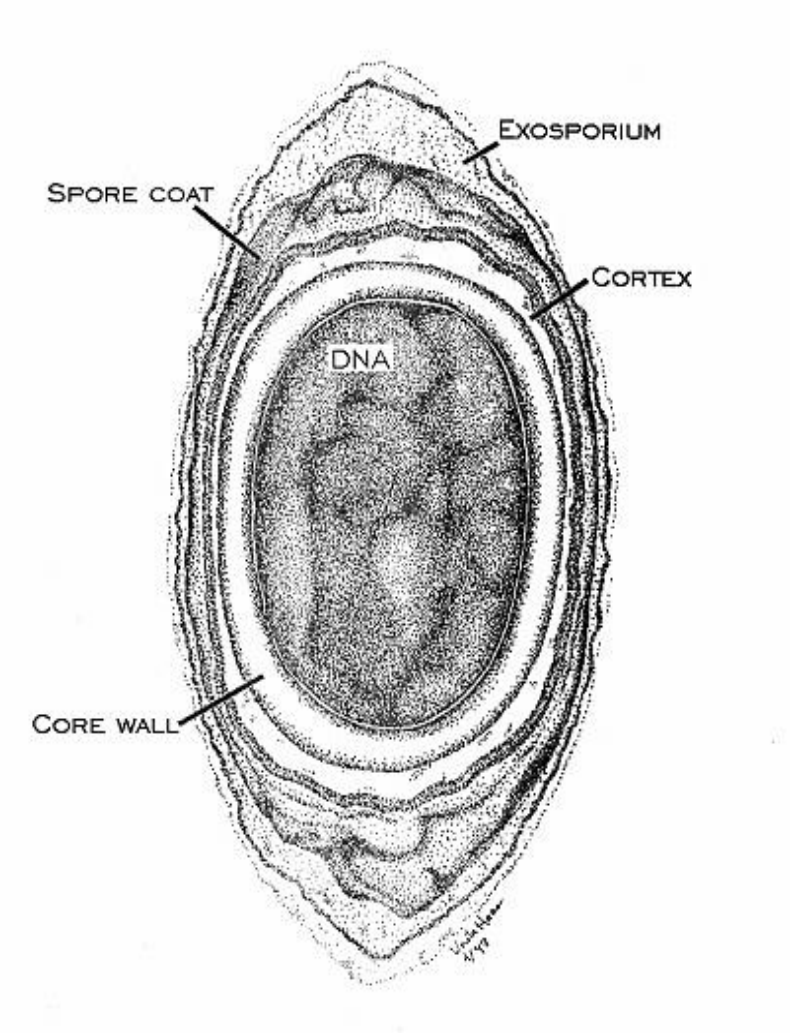
Testing Against Fungal Spores

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



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

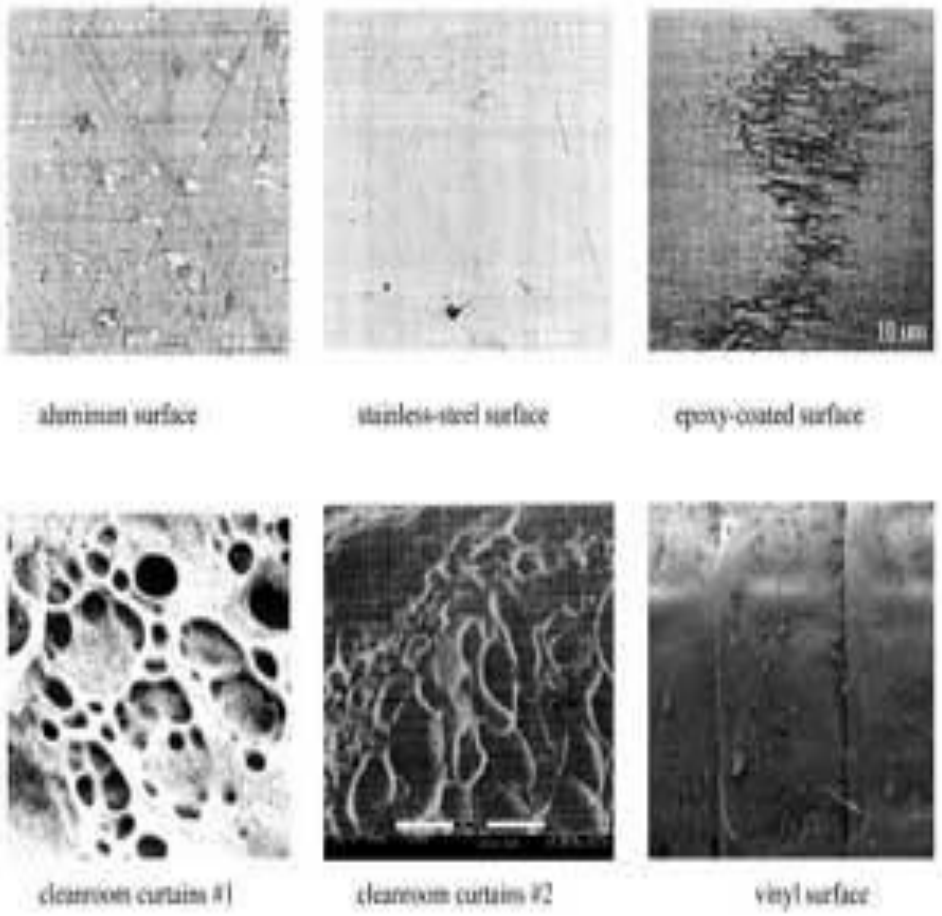


Surface/Coupon Issues

- 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

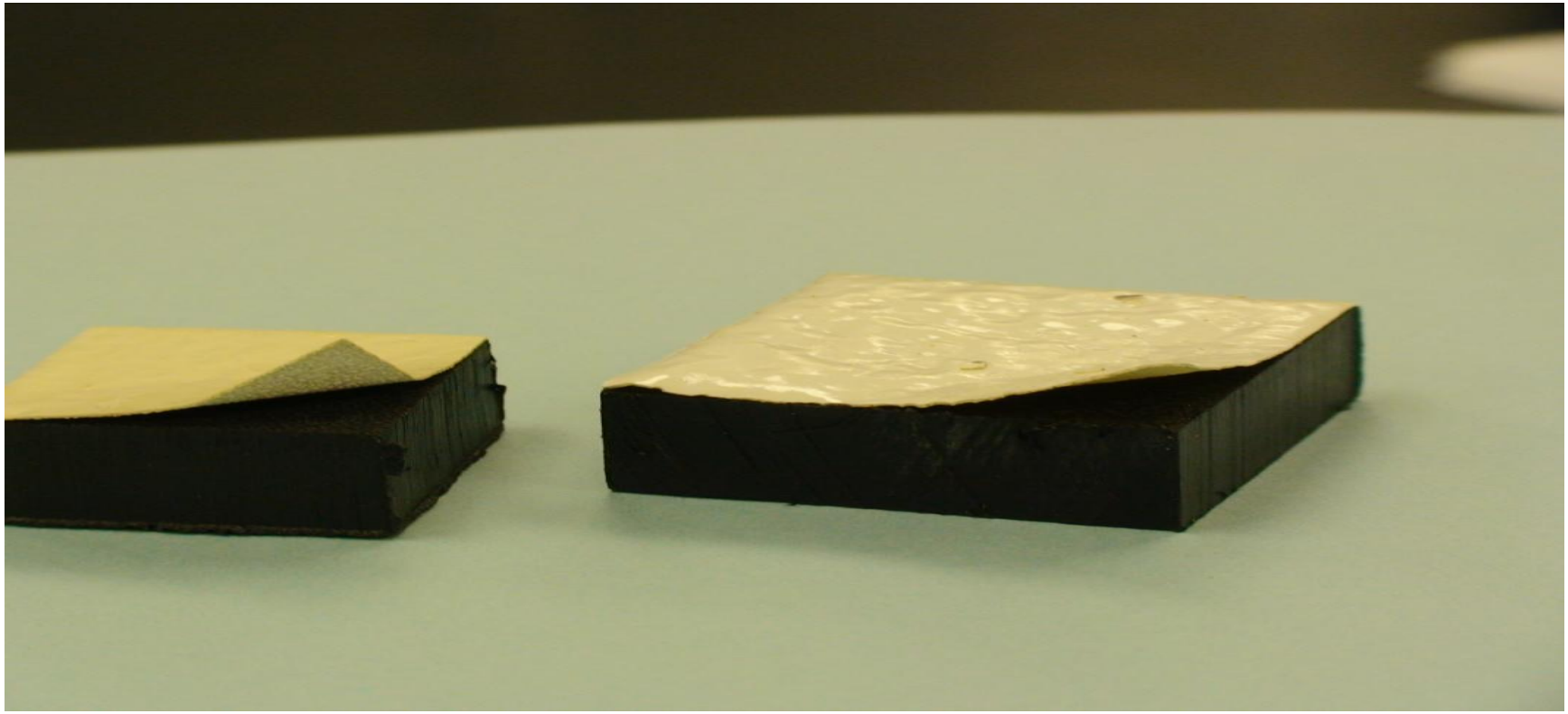
Surface Type and Condition

- 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

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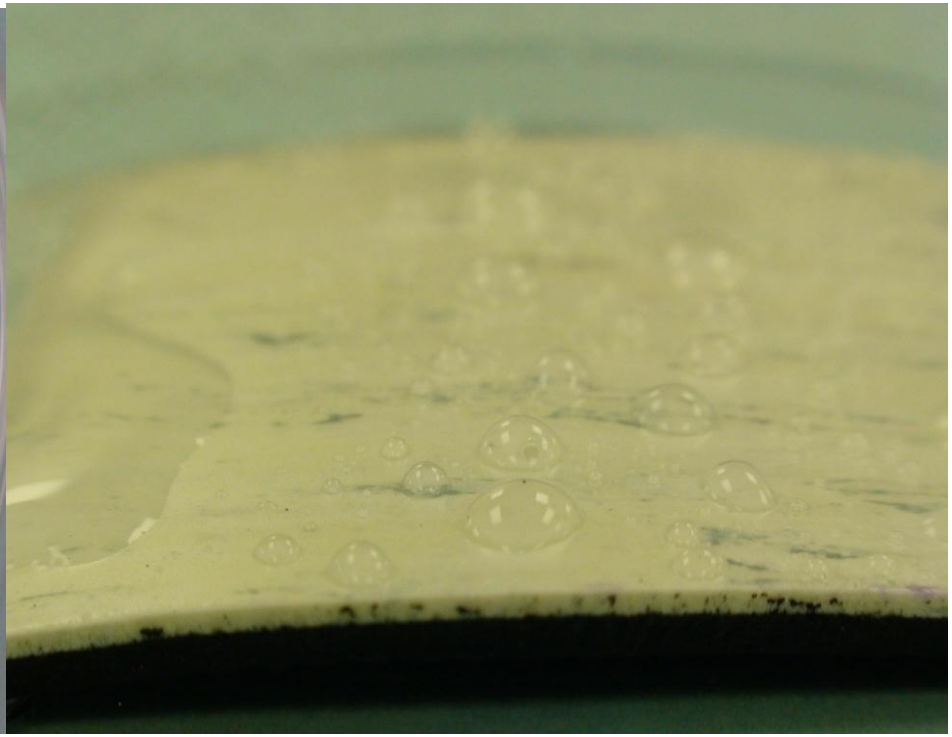
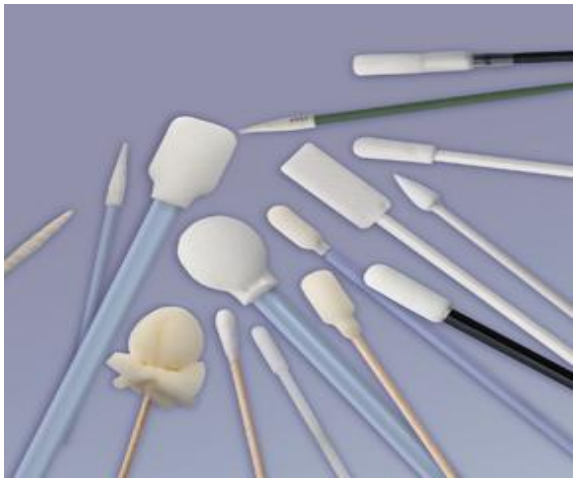


Recovery Method Issues



- 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

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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 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

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- 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

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THANK YOU!

Questions & Answers

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