Disinfectant Testing and Test Methods

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PDA Parenteral Drug Association

PDA Aseptic Manufacturing Excellence Conference 2024

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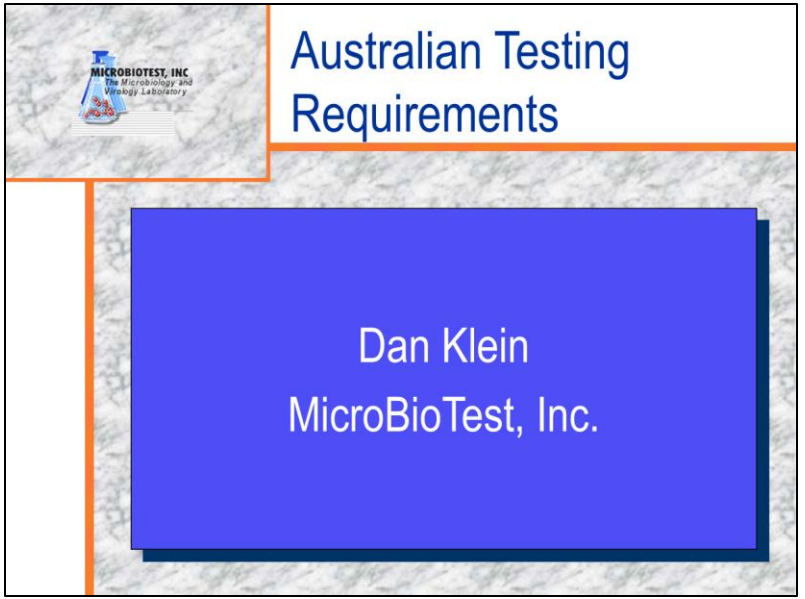
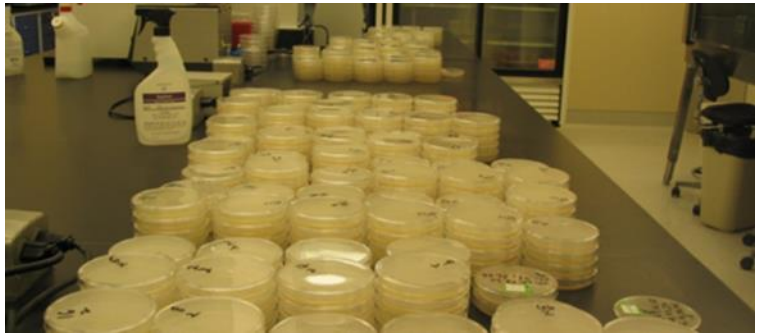
Disinfectant Testing and Test Methods

- Long history of method development and disinfectant testing
  - R&D and Contract Lab Management
  - AOAC Committee M Chair
  - ASTM E35.15
  - First big presentation was on testing
    - CSMA (then CSPA, then HCPA)
    - June, 1999



Disinfectant Testing and Test Methods

Supplier / Registration Testing



- US Environmental Protection Agency
- Europe and Rest of World

Qualification Testing

- Disinfectant Efficacy Testing (DET)
- In situ Evaluations

US EPA

- Methods Typically taken from AOAC Chapter 6, Disinfectants
  - Primarily qualitative
- Pass/Fail Criteria differ for bacteria, TB, fungi and spores
  - Developed in the '50's for *S. aureus* outbreaks

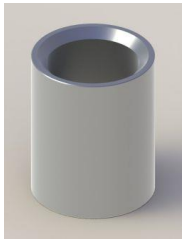
Difficult to change

CLAIM	TEST REPLICATES	TEST METHOD(S)	TEST ORGANISMS	PASS/FAIL CRITERIA
Bactericidal	60 carriers per lot per microorganism 3 lots of product	AOAC Use-Dilution Method, (955.15), (964.02)	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442 Additional supplemental bacteria as required by claim	*S. aureus*, ≤3/60 positive *P. aeruginosa*, ≤6/60 positive All others, 0/10 positive
Fungicidal	Suspension test or 10 carriers per lot 2 lots of product	AOAC Fungicidal Activity Test (955.17) Modified AOAC Use-Dilution	*Trichophyton interdigitale* ATCC 9533	Complete kill / No Growth
Tuberculocidal	10 carriers per lot 2 lots of product	AOAC Tuberculocidal Activity Test (965.12)	*M. tuberculosis* var *bovis* (BCG)	No positive carriers
Virucidal	1-2 carriers per lot	ASTM E-1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces	Specific virus claimed	≥3.0 log10 reduction
Sporicidal	60 carriers per lot per surface (x2) per microorganism 3 lots of product	AOAC Sporicidal Activity of Disinfectants (966.04)	*Bacillus subtilis* ATCC 19659 *Clostridium sporogenes* ATCC 3584	Complete kill on all carriers
Sanitizer for Non-Food Contact Surfaces	3 lots of product	ASTM E-1153 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 4352 -or- *Enterobacter aerogenes* ATCC 13048	≥3.0 $\log_{10}$ reduction

US EPA

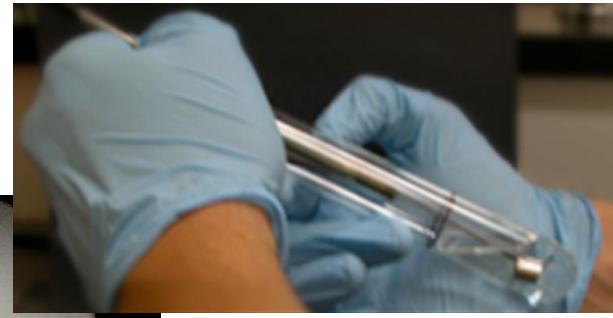
REGULATORY TESTING

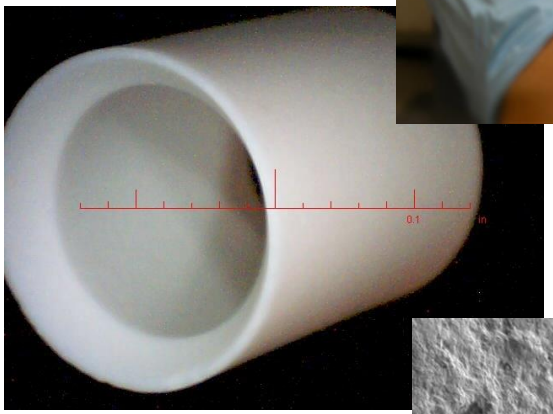
- United States (EPA)
- All microorganisms on label must be tested
  - Bactericidal
    - *S. aureus*
      - 60 carriers with >10^5, complete kill in 57+/60 x 3 lots
    - *P. aeruginosa*
      - 60 carriers with >10^5, complete kill in 54+/60 x 3 lots
- Challenging to pass
- Challenging to test
- Not directly indicative of real-world conditions

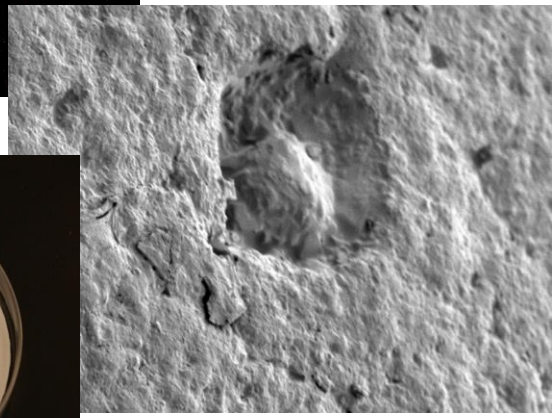


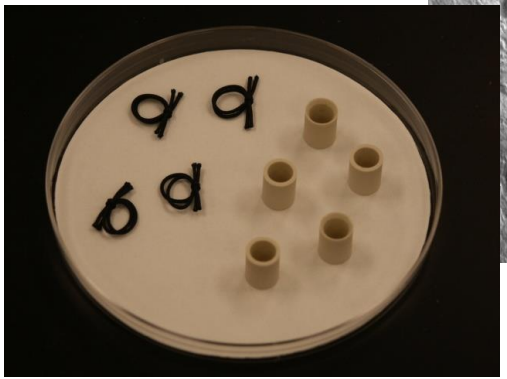
US EPA

- AOAC Sporicidal Test is even worse
- 0 positives allowed
  - 3 Lots
  - 60 suture loops
  - 60 porcelain penicylinders
  - *B. subtilis*
  - *C. sporogenes*
- 0 Positives allowed in 720 tested carriers
  - Qualitative test
- Propensity for false positives









STERIS

Europe and Rest of World

- EU Methods divided into 3 tiers
  - Phase 1
    - Basic suspension tests
  - Phase 2
    - Simulation studies
  - Phase 3
    - Tests under practical conditions
- TGA
  - TGA Disinfectant Test (suspension)
  - Hard Surface Test (increased carriers)

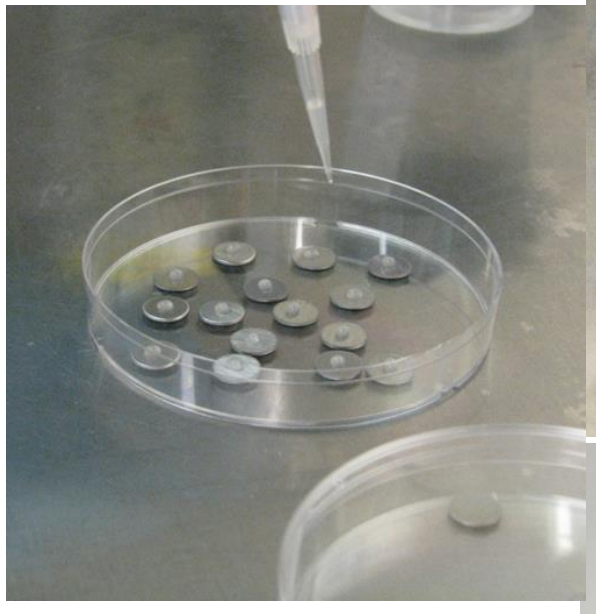
	CLAIM	TEST METHOD(S)	TEST ORGANISMS	PASS/FAIL CRITERIA
Basic Suspension Tests	Bacteria	EN 1040	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538	≥5.0 $\log_{10}$ reduction
	Fungi	EN 1275	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥4.0 $\log_{10}$ reduction
	Spores	EN 14347	*Bacillus subtilis subsp. spizizenii* ATCC 6633 *Bacillus cereus ATCC 12826*	≥4.0 $\log_{10}$ reduction
Quantitative Suspension Tests	Bacteria	EN 1276	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541	≥5.0 $\log_{10}$ reduction
	Fungi	EN 1650	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥4.0 $\log_{10}$ reduction
	Spores	EN 13704	*Bacillus subtilis* ATCC 6633	≥3.0 $\log_{10}$ reduction
Hard Surface Test	Bacteria	EN 13697	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541	≥4.0 $\log_{10}$ reduction
	Fungi		*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥3.0 $\log_{10}$ reduction

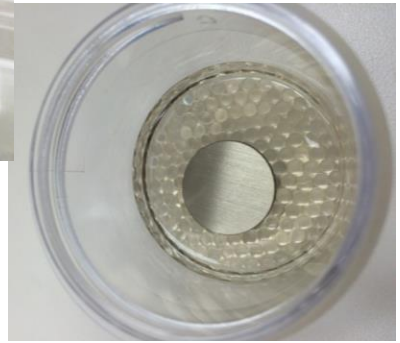
*This table does not intend to outline all available claims or test conditions available for disinfectants within REACH guidelines; it only highlights some common base claims.

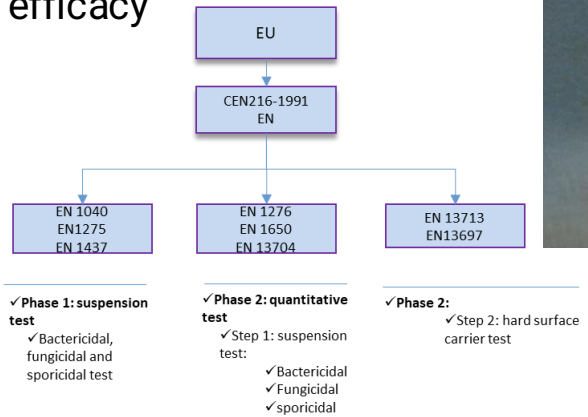
Europe

EN 13697

- Basic Efficacy Test on a hard surface
- Does not work well for RTU products
  - 80% dilution
- 5 log reduction value. 3 log reduction for fungicidal efficacy





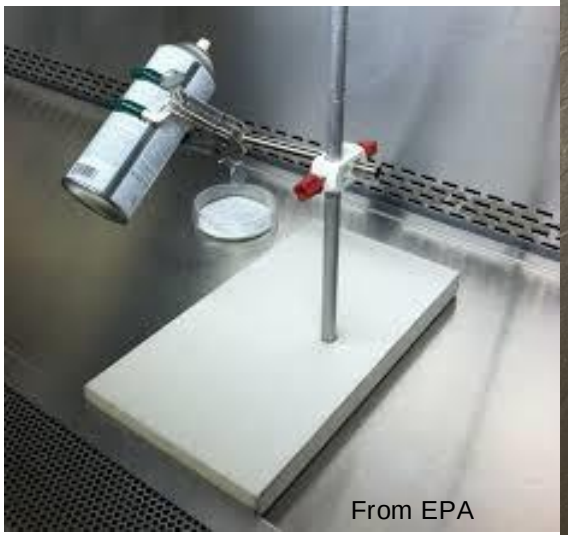


Spray Testing

US Germicidal Spray Test

- Qualitative test
  - 60 slides for *S. aureus* and *P. aeruginosa*, 3 lots
  - 10 slides, 2 lots for supplementals

Challenges with reproducibility


From EPA

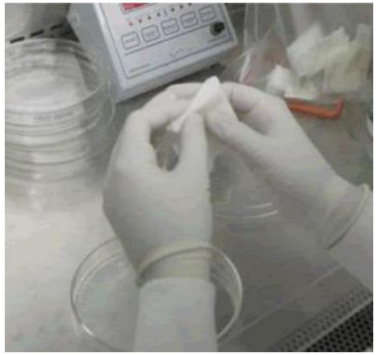
Wipe Testing

EN 16615, European Wipes Test

- 4 FIELD TEST
  - 2.5 kg weight (for pressure)
  - Field #1 is inoculated
- *S. aureus*, *P. aeruginosa*, *E. hirae*
- 5 log reduction on test field 1 and ≤ 50 CFU on fields 2 – 4

US Modified Germicidal Spray Test

- “Origami” method
- 10 Slides per wipe
- QPM under development



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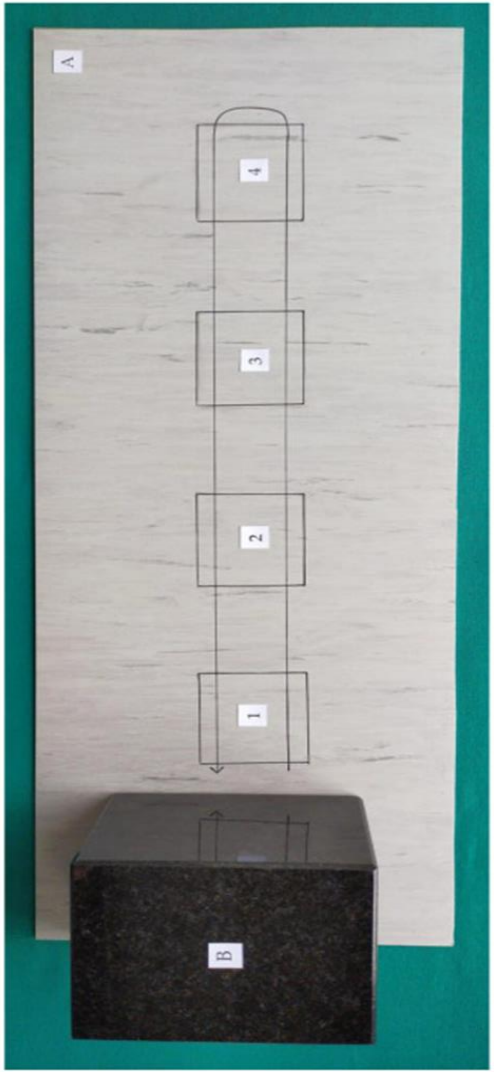
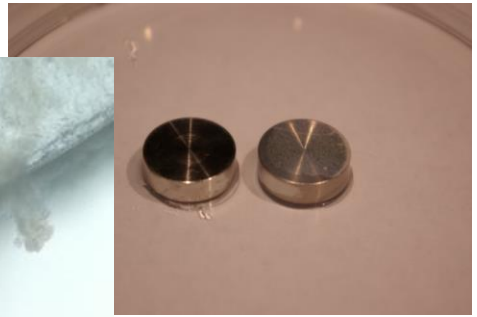


Biofilm and Other Testing

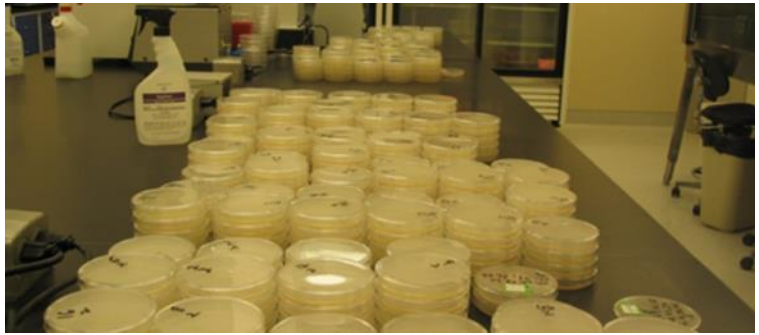
- CDC Reactor / ASTM Single tube method
- *Pseudomonas aeruginosa and Staphylococcus aureus*
  - 3 lots on three separate days
- 6 log reduction on five test coupons
- Maximum 10-minute contact time

Other tests include sanitizers, fabric, carpet, air, etc.



Disinfectant Testing and Test Methods

Supplier / Registration Testing

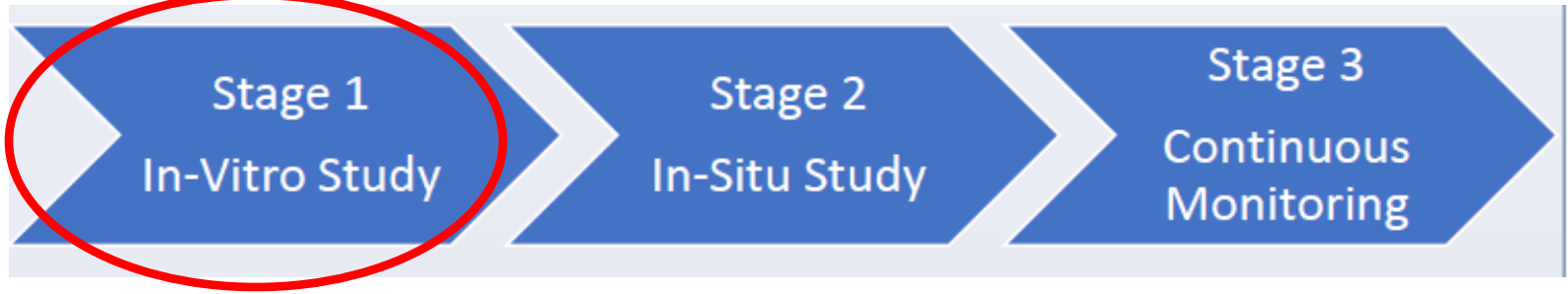


- US Environmental Protection Agency
- Europe and Rest of World

Qualification Testing

- Disinfectant Efficacy Testing (DET)
- In situ Evaluations

Disinfectant Qualification Testing



- In-vitro testing/disinfectant efficacy test (DET)
  - Laboratory study that looks at representative cleanroom surfaces and microorganisms

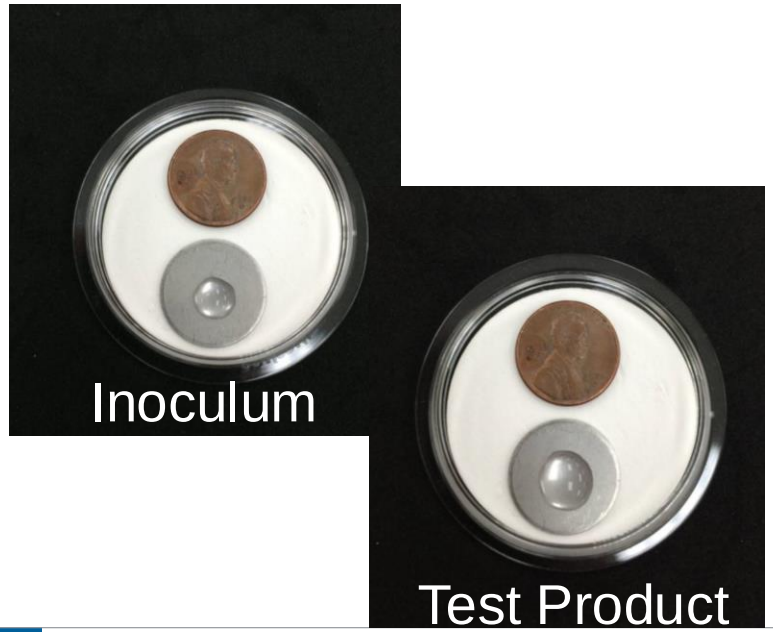
Disinfectant Efficacy Test

- Infinite number of microorganisms
- Nearly infinite number of test method variations
- Must understand real-world surfaces and conditions
- Must choose the best method available
- Must design the study to avoid misleading results
- Must select representative strains
  - American Type Culture Collection (ATCC) versus environmental monitoring (EM) isolates

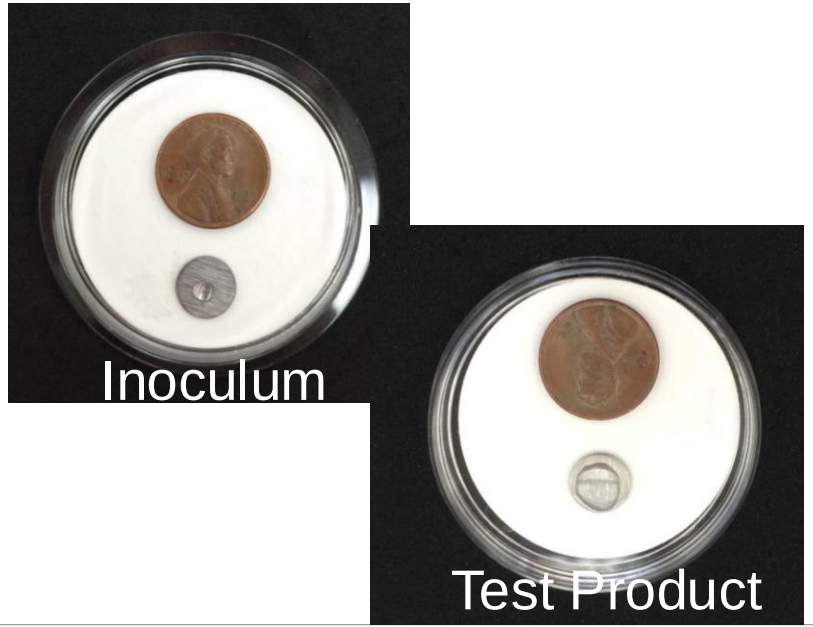
	Microorganism	Examples
More Resistant	Prions	Scrapie, Creutzfeld-Jacob disease, Chronic wasting disease
	Bacterial Spores	*Bacillus, Geobacillus, Clostridium*
	Protozoal Oocysts	*Cryptosporidium*
	Helminth Eggs	*Ascaris, Enterobius*
	Mycobacteria	*Mycobacterium tuberculosis, M. terrae, M. chelonae*
	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

Disinfectant Efficacy Testing

EN 13697



ASTM E 2197



Common Causes of Failure

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

Common Causes of Failures

- General Causes of failures
  - Improper dilution of concentrated disinfectant
  - Generating data that is not useful
    - Using AOAC test methods for coupon studies
  - Ineffective chemistries
    - Testing alcohol or disinfectants without sporicidal claims against bacterial endospores
  - Contact time too short
    - Inadequate wet contact with inoculum

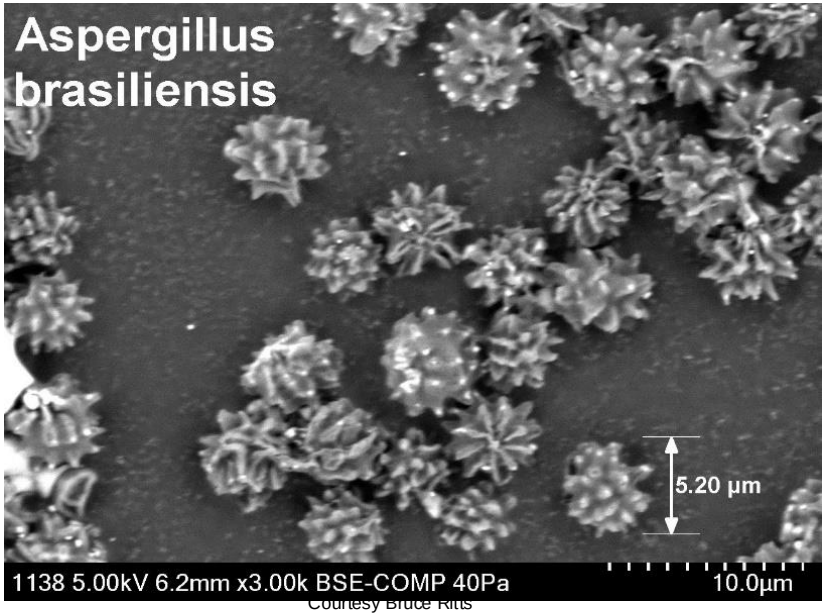
Common Causes of Failures

- Neutralization Problems
  - Incomplete neutralization
    - Non-dilutional series of plates
    - Uneven distribution of microorganisms
    - Results in over-estimation of activity
  - Any potential toxicity of neutralizer
- ASTM 1054

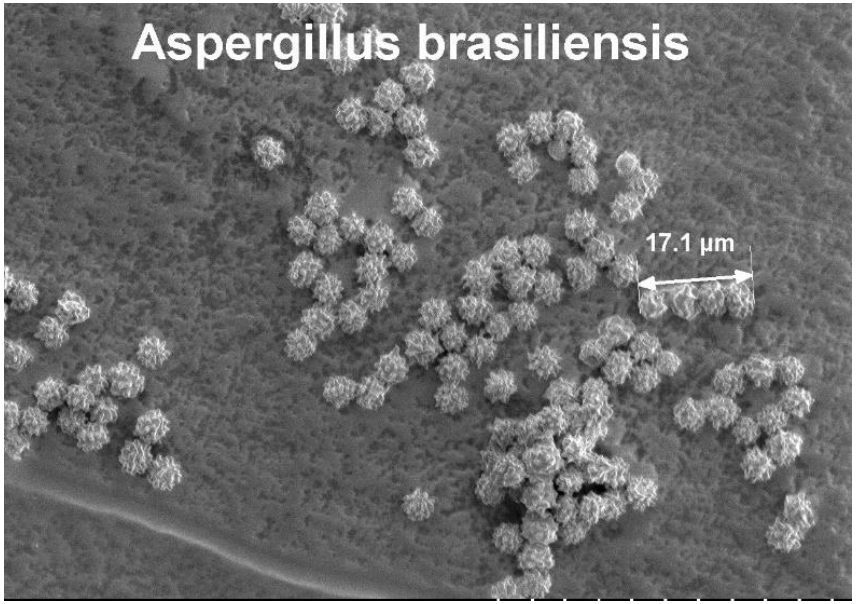
Common Causes of Failures

- Inoculum Problems
  - Making sure bacteria is at the right phase of growth
  - Clumping of the inoculum
  - Appropriate titers
    - Low titers
      - Desiccation impact
    - Too high can be unrepresentative
  - Hyphae elements in fungal suspension, isolate the fungal spores with fritted filter or glass wool (test spores and not mycelial mat)

Aspergillus brasiliensis



Courtesy Bruce Ritis



Common Causes of Failures

- 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:

Common Causes of Failures

- 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

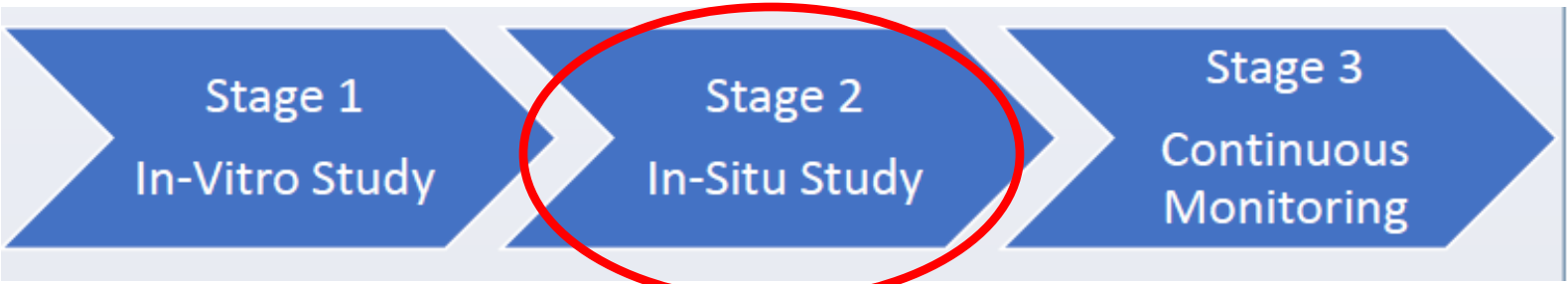
Disinfectant Efficacy Test (DET) Study Tips

- AOAC methods are inappropriate for this testing (but some elements can be of value)
- EN-13697 (2019) and ASTM E2197-17 offer valuable insight into quantitative surface testing
- USP 43 <1072> is useful in determining log reductions
- Combining physical removal and chemical kill in one study is not recommended
  - Test the disinfectant, not spraying or wiping efficacy
- Using a contract lab to perform testing sounds easy but still requires time, effort, and vigilance
- Smaller coupons allow for better enumeration
- Auditing the contract lab is very useful

Requalification of DET

- 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

In Situ Evaluations



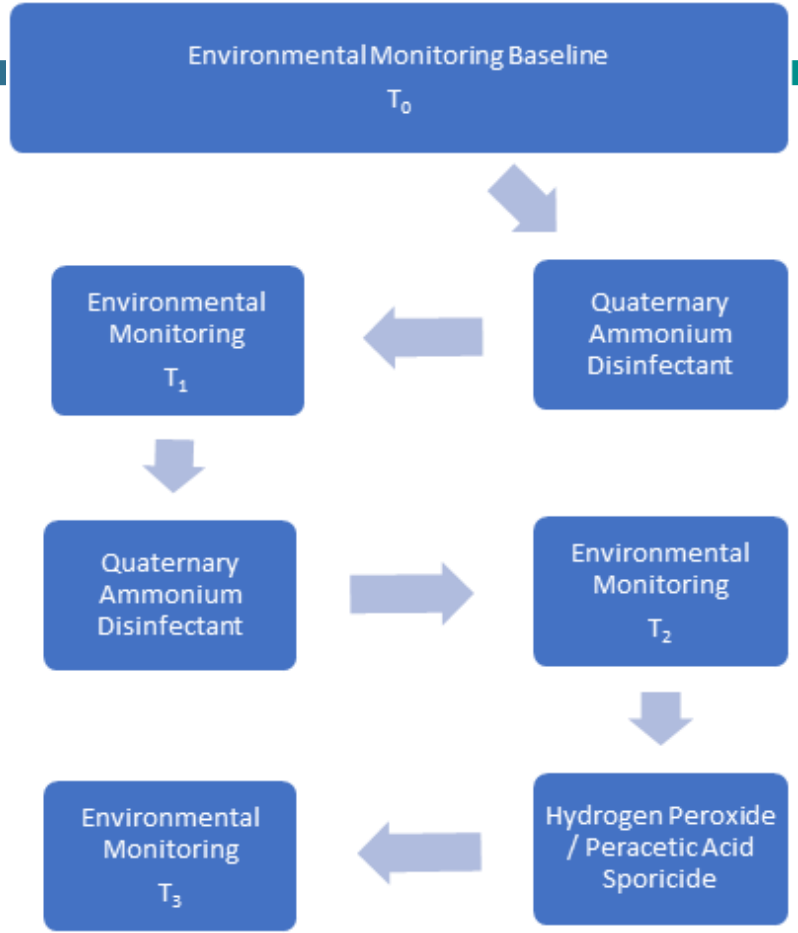
- In situ evaluation/triple clean
  - New cleanroom
  - At shutdown
  - After construction
  - After a power failure
  - After a worst-case event (e.g., a natural disaster)

Triple Cleaning

- No unified definition
- PDA TR "**Facilities should strongly consider having special start-up cleaning and disinfection programs in place following "shutdowns" or when significant construction is performed.**"
- 2X Disinfectant and 1X Sporicide

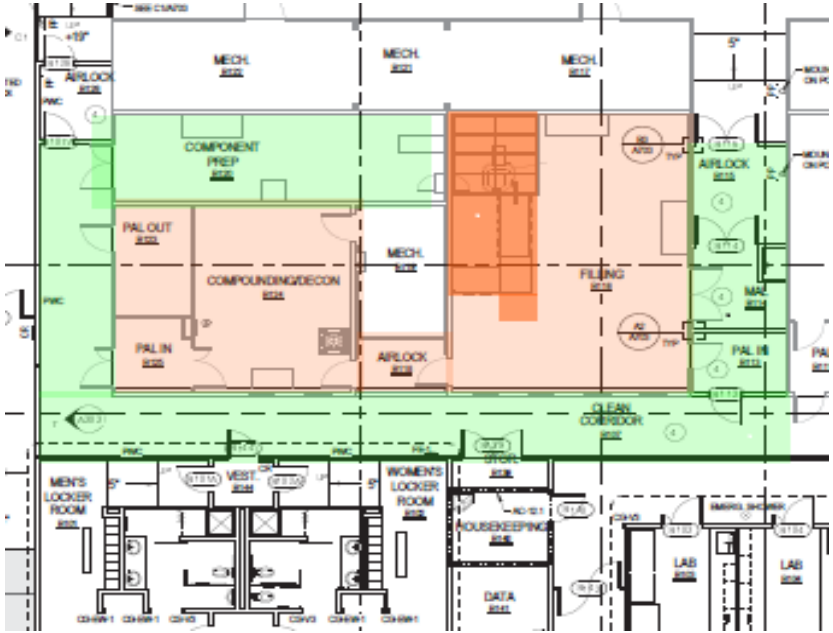
In Situ Evaluations

- Use actual cleaning procedure SOPs (update prior to validation study)
- “Worst-case” conditions
  - Higher microbial load
- Compare environmental data before and after procedures
- Should include data from more than one cleaning event



In Situ Evaluations

- During triple clean, sample critical areas of the facility
- The environmental monitoring can consist of RODAC® plating, settle plating, active air sampling and swabbing at these worst-case locations in the cleanrooms
- Microbiological data is never exact, but a positive observed trend can show that a simulated regimen of disinfectant/disinfectant / sporicide is effective under use conditions



In Situ Evaluations

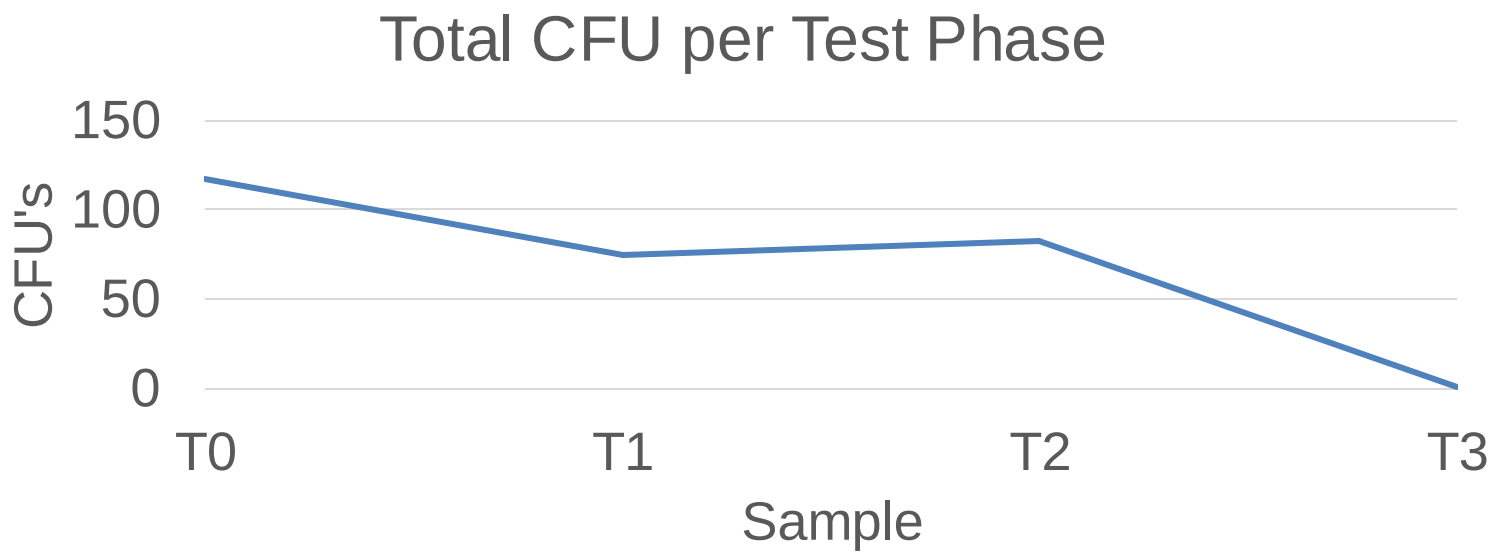
Summary: Sample Collection and Test Results													
Room	ISO Class	Samples				CFU count				CFU/Plate			
		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
B107 Clean Corridor	8	16	16	16	16	10	8	10	0	0.63	0.50	0.63	0.00
B120 Prep Room	8	12	12	12	12	9	5	6	0	0.75	0.42	0.50	0.00
B123 Material Entrance	7	5	5	5	5	0	0	0	0	0.00	0.00	0.00	0.00
B125 Aseptic Gowning	7	6	6	6	6	4	3	0	0	0.67	0.50	0.00	0.00
B124 Compounding Room	7	16	16	16	16	15	7	5	0	0.94	0.44	0.31	0.00
B118 Pass Through	7	6	6	6	6	4	2	3	0	0.67	0.33	0.50	0.00
B116 Filling Suite	7	19	19	19	19	24	30	21	1	1.26	1.58	1.11	0.05
B116 Laminar Flow Hood	5	3	3	3	3	2	0	0	0	0.67	0.00	0.00	0.00
B116 Behind Curtain	5	10	10	10	10	10	2	0	0	1.00	0.20	0.00	0.00
B116 Fill Machine	5	14	14	14	14	29	3	0	0	2.07	0.21	0.00	0.00
B116 Isolator Finger Tips	5	13	13	13	13	4	0	0	0	0.31	0.00	0.00	0.00
B114 Material Exit	7	6	6	6	6	0	0	0	0	0.00	0.00	0.00	0.00
B113 Personnel Exit	7	6	6	6	6	6	15	37	0	1.00	2.50	6.17	0.00
Overall	NA	132	132	132	132	117	75	83	1	0.89	0.57	0.62	0.01

Micro data is never perfect – outliers should be addressed

"The investigator theorized that the outlier could be a result of unauthorized foot traffic during the study."

Summary: Sample Collection and Test Results													
Room	ISO Class	Samples				CFU count				CFU/Plate			
		T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
B116 Behind Curtain	5	12	12	12	12	20	1	2	1	1.67	0.08	0.17	0.08
B116 Filling Suite	7	21	21	21	21	40	5	6	3	1.90	0.24	0.29	0.14
B118 Air Lock	7	6	6	6	6	5	1	3	1	0.83	0.17	0.50	0.17
B124 Compounding Room	7	18	18	18	18	17	18	1	2	0.94	1.00	0.06	0.11
B125 Personnel Entrance	7	6	6	6	6	11	13	1	27	1.83	2.17	0.17	4.50
B123 Material Entrance	7	5	5	5	5	4	2	0	0	0.80	0.40	0.00	0.00
B120 Component Prep	8	13	13	13	13	21	18	18	4	1.62	1.38	1.38	0.31
B113 Personnel Exit	8	6	6	6	6	10	2	0	0	1.67	0.33	0.00	0.00
B114 Material Exit	8	6	6	6	6	2	1	3	0	0.33	0.17	0.50	0.00
B107 Clean Corridor	8	18	18	18	18	55	51	47	4	3.06	2.83	2.61	0.22
Fill Machine	5	20	20	20	20	11	4	0	0	0.55	0.20	0.00	0.00
Isolator Glove Fingertips	5	13	13	13	13	1	0	0	0	0.08	0.00	0.00	0.00
Overall	NA	144	144	144	144	197	116	81	42	1.37	0.81	0.56	0.29

In Situ Evaluations



- Provides excellent in situ data mimicking a disinfection program

Industry Guidance

- Points to remember are that disinfectants are less effective against the higher numbers of microorganisms used in laboratory challenge tests than they are against the numbers found in clean rooms
- new verbiage from the not-yet-released revised USP <1072>: “Qualifying the effectiveness of cleaning and disinfection processes in situ is a more reliable and accurate measure of the cleaning and disinfection program’s effectiveness than surface challenge tests. Qualification studies demonstrate that the cleaning and disinfection procedures performed by trained operators will consistently result in the required particulate and microbial cleanliness that returns the facility to its classified state as suitable for the intended use.”

In-Situ Field Trial

"From my perspective, I am not opposed to *in-situ* field trial data and being mindful of "most challenging" conditions (my term to supplant the use of term worst case), which may in fact demonstrate, perhaps a bit more robustly, the disinfectant cleaning evaluation / validation.

I would offer if I was evaluating an *in-situ* disinfectant studies and the data supporting the scientific conclusions that the disinfectants were acceptable, I would hard pressed to point out that the *in-situ* study was not acceptable. Through the years, many have heard me say, ad nauseum, if the scientific data support the scientific conclusion, there shouldn't be a concern. Ultimately, it comes down to the support data."

Thomas Arista 7/21/2022

Disinfectant Testing and Test Methods

Supplier / Registration Testing

- US Environmental Protection Agency
- Europe and Rest of World

Qualification Testing

- Disinfectant Efficacy Testing (DET)
- In situ evaluations

Thank You!

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