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Disinfectant Efficacy Testing and Validation

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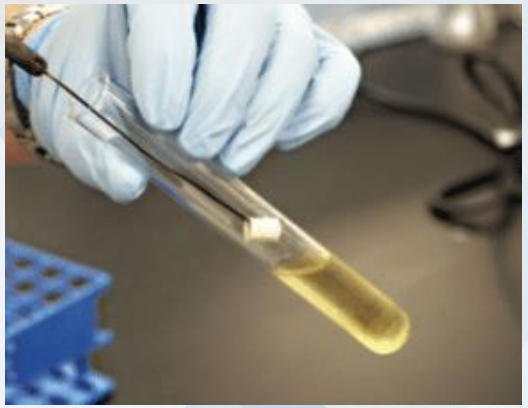
Outline

- Microbial Control
- Standards and Methods
- *In vitro* Methodology Considerations
  - Microorganisms
  - Substrates/Test Surfaces
  - Disinfectants
  - Neutralization
- General Coupon Study Outline

Microbial Control

In vitro testing – artificial environment or laboratory testing

- Disinfectant manufacturers are required to register with EPA using AOAC methodology
- Suspension testing
- Carrier/Coupon testing, Disinfection Validation
- No one method is universally used or required



In situ testing – in natural or original position

- Actual cleaning procedures
- Before and after institution of new regimen
- Following shutdowns
- Construction
- Is it actually working

Environment monitoring

- Data trending
- Identification of microorganisms

Standards and Methods

USP <1072>

- 2 log (spores) and 3 log (everything else)

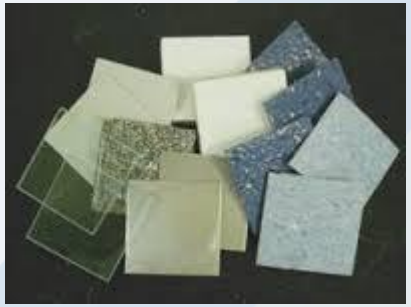
PDA Technical Report 70

ASTM

- Time Kill Method (E2315)
- Sanitizer Method (E1153)
- Quantitative Carrier Method (E2111 & E2197)
- Biofilm Method (E2562 and E2871)
- Standard Guide for Evaluation of Cleanroom Disinfectants (E2614)

EN

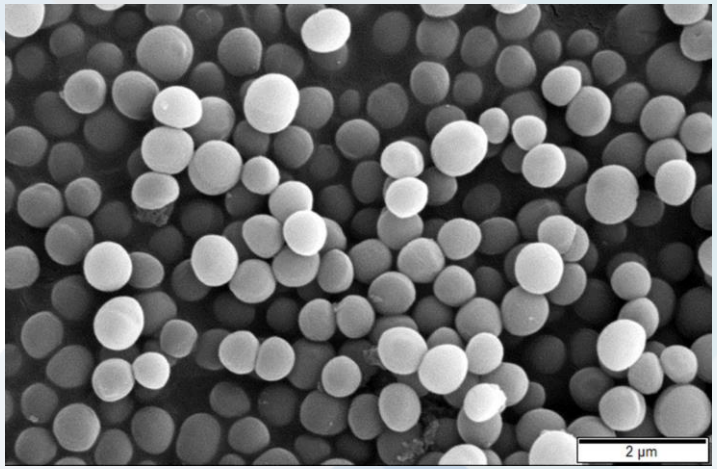
- 1040 – Bacterial suspension test
- 1276 – Bacterial suspension test
- 1650 – Fungal suspension test
- 13704 – Sporicidal suspension test
- 13697 – Carrier test



Microorganism Selection

ATCC microorganisms could be included, but environmental isolates must be included

- Gram positive rod (spore former)
- Gram positive rod (non-spore forming)
- Gram positive cocci
- Gram negative
- Yeast
- Mold (spore formers/conidia)



Risk-based Matrix approach

- Most commonly recovered organisms
- Location
- Most Resistant Organisms (MRO)
- Resistance profile based on disinfectant selection

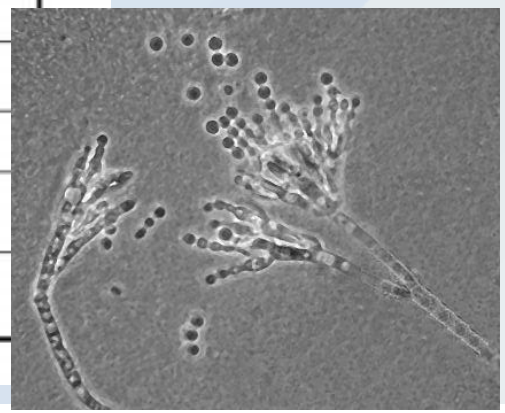
Testing Considerations

- Drying time
- Organism preparation (Mold and bacterial spores)
- Inoculum concentration relative to acceptance criteria

Hierarchy of Resistance

	Microorganism	Examples
More Resistant	Prions	Scrapie, Creutzfeld-Jacob disease, Chro 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

Aspergillus brasiliensis
Aspergillus fumigatus
Stachybotrys chartarum
Penicillium chrysogenum
Trychophyton mentagrophytes



* McDonnell G. Antisepsis, disinfection and sterilization: types, action and resistance. Washington, DC: ASM Press; 2007.

Microorganism Selection

Table 1 – USP <1072>

Types of Microorganisms	Examples
Bacterial spores	*Bacillus subtilis* and *Clostridium sporogenes*
Mycobacteria	*Mycobacterium tuberculosis*
Nonlipid-coated viruses	Poliovirus and rhinovirus
Fungal spores and vegetative molds and yeasts	*Trichophyton*, *Cryptococcus*, and *Candida* spp.
Vegetative bacteria	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* spp.
Lipid-coated viruses	Herpes simplex virus, hepatitis B virus, and HIV

Substrates/Test Surfaces

Cleanroom disinfectant validations – representative materials

- Risk based as well (worst case, most contaminated, commonly touched)
- Coupon Size
- Recovery off surfaces
- Surface Conditions and Preparation

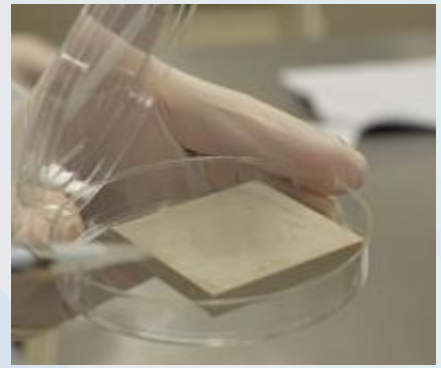
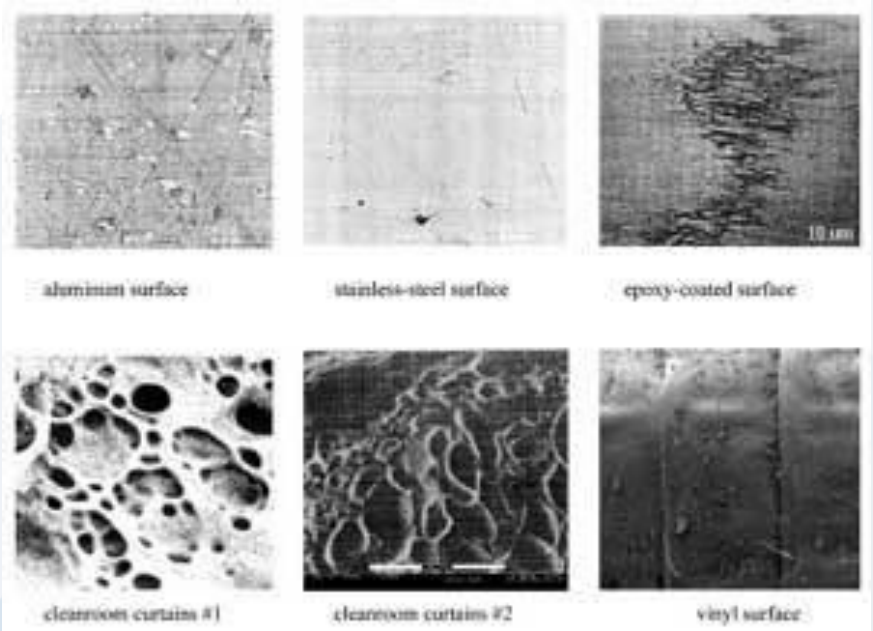


Table 6 – USP <1072>

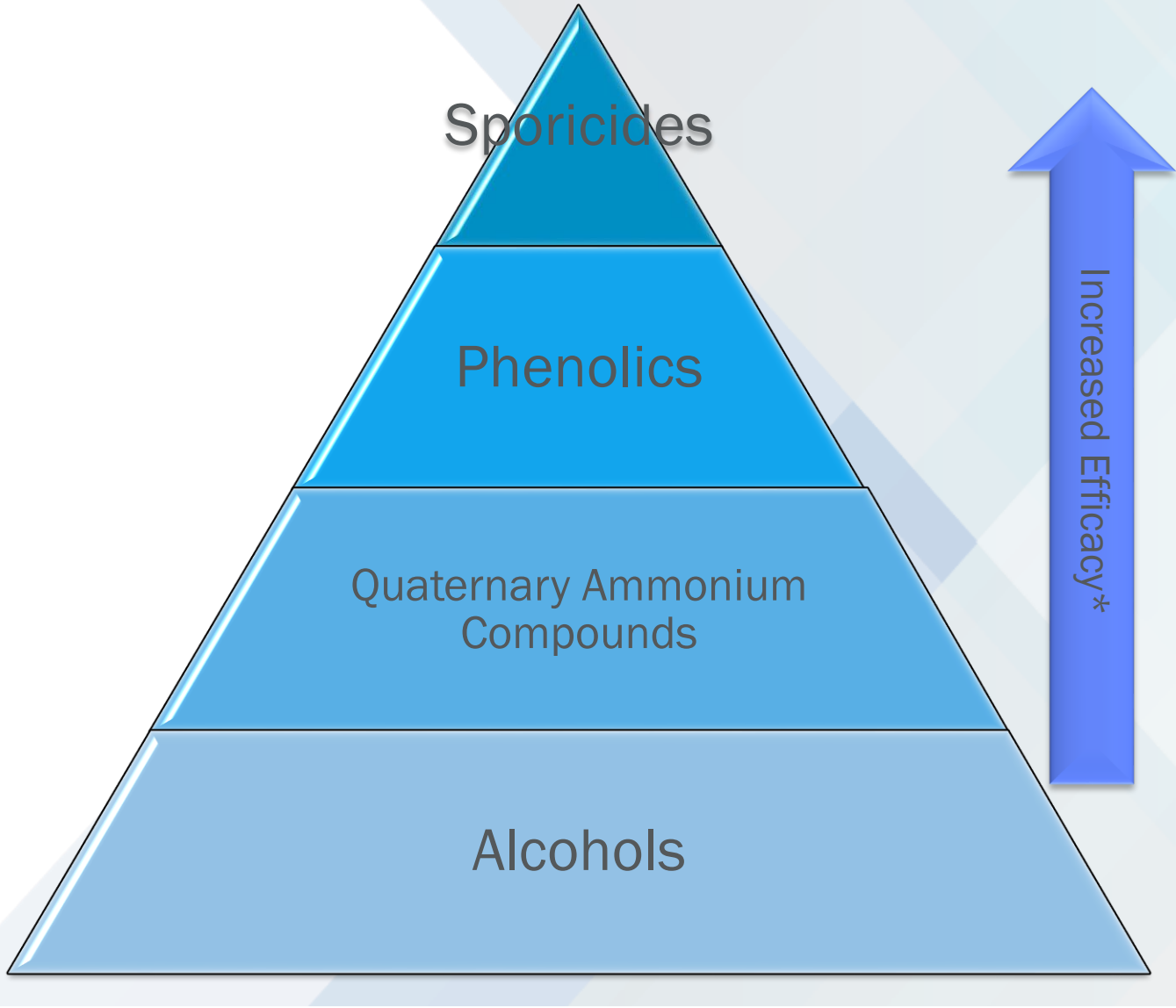
Material	Application
Stainless steel 304L and 316L grades	Work surfaces, filling equipment, and tanks
Glass	Windows and vessels
Plastic, vinyl	Curtains
Plastic, polycarbonate	Insulation coating
Lexan® (plexiglass)	Shields
Epoxyl-coated gypsum	Walls and ceilings
Fiberglass-reinforced plastic	Wall paneling
Tyvek®	Equipment Wraps
Terazzo tiles	Floors



*Vellutato, "Developing compliant and effective cleaning and disinfection methodologies in GMP controlled environments"

Disinfection Selection

- Temperature
- Contact time
- Concentration (or RTU)
- Use-life
- Sterile offering
- Organic matter
- Water Quality
- Safety (PEL/STEL)
- Corrosive to materials
- Surfactancy
- Rotation



Disinfection Selection

USP <1072> – Table 2

Chemical Type	Classification	Example
Aldehydes	Sporicidal agent	2% Glutaraldehyde
Alcohols	General purpose disinfectant, antiseptic, antiviral agent	70% IPA
Chlorine and NaOCl	Sporicidal agent	0.5% Sodium Hypochlorite
Phenolics	General purpose disinfectant	500 µg per g Chloroscresol, 500 µg per g chloroxylenol
Ozone	Sporicidal agent	8% Gas by weight
Hydrogen Peroxide	Vapor phase sterilant, liquid sporicidal agent, antiseptic	4 µg per g vapor, 10%-25% solution, 3% solution
Substituted diguanides	Antiseptic agent	0.5% Chlorhexidine gluconate
Peracetic acid	Liquid sterilant, vapor phase sterilant	0.2% Peracetic acid, 1 µg per g peracetic acid
Ethylene oxide	Vapor-phase sterliant	600 µg per g Ethylene oxide
Quaternary ammonium	General purpose disinfectant, antiseptic	Concentration dependent on application, Benzalkonium chloride
β-Propiolactone	Sporicidal agent	100 µg per g β-Propiolactone

Neutralization Scheme/Design

Neutralizing Agent – An agent that inhibits the antimicrobial properties of a disinfectant/sporicidal agent without impairing the recovery of viable microorganisms

- Physical dilution (ratio) and chemical inactivation (broth and agar)
- Do not want static or -cidal activity after the contact time.
- The neutralization control should be performed in the same ratio and mimic the study design with an appropriate contact time.

Components that should be included in the Neutralization Control

- Neutralization baseline (inoculum)
- Neutralization toxicity
- Neutralization test
- All organism and all actives

Acceptance Criteria

- Neutralization inoculum <100 CFU recovered
- Neutralization recovery of 70%

Neutralizer Examples

Disinfectant	Neutralizing Agent
Alcohols	Dilution or polysorbate 80
Glutaraldehyde	Glycine and sodium bisulfite
Sodium hypochlorite	Sodium thiosulfate
Chlorhexidine	Polysorbate 80 and lecithin
Mercuric chloride and other mercurial	Thioglycolic acid
Quaternary ammonium compounds	Polysorbate 80 and lecithin
Phenolic compounds	Dilution or polysorbate 80 and lecithin
Hydrogen peroxide	Catalase

General Coupon Study Outline

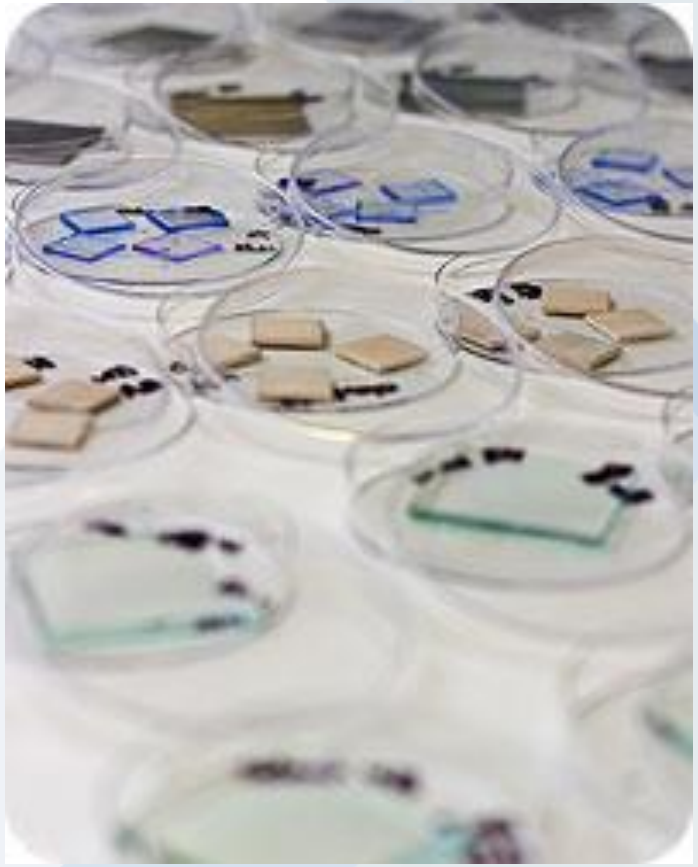
1. Inoculate chosen coupon with the appropriate **volume and titer** of challenge organisms and then **dry coupons** according to method chosen (perform testing in triplicate).
2. Apply test product according to situation and/or method chosen (spray, immerse, **cover**).
3. Just before **contact time** has elapsed, **"remove"** the coupon from the test product and at the elapsed contact time place the coupon into a tube containing an appropriate **chemical neutralizer**.
4. **Sonicate and vortex** to recover organisms from coupon surface. (other recover methods including swab method and contact plates).
5. Perform serial dilutions in order to capture a countable number of organisms.
6. Plate 1 mL from each dilution tube into a sterile labeled Petri dish.
7. **Pour plate** using the appropriate agar media for growth and additional neutralization if needed.
8. Incubate plates for appropriate time and temperature before reading.

Note: There are quantitative and qualitative methodologies

In vitro Methodology Considerations

Estimate the *in vitro* efficacy when reproducing surface disinfection conditions include:

- Worst Case
- Microorganisms
- Substrates/Test Surfaces
- Disinfectants
- Neutralization
- Frequency of Disinfection
- Frequency of Testing



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Thank You!

Questions?