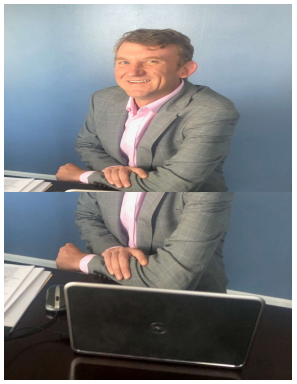
STERIS
Life Sciences

CONTAMINATION CONTROL IN CLEANROOM MANUFACTURING

Dan Klein, Senior Manager, Technical Services

Biography



Dan Klein

Sr. Manager, Technical Services
STERIS Corporation

Dan has over two decades of industry experience, including working in research and development for microbiology and clinical affairs. He has also managed a contract testing laboratory. Dan holds a master's degree in biology and a bachelor's degree in microbiology

At STERIS Life Sciences, Dan provides technical expertise to Customers and helps troubleshoot and solve contamination issues. He supports Customers with a variety of critical processes and general understanding of the latest developments in the industry. He frequently presents data and other information at international industry meetings and sponsored events.

Throughout his professional experience, Dan has authored numerous industry articles and book chapters in peer-reviewed journals related to disinfection and sterilization.

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CLEANROOM CONTAMINATION CONTROL

Best Practices for Contamination Control

- Preventing ingress or egress of problematic microorganisms

Contamination Control Challenges

- Mold and Bacterial Spore Excursions
- Biofilm and Biofilm remediation

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CONTAMINATION CONTROL

ISO 14644-1 Cleanroom Standards

Class	Maximum Particles/m³						FED STD 209E equivalent	EU
	>=0.1 µm	>=0.2 µm	>=0.3 µm	>=0.5 µm	>=1 µm	>=5 µm		
ISO 1	10	2						
ISO 2	100	24	10	4				
ISO 3	1,000	237	102	35	8		Class 1	
ISO 4	10,000	2,370	1,020	352	83		Class 10	
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100	A/B
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1,000	
ISO 7				352,000	83,200	2,930	Class 10,000	C
ISO 8				3,520,000	832,000	29,300	Class 100,000	D
ISO 9				35,200,000	8,320,000	293,000	Room Air	

Pharmaceutical Cleanrooms

What is ISO 8 cleanroom classification? Cleanroom Technology. Nov, 2021

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CONTAMINATION CONTROL

USP 43 <1116> Suggested Initial Contamination Recovery Rates in Aseptic Environments

Microbial recommendations for different ISO classes can vary slightly

Environmental Monitoring is key to maintaining microbial control

Frequency and types of disinfectants and sporicides will vary based on ISO classification

	Air sample (%)	Settling plates 90mm (4 hrs) (%)	Contact plates 55mm (cfu/plate)	Glove print 5 fingers (cfu/glove)
Isolator/ RABS	<0.1	<0.1	<0.1	<0.1
ISO 5	< 1	< 1	< 1	< 1
ISO 7	< 5	< 5	< 5	< 5
ISO 8	< 10	< 10	< 10	< 10

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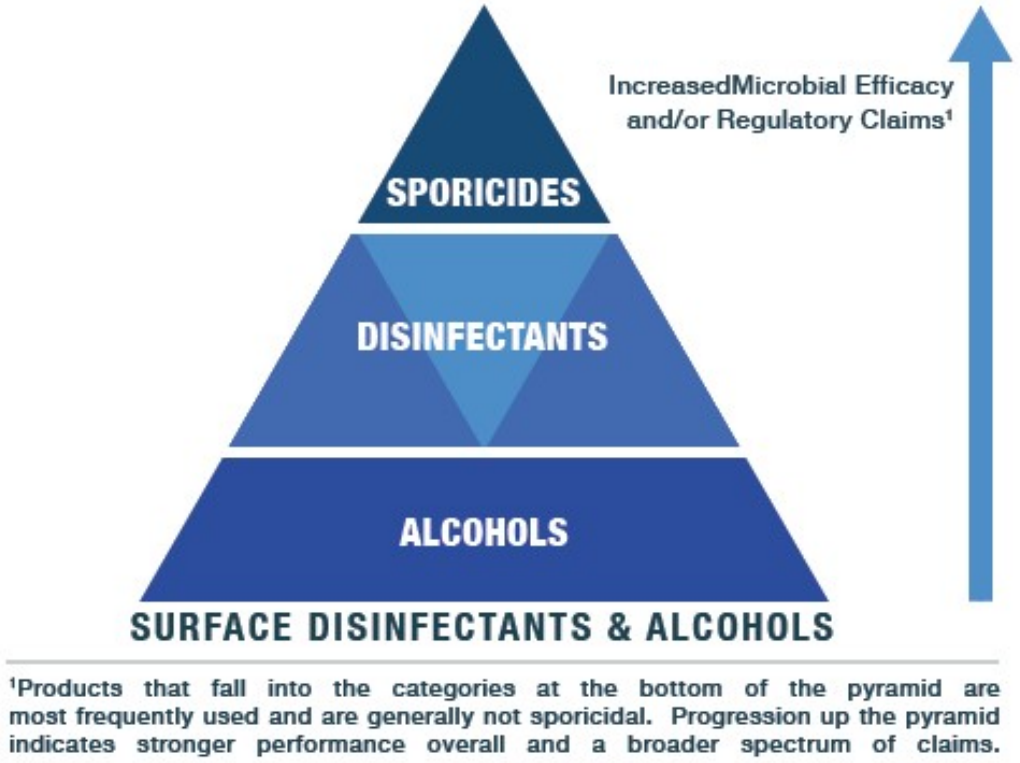
CONTAMINATION CONTROL

- EPA Classifications
  - Sterilizer (Sporicide)
    - Proper use results in 100% kill of all microorganisms, including bacterial endospores (*B. subtilis*, *C. sporogenes*)
    - 6 Log reduction
  - Disinfectant
    - Proper use results in 100% kill of vegetative bacteria, target viruses and target fungi
  - Sanitizer
    - 3-Log reduction Non-Food Contact Surfaces



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CONTAMINATION CONTROL

Should be risk based and data driven based on current best practices

- Pre-cleaning – None, unless there is excessive soil
- Disinfectant – Should be able to handle some soil and have broad spectrum efficacy
- Sporicide – Based on Environmental Monitoring data
  - Use for pass-through items
- Rinsing – Goal is Visually Clean
  - WFI or Alcohol on a routine basis, based on experience or risk assessment
  - Cleaners can be used for buildup or after shutdowns

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CONTAMINATION CONTROL

BEST PRACTICES FOR A DISINFECTION PROGRAM

- Rotation of 1 Disinfectant and 1 Sporicide
- “pharmaceutical & biotechnology industries have moved away from the rotation of 2 disinfecting agents. This formerly common practice led to high residue levels and subordinate efficacy performance…The rotation of a disinfectant with a sporicide is superior to the use of rotations of multiple disinfectants.” (PDA TR No. 70)
- Frequency of use dependent on ISO Classification

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CONTAMINATION CONTROL

	Daily	Weekly	Monthly	Yearly
Controlled Area				
Floors	X	X		
Ceilings				X
Walls			X	
Fixtures/Equipment			X	
Class 100,000 (ISO 8)				
Floors	X			
Ceilings				X
Walls			X	
Fixtures/Equipment		X	X	
Class 10,000 (ISO 7)				
Floors	X			
Ceilings			X	X
Walls		X		
Fixtures/Equipment	X			
Class 100 (ISO 5)				
Floors	X			
Ceilings	X			
Walls	X			
Fixtures/Equipment	X			

CONTAMINATION CONTROL

Grade A (ISO 4.8) or B (ISO 5 at rest, ISO 7 in operation)

ISO 5 requires frequent decontamination using sterile disinfectants and sporicides for use

Surface	Method	Cleaning Agent	Frequency	Rinse
External Hoods • Back, Sides, Top	Wipe	Sterile disinfectant with surfactant	Daily	Sterile WFI or 70% IPA as needed to remove residue buildup
• Door, Sliding Panel	Wipe	Sterile disinfectant with surfactant	Daily	
		Sterile Sporicide	Weekly or in response to microbial monitoring	
Inside Hood or Curtain • Work Surface • Sidewalls • Apparatus/Critical Surfaces	Wipe	Sterile disinfectant with surfactant	Daily, preuse and postuse	
		Sterile Sporicide	Weekly or in response to microbial monitoring	
• Curtains	Wipe or Mop	Sterile disinfectant with surfactant	Daily	
		Sterile Sporicide	Weekly or in response to microbial monitoring	
Adjacent Flooring and Walls	Mop	Sterile disinfectant with surfactant	Daily, between lots and shifts	
		Sterile disinfectant with surfactant followed by a sterile sporicide, as necessary	Weekly or in response to microbial monitoring	

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CONTAMINATION CONTROL

Grade C (ISO 7 at rest, ISO 8 in operation)

Surface	Method	Cleaning Agent	Frequency	Rinse
Floors • Normal Traffic Paths	Mop	Disinfectant with surfactant	Daily after transfers	As needed to remove residue buildup
• Proximity to Open Process or Transfer Areas		Disinfectant with surfactant followed by a sporicide	Weekly or monthly, if necessary	
Walls • General	Wipe or Mop	Disinfectant with surfactant followed by a sporicide, if necessary	Weekly or monthly	
• Door Plate		Disinfectant with surfactant	Daily	
Equipment • Shelving • Portable Tanks • Processing Items	Spray or Wipe	Disinfectant with surfactant	Before and after use	
• Carts (wheels)		Sporicide		
Other Surfaces • Furniture	Spray or Wipe	Disinfectant with surfactant	Daily	
• Chair (wheels)		Sporicide		

CONTAMINATION CONTROL

Grade D (ISO 8 at rest)

Surface	Method	Cleaning Agent	Frequency	Rinse
Floors • Around Drains • Foot Traffic Paths • Spill Areas • Access Ports	Mop	Disinfectant with surfactant	Daily at shutdown, between process changeover	Not necessary after each application†
Walls, Ceilings • General	Wipe or Mop	Disinfectant with surfactant	Monthly	Not necessary after each application†
• Doors, Handles, High-Traffic Areas	Wipe or Mop	Disinfectant with surfactant	Daily	
Equipment • Adjacent to Access Port	Spray or Wipe	Disinfectant with surfactant	Daily during processing	As needed to remove residue buildup
• Surface Upstream Airflow Path to Process Opening			Weekly	
Other Surfaces • Sinks • Benches • Trash Containers	Wipe	Disinfectant with surfactant	Daily	Not necessary after each application†

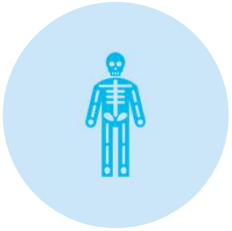
A sporicidal agent must be used quarterly, semi-annually or as needed in response to microbial monitoring.[5,6]

† Any contamination control program should incorporate a residue removal component. See the Residue Removal Section for details.

CNC (Controlled, Not Classified) Area Cleaning Frequency

Hallways and Floors --- Mop daily ---Rinse as needed

Walls and Ceilings--- Mop monthly—Rinse as needed



Equipment (carts, racks, trash receptacles, etc.)---Wipe weekly---Rinse as needed

Rinsing is based on visual observation and safety

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CLEANROOM CONTAMINATION CONTROL

Best Practices for Contamination Control

- Preventing ingress or egress of problematic microorganisms

Contamination Control Challenges

- Mold and Bacterial Spore Excursions
- Biofilm and Biofilm remediation

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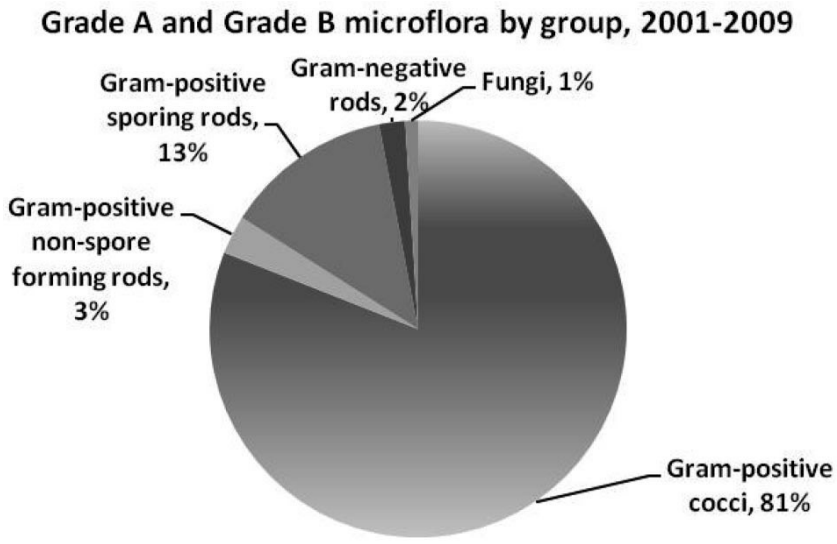
Hot Topics: Mold and Bacterial Spore Excursions

- Hard to Kill Microbes
  - Bacterial Spores
  - Fungi

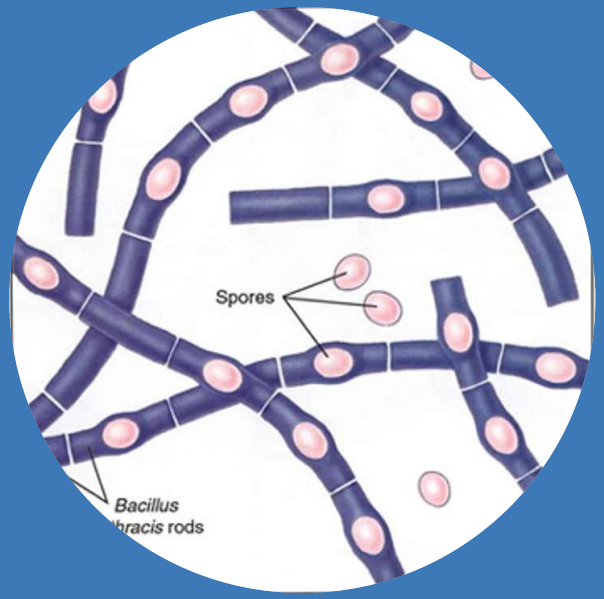


T. Sandle, PDA J Pharm Sci and Tech 2011, 65:392-403
A Review of Cleanroom Microflora: Types, Trends, and Patterns

	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

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

Hot Topics: Bacterial Spore Excursions



Sources: Carried into cleanrooms via Materials, Equipment, Personnel

- Indicative of weak transfer procedures, pass-thru items imperfect aseptic controls (barriers, HVAC, seals, pressure differentials)

- Typically found in the soil

Highly Resistant to Disinfection

Hot Topics: Bacterial Spore Excursions

- All spores are hard to kill
  - Some harder than others
- Require use of a sterilant / sporicide
  - Contact time is essential
- Strains can vary in susceptibility

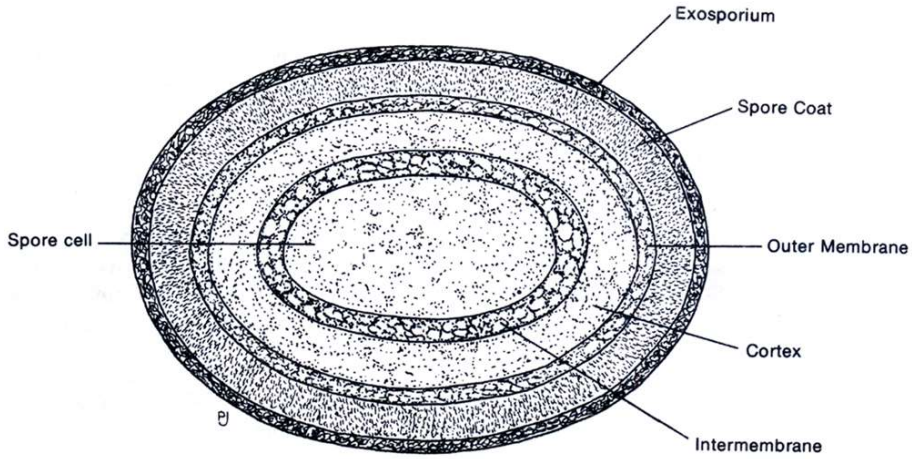
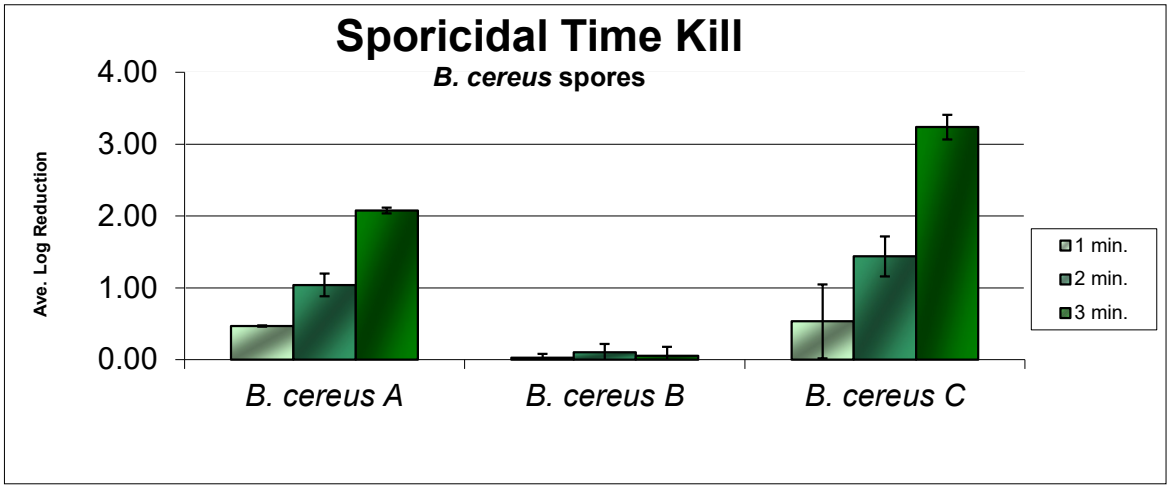
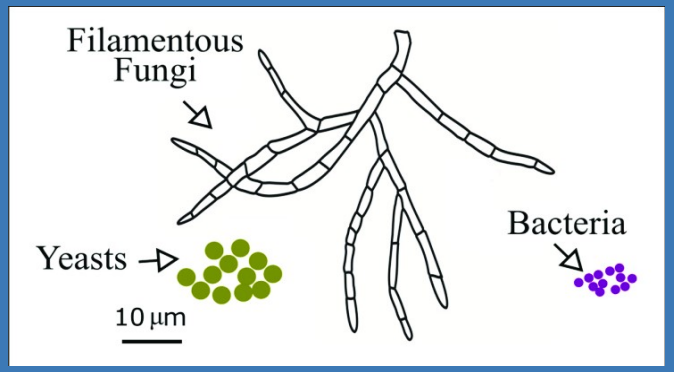


Fig. 8.1. Endospore



Hot Topics: Mold Excursions



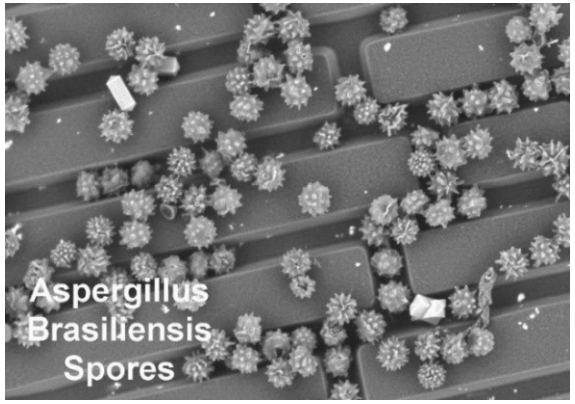
Fungi spread easily through air

Species that produce fungal spores are harder to kill

- *A. brasiliensis*
- *Chaetomium globosum*

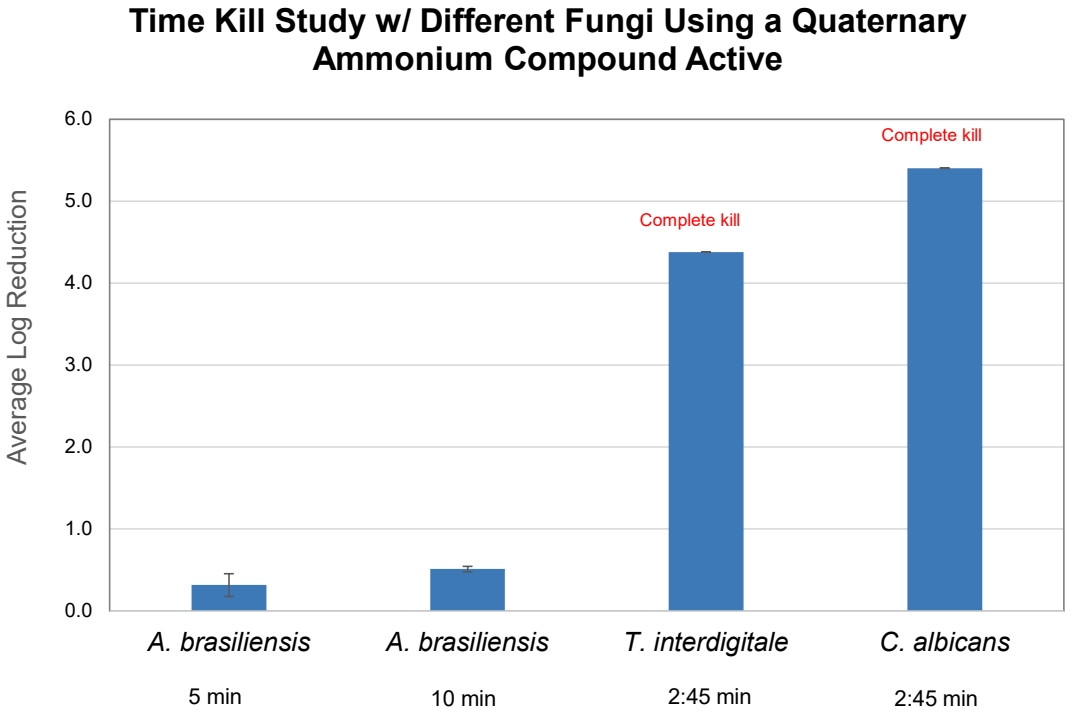
Reservoir / Source

- Drywall
- Cardboard
- Wood
- Refrigeration conditions



Hot Topics: Mold Excursions

- Fungal spores can be challenging to disinfect
  - Formulation matters
- *T. mentagrophytes* is the fungi used to make fungicidal claims
- May require a sporicide even with fungicidal disinfectants



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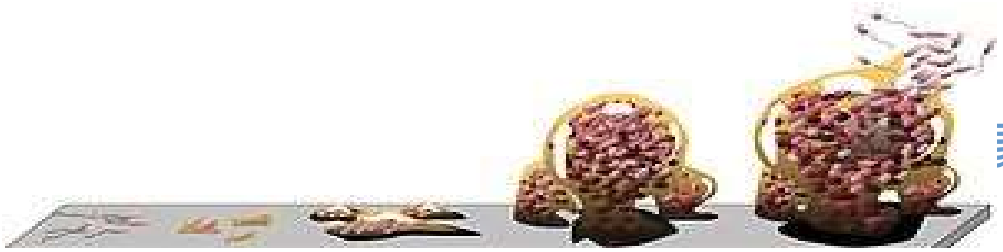
Hot Topics: Biofilm and Biofilm remediation

- Microorganisms do not typically exist as single cells
  - Biofilm as a survival mechanism
  - Reported to be 10X – 100X more resistant to disinfection
- Extracellular Polymeric Substance (EPS) is a slimy matrix that consists of polysaccharides, proteins, nucleic acid and lipids to aid survival
- EPS can be harder to clean than process residues
- Residual organic material can reduce efficacy of biocides
- Residual EPS can reduce penetration of biocides
- Removal of EPS prior to sanitization or disinfection is critical



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Hot Topics: Biofilm and Biofilm remediation

- Two-Step Process
  - Cleaning of the vessel with 5% Alkaline Cleaner at 60C
  - Then sporicidal soak with a 3% solution of a concentrated Peracetic acid / Hydrogen peroxide sporicide
- Look for any design or system engineering flaws
  - Eliminate the possibility of any dead legs and any leg where turbulent flow is not confirmed
  - Confirm the slope of the piping is conformed to engineering best practice. i.e. slope verification audit
  - Eliminate the possibility of any non-turbulent pressure valves such as solenoid valves where liquid is stagnant
- Look for any damaged materials or surfaces that may aid in biofilm formation
  - Rouge or rouged surfaces
  - Damaged valves or membranes

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CONCLUSIONS

- Delivering safe and effective products requires a well-designed cleanroom contamination program
- Pharmaceutical, Medical Device and Biopharma industries continue to evolve and grow with new innovations and new regulations
- Rapid Growth in ATMP (advanced therapeutic medicinal products) and vaccine industries creates training challenges and opportunities
- New Regulations include EU GMP Annex 1: Manufacture of Sterile Medicinal Products which require a holistic Contamination Control Strategy (CCS)
- Challenges remain, including mold and bacterial spore excursions and biofilm
- Many similarities exist in the production of safe, unadulterated pharmaceutical products and the needs for safe, unadulterated space missions

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Questions?

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