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Why Disinfectant Qualification Studies Fail?

PDA, San Diego
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About the Speaker

Ziva Abraham is the CEO of Microrite Inc. consulting and has over 30 years of academic, research, clinical and industrial experience in microbiology, and quality assurance. Ziva has received her Master's Degree in microbiology with a focus on Mycology and has conducted research on developing microbial Insecticides using entomogenous bacteria and fungi for her PhD degree. Her career also includes founding and managing clinical laboratories for Maccabi Medical in Israel. She has trained personnel from various industries in microbiology techniques and methods. She founded Microrite consulting to address the varied contamination issues she was witnessing in the industry.

Plan of Attack on Planktonic Microbes

Know your enemy before you attack!

Confusion with Definitions

Sporicide: Chemical agent that kills all microorganisms including bacterial and fungal spores

Disinfectant: Chemical agent that kills microorganisms but not necessarily spores

Germicide: Chemical agent that kills microorganisms

Bactericide: Chemical agent that kills bacteria but not necessarily spores

Fungicide: Chemical agent that kills fungi

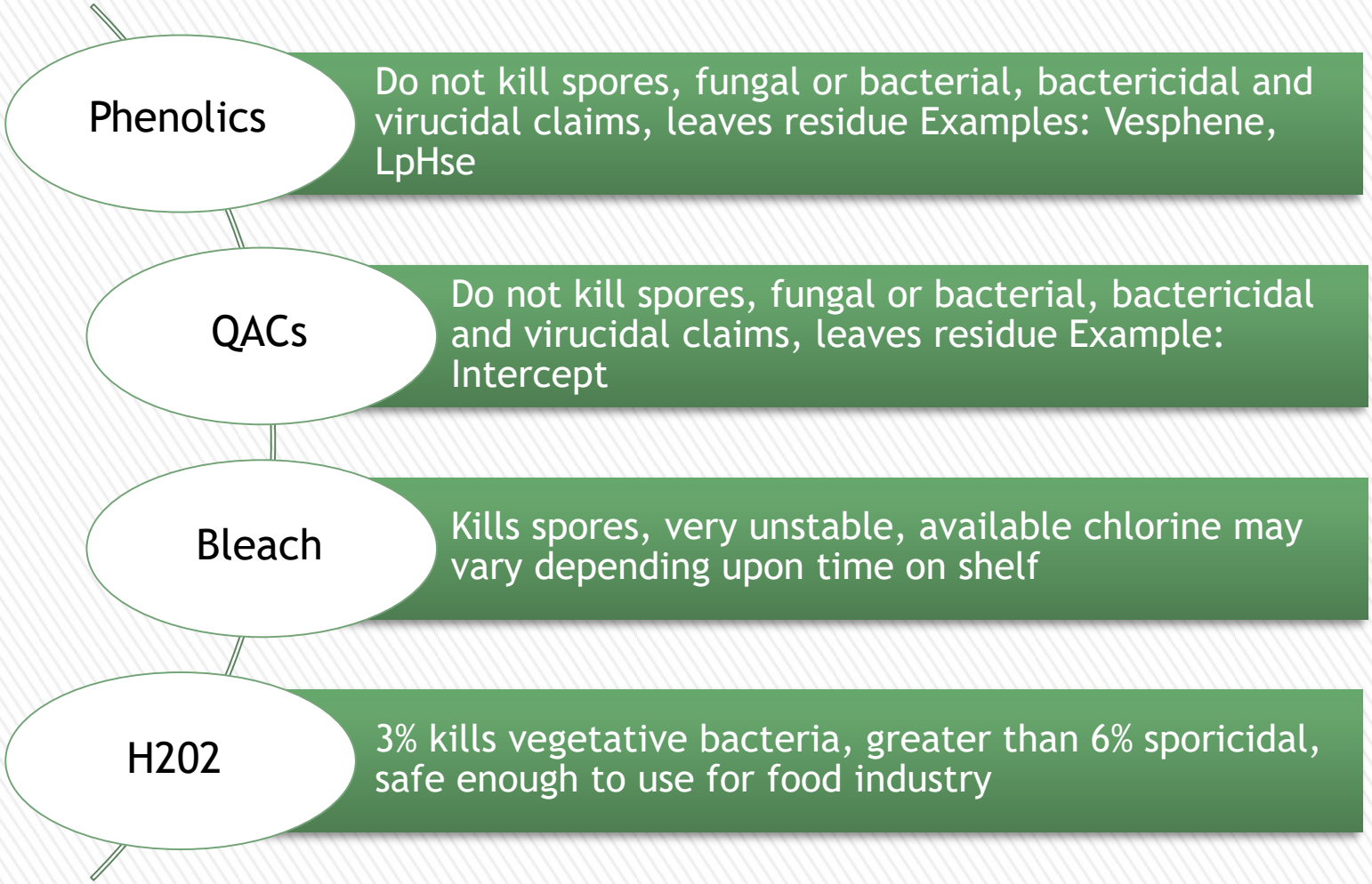
Virucide: Chemical agent that kills viruses

Cleaning agent: Agent with surfactant

Surfactant: Agent that breaks surface tension

Sanitizer: Reduces the number of microbes

Knowledge Gap



Knowledge Gap

PAA Chemistries

Mixture of peracetic acid and H2O2 forms a potent sporicidal agent

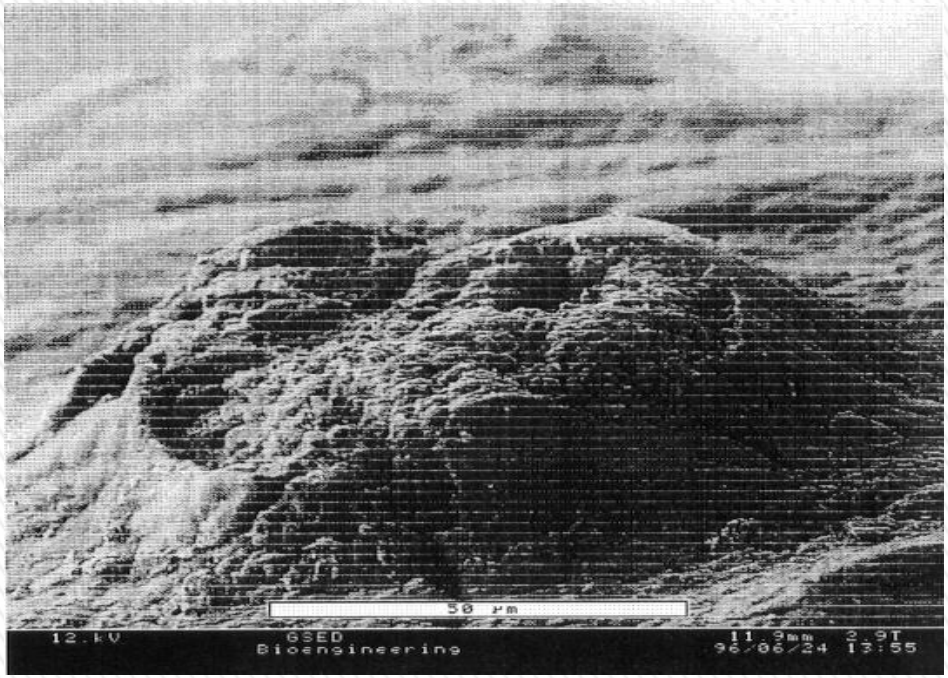
Aldehydes

Formaldehyde and Gluteraldehyde potent sporicides, highly carcinogenic

Alcohol

Sanitizer, 30% water required to seep through cell wall barrier and disrupt cell contents, some bacteria may use it as carbon source (pseudomonads)

Phenolic residue



Regulatory Bodies

Regulatory Bodies

- Disinfectants are classified by FDA, EPA,AAMI and CDC mainly for medical device industry
- OSHA oversees the health and safety aspects
- Disinfectants used for hard surfaces are registered with EPA

Required testing before marketing disinfectants:

- Simulated Use Testing
- Actual Use Testing
- Biocompatibity data-evaluation of residue
- Toxicity data
- Material Compatibility Data
- Chemical Indicator

Testing for Manufacturers

AOAC Methods

Disinfectants must pass the tests described in the AOAC Manual in order to be approved by the Environmental Protection Agency.

AOAC Tests Include:

- Spray Products
- Phenol Coefficient
- Use Dilution
- Hard Surface Carrier
- Tuberculocidal Activity
- Sporicidal Activity
- Fungicidal Activity

What Organisms are tested for label claim?

T. mentagrophytes for fungicidal claim
EU methods require a mold *A. niger* and a yeast *C. albicans*

AOAC for bacterial is *S. cholerasuis*, *S. aureus* and *P. aeruginosa* also common for many different regions and agencies

AOAC for sporicidal is an aerobic *B. subtilis* and an anaerobic *C. sporogenes*

AOAC for tuberculocidal is *M. bovis*; *M. smegmatis* can be used as a presumptive test but its resistance is much weaker to germicides than *M. bovis* or *M. terrae*

AOAC for virucidal is not set, they recommend following the EPA method which generally uses HBV, HCV, Herpes simplex, HIV and influenza

FDA uses polio virus II and an enveloped virus one selects while EU calls for polio virus I and adenovirus V

Why qualify disinfectants if they are already tested?

Label claim testing does not address all microorganisms found in cleanrooms

Cleanroom flora much diverse

Clinically relevant organisms such as *S. aureus*, *Salmonella* or *Trichophyton* not commonly encountered in cleanrooms

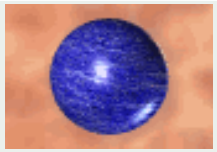
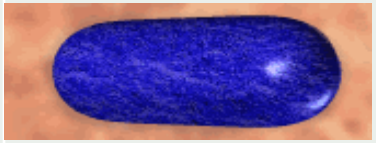
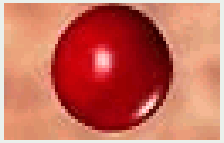
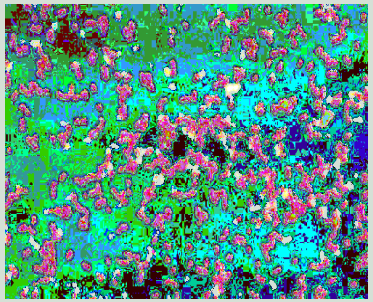
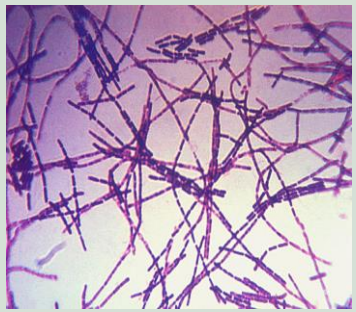
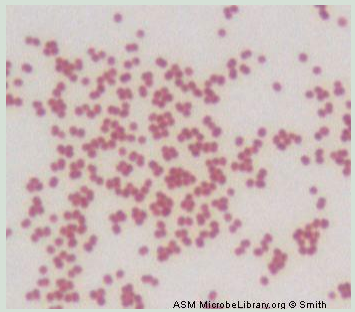
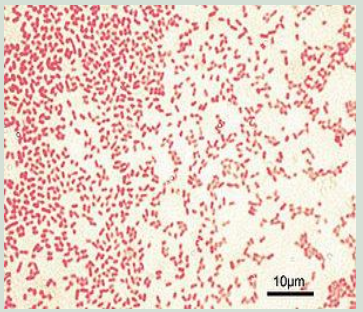
Aspergillus is not the toughest spore to kill

Many fungi are harder to kill that those tested

Bacillus cereus is tougher than the bacillus tested

Label claims can be misleading!

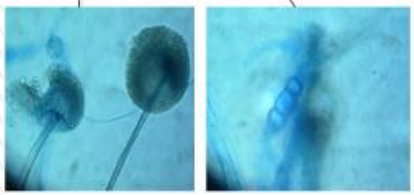
Bacterial Contamination

Gram Positive		Gram Negative	
Cocci	Rods	Cocci	Rods
			
			

Fungi Found in Cleanrooms

FUNGI

- Zygomycota
  - Asexual
  - Sexual



- Ascomycota
  - Sexual

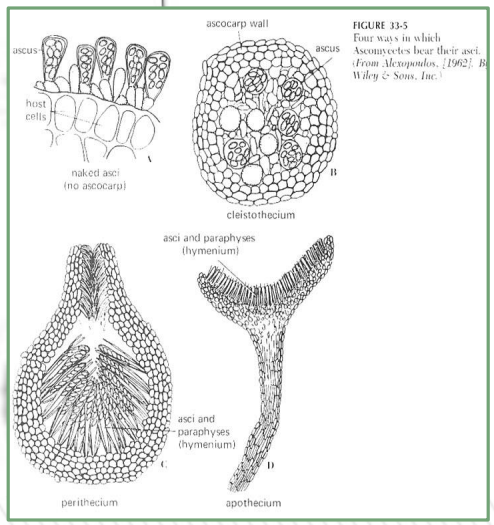
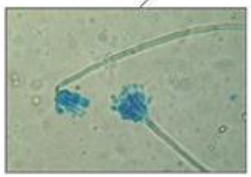


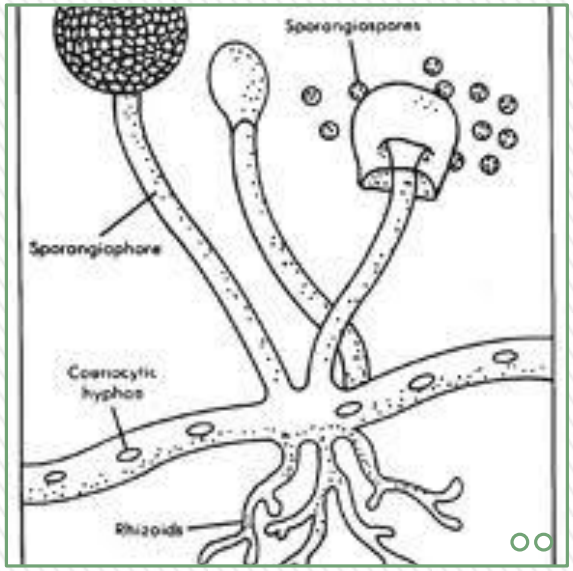
FIGURE 33-5
Four ways in which Ascomycetes bear their asci. (From Alexopoulos, [1962]. Wiley & Sons, Inc.)

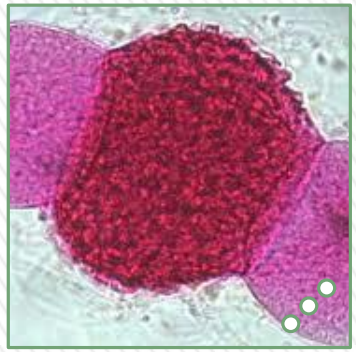
- Basidiomycota

- Deutoromycota
  - Hyalaine
  - Colored



The tough ones





Rhizopus Microscopic Structure Zygospore

The tough ones

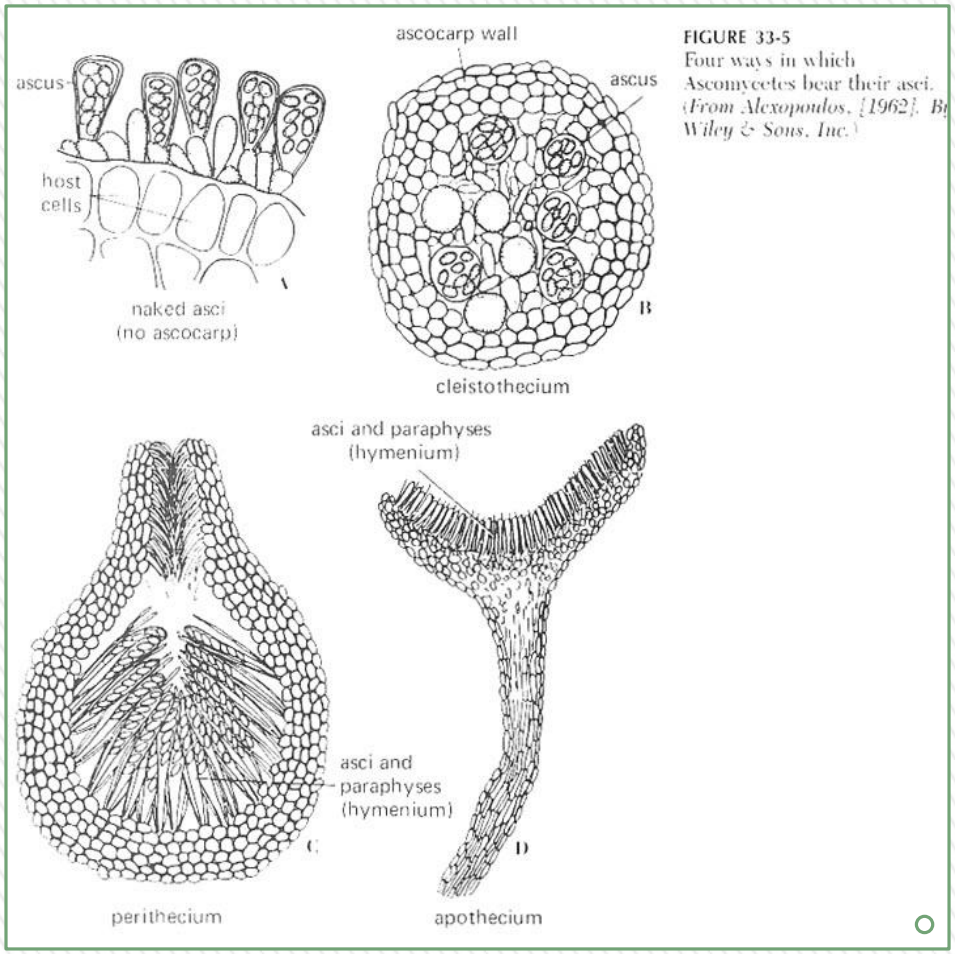


FIGURE 33-5
Four ways in which Ascomycetes bear their asci. (*From Alexopoulos, [1962].* By Wiley & Sons, Inc.)

Ascocarps Perithecium Ascospores

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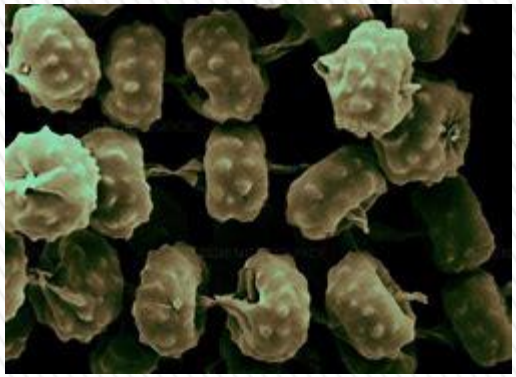
The gap still exists

NEW EUROPEAN STANDARD- **EN 13697** April 2015

Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional area

Aspergillus brasiliensis (ex A. niger):
“presence of a high concentration (at least 75 % of spiny spores) of characteristic mature spores, i.e. spiny spores (versus smooth spores)”



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Material Compatibility Overlooked

- Material compatibility testing is performed during registration
- Toxicity is also tested
- OSHA regulations are defined
- Recommended dilutions are tested for material compatibility
- More is not better:
  - Can erode surfaces
  - May not go through the cell barrier
- Lower dilutions do dot help
  - Dilution factors noted on label have been tested

Material Compatibility

Material	Effect of		
	Minncare concentrate	3% Minncare Solution	1% Minncare Solution
Non Porous Materials			
ABS	B	A	A
Acrylic	A	A	A
C-PVC	A	A	A
Polyoxymethylene	A	A	A
Polyphenylene Oxide/Polystyrene	A	A	A
Polyamide	C	B	A
Plexiglass	A	A	A
Polycarbonate	A	A	A
Polyethylene	A	A	A
High-Density Polyethylene	A	A	A
UHMW Polyethylene	A	A	A
Polypropylene	A	A	A
Polysulfone	A	A	A
Polyurethane	NR	B	A
PVC (rigid)	A	A	A
Polyvinylidene Fluoride	A	A	A
Polytetrafluoroethylene	A	A	A
Anodized Aluminum	B	B	B
Copper	NR	NR	NR
Brass	NR	NR	NR
Stainless Steel	A	A	A
Elastomers and Epoxys			
Buna-N	NR	B	B
EPDM	NR	A	A
Ethylene Propylene (EPR)	B	A	A
Latex	NR	B	B
2-Chlorobutadiene	NR	NR	NR
Silicone	A	A	A
Vinylidene Fluoride	B	B	A
Epoxy Adhesive	NR	B	A
Epoxy Paint	NR	NR	NR

Material Compatibility

A=Compatible, no significant effects noted

B=Material exhibited some minor reactions

C=Significant reaction-material still some performance qualities

NR=Exposure results in material failure

Material Compatibility

Case Study

Client found *Chaetomium globosum* on a belt in the filling line.

They performed DE study; however the fungus could not be killed at routine or extended contact time

Client decided to increase the concentration of SporKlenz from 1:100 to 1:50 dilution

End result: walls were eaten up, floors with pits

Panic: SporKlenz is not good for the facility, stopped using Sporklenz

Chemical Compatibility Overlooked

Cellulose and Bleach interaction
Cellulose and H2O2 interaction

Bleach (sodium hypochlorite) definitely reacts with cellulose. It was the first treatment used to bleach wood and paper to make it turn white. The problem with this is the efficacy against organisms will be used quickly and lost, bleach with a higher pH will react slower but also takes longer to kill microbes. This happens with H2O2 as well, but with the addition of peracetic acid the ability to power through organics remains high.

Chemical Compatibility Overlooked

Bleach and QAC interaction

Chemical Reactions from Mixing Bleach and Ammonia
Mixing bleach and ammonia is extremely dangerous, since toxic vapors will be produced. The primary toxic chemical formed by the reaction is chloramine vapor, with a potential for hydrazine formation.

References and Publications

Suitability of Methods

- AOAC Methods not suitable for Pharmaceutical Industry
- No one method used or recommended
- Minimum guidance in:
  - USP <1072> Disinfectants and Antiseptics
  - ASTM: E1054 – 02; Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents
  - ASTM Standard: E2614 – 08, Standard Guide for Evaluation of Cleanroom Disinfectants
  - DS/EN 13697:2015

Disinfectant qualification methods commonly used in Industry:

- Tube Dilution Method
- Coupon Methods

Planning Gaps

The most important consideration when planning a study is understanding that this study cannot prove efficacy of cleaning procedures

- Cleaning procedures translate to chemical kill by disinfectant activity and physical removal by mops, and wipes
- This study should be undertaken to prove the chemical kill of disinfectants on various surfaces for a battery of organisms at the manufacturer recommended or other contact time points
- The chemical kill of microorganisms in the cleanroom occurs by choosing the right dilution of disinfectant, a rotation between non-sporicidal and sporicidal disinfectants, and cleaning agents
- Physical removal of microorganisms from cleanroom surfaces is attained by adequate coverage of the surfaces cleaned by the disinfectant/cleaning agent using good quality cleaning mops that can physically remove the microorganisms during the disinfection and cleaning procedure

Planning Disinfectant Qualification

Common factors no matter which method you choose

- Choice of disinfectants
- Choice of organisms
- Purity of organisms
- Maintenance of organisms
- Choice of hard surfaces
- Sterilization of hard surfaces
- Media growth promotion and sterility for buffers performed
- Enumeration for target inoculum
- Maintenance of target inoculum
- Daily inoculum verification
- Method validation
- Recovery study and recovery loss calculation-in case of hard surfaces
- Calculating log reduction

Choice of Organisms

Common Errors

- Trends for in-house isolates not available
- Test each new isolate
- Test all USP challenge organisms
- Test only in-house isolates
- Process organism not tested
- Don’t test what you don’t see in the facility
- Trust disinfectant label for fungicidal activity and test hard to kill mold
- Keep on retesting at manufacturer recommended contact time with no success
- Establish unattainable acceptance criteria

Disinfectants tested

- Testing should be performed using only those disinfectants used in the cleanroom on specific surfaces
- Those used for other areas need not be tested
- It is best to test around the expiry date of:
  - Concentrate
  - Diluted Test
- Testing for expiry is a challenge as the disinfectant may exceed its expiry during testing
- Testing is performed using dilutions used for cleanroom cleaning
- Ensure that disinfectant dilutions are those recommended
- Always test recommended dilution

Dilution Errors

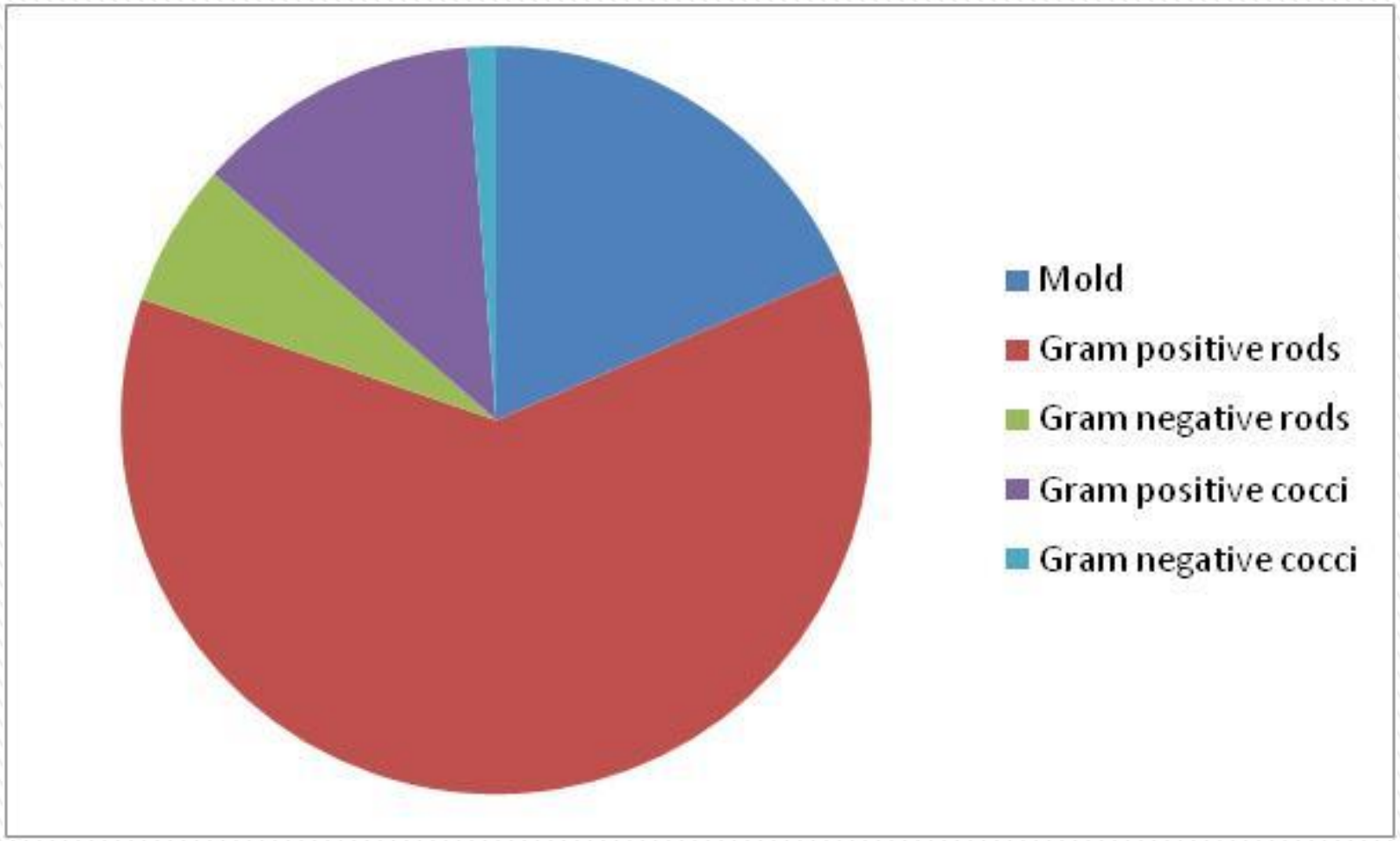
- Dilutions not verified
- Dilution errors lead to erroneous results
- Analyst errors in dilution

Case Study

- Concentrated and RTU SporKlenz was sent for testing to a contract laboratory
- RTU showed efficacy on vegetative bacteria, spore formers as well and monilaceous fungi
- Concentrated SporKlenz failed all the above

What really happened?

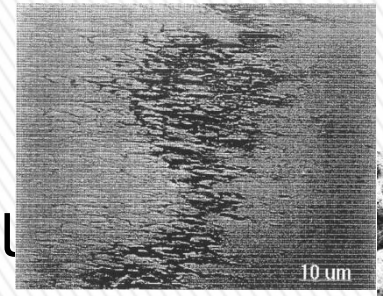
Predominant organisms not known

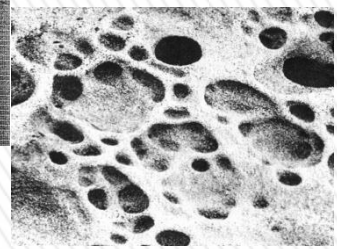


Surface characteristics?

Hard Surfaces Evaluation

Those that exhibit surface tension- glass, plexiglass, stainless steel





- Those that soak- wall, floor
- Those that cannot be sterilized-wall
- Those that warp on sterilization-vinyl, linoleum
- Those that are rough-powder coated stainless steel, wall

The method selected should be able to recover from all types of surfaces

Sterilization of these representative surfaces should be thought through

Outsourcing challenges

- Does the contract laboratory have experience in performing this study?
- What method are they using?
- Do they have references?
- Who will be working at the bench?
- Who will be reviewing data?
- Will they send you sets of data or an entire test report?
- If an error is made, who is responsible?
- If the data is very variable, how will it be handled?
- What is the cost?

Conducting the study in-house?

- Is there expertise in-house?
- Can in-house personnel be trained?
- Will management agree to training and execution costs?
- Will temps be hired to execute the study?
- Why will train these analysts?
- Will they know when data is erroneous?
- Will they or the reviewer be able to identify execution errors?
- Will conduction the study in-house be overwhelming?
- How then could it be broken into parts?
- How to prioritize?

Dispel myths before you begin

- There is a major misunderstanding of the practical use or simulated use testing when applied to coupon studies
- It is important to keep in mind that disinfectant qualification studies are performed to evaluate the chemical efficacy of the disinfectant, while cleaning is the physical removal of bioburden from the surfaces
- The disinfectant kills and the cleaning process removes
- The efficacy of both disinfection and cleaning is measured by trending environmental monitoring data and ensuring that the disinfection/sanitization and cleaning program is effective

Dispel myths before you begin

- These studies are often confused with cleaning validation
- Cleaning validation is performed to ensure that the product is removed during the equipment cleaning process to avoid product cross contamination
- Disinfectants are qualified to ensure that the disinfectants chosen are capable of killing or reducing bioburden on cleanroom surfaces
- This confusion had led many to simulate cleaning practices using coupons and using squeegees or wipes leading to erroneous results
- If such methods are used, chemical action of the disinfectant and the physical action of removal yields very good log reduction for spores and other hard to kill organisms even with disinfectants that are not capable of such efficacy
- This leads to choice of wrong disinfectants due to false data and ultimately contamination issues

Tube Dilution Method (Suspension Test)

Benefits

Less time consuming than testing on coupons

Can be quantitative or quantitative depending upon the inoculum

Drawbacks

Does not prove that the disinfectant is effective on hard surfaces

Materials used for higher inoculum as expensive as coupon study
Only sterilizable coupons such as stainless steel and glass can be used for both methods

Coupons like wall material will introduce contamination which may make reading surviving CFUs a challenge in both methods

If large quantity of neutralizing broth is used and only 1 mL is used for pour plating or spread plating, the data may not be dependable

Coupon Method (contact plates)

Benefits

Less time consuming among the representative Hard Surface Studies

Drawbacks

Very inconsistent and may be able to prove a 3 log reduction due to limited number of colonies that can be counted on 25 cm. sq. area

Hard to enumerate fungi and spreaders

Not always quantitative (with TNTC growth, the CFUs cannot be counted)

Coupon Method(swab recovery method)

Benefits

- All representative hard surfaces can be tested
- Complete quantitative study

Drawbacks

- Extensive and time consuming

Coupon Method (rinse method)

Benefits

- All representative hard surfaces can be tested
- Complete quantitative study

Drawbacks

- Extensive and time consuming
- Inconsistent recoveries as the pressure applied during rinses may vary

Coupon Method (manual dislodging method)

Benefits

- All representative hard surfaces cannot be tested
- Coupons that cannot be sterilized may add contaminants
- Complete quantitative study

Drawbacks

- Extensive and time consuming
- Low and inconsistent recoveries in dislodging method
- Loss of CFUs as they can adhere to coupons and bags
- Wall coupons cannot be steam sterilized
- Mold growth may take over
- Cannot be vortexed

Application Errors

Covering the entire area where inoculum is applied may be attained by:

- Spraying
- Flooding
- Spreading without removal of inoculum

Special attention should be paid to surface tension which prevents the disinfectant from completely covering a hard surface

Neutralization of Disinfectant

Disinfectant	Neutralizer(s)
Gluteraldehyde	Sodium Sulphite, Bisulphite
Chlorine	Sodium Thiosulphate
Phenolics	Tween (polysorbate)
QAC	Lecithin, Polysorbate
Hydrogen peroxide	Catalase

TSA and SDA contact plates contain lecithin and polysorbate 80

Letheen Broth contains lecithin and polysorbate 80

DE neutralizing agar and broth contain all above mentioned neutralizers- DE is not used in industry

Time ‘0’ Study or Inoculum Verification

- Stored inoculum may drop in viability
- Time ‘0’ Study verifies the inoculum count on the day of testing
- Time ‘0’ is performed at the end of efficacy when the same target inoculum is used over days
- Efficacy must be repeated if Time ‘0’ drops too low (where 3 log reduction cannot be calculated)

Calculations

Calculation of Target Inoculum
Calculate target inoculum in the enumeration study by counting CFU on the plate where the count is less than 300 CFU and multiplying with the dilution factor

Calculation of Time "0" Inoculum
Calculate Time '0' inoculum by counting CFU on the plate where the count is less than 300 CFU and multiplying with the dilution factor

Calculation of Log Reduction
The log of the average CFU recovered from the efficacy study is subtracted from the log of those recovered on the positive control

Common errors

- Target inoculum un attainable for certain fungi
- Drop in inoculum
- Disinfectant does not cover organisms of hard surface due to surface tension
- Variability within replicates
- Data does not represent disinfectant's capability
- Disinfectant neutralized on coupon- some hard surfaces exhibit surface tension

Neutralization Study

Disinfectants act beyond 10 minutes

Wiping down surfaces after a set contact time does not help

- To verify that the residue on surface does not interfere with recovery of organisms during environmental monitoring
- The residue from disinfectants does not exert bacteriostatic / fungistatic effect on surviving microorganisms
- To verify that the neutralizers in the contact plates are adequate to neutralize the active ingredients in the disinfectant residue
- The time lapse between cleaning and sampling is appropriate for recovery of organisms

Chemical Kill vs Physical Removal

Chemical Kill

- Choice of disinfectants
- Rotation of disinfectants
- Wipedown procedures
- Disinfectant qualification

Physical Removal

- Choice of mops - ergonomic and reaching hard to clean areas
- Choice of mop heads – capacity to hold and dispense adequate disinfectant
- Choice of adequate wipes to physically remove particulates and residue

Significance of contact time

- Disinfection needs both chemical kill and physical removal
- Surfaces are irregular and differ in time taken to absorb or dry
- Adequate application of disinfection without soaking is the key
- Ability of supplies to cover all surfaces evenly
- Ability of supplies facilitate physical removal
- Good coverage and maximum contact time= reduction of contamination

Disinfectant qualification errors

- There is no one method for qualifying disinfectants
- Companies may depend on contract laboratories for these studies
- Many laboratories may try to mimic cleanroom
- cleaning which may lead to erroneous data
- If these findings are applied to the cleaning program contamination is inevitable

483 Observations

- Systematic facility cleaning for mold was not initiated in a timely manner. Systematic cleaning was initiated after several months of environmental excursions for mold throughout the manufacturing areas, including aseptic areas

- Disinfectant effectiveness studies against representative microorganisms and/or specific in-house isolates were not conducted for cleaning agents used in your facility to disinfect production areas, including aseptic areas

483 Observations

- Your response to this observation appears adequate; however, we are unable to determine if this response is adequate without review of the final summary with the included data for VP-XXX, Addendum 3, "Disinfectant Efficacy Verification for Hard Surfaces." This information will be reviewed during the next establishment inspection

- All surfaces that are used in critical processing and manufacturing areas were not evaluated in the "Disinfectant Efficacy Verification for Hard Surfaces" VP-XXX-PV approved: xx/xx/xxxx

- Your response to this observation is not adequate. The **(b)(4)** work-top surface is an area that is monitored and is located in a classified area. Therefore, the effectiveness of its cleaning should be evaluated just as the other surfaces

483 Observations

- The qualification of your disinfectant **(b)(4)** failed to demonstrate that it is suitable and effective to remove microorganisms from different surfaces. Specifically, this disinfectant failed to meet qualification criteria when challenged with multiple organisms

- Your disinfectant qualification for **(b)(4)** and **(b)(4)** bi-spore disinfectants documented that the log reduction criteria (Bacteria ≥ 4, Fungi ≥ 3) was not met when challenged with multiple organisms in a variety of surfaces. After disinfection, you recovered Micrococcus luteus on vinyl, **(b)(4)**, stainless steel, glass, and wall laminate and *Enterobacter cloacae, Rhodococcus sp, Burkholderia cepacia, Pseudomonas aeruginosa, Methylobacterium mesophilicum and, Acinetobacter lwoffi* on glass

- However, your procedures for routine cleaning of the aseptic manufacturing area continue to require the use of unqualified disinfectants during days **(b)(4)** through **(b)(4)** of your disinfectant program

QUESTIONS

Email me if you have any follow up questions

Email: Zabraham@microrite.com