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LUNCH AND LEARN

Microbes and Pharmacies:
The CGMP of Contamination Control
October 9, 2015

Featured Speaker: **Scott Sutton, Ph.D.**
The Microbiology Network
N. Chili, New York

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Event Code

Code will be provided at the end of today's activity
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3

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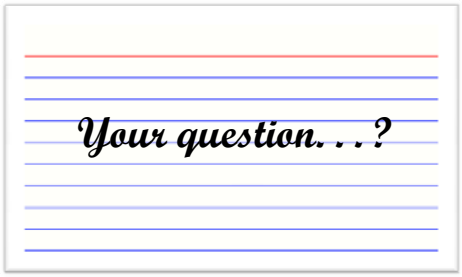
- Submit your questions to your site manager.
- Questions will be answered at the end of the presentation.



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Resources

- Visit www.ProCE.com/PharMEDiumRx to access:
  - Handouts
  - Activity information
  - Upcoming live webinar dates
  - Links to receive CE credit


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Webinar Information

Questions? Email us at info@proce.com or call our office at 630.540.2848.

Section 503B of the Food, Drug and Cosmetic Act: What does it mean?
Live webinar date: March 14, 2014 - 11:30am - 12:30pm CT

Activity Information | Download Handout *Coming Soon* | Live Webinar CE: Post-Test/Evaluation *Coming Soon* | On-Demand Webinar CE: Post-Test/Evaluation *Coming Soon*

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Disclaimer

- I am an independent consultant.
- I have been involved with USP, PDA, ASM, *etc.* for many years.
- I do not represent any organization in this presentation.
- Opinions expressed in this presentation are mine alone, and should not be interpreted as the policies, positions or whims of any other organization.

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Webinar Outline

- Microbial Life
  - Types
  - Prevalence
  - Nutrition
- Contamination Control
  - Facility
  - Process

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Microbial Life

Types of Microorganisms

- Common pharma considerations:
  - Bacteria
    - Gram Positive
    - Gram Negative
  - Yeast
  - Mold
  - Virus
  - Prion

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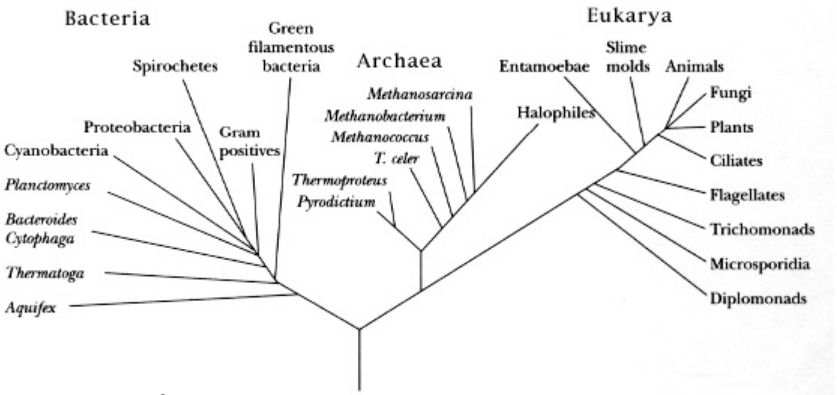
Microbial Life



Types of Microorganisms

Phylogenetic tree based on 16S rRNA sequence analyses

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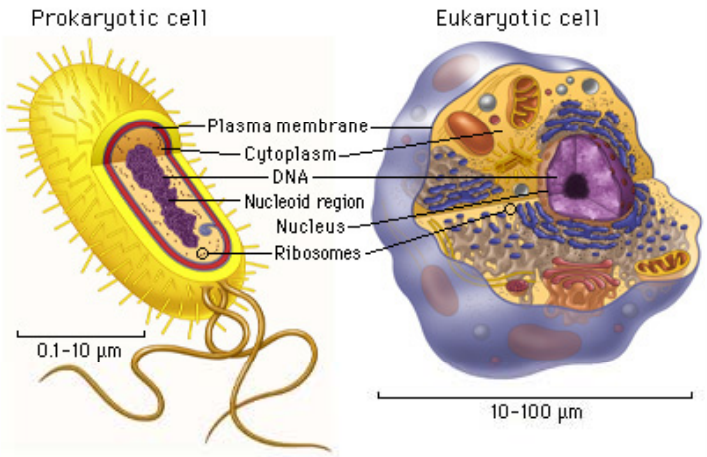
Microbial Life



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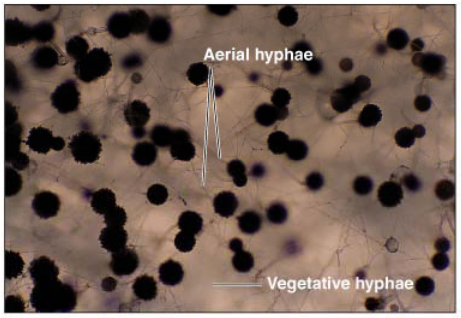
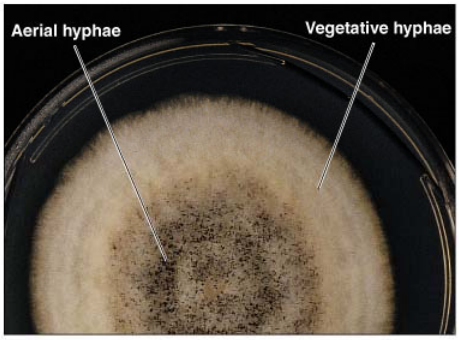
Fungi



(a) ***Aspergillus niger***



(b) ***A. niger on agar***

- Two types of hyphae – aerial hyphae to generate sexual spores vs vegetative hyphae used to harvest food.
- Fungal spores are not armored survival bunkers (like bacterial spores) but rather reproductive mechanisms

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Microbial Life

Yeasts

- Unicellular fungi
- Budding yeasts-uneven cell division
  - Protuberance forms -bud
  - Nucleus divides & one goes into bud
  - Cell wall material separates bud from mother cell

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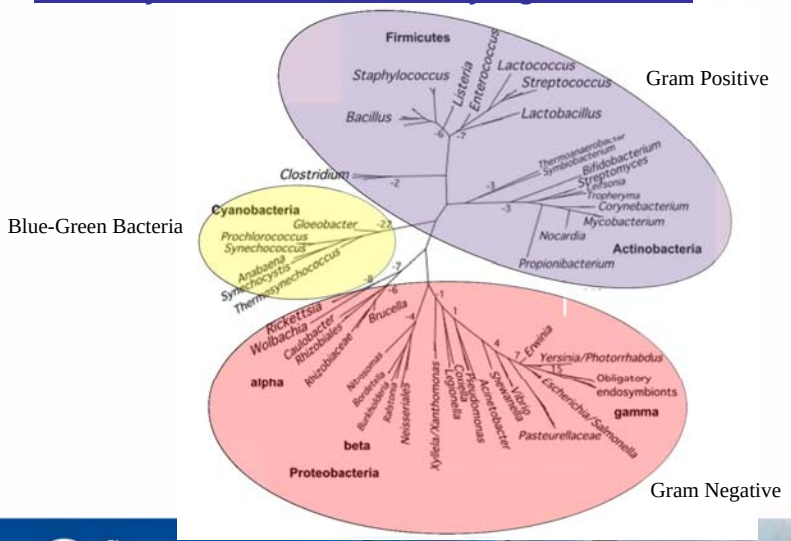
Microbial Life

Procaryotes - Bacterial Phylogenic Tree



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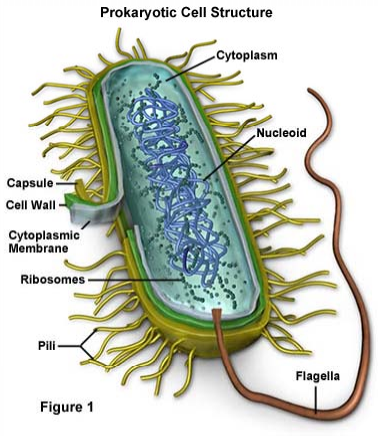
Microbial Life

Procaryotes

- Peptidoglycans in Cell Walls
- No nucleus
- DNA in closed loop
- Different ribosomes; 70S opposed to 80S in eucaryotes
- Different DNA synthesis enzymes
- No membrane-bound organelles
- Diversity of energy-yielding reactions (*eg* N_2 fixation)



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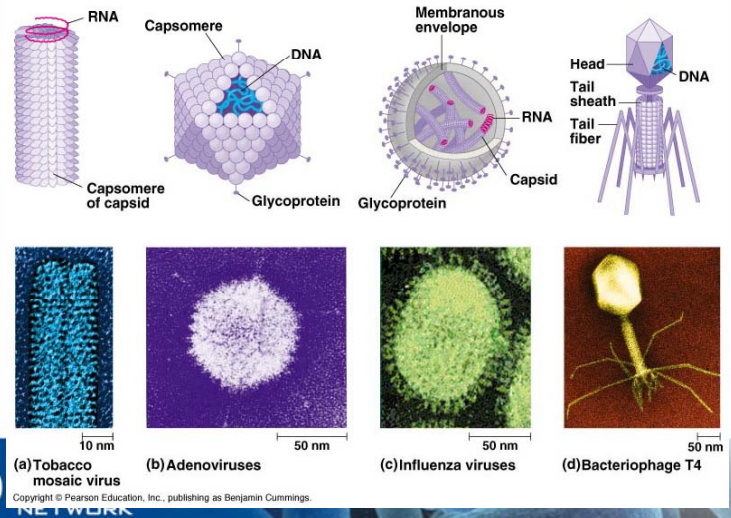
Microbial Life

Virus



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Microbial Life

Size – What Scale?

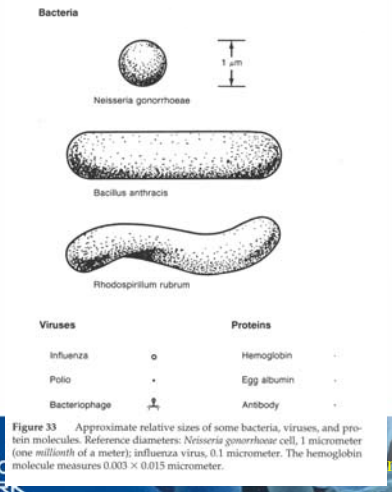

Figure 33 Approximate relative sizes of some bacteria, viruses, and protein molecules. Reference diameters: *Neisseria gonorrhoeae* cell, 1 micrometer (one *millionth* of a meter); influenza virus, 0.1 micrometer. The hemoglobin molecule measures 0.003 × 0.015 micrometer.

From: Microbes: An Invisible Universe by H Gest. ASM Press. 2003

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Microbial Life –Where?

- Common Garden Soil
  about $5x10^9$ (5,000,000,000 or 5 billion) per teaspoon
- Human Skin
  2,500,000 ($2.5x10^6$ or 2.5 million) per inch
- Human Saliva
  10^9 per mL
- 1 sneeze
  20,000
- Human Feces
  1×10^{11} (100,000,000,000 or 100 billion) per gram

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Microbial Life

- It is estimated that 500 to 1000 species of bacteria live in the human gut (Sears CL. 2005. A dynamic partnership: celebrating our gut flora. *Anaerobe*. 11(5):247-51)
- A roughly similar number on the skin.
- More than 100 trillion bacterial cells associated with our bodies (1×10^{11}). Our bodies are made of only some several trillion human cells ($\sim 10^{9}$)

We are somewhat outnumbered

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Microbial Life - Nutrition

- ENERGY
- NITROGEN
- CARBON
- PHOSPHOROU… POTASSIUM & SODIUM.
- CALCIUM
- WATER
- MINERALS …COBALT *etc*. (trace metals)

Energy may be light (the sun or lamps) or inorganic substances like sulfur, carbon monoxide or ammonia, or preformed organic matter like sugar, protein, fats etc. Without energy life can not exist and quickly dies or becomes inactive.

Nitrogen may be nitrogen gas, ammonia, nitrate/nitrite, or a nitrogenous organic compound like protein or nucleic acid.

Carbon can be in the form of carbon dioxide or monoxide, methane, or complex organic material

Most cells require calcium in significant quantities, but some seem to only need it in trace amounts.

All life requires liquid water in order to grow and reproduce – even fungi require free water above an A_w of 0.6

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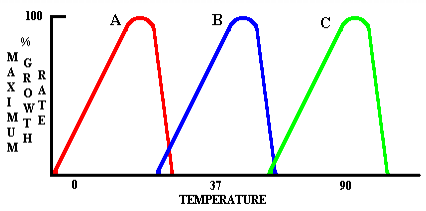
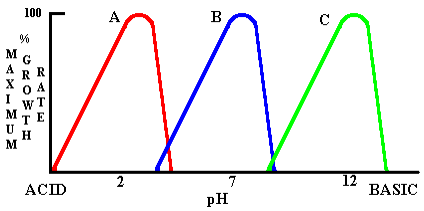
Microbial Life – Environmental Requirements





A. Psychrophiles
B. Mesophiles
C. Thermophiles

A. Acidophiles
B. Neutrophiles
C. Alkalinophiles

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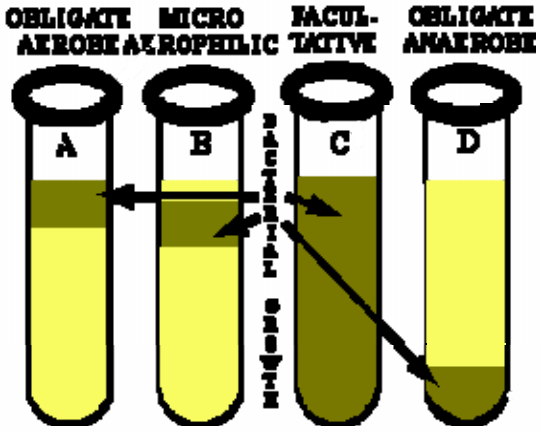
Microbial Life - Oxygen



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Microbial Life

Important Aspects of Microbial Nutrition

- Microorganisms need food and water (even the photosynthetic ones need nutrients)
- Microorganisms have temperature ranges Range has lower and upper limit
- Microorganisms have pH ranges
- Microorganisms may require oxygen, may be able to tolerate it, or may require its absence

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Webinar Outline

- Microbial Life
  - Types
  - Prevalence
  - Nutrition
- Contamination Control
  - Facility
  - Process

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Contamination Control

	Validation	Control	Monitoring
Facility	Qualification of the Clean Room area and HVAC System	Maintenance of Facilities Sanitization; Revision of Barriers, Traffic Patterns, or Air Balance	Environmental Monitoring (EM)
HVAC	Qualification of the Clean Room area and HVAC System	Certification and Preventative Maintenance (PM) of System; Repair of HEPA Filters	EM
Water	Qualification of Water System	Certification and PM Regular Sanitization of System	Bioburden Monitoring of Water System
Equipment	Qualification of the Equipment as Suitable for its Intended Use	Certification and PM Regular Sanitization	EM Finished Product Release Testing
Sanitization	Validation of Cleaning, sanitization and sporicidal treaments	Regular cleaning and sanitization of facilities and equipment	EM
Personnel	Proficiency Criteria Participation in Media Fills Trending Data by Operator	Training Discipline	Personnel Monitoring Trending Data by Operator
Process	Process Validation	Acceptance Testing of Raw Materials and Containers	In-process Bioburden Monitoring Finished Product Release Testing

Sutton, S. 2015. Bioburden Contamination Control: A Holistic Overview. *Amer Pharm Rev - Endotox Suppl.* pp 20-24

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Facility Control

- Qualification of Cleanrooms
  - Classification
  - Smoke Studies
- Control
  - Facility Maintenance
  - Gowning
  - Traffic Patterns
  - Sanitization
- Monitoring
  - Environmental Monitoring

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Control by Facility Design

- Separation of clean and dirty operations
- Controlled access to areas
- Expoxied floors and walls – easy to clean and prevent nooks for bacteria to hide
- Quarantine areas
- PEC and Laminar HEPA Airflow

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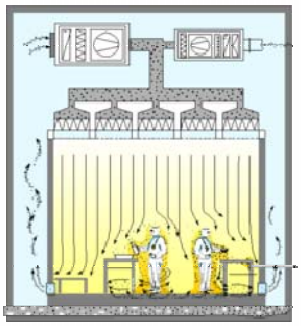
Important Consideration

First Air



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Control by Procedure

- Validate all bulk holding times
- Training
- Regular Cleaning and Disinfection
- Trending of EM data to demonstrate compliance

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Facility Control

- Qualification of Cleanrooms
  - Classification
  - Smoke Studies
- Control
  - Facility Maintenance
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  - Sanitization
- Monitoring
  - Environmental Monitoring

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How are Bacteria Spread?

- Air
- Water
- People
  - Skin
  - Hair
  - Clothes
  - Mucous Membranes
- Particles
- Surfaces

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People are The Major Source of Contamination in a Cleanroom

- Sources
  - Skin
  - Hair
  - Clothing
  - Shoes
  - Makeup
- Illness
- Open rash or sore

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Methods of Spread

- Dispersion
  - Generate particulate matter
  - Viable microorganisms attached to particles (dead skin, hair, fibers)
- Touch Contamination
- Aerosols

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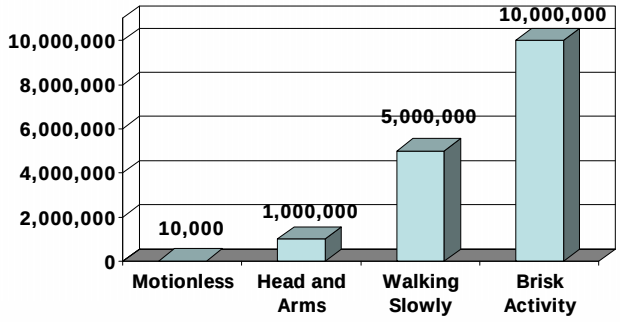
Personnel Shedding – Particles by Activity



Reinmuller, B and B Ljungqvist. 2000. Evaluation of Cleanroom Garments In a Dispersal Chamber - Some Observations. *Euro J Parenteral Soc* 5(3):55-58

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People

- Body is covered with millions of skin flakes
- Flakes are constantly shedding
- Gown captures
- Glove Captures

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Control of People

- Gown/PPE
- Sanitization
- Procedures

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Cleaning/Disinfection

- Must sanitize/disinfect cleanrooms regularly
- EM program shows efficacy of program
- Activity of Disinfectants
  - Cleansing
  - Kill

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Validation of Sanitization

- ***In vitro* Study**
  Suspend organisms in sanitizer/sporicide – include several from facility. Test for survivors with time.
- **Carrier Study**
  Inoculate a 2x2 inch coupon (different materials of construction) with challenge organism (Include facility isolates). Apply sanitizer/ sporicide to and let sit for dwell time. Recover.
- ***In vivo* study**
  Test out in model room – show decrease in organisms
- **EM trending confirmation**

More information in USP <1072>

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Control of People

- Gown/PPE
- Sanitization
- Procedures

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Facility Control

- Qualification of Cleanrooms
  - Classification
  - Smoke Studies
- Control
  - Facility Maintenance
  - Gowning
  - Traffic Patterns
  - Sanitization
- Monitoring
  - Environmental Monitoring

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Components of an EM Program

- Air
- Surface
- Personnel
- Water (Utilities)
- Sanitization Efficiency

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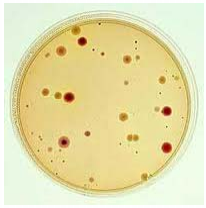
EM – Air Monitoring

Passive Monitoring – Settle Plates



Active Air Monitoring

RCS

SAS Sampler

Air Ideal

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Surface/Personnel Sampling Technologies

- Rodac plates
- Swabs
  - Cotton
  - Calcium alginate
  - Nylon

Note: There is no regulatory requirement nor scientific reason to determine recovery efficiency of surface sampling methods. TRENDS are what are important

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Water - Microbial Considerations

- Identification of Microorganisms
  - Recommended
- Alert and Action Levels
  - Alert Level: process drifting from normal operation condition if exceeded
  - Action Level: process drifting from normal operating range, if exceeded
  - Corrective Action even when product quality might not be compromised

USP <1231> Water for Pharmaceutical Purposes

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Webinar Outline

- Microbial Life
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Contamination Control

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Process Control

- Process Validation
  - Qualified Equipment
  - Qualified Hold Times
  - Media Fills
- Process Control
  - Use of Controlled Raw Materials
  - Personnel Training
- Process Monitoring
  - In-process monitoring
  - Finished Product Testing

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Process Validation

- Qualified Equipment
  - Fillers
  - Cappers
  - Balances
  - pH Meters
- Qualified Hold Times
  - Clean Hold - Equipment
  - Dirty Hold – Equipment
  - Process Hold
- Media Fills

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Sterilization Validation / Media Fills

- Sterilization Validation; or
- Media Fill (for aseptic fills)
  - Must include worst case conditions
  - Mimic individual processes
    - Process Steps
    - Number filled
    - Duration
  - Performed twice a year per operator

FDA. 2004. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice

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Process Control

- Process Validation
  - Qualified Equipment
  - Qualified Hold Times
  - Media Fills
- Process Control
  - Use of Controlled Raw Materials
  - Personnel Training
- Process Monitoring
  - In-process monitoring
  - Finished Product Testing

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21 CFR 211.110 Sampling and testing of in-process materials and drug products.

(a) To assure batch uniformity and integrity of drug products, written procedures shall be established and followed that describe the in-process controls, and tests, or examinations to be conducted on appropriate samples of in-process materials of each batch. Such control procedures shall be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product. Such control procedures shall include, but are not limited to, the following, where appropriate:

(1) Tablet or capsule weight variation;

(2) Disintegration time;

(3) Adequacy of mixing to assure uniformity and homogeneity;

(4) Dissolution time and rate;

(5) Clarity, completeness, or pH of solutions.

(6) Bioburden testing.

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Process Control

- Process Validation
  - Qualified Equipment
  - Qualified Hold Times
  - Media Fills
- Process Control
  - Use of Controlled Raw Materials
  - Personnel Training
- Process Monitoring
  - In-process monitoring
  - Finished Product Testing

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Finished Product Testing

- Bacterial Endotoxin Testing (USP <85>)
- Sterility Tests (USP <71>)
- Antimicrobial Effectiveness Testing (USP <51>)

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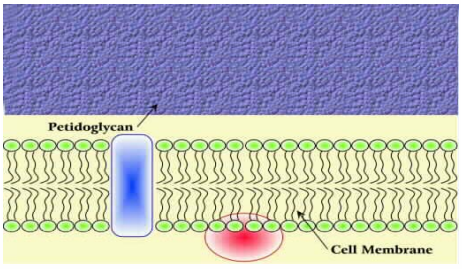
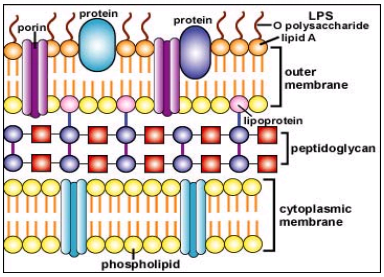
Schematics of Cell Wall Components



Gram Positive Structure



Gram Negative Structure

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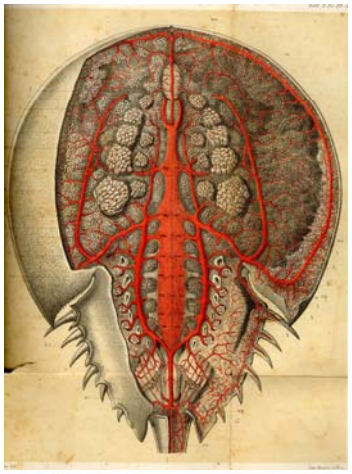
Limulus Polyphemus
Limulus Amebocyte Lysate

- Hemocyanin not Hemoglobin
  - Blue Blood – copper not iron



LAL - Limulus Amebocyte Lysate

The aqueous extract obtained after lysis of the blood cells of the horseshoe crab *Limulus polyphemus*

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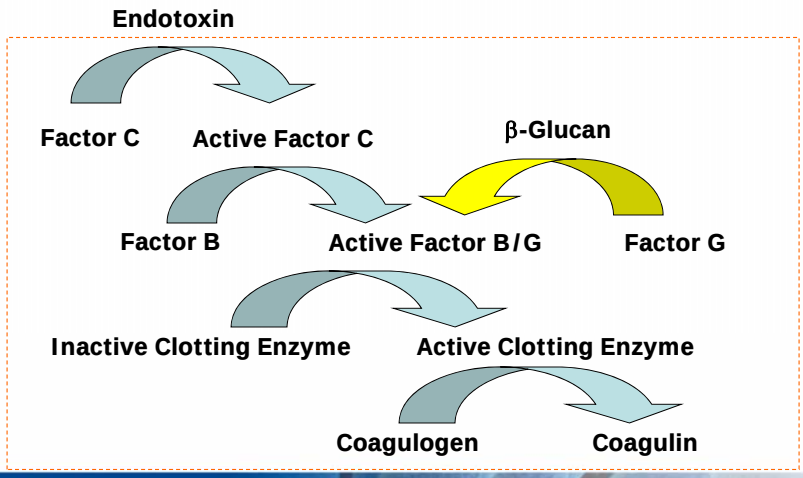
LAL Coagulation Cascade



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USP <85> Bacterial Endotoxins Test Types of Testing

- Gel-Clot
  - Limit test
  - Semi-quantitative
- Photometric
  - Turbidimetric
    - Kinetic
    - End-point
  - Chromogenic
    - Kinetic
    - End-point

Only accurate to +/- 2X reading

BET Qualified by Inhibition / Enhancement Study

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Finished Product Testing

- Bacterial Endotoxin Testing (USP <85>)
- Sterility Tests (USP <71>)
- Antimicrobial Effectiveness Testing (USP <51>)

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Sterility Testing

- Two separate tests
  - Membrane Filtration
  - Direct Transfer
- Number of units tested and volume tested per unit are mandated in test
- Method Suitability mandated in test
- Requires Growth
  - Incubation period - 14 days
  - 2 media & 2 temperatures

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Membrane Filtration

- Filter required amount of product through two filters (qualified with Method Suitability)
- Neutralize/Rinse
  - 3 100 mL volumes suggested
  - Formulations for dilution fluids suggested
- One filter into Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25°C for 14 days
- One filter into Fluid Thioglycollate Medium (FTM) – incubate at 30-35°C for 14 days

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Direct Inoculation

- Place required amount of product into sufficient recovery medium (with neutralizers?) (qualified with Method Suitability)
  - Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25°C for 14 days
  - Fluid Thioglycollate Medium (FTM) – incubate at 30-35°C for 14 days

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Finished Product Testing

- Bacterial Endotoxin Testing (USP <85>)
- Sterility Tests (USP <71>)
- Antimicrobial Effectiveness Testing (USP <51>)

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Antimicrobial Efficacy Testing

USP <51>

- Designed to demonstrate the ability of a multidose product to withstand microbial challenge.
- High-level challenge, regular sampling of the challenge suspension for survivors
- Method Suitability mandated in test

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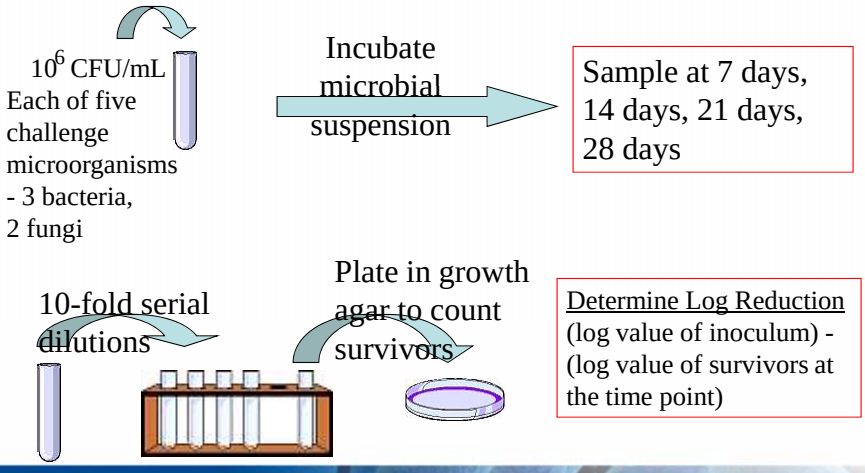
Antimicrobial Efficacy Testing (AET)



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AET Categories

Category	Product Description
1	Injections and other parenterals, otic, sterile nasal products, and opthalmics
2	Topical Products
3	Oral Products
4	Liquid Antacids

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Scope of AET

- It can
  - Provide relative estimates of the biological activity of a preservative system in a particular formulation at a particular time.
- It cannot
  - Predict the preservative efficacy of the multidose finished product in all patients hands under all conditions.

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Webinar Review

- Microbial Life
  - Types
  - Prevalence
  - Nutrition
- Contamination Control
  - Facility
  - Process

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Thank you for your attention

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