Lecture 21
BT 206
20/03/2023

Measuring Microbial Growth,

Direct Counting

- Growth is measured by the change in the number of cells over time. Cell counts done microscopically measure the total number of cells in a population, whereas **viable** cell counts (plate counts) measure only the living, reproducing population.

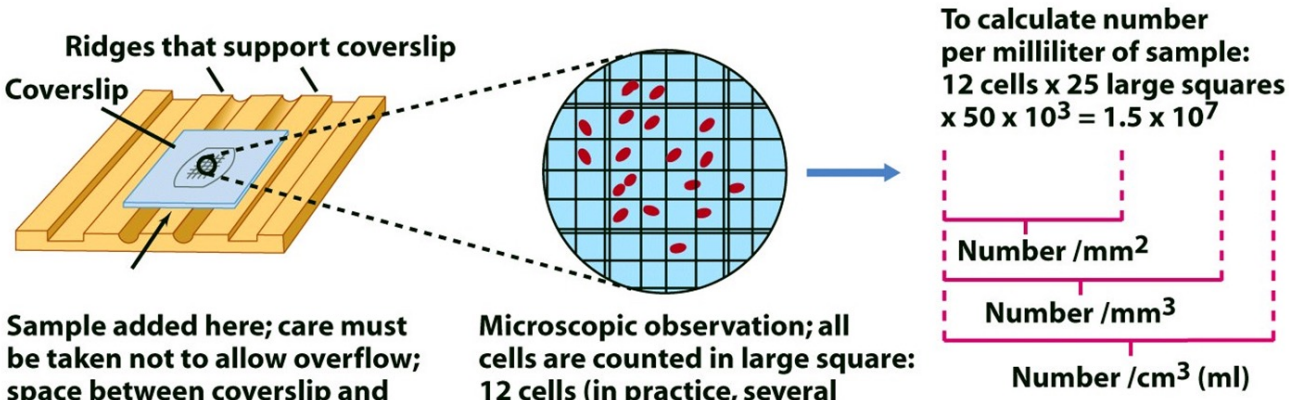


Figure 6-9 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Measuring Microbial Growth,

Viable count: Viable cell is one that is able to divide and form offspring. Done on plate count in two ways:

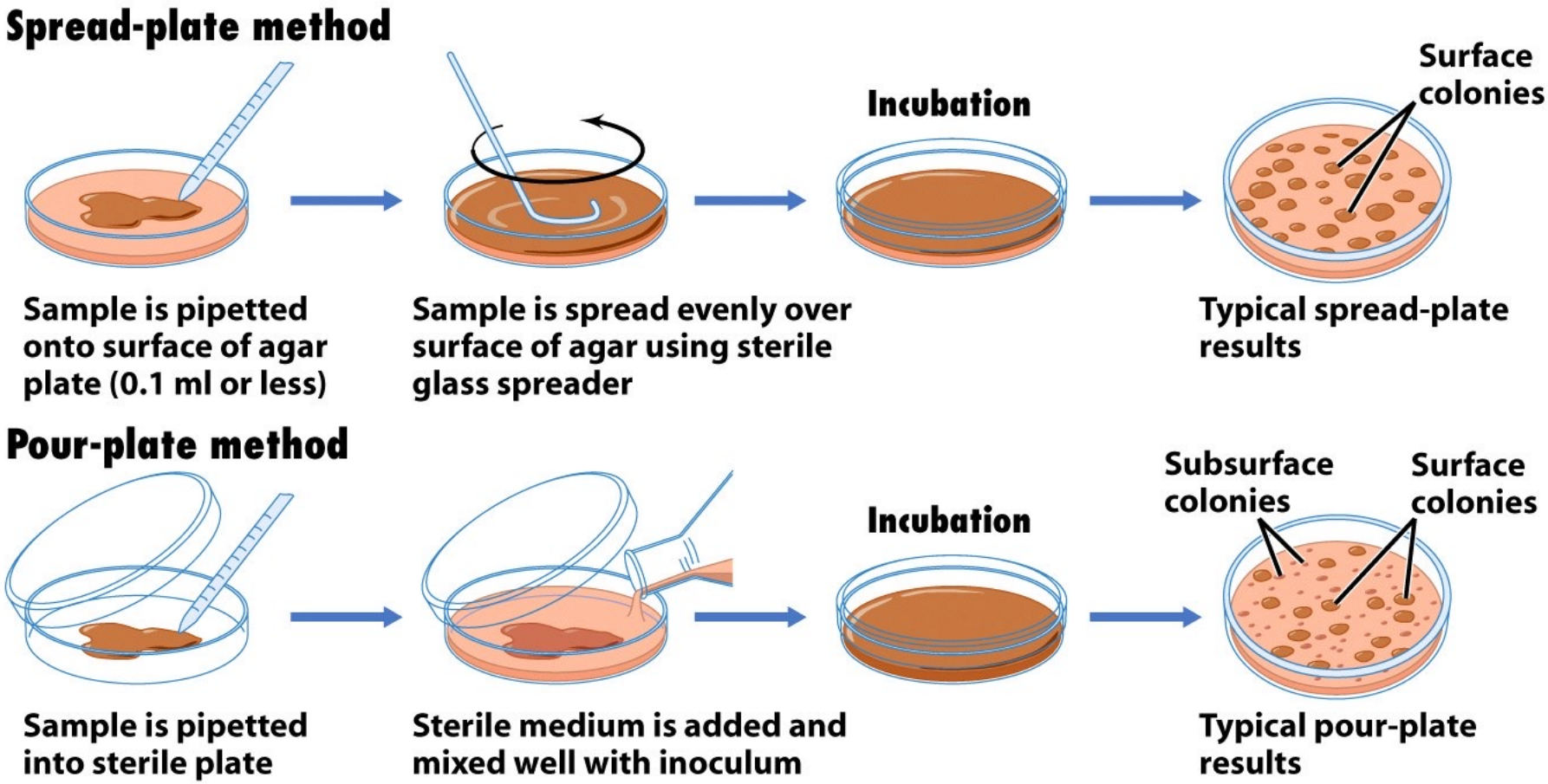


Figure 6-10 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Lecture 22
BT 206
21/03/2023

Measuring Microbial Growth

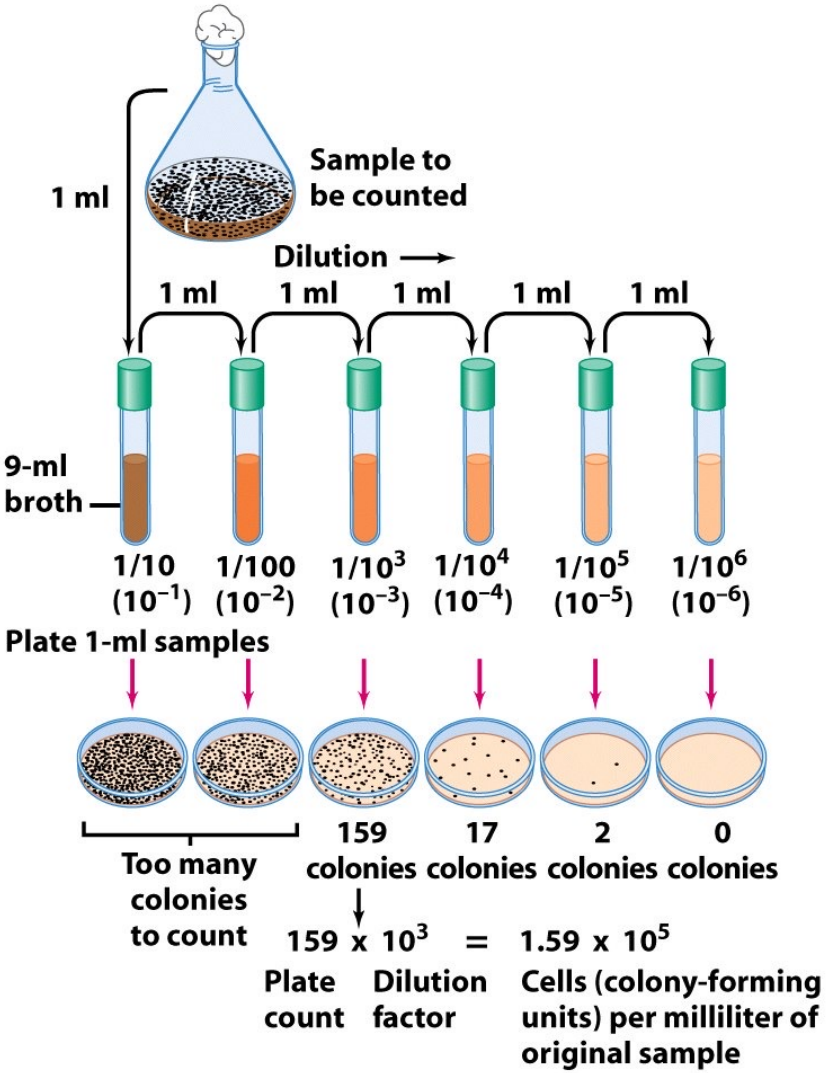


Figure 6-11 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Measuring Microbial Growth,

Indirect Counting

- Turbidity measurements are an indirect but very rapid and useful method of measuring microbial growth. However, to relate a direct cell count to a turbidity value, a standard curve must first be established.

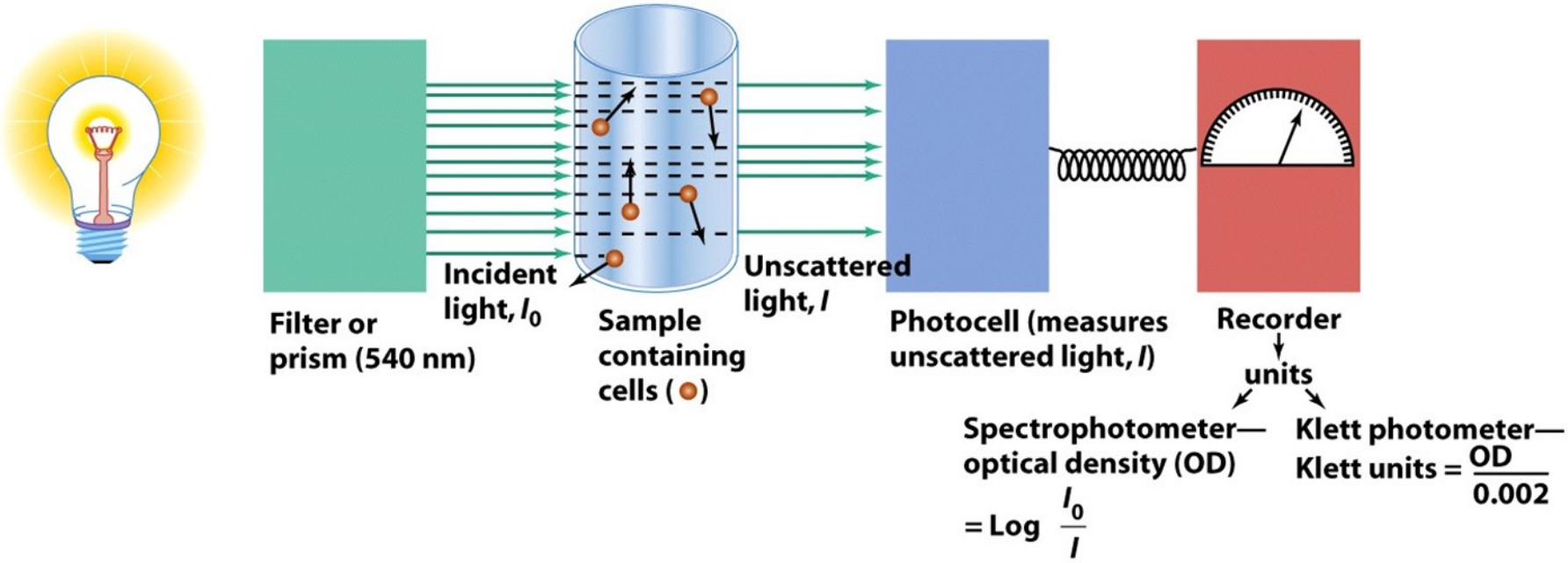


Figure 6-12a Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Measuring Microbial Growth,

Indirect Counting…

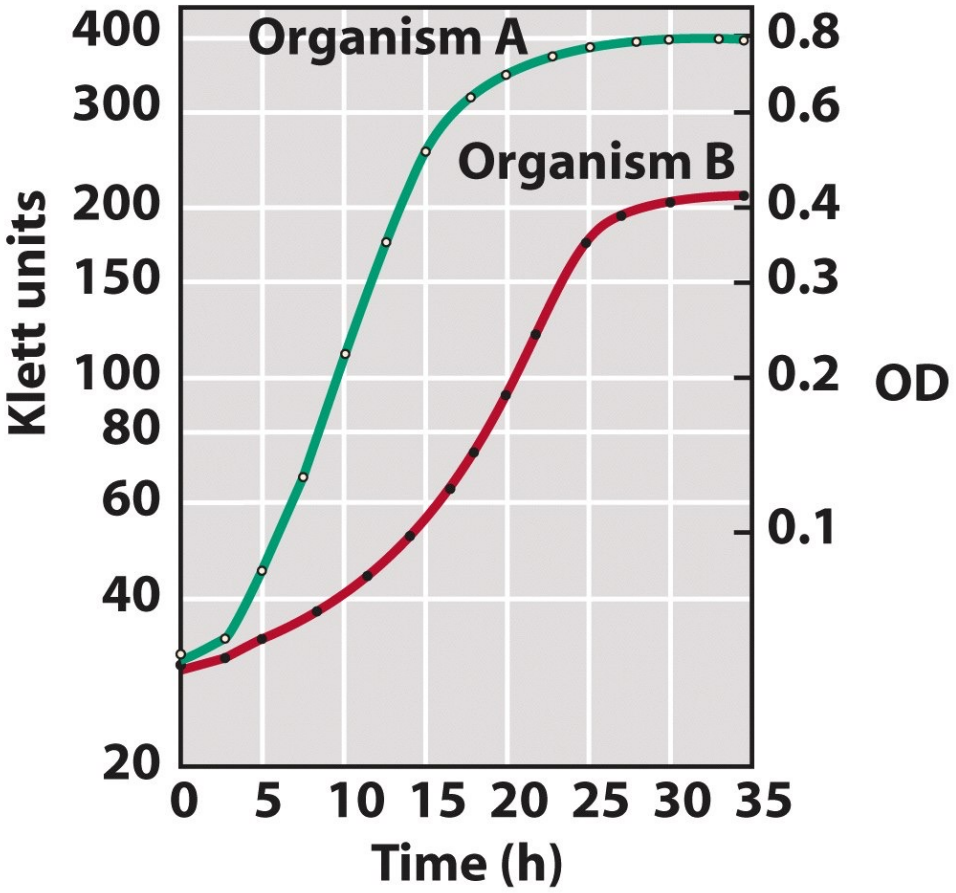


Figure 6-12b Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Targeted plate counts:

- Use of highly selective culture media and growth conditions
- Allows one to target only particular species in a mixed population of microorganisms present in the sample.

➢ Example a complex medium containing 10% NaCl is useful in isolating species of *Staphylococcus* from skin.

- Practical application: In food industry viable counts done on both Complex and Selective media allows for both quantitative and Qualitative assessment of microbial load.

The great plate count Anomaly:

- Direct microscopic counts of natural samples typically reveal far more organism than are recoverable on plates of any single culture medium
  - ➢ Reason: Microscopic methods may count dead cells as well;
  - ➢ Different organism require different nutrient and growth condition
  - ➢ One medium and one set of conditions may be best expected to support the growth of only a subset of microorganism in the natural sample
- Thus, plate count can be highly unreliable when used to assess total cell numbers of natural samples, such as soil and water

The Control of Microbial Growth

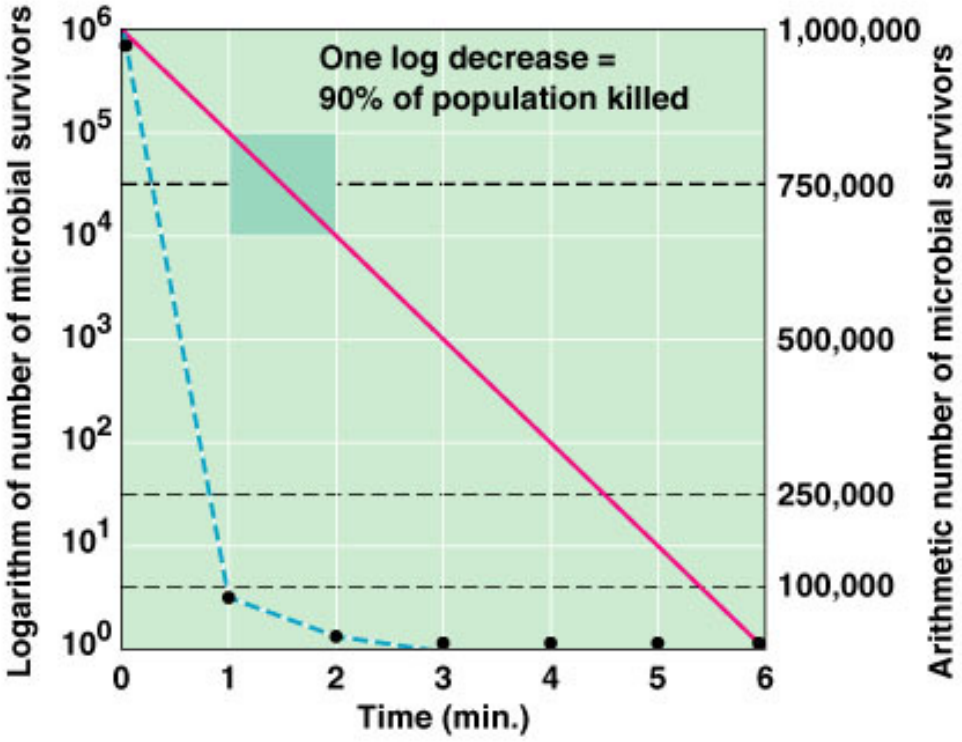
- Sepsis refers to microbial contamination.
- Asepsis is the absence of significant contamination.
- Aseptic surgery techniques prevent microbial contamination of wounds.

Terminology

- Sterilization: Removal of all microbial life
- Commercial Sterilization: Killing *Clostridium botulinum* endospores
- Disinfection: Removal of pathogens
- Antisepsis: Removal of pathogens from living tissue
- Degerming: Removal of microbes from a limited area
- Sanitization: Lower microbial counts on eating utensils
- Biocide/Germicide: Kills microbes
- Bacteriostasis: Inhibiting, not killing, microbes

The rate of microbial death

- Bacterial populations die at a constant logarithmic rate.



Bacterial cells are dying at a rate of 90% each min

Figure 7.1a

Effectiveness of antimicrobial treatment depends on:

Factors influencing the effectiveness of antimicrobial treatments:

- Number of microbes
- Environment (organic matter, temperature, biofilms)
- Time of exposure
- Microbial characteristics

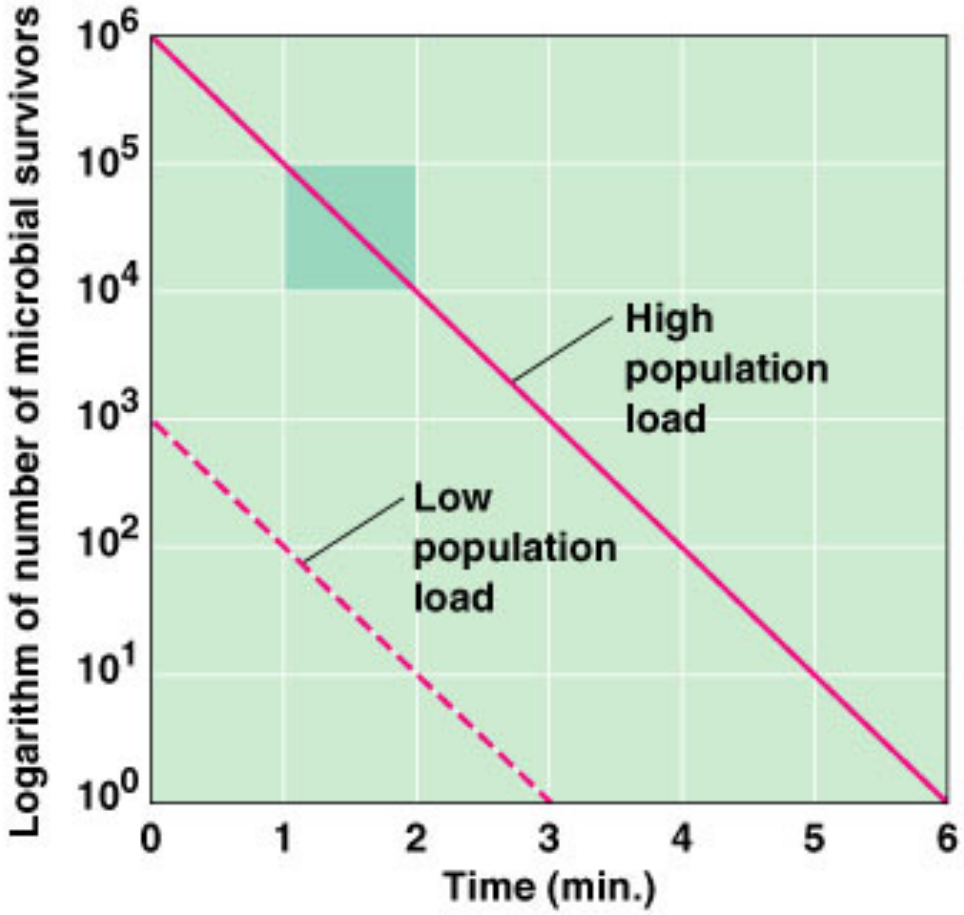


Figure 7.1b

Actions of Microbial Control Agents

- Alternation of membrane permeability
- Damage to proteins
- Damage to nucleic acids

Physical Methods of Microbial Control

- Heat
  - **Thermal death point** (TDP): **Lowest temperature** at which all microorganism in a particular liquid culture are killed in 10 min.
  - **Thermal death time** (TDT):**Minimum length of time** to kill all bacterial cells in particular liquid culture to be killed at a given temperature
  - **Decimal reduction time** (DRT): **Minutes to kill 90%** of a population at a given temperature

Heat

- Moist heat denatures proteins
- Autoclave: Steam under pressure

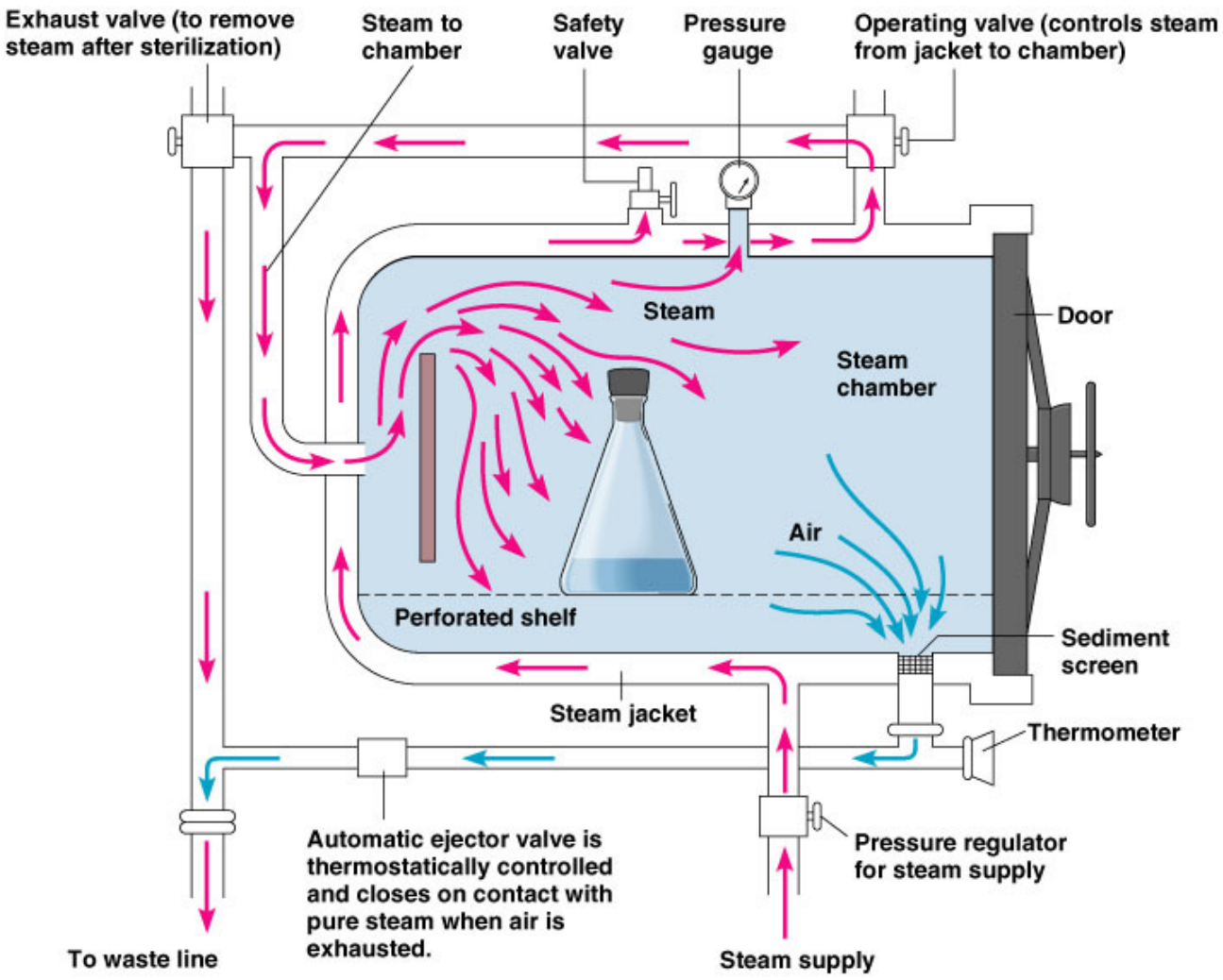


Figure 7.2

Lecture 23
BT 206
22/03/2023

Physical Methods of Microbial Control

- Pasteurization reduces spoilage organisms and pathogens
- Equivalent treatments
  - 63°C for 30 min
  - High-temperature short-time 72°C for 15 sec
  - Ultra-high-temperature: 140°C for <1 sec
  - ➢ Thermoduric organisms survive during pasteurization

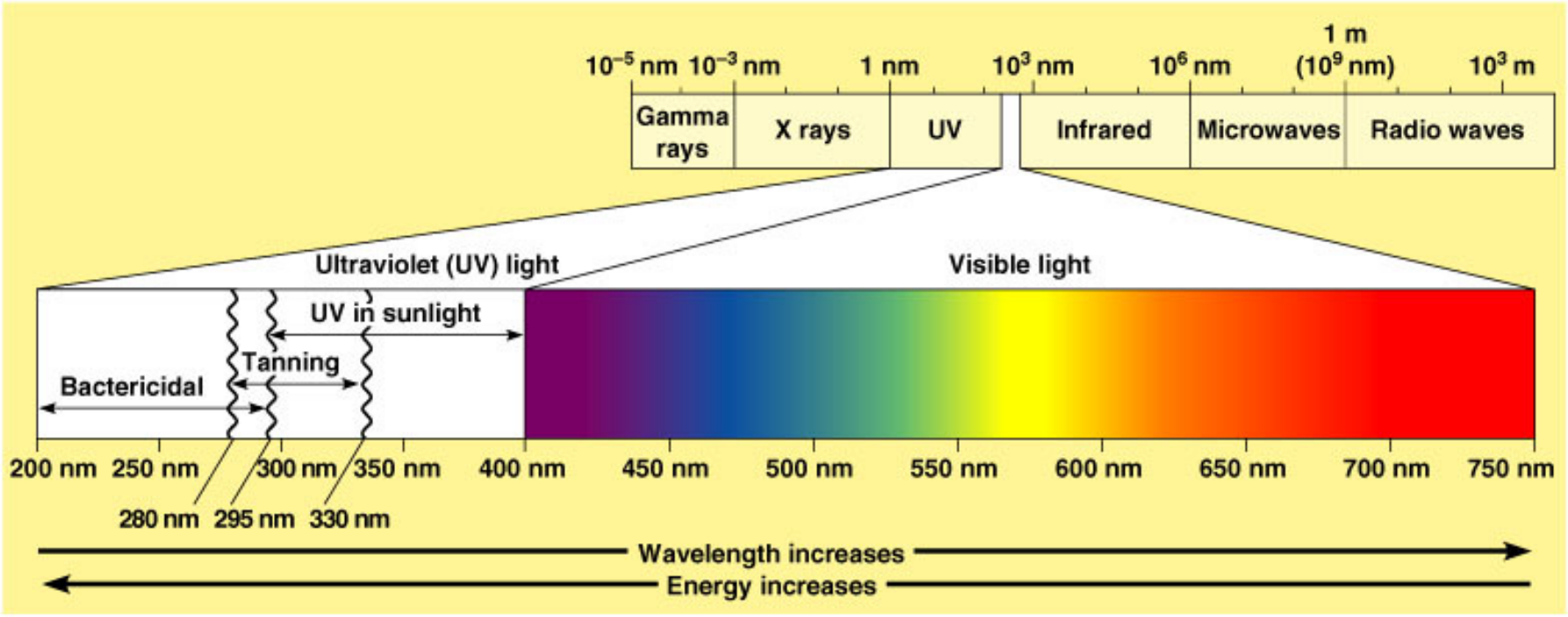
Physical Methods of Microbial Control

- Dry Heat Sterilization kills by oxidation
  - Flaming
  - Incineration
  - Hot-air sterilization

	Hot-air	Autoclave
Equivalent treatments	170°C, 2 hr	121°C, 15 min

Physical Methods of Microbial Control

- Filtration removes microbes
- Low temperature inhibits microbial growth
  - Refrigeration
  - Deep freezing
  - Lyophilization
- High pressure denatures proteins
- Desiccation prevents metabolism
- Osmotic pressure causes plasmolysis
- Radiation damages DNA
  - Ionizing radiation (X rays, gamma rays, electron beams) : water ionization to form hydroxyl radicals
  - Nonionizing radiation (UV): Form thymine dimers (Pyrimidine bases form bond)
  - (Microwaves kill by heat; not especially antimicrobial)



- *Antimicrobial effect of sunlight is due to formation of singlet oxygen.*
- *Pigments produced by some bacteria provide protection*

Figure 7.5

Lecture 24
BT 206
23/03/2023

Chemical Methods of Microbial Control

- Principles of effective disinfection
  - Concentration of disinfectant
  - Organic matter
  - pH
  - Time
- Evaluating a disinfectant
  - Use-dilution test
    1. Metal rings dipped in test bacteria are dried
    2. Dried cultures placed in disinfectant for 10 min at 20°C
    3. Rings transferred to culture media to determine whether bacteria survived treatment

    4. *Salmonella cholerasuis, Staphylococcus aureus*, and *Pseudomonas aeruginosa* are the three bacteria used in this test.

Chemical Methods of Microbial Control

- Evaluating a disinfectant
  - Disk-diffusion method

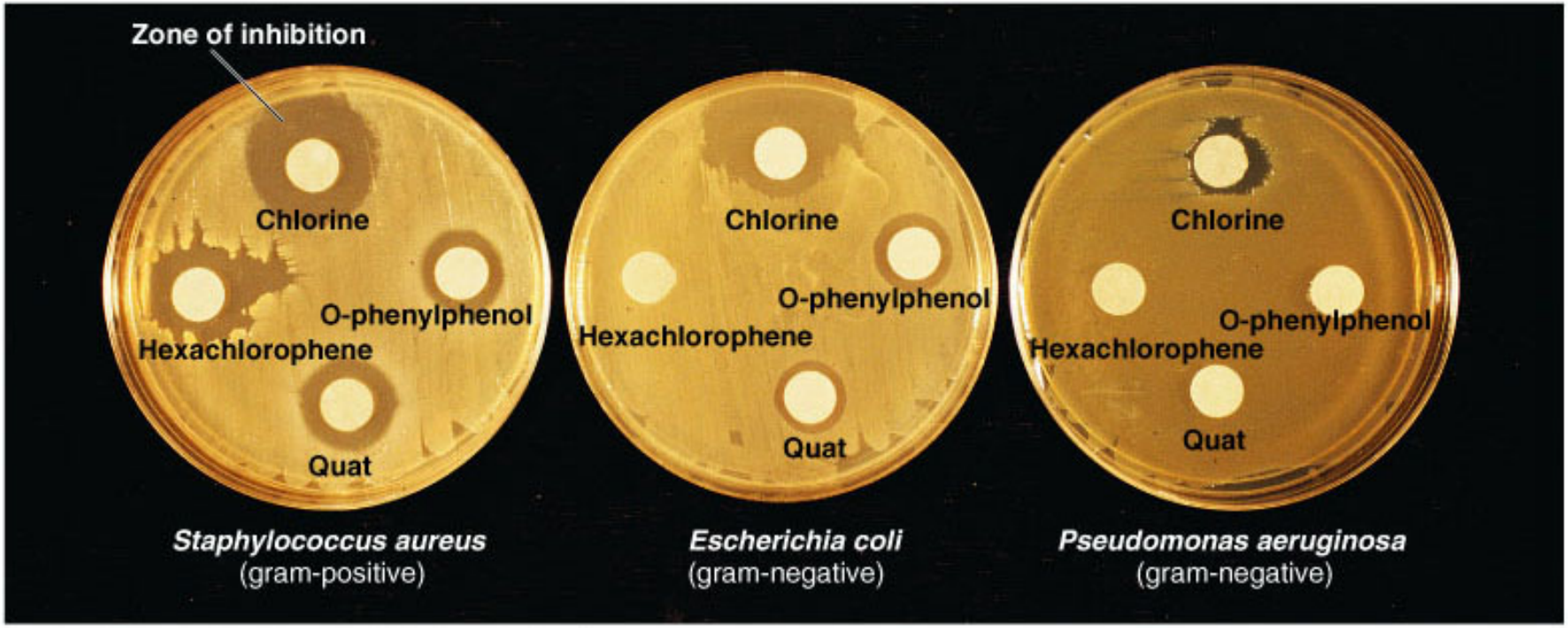
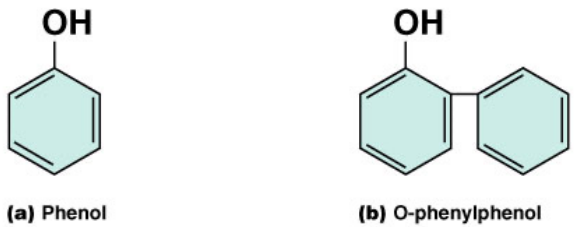


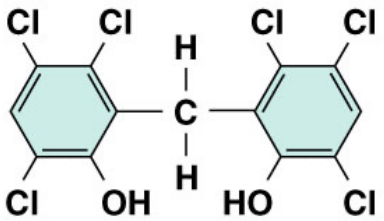
Figure 7.6

Types of Disinfectants

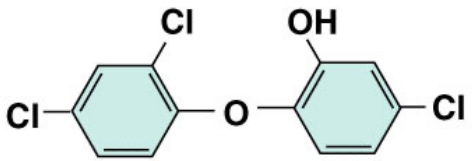
- Phenol
- Phenolics (derivatives of phenol). Lysol/ Cresols (derived from coal tar)
- Bisphenols. Hexachlorophene (lotion for newborn), Triclosan (in soaps)
  - Disrupt plasma membranes
  - Triclosan inhibits an enzyme needed for biosynthesis of fatty acid
- Biguanides. Chlorhexidine (combined with alcohol/detergent)
  - Disrupt plasma membranes
  - Microbial control on skin and mucous membrane
  - Biocidal against vegetative bacteria and yeasts



(a) Phenol (b) O-phenylphenol



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Figure 7.7

Types of Disinfectants

- Halogens. Iodine, Chlorine
  - Oxidizing agents
  - Bleach is hypochlorous acid (HOCl)
  - Calcium hypochlorite (dairy equipment & restaurant eating utensils) and Sodium hypochlorite (NaOCl eg Clorox)
  - Chlorine dioxide (to kill endospores of anthrax)
  - Iodine as Tincture (solution in aqueous alcohol)
  - Iodophor (combination of iodine and an organic molecule) eg. Betadine, or Povidone-iodine. Povidone is surface active iodophore.
  - Chlorine dioxide: gaseous in nature for anthrax endospores
  - Chloramines: Chlorine +Ammonia.: very stable

Types of Disinfectants

- Alcohols. Ethanol, isopropanol
  - Denature proteins, dissolve lipids
  - Effectively kills bacteria and fungi but not endospores and non enveloped viruses.

TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes***

Concentration of Ethanol (%)	Time (sec) 10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.

Types of Disinfectants

- Heavy Metals. Ag, Hg, Cu
  - Oligodynamic action:

    In oligodynamic action, **metal ions concentrate on the surface of the living object (bacteria, algae, and so on)**, which causes blocking of the free carbonyl and sulfhydryl groups of the surface structures. Oligodynamic action extends to enzyme systems and even to the activity of inorganic catalysts.

  - Denature proteins
  - Silver-sulfadiazine (topical cream for burns)
  - Surfacine (surface application on animate and inanimate)
  - Copper sulfate (algicide)
  - Zinc chloride (mouthwashes)
  - Zinc oxide (antifungal in paints)

BT 206 Microbiology 3-0-0-6

Syllabus

Introduction to microbiology and study of microorganisms: Scope of Microbiology; History of Microbiology: Spontaneous generation; Germ theory of diseases; Cell theory; Contributions of Antonie van Leuwenhoek, Joseph Lister, Robert Koch, Louis Pasteur, Edward Jenner, John Tyndall, Sergei N. Winogradsky, Alexander Fleming, etc; Microbial cell structure and function: General account of cell size, arrangement, shape; capsule, slime, pili, spores; structure and function of gram negative & gram-positive cell wall and membrane; periplasmic space; brief account of viruses; mycoplasma, eukaryotic microbes.

Microbial taxonomy: Taxonomy: principle and its types; classical approach: numerical, chemical, serological and genetic; bacterial taxonomy: Bergey's manual of Systematic Bacteriology (eubacteria and archaebacteria)

Methods and techniques in Microbiology: Microscopy: Principles; light microscope, phase contrast, dark field, bright field, fluorescent, interference microscope (stereo microscope); confocal microscopy; electron microscope (TEM and SEM).

Nutrition, growth and culturing: Microbiological media, composition and types; selective and differential media; growth curve, growth kinetics; influence of environmental factors on microbial growth; nutritional groups of bacteria; overview estimation of microbes - direct microscopic count, turbidometric assay; indirect method - CO2 liberation, protein estimation; sterilization and disinfection.

Microbial metabolism: Carbohydrate catabolism; anaerobic respiration, fermentation; protein and lipid catabolism; biosynthesis of purines, pyramidines, peptidoglycan, amino acids, lipids.

Microbial genetics: DNA replication in bacteria, fundamentals of gene regulation; mutations and DNA repair; plasmids, transformation, conjugation, transduction; Fundamentals of microbial genomics, metagenomics; Introduction to metagenomics; Scope and applications of genomics and metagenomics;.

Applications/Role of microbes: Applications in agriculture; environment; industry; health and disease. microbe interactions; mechanisms of pathogenicity.

Text Books

1.G. Tortora, B. Funke and C. Case,Microbiology,An Introduction (International Edition), 8th Ed, Pearson Education, 2003.

2. M. Madigan, J. Martinko ad J. Parker, Brock Biology of Microorganisms, 10th Ed, Prentice Hall, 2002.

References

1. R. Y. Stanier, J. L. Ingraham, M.L. Wheelis and P. R. Painter, General Microbiology, 5th Ed, Macmillan Press, 1987.

2. L. M. Prescott, J. P. Harley and D. A. Klein, Microbiology, 6th Ed, McGraw Hill, 2005.

3. J. G. Black, Microbiology: Principles & Explorations. 5th Ed, John Wiley & Sons Inc., 2002.Benjamin Lewin, Genes VIII (International Edition), Pearson Education, 2004.

Lecture 25
BT 206
24/03/2023

Types of Disinfectants

- **Surface-Active Agents or Surfactants**: decrease surface tension among molecules of a liquid

Soaps /Detergents	Degerming by emulsification
Acid-Anionic Sanitizers	. Anion of the molecule react with plasma membrane. Sanitizing dairy utensils and equipments
Quaternary Ammonium Compounds: Widely used Cationic detergents (Quats)	Bactericidal, Denature proteins, disrupt plasma membrane -eg Zephiran (Benzalkonium Chloride) and Cepacol (Cetylpyridinium Chloride)

Types of Disinfectants

- Chemical Food Preservatives
  - Organic Acids
    - Inhibit metabolism
    - Sorbic acid, benzoic acid, calcium propionate
    - Control molds and bacteria in foods and cosmetics
  - Nitrite prevents endospore germination and preserve the red color of meat by reacting with blood component of meat.
  - Sodium nitrite and nitrate are added to meat products

    (Nitrosamines are carcinogenic)
  - Antibiotics. Nisin (a bacteriocin) and natamycin (a pimaricin) prevent spoilage of cheese

Types of Disinfectants

- Aldehydes
  - Inactivate proteins by cross-linking with functional groups (–NH_2, –OH, –COOH, —SH)
  - Glutaraldehyde, formaldehyde
  - These are used by morticians for embalming
  - Gluteraldehyde when used in 2% solution (cidex) : bactericidal, tuberculocidal, virucidal in 10 min.
- Gaseous Sterilants
  - Denature proteins
  - Ethylene oxide
  - Kills all microbes and endospores but requires lengthy exposure period (4 -18 h)
  - Ethylene oxide: explosive in nature but highly penetrating. Often mixed with nitrogen or carbon dioxide.
  - Propylene oxide and β-propiolactone.

Types of Disinfectants

- Peroxygens
  - Oxidizing agents
  - O_3, H_2O_2, peracetic acid
  - Benzoyl peroxide: wounds infected with anaerobic pathogens (like acne)
  - Peracetic acid : one of most effective liquid chemical sporicides available and also considered as sterilant.

Microbial Characteristics and Microbial Control

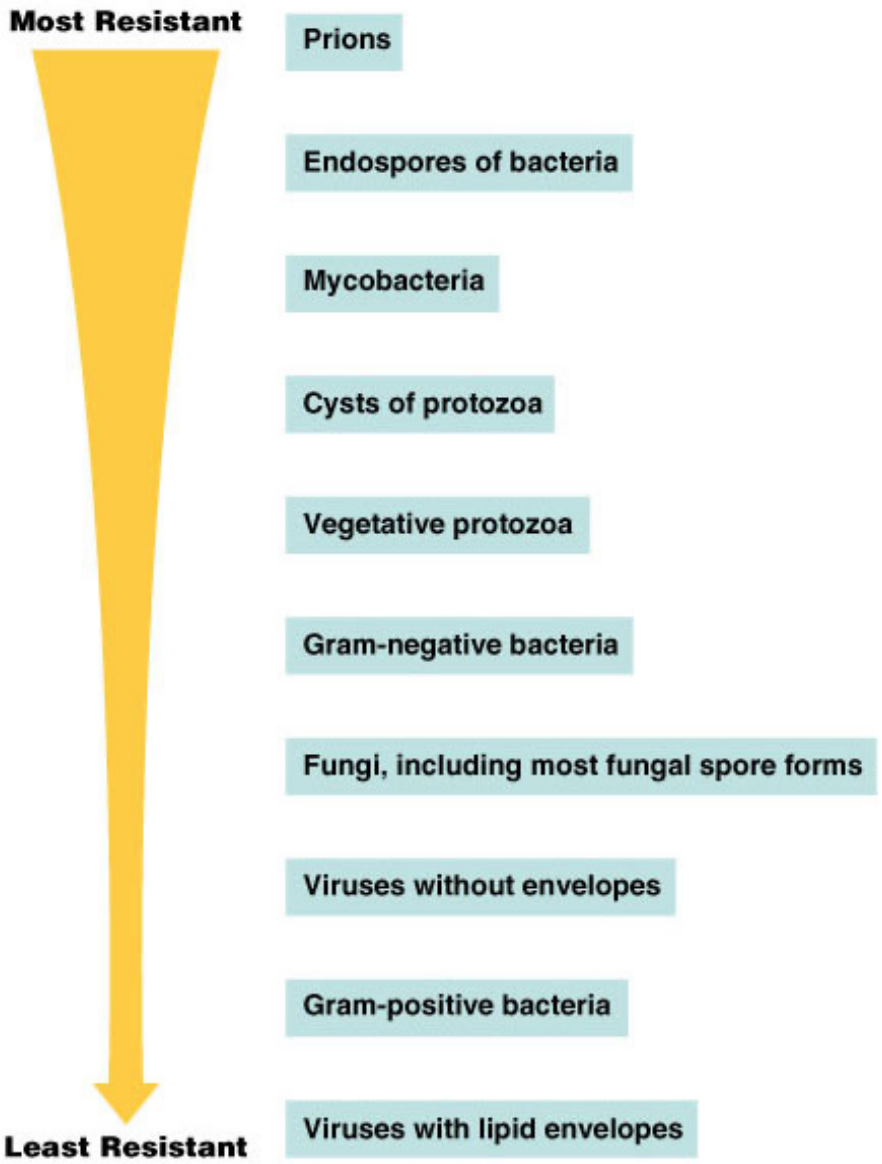


Figure 7.11

Microbial Characteristics and Microbial Control

Chemical agent	**Effectiveness against**	
	Endospores	*Mycobacteria*
Phenolics	Poor	Good
Quats	None	None
Chlorines	Fair	Fair
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good