Sterilization & Disinfection

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Sections in Medical Microbiology & Immunology

- Chapter 2, pages 12-13
  - This section covers endospores
- Chapter 13, pages 98-101
Sterilization and Disinfection

- **Sterilization**
  - Kill all forms of microbial life
  - Desirable but not always feasible

- **Antisepsis**
  - Prevent sepsis (infection) by killing infectious microorganisms

- **Disinfection**
  - Same as antisepsis, but applied to inanimate objects

- **Sanitize**
  - Reduce the number of microorganisms
EPA Definitions

- **Sterilizers (Sporicides)**
  - Used to eliminate all forms of microbial life including fungi, viruses, bacteria and their spores

- **Disinfectants**
  - Used on hard inanimate surfaces to destroy or inactivate infectious fungi and bacteria but not necessarily spores

- **Sanitizers**
  - Used to reduce, but not necessarily eliminate, microorganisms from the inanimate environment to levels considered safe by public health code

- **Antiseptics and Germicides**
  - Used on living humans or animals to prevent infection by inhibiting the growth of microorganisms. They are considered drugs and are regulated by the FDA
Sterilization and Disinfection

- These agents are non-selective
  - Damage human cells as well as microorganisms
- Limited therapeutic use
- Not absolute and the effect depends on the environment and condition of the bacteria
Destruction of Microorganisms

- Destruction of all microorganisms is not equivalent to sterilization
  - Killing microorganisms in an intravenous solution can release pyrogenic compounds and cause toxic shock
- Solutions should be sterilized so that bacteria never have a chance to grow
Death Rates During Sterilization are Exponential

- Killing of *E. coli* by phenol
- Typical of most bacteria
- Phenol is the standard
Death of Spores is also Exponential but Much Slower

- Killing of *B. subtilis* spores by phenol
- Specific rate constant is 1000 fold less
- True for all means of sterilization
- Killing spores is a major problem
Killing of Bacterial Spores

- Relatively resistant to killing by all means of sterilization

- Spores of *B. subtilis* are like spores of other bacteria, including pathogens such as:
  - *Clostridia* (botulinum, tetani)
  - *Bacillus anthracis*

- Problem of immense importance
Bacterial Spores

- Endospores are formed in response to nutrient depletion
- Composition of spores is distinct
  - Spores contain everything necessary to regenerate vegetative cells
- Spores are much more resistant to sterilization
- Basis of resistance is extremely low water content
  - Due to presence of dipicolinic acid
Dipicolinic Acid

- Responsible for the low H$_2$O and high Ca$^{2+}$ content of spores
- Unique in spores
- Chelates Ca$^{++}$
- Stabilizes DNA by intercalation
Sporulation and Germination

- Sporulation initiated by starvation
- Specific biochemical signal
  - GTP deficiency
  - Unfavorable environment leads to decreased amino acids, causing increased ppGpp, which inhibits GTP synthesis
Genetic Regulation of Sporulation

- The sporulation switch
- Specialized sigma (σ) factors
- σ factors are initiating proteins associated with RNA polymerase
  - σ29 is sporulation specific factor in *B. subtilis*
  - σ29 directs transcription of sporulation specific genes
  - σ55 is the factor for vegetative growth
Regeneration of Vegetative Cells

- Activation
- Germination
- Outgrowth
Regeneration of Vegetative Cells

- **Activation**
  - Generally by heat or chemicals
  - Mechanism is not known
  - Inactivation of a critical protein is one possibility

- **Germination**

- **Outgrowth**
Regeneration of Vegetative Cells

- Activation

- Germination
  - Irreversible
  - Requires Water
  - Accompanied by loss of resistance
  - Does not require nucleic acid or protein synthesis

- Outgrowth
Regeneration of Vegetative Cells

- Activation
- Germination
- Outgrowth
  - Active biosynthesis
  - Ordered transcription and translation
Extrapolation is Dangerous

- The approach to 0 is asymptotic.
- There is no absolute time when 0 microorganisms remain.
- Only the probability that a sample will have 0 microorganisms can be stated.

**B. subtilis**

Spores

Survivors

- Hr in 5% Phenol at 33 C
Extrapolation is Dangerous

- Quotation of odds is the best that can be done
- Even the predictions may be inaccurate
  - Kinetics vary with low populations
  - Kinetics are affected by composition of suspending medium (for example, aggregates of bacteria can survive longer)
Extrapolation is Dangerous

- First poliovirus vaccine (Salk vaccine) was a killed virus vaccine
  - Virus was treated for 1 week in 1:4000 formalin at pH 7 and 37°C
  - Aggregation of particles resulted in decreased killing of the virus

- 200 cases of paralytic polio and 11 deaths due to live virus in a batch of Salk vaccine
Chemical Agents that Damage the Cell Membrane

- Surface active compounds
- Phenolic compounds
- Alcohols
Surface Active Compounds

- Detergents
- Cationic agents, such as Zephiran
- Anionic agents, such as SDS
  - Never combine cationic and anionic agents (they neutralize each other)
- Nonionic agents, such as Tween 80
  - These are not very effective, and can even serve as nutrients for bacterial growth
Phenolic Compounds

- These are the standards to which all others are compared
- Alkyl and chloro phenols
  - Lysol (brand name disinfectant)
  - Triclosan (antibacterial soaps, deodorants, toothpaste, consumer products)
- Halogenated diphenyls
  - Hexachlorophene (soap withdrawn from over-the-counter sales in 1973)
Alcohols

- Ethanol
  - Concentrations between 50-70% are optimal for killing
  - 100% is not effective
- Isopropanol
  - More effective than ethanol, but more toxic
- Witch hazel
  - Alcoholic solution of an extract from the leaves and bark of the “witch hazel” tree

*Alcohols are not suitable for sterilizing surgical instruments*
Denaturation of Proteins

- Organic acids
  - benzoic and proprionic acids
- Preservatives in food and pharmaceuticals
- Alkyl esters of organic acids act like alkyl-substituted phenols
  - Nontoxic orally because they are rapidly hydrolyzed to p-hydroxy-benzoate
Modification of Proteins and Nucleic Acids

- Heavy metals
- Oxidizing agents
- Dyes
- Alkylating agents
Heavy Metals

- High affinity for sulfhydryl (SH) groups
- Effective at very low concentration (1 ppm)
- Reversed by sulfhydryl compounds
- Mercurials (mercurochrome)
- Silver compounds
  - Silver nitrate is used in the eyes of newborns to prevent Gonococcal infection
  - Silver sulfadiazine is used to prevent infection of the skin in burn patients
Oxidizing Agents

- Iodine combines with proteins and iodinates tyrosine residues
- Iodine preparations
  - Tincture of Iodine (2% I\(_2\) + 2% NaI in 50-70% ethanol)
  - Iodophores (I\(_2\) in a surfactant) – Betadyne
  - Potassium Iodide (KI) is used, but it is painful and destructive. Combining a detergent makes it less irritating.
- Effective against spores
Oxidizing Agents

- Chlorine (Cl₂) and hypochlorites
  - Chlorox (bleach)
  - Yield hypochlorous acid (HOCl)
  - Cl₂ + H₂O → HCl + HOCl

- Hydrogen peroxide and organic peroxides
  - Sensitivity to H₂O₂ depends on the absence of catalase activity
  - 3% solution was used for contact lenses
Dyes

- Triphenylmethanes
  - Mode of action undefined
  - Topical treatment for skin
  - Brilliant green, malachite green, crystal violet

- Acridines
  - Wound antisepsis
  - Mutagenic because they insert in DNA
  - Proflavine, Acriflavine
  - Carcinogenic
Alkylating Agents

- Active against spores at levels equivalent to those necessary to kill vegetative cells
- Formaldehyde
- Glutaraldehyde
- Ethylene oxide
Formaldehyde

- Reacts with $\text{CO}_2^-$, OH and SH groups
Formaldehyde

- Aqueous solution is formalin (37% solution)
- 0.2 – 0.4% is used to inactivate virus to make vaccines (remember the Salk vaccine)
- Used as a gas for decontamination
- Carcinogenic
Glutaraldehyde:

- Reacts with SH and NH groups
- 10 times as effective as formaldehyde
- Cold sterilant for surgical instruments

\[
\text{CH}_2\text{CHO} \quad \text{CH}_2 \quad \text{CH}_2\text{CHO}
\]
Ethylene Oxide

- Forms hydroxyethyl derivatives
  - $\text{CO}_2^-$, OH, SH, & phenolic groups of proteins
  - $\text{PO}_4$ & N of nucleic acids

- Biohazard

\[ \begin{array}{c}
\text{H}_2\text{C} \\
\text{O} \\
\text{H}_2\text{C} \end{array} \]
Physical Agents

- Heat
- Freezing and thawing
- Radiation
- Filtration
Heat

- The physical agent that is used most often, especially in a medical setting
- Sterilization with heat is strongly dependent on time, temperature, pressure, and H₂O
- Sterilization is slower in the dry state
  - Proteins lose polar groups and become resistant to denaturation
## Minimal Requirements for Sterilization

<table>
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<th>Time (min)</th>
<th>Pressure (lb/sq ft)</th>
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Other Methods Using Heat

- **Tyndallization**
  - A fractional sterilization method
  - Heat to 80° - 100°C for 30 minutes on 3 consecutive days
  - Spores are activated during each heating cycle, and then killed during the next cycle

- **Pasteurization**
  - Reduces the number of microorganisms and kills most pathogens
  - Heat to 62°C for 30 minutes
  - Used for milk (developed by Pasteur for wine)
Freezing and Thawing

- Not reliable
- Used to preserve bacteria in the laboratory
- Should never be used for sterilization
Ultraviolet Radiation

- Produces pyrimidine dimers in DNA
- Damage can be repaired by photoreactivation or SOS repair
- Low penetrating power limits use
- Can cause damage to eyes and skin
- Used for sanitizing rooms and tissue culture hoods
Ionizing Radiation

- $\gamma$-rays, x-rays and accelerated electrons

- Direct Effect
  - Energy directly damages macromolecules
  - Kills spores

- Used for sterilization of surgical supplies and food, killing spores because of the direct effect
Ionizing Radiation

- **Indirect effect**
  - From ionization of H$_2$O:
    - H$_2$O + E → H$_2$O$^+$ + e$^-$
    - H$_2$O$^+$ + H$_2$O → H$_3$O$^+$ + ·OH
    - e$^-$ + H$_2$O → OH$^-$ + H·
  - If O$_2$ is present, H$_2$O$_2$ and organic peroxides are also formed
  - Not effective against spores
Filtration

- Bacteria and larger microorganisms are easily removed from liquids
  - Effective pore size of 0.22 microns
- Removal of viruses requires ultrafiltration, which is feasible only for highly specialized materials
  - Requires high pressure because of the small pore sizes of the membranes