Process of Removal and Inactivation of Microorganisms
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Course Overview

I. Regulatory Requirements for Control of microorganisms
   - U.S. and EU requirements
   - Regulatory concerns for specific microorganisms
   - Contamination issues associated with microorganisms

II. Process Remove and Inactivation of Microorganism
   - Procedures for removing and controlling of virus/bacteria and fungi
   - Test method used to detected removal
   - Developing acceptance criteria
   - Valuation of the effectiveness of removal process

III. Validation and Monitoring of Removal Process
   - Validation of methodology for removal and/or inactivation of microorganisms
   - Developing disinfectant, sterilization and cleaning program
   - Routine monitoring program for microorganism removal

IV. Interactive Exercise
   - Participants walk through a risk assessment program to determine most effective method for removal and/or sterilization of microorganisms.
Disinfection/Sanitization Program

- CFR211.56 (b)

  “There shall be written procedures assigning responsibilities for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities; such written procedures shall be followed.”
Control of Microbial Growth

Effect of two basic ways:
1. By Killing Microorganisms
2. By inhibiting the Growth of Microorganisms

Usually involves the use of:
1. Physical Agents
2. Chemical Agents
Level of Resistance

1. Endospores
2. Mycobacteria
3. Fungal Spores
4. Small Non-envelopedViruses
   - Polio, Rotavirus, Rabies
5. Vegetative Fungal Cells
6. Enveloped Viruses
   - Herpes, Hepatitis B & C, HIV
5. Vegetative Bacteria
Principles of Microbial Control (Definitions)

- **Prevention / Control of Growth** to Prevent Infection and Spoilage
**Sterilization** / Complete Destruction

- No degrees of sterilization (*All or Nothing*)

- Utilizes
  a. Heat
  b. Radiation
  c. Chemicals
  d. Physical Removal
Once something is sterilized, it will remain sterile if properly sealed. Sterilization is performed on surgical equipment, filter housing, and certain sterile manufacturing equipment in order to prevent the spread of microorganisms.
Principles of Microbial Control

- Disinfection / Reducing Growth Nonliving Surfaces
Principles of Microbial Control

- **Disinfection** may not necessarily eliminate spores or all of the microorganisms from an object or environment.

- While disinfection is not as extreme as sterilization, but it is considered to be an adequate level of cleanliness for most situations.

- Examples of disinfectants include iodine solution, copper sulfate, ozone, and chlorine gas.
Principles of Microbial Control

- **Sanitize**

  Sanitizing is the process of reducing the number of microorganisms that are on a properly cleaned surface to a safe level. A safe level is defined as a 99.999% reduction of the number of disease microorganisms that are of public health importance. Sanitizing is accomplished by using either heat, radiation, or chemicals.
Principles of Microbial Control

- **Antiseptic** -- an agent used against sepsis or putrefaction in connection with human beings or animals.
- **Disinfectant** -- an agent that is applied to inanimate objects; it does not necessarily kill all organisms.
- **Sanitizer** -- an agent that reduces the microbiological contamination to levels conforming to local health regulations.
- **Germicide** -- an agent that destroys microorganisms.
- **Bactericide** -- an agent that causes the death of a specific group of microorganisms.
- **Bacteriostat** -- an agent that prevents the growth of a specific group of microorganisms but does not necessarily kill them.
- **Sanitization** -- the process of reducing microbiological contamination to a level that is acceptable to local health regulations.
- **Sterilization** -- the process of destroying all microorganisms.
Principles of Microbial Control

- **Cide** – Suffix Meaning to **Kill**
- **Stat** – Suffix Meaning to **Inhibit**
- **Sepsis** – Bacterial Contamination
- **Asepsis** – Lack of Bacterial Contamination
Rate of Microbial Death

- Bacteria Usually Die At a Constant Rate
- Plotted Logarithmically This Will Give a Straight Line

![Graph showing logarithm of number of microbial survivors against time (minutes). The graph demonstrates a straight line and indicates that one log decrease equals 90% of the population killed.](TF7LF0~6.JPG)
Time to Kill in Proportion to the Population Size

- Large Numbers Require Greater Time
- Small Numbers Require Less Time
Susceptibilities Vary

- Endospores are Difficult to Kill
- Organic Matter May Interfere with Heat Treatments and Chemical Control Agents
Control Agents Act By

- **Alteration of Membrane Permeability**
  - Susceptibility of membrane is due to its lipid and protein composition
  - Control Agents can alter permeability

- **Damage to Proteins and Nucleic Acids**
  - Break hydrogen and covalent bonds in proteins
  - Interfere with DNA, RNA, Protein Synthesis
Physical Methods

- Heat
- Filtration
- Low Temperatures
- Desiccation
- Osmotic Pressure
- Radiation
Heat

- Most Frequent and Widely Used.
- Always Consider
  1. Type of Heat
  2. Time of Application
  3. Temperature
- Endospores are the most heat resistant of all cells.
- Denatures Enzymes
- **Moisture improves heat penetration**, making sterilization by moist heat more effective then dry heat.
- Includes boiling and autoclaving.
Thermal Death Point

- **Thermal Death Point (TDP)** / Lowest Temp to Kill All the Bacteria in a Broth in 10 Minutes.

- This aspect of thermal death is useful in purifying water via boiling.

- **Boiling** -- Kills Many Vegetative Cells and Inactivates Viruses Within 10 Minutes (30 Minutes to be Safe) but has no effect on spores.
Dry Heat (Hot Air Oven)

- 160° C for 2 Hours or 170° C for 1 hour
- Used for
  a. Objects That Won’t Melt
  b. Glassware
  c. Metal
Filtration

- The passage of a liquid or gas through a filter with pores small enough to retain microbes.
- Especially important to sterilize solutions which would be denatured by heat (antibiotics, injectable drugs, amino acids, vitamins.)
HEPA Filters

- HEPA filters are High-Efficiency Particulate Air filters designed for the filtration of small particles. Certified HEPA filters must capture a minimum of 99.97% of 0.3 microns contaminants.
Low Temperatures

- Decreasing Temperature Decreases Chemical Activity
- Low Temps are **Not** Bactericidal
Desiccation

- Disrupts Microbial Metabolism
- Stops Growth / Microbes Are Still Viable
- Freeze-drying / Dehydration
- Viruses and Endospores Can Resist Desiccation
Radiation

- Acts by destroying DNA or damaging it.
- Its efficiency is dependent on the wavelength, intensity, and duration.
The most lethal type of radiation is ultraviolet radiation with a wavelength of 260 nm. This is the wavelength most actively absorbed by DNA. It is useful for disinfecting surfaces, air and liquids.
Studies have shown that irradiating microorganisms like *E. coli* and *Salmonella* may give rise to even more dangerous, radiation-resistant strains of bacteria.
Microwaves

- Kill Microbes Indirectly with Heat.
- Can denature organic material.
- Only effective on plastic components not metals
Chemical Control Methods

- Phenols and Phenolics
- Biguanides
- Halogens
- Alcohols
- Heavy Metals and Their Compounds
- Surface-Active Agents
- Quaternary Ammonium Compounds
- Aldehydes
- Antibiotics
Two Conditions Influence the Effectiveness of Chemical Disinfectants

1. Type of Microbe
   - G+ More Susceptible to Disinfectants
   - Pseudomonands Can Grow in Disinfectants and Antiseptics
   - *M. tuberculosis* is Resistant to Many Disinfectants
   - Endospores Most Resistant

2. Environment
   - Organic Matter and Increased Temp
Evaluating a Disinfectant

- Old Standard is the **Phenol Coefficient Test**
  (FYI -- The phenol coefficient is the value obtained by dividing the highest dilution of the test solution by the highest dilution of phenol that sterilizes the given culture of bacteria under standard conditions of time and temperature.)
Cleaning/Sanitization is a regulatory requirement

Cleaning should not be confused with disinfection

A cleaning program is not complete without contamination control
An effective cleaning and sanitization process is attained by:

- Treatment with strong acids and bases
- Use of high velocity hot water or steam
- Use of detergents and/or sanitizers
- Rinsing with high quality water (Purified or WFI)
- Use of solvent rinses
- Drying at elevated temperatures
Product Classification

- **Sanitizers**
  - proper use results in 99.9% (3 log) reduction of bacteria
  - cannot handle soil; use on pre-cleaned surfaces

- **Disinfectants**
  - proper use results in 100% (> 4 log) reduction of bacteria and yeast. Limited reduction for mold.
  - most have surfactants/cleaning ability

- **Sterilants**
  - proper use results in 100% (> 6-7 log) reduction of all microorganisms, including bacterial spores.
  - require application on pre-cleaned surfaces
Sanitizers

Alcohols

- Ethyl and Isopropyl (70-85% conc.)

- Gloves, work surfaces, equipment, instruments, rinse

- No residue, not sporicidal, non-corrosive, weak inactivation by soil

- Flammable, volatile, toxic
Disinfectants

Phenolics

- Typical Conc. (0.15-5.0%)
- Work surface, floors, walls, equipment (non-product contact)
- Residue (residual activity), corrosive, not sporicidal, biodegradable, cleaning ability, fungicidal activity
Disinfectants

Quaternary Ammonium Compounds

- Typical Conc. (0.1-2.0%)
- Work surface, floors, equipment (non-product contact)
- Pre-clean and decontamination
- Residue, corrosive, not sporicidal, not soil tolerant/inactivated by detergents, weak fungicide, less active against gram (-) rods, tend to foam
Disinfectants

Chlorines

Sodium Hypochlorites
- Typical Conc. (500-5000 ppm)
- Floor drains, glassware & equipment (non-product contact)
- Residue, corrosive, sporicidal, fungicidal, not soil tolerant
- Poor stability, incompatible w/ some detergents

Chlorine Dioxide
- Animal facilities, water treatment, slime control
- Gaseous form used for decontamination of isolators
- Sporicidal, very reactive/must be prepared at site of application
- Not compatible with phenolics
Sterilants

Hydrogen Peroxide
- Typical Conc. (3-6%)
- Work surface, floors, equipment, fogging (VHP)
- No residue, corrosive, sporicidal (> 6% conc), fungicidal
- Can damage rubber, plastic and fabrics
- Most effective in combination with peracetic acid

Peracetic Acid
- Typical Conc. (0.2% Liq)
- Work surface, floors, equipment (non product contact), fogging (VPPA)
- Residue, corrosive, sporicidal, fungicidal
- Most effective in combination with hydrogen peroxide
Sterilants

Aldehydes

Formaldehyde
- Typical Conc. (3-8%)
- Mainly fogging applications
- Residue, non-corrosive to metals, slow sporicidal activity, fungicidal
- Toxicity issues

Glutaraldehyde
- Typical Conc. (2-5%)
- Ideal for liquid immersion of contaminated instruments & equipment, compatible with detergents
- Residue (yellow film), corrosive, sporicidal, fungicidal
- Toxicity issues
Disinfectant Selection

(USP <1072> Disinfectants and Antiseptics, PF,

- Population and types of organisms
- Spectrum of activity of disinfectant
- EPA registrations
- Method of application
- Contact time
- Nature and surface to be disinfected
- Compatibility of surface with disinfectant
Disinfectant Selection

(USP <1072> Disinfectants and Antiseptics, PF,

- Corrosiveness of disinfectant
- Organic compounds present on surface
- Operator safety
- Compatibility with cleaning agents
- Planned rotation of disinfectants
- Needed steps to avoid contamination of pharmaceutical products by disinfectant
- Need for residual bactericidal activity
Disinfectant Selection - Other Factors to Consider

- Quality, sterility, and stability of product
- Ease of application
- Cleaning ability
- Supporting vendor documentation
- Cost and availability
- Factors that may affect performance (temp., organic matter, contact time, pH, etc…)
- Regulatory expectations/regulations
All germicidal cleaners fall under the FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) as amended (1977) and are administered by EPA (Environmental Protection Agency).

EPA requirements

- safety: directions for use and disposal
- efficacy: per AOAC (Association of Official Analytical Chemists) Official Methods of Analysis
Preparation and Use of Disinfectants

Preparation and Storage

- EC Guide, Annex 1, 38

“Disinfectants and detergents should be monitored for microbial contamination: dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilised. Disinfectants and detergents used in grade A and B areas should be sterile prior to use.”
Preparation of Disinfectants

- **Preparation**
  - concentration is critical
  - grade of water should be process-grade
  - storage of disinfectant solutions
  - filtration needs and filter compatibility
Use of Disinfectants

- Method of application
  - spray, wipe, mop
  - 2-bucket system
  - handling of mop head and squeegees
  - rinsing (as needed to control residue)
Use of Disinfectants

- Cleaning Personnel
  - contract service vs manufacturing operators
  - training and monitoring for cleaning crew
Current regulatory expectations for use of disinfectants

- Vendor Qualification
- Procedure for product acceptance and rejection
- Detailed SOPs and documentation for the preparation and use of disinfectants
- Limit application area
  - top to bottom & cleanest to dirtiest
Current regulatory expectations for use of disinfectants

Rotation

- EC Guide, Annex 1, 37
  “Where disinfectants are used, more than one type should be employed. Monitoring should be undertaken regularly in order to detect the development of resistant strains.”

- ISO Recommendation

- Industry Practice
Current regulatory expectations for use of disinfectants

- **Disinfectants Qualification**
  - Risk Assessment
  - Studies to evaluate the effectiveness of the disinfectants as they are used and prepared
  - Studies to evaluate the storage conditions of disinfectants for possible loss of efficacy
  - Use of environmental isolates in the qualification studies
  - Neutralization studies

..\Disinfectant Effectiveness Matrix (version 1) Updated.xls
Disinfectant Qualification - Studies

**In-Situ**
- Use actually cleaning procedures
- Monitoring pre and post cleaning at worst-case conditions/document activities
- Increased number of sample sites
- Compare EM data before & after

**In-Vitro**
- Surface Tests (use of coupons or pieces of equipment/material)
- Carrier Tests (AOAC method)
- Use-Dilution Tests (modified AET)
Typical Test Organisms

- *Escherichia coli* - ATCC 8739
- *Pseudomonas aeruginosa* - ATCC 9027
- *Staphylococcus aureus* - ATCC 6538
- *Bacillus subtilis* - ATCC 6633
- *Candida albicans* - ATCC 10231
- *Aspergillus niger* - ATCC 16404
- Environmental isolates
Disinfectant Qualification

**Surface Test**

- Preferred method by inspectors
- No guidance document available
- Must select types of surfaces (coupons)
- Variable microbial challenge level
- Quantitative method
- Must select contact times (1-5-10 minutes)
- Variable test conditions (wet vs dry)
- Sampling/recovery technique (swab, rinse, contact plate)
- Need neutralization studies
Disinfectant Qualification

Carrier Test

- AOAC method to qualify disinfectants
- Use of glass penicylinders
- Qualitative method: pass/fail test
- May use a modified carrier test to quantitate the recovery obtained
- Need neutralization studies
- Time consuming and technique dependent
Disinfectant Qualification

Use-Dilution Test

- Modified AET method
- Must choose contact times (10 minutes)
- Quantitative method
- Recovery Method (MF technique)
- Neutralization studies
Disinfectant Qualification - User’s Approach/Decisions

- Type of agent and materials/surfaces and sites to be evaluated.
- Testing protocol (In-vitro vs In-situ)
- Types of challenge organisms
- Contact times
- Sampling methods (swab, rinse, or contact plate)
Disinfectant Qualification - User’s Approach/Decisions

- Neutralization and Recovery studies
- Acceptance criteria should be based on industry guidelines and application of the product
- Change control
- Re-qualification needs
Disinfectant Qualification - Acceptance Criteria

- **Surface Tests**
  - ≥ 3-log reduction vegetative bacteria and fungi
  - ≥ 2-log reduction spore-forming bacteria

- **Use-Dilution Tests**
  - ≥ 4-log reduction (disinfectant properties)
  - ≥ 6-log reduction (sporicidal properties)

- **Carrier Test**
  - AOAC criteria

- **In-situ Test**
  - counts not to exceed alert levels
Disinfectant Qualification

Which method is the best?

- Company’s Objectives
- Cost considerations
- Type of disinfectant
- Application of disinfectant
- Environment to be decontaminated
  - Combination of tests
KEY FACTORS IN DISINFECTANT EFFICACY

• Concentration of Disinfectant
• Number and Location of Microorganisms
• Resistance of Microorganisms
• Contact Time
• Physical and Chemical Factors
  – Example: pH, temperature, organic contaminants
• Potential surface damage
Questions?
Thank You!!