SAND2012-2459P

Decontamination

Draft Slide Deck – December 2011

BIOSECURITY ENGAGEMENT PROGRAM

CBEP
COOPERATIVE BIOLOGICAL ENGAGEMENT PROGRAM

Introductions

- Instructors
- Students
  - Your name?
  - Where are you from?

Action Plan(pg X)

By the end of this lesson, I would like to:					
KNOW		FEEL		BE ABLE TO DO	

Your learning doesn't stop with this lesson. Use this space to think about what else you need to do or learn to put the information from this lesson into practice.

What more do I need to know or do?	How will I acquire the knowledge or skills?	How will I know that I've succeeded?	How will I use this new learning in my job?

Use space on back, if needed

Objectives

- Discuss the differences between disinfection, decontamination, and sterilization.
- Discuss the various decontamination methods used for surface and area decontamination.
- Explain how validation of the decontamination procedure is conducted and be able to interpret the results.

Biorisk Management Review

Risk Mitigation Strategies Review

Definitions

- ***Sterilization*** - act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms. The definition is categorical and absolute - an item either is sterile or is not.

- ***Disinfection*** - Generally less lethal process than sterilization. It is the elimination of nearly all recognized pathogenic micro-organisms but not necessarily all microbial forms (e.g., bacterial spores).

Definitions, continued

- ***Antiseptic*** - a substance that prevents or arrests the growth or action of microbes, either by inhibiting their activity or by destroying them
  - "septic" – containing disease causing organism, anti - remove

- ***Decontamination*** – A process to remove contamination. Decontamination renders an area, device, item, or material safe to handle, that is, reasonably free from a risk of disease transmission.

Methods of Decontamination

- Chemical (e.g., bleach)
- Thermal (e.g., autoclave)
- Filtration (e.g., HEPA filter)
- Radiation (e.g., UV light)

Classes of Chemical Disinfectants

- Halogens (Chlorine, Iodophors)
- Aldehydes (Glutaraldehyde/Formaldehyde)
- Phenolics
- Alchohols
- Acids (Peracetic acid) & Alkalis (NaOH)
- Oxidizing Agents (Hydrogen peroxide)
- Quaternary Ammonium compounds
- Biguanidines (Chlorhexidine)

The Ideal Chemical Disinfectant

- You are looking for the perfect chemical disinfectant .
- In your small group, list all of the properties of the ideal chemical disinfectant.
- List one property per sticky note.

Factors affecting disinfection

- In your small group, consider the conditions and factors that might affect how well a chemical disinfectant will work.
- Write each factor on an individual sticky note.

Factors Affecting Disinfection

- Number of microorganisms
- Location of microorganisms
- Innate resistance to the disinfectant
- Concentration and potency of the disinfectant
- Physical and chemical factors
- Presence of organic matter
- Duration of exposure
- Biofilms

Resistance to Disinfectants

Resistant

Prions (agents causing Creutzfeldt-Jakob Disease)
Bacterial spores (*Bacillus anthracis*)
Coccidia (*Cryptosporidium*)
Mycobacteria (*M. tuberculosis*)
Nonlipid or small viruses (polio, coxsackie)
Fungi (*Aspergillus, Candida*)
Vegetative bacteria (*E. coli, S. aureus*)
Lipid or medium-sized virus (HIV, herpes, hepatitis B)

Susceptible

Environmental Factors

- Dried spills (from media, buffers) may limit contact between the disinfectant and the target organism.
  - Pre-cleaning usually necessary for spills
- Dirt, grease and oils - all can protect the organisms.
  - Grease and oils will repel water based disinfectants.

Product Factors

- Age of the product/solution
- Method of application
    - spray vs. wipe
- Rate of application
- Storage conditions
  - Opaque vs. clear containers

BREAK

Properties of Chemical Disinfectants

- The instructor will assign your group a chemical disinfectant to research.
- In your small group, review the resource material provided in your workbook.
- Complete the template in your workbook that includes the following information and be prepared to report to the group.
    - Mode of action
    - Typical concentration used
    - Uses in the laboratory
    - Advantages
    - Limitations/Disadvantages

Criteria	Report
Name of Chemical Disinfectant:	
Mode of Action	
Typical Concentration used	
Uses in the Laboratory	
Advantages	
Limitations/Disadvantages	

Choosing a Chemical Disinfectant

- In your small group:
  - Read the scenario (5 minutes)
  - Discuss and select an appropriate disinfectant for use in the scenario (5 minutes)
  - Using the template in your workbook, write an SOP for using the disinfectant in the scenario (15 minutes).

Scenario

- A researcher plans to grow various strains of *Bacillus cereus* (a potential foodborne pathogen closely related to *B. anthracis*) on petri dishes.
- Individual colonies will then be used to inoculate liquid broth cultures of up to 500 mLs. The cultures are grown in glass reusable Erlenmeyer flasks in a shaker incubator.
- Cultures will be transferred to plastic disposable tubes to be spun down in a centrifuge. The pellet will be washed, collected and analyzed for toxin production. This will involve the use of micropipettes, glass slides, and various stains and reagents.
- Sub cultures will be lyophilized for storage in small (<1ml) cryovials and stored in the freezer.
- ***How will lab surfaces and reusable materials be disinfected?***

Standard Operating Procedure for:	
Conditions	
Who should use the SOP?	
When should it be used?	
Why should the SOP be used?	
Where should it be used?	
Context	
Input(s):	Contaminated surfaces and reusable materials
Output:	Disinfected surfaces and reusable materials
Preparation required:	
Actions (steps required to move from the input to the output)	
Step 1	
Step 2	
Step 3	
Step 4	
Step 5	

"Evaluating" your SOP

- Give your SOP to another small group for evaluation.
- Read the SOP you've been asked to evaluate (5 minutes) and answer the following questions (10 minutes):
  - Did you understand the SOP?
  - Is it physically possible to follow the SOP?
  - What questions do you have?
  - What suggestions might make the SOP easier to understand and follow?
- If time allows, come to a class-wide consensus on the SOP to be used.

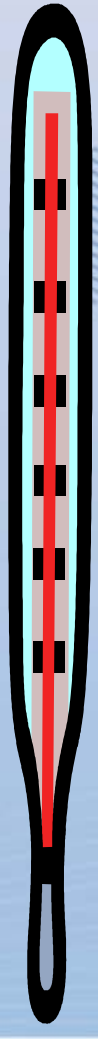
BREAK

Additional Methods of Disinfection

- Thermal
  - Autoclave
  - Incinerator
- Filtration
- Radiation
  - Non-ionizing (UV light, microwave)
  - Ionizing (E-Beam, gamma and x-rays)

Autoclaves

Heat Kills!



- 160 °C Spores killed 2 hrs dry heat
- 134-138 °C Prions inactivated
- 121 °C Spores killed in 2 min (autoclave)
- 100 °C Only spores survive after 10 minutes
- 82 °C Bacteria killed 3 secs (pasteurization)
- 72 °C Bacteria killed 17 secs
- 63 °C Bacteria killed in 30 mins
- 56 °C HIV inactivated 30 mins
- 41 °C Protein denaturing starts
- 37 °C Body temperature
- 20 °C Room temperature

Principles of Autoclave Sterilization

- Direct exposure to steam at the required temperature and pressure for a specific time
  - 121 °C – 123 °C
  - 15 psi; 1.05 kg/cm2
- Time required depends on the nature of the material to be sterilized. (Generally 1 hr for waste)

Steam Penetration

- Steam must directly contact all areas of the load (bags should be loosely gathered)
- If the steam can't penetrate a dry container, you have dry heat, which takes much longer to achieve kill.
- Add ~ 50 - 250 ml of water to bags prior to autoclaving to facilitate steam saturation

Activity: When to Autoclave?

- Work with your group to develop a list of the advantages and disadvantages for using an autoclave to decontaminate laboratory materials.
- Complete the template in your workbook.
- Based on your answers:
  - When would using an autoclave be advantageous?
  - When would another method be preferable to autoclaving?

Autoclave Safety

- Follow manufacturers' guidelines
- Do not open pressurized chamber
- Avoid standing directly in front when opening
- Establish a preventative maintenance schedule and annual inspection by certified technician
- Wear appropriate PPE
- Careful – liquids are hot
- Open door slowly, allow steam to vent before opening fully

Autoclave safety

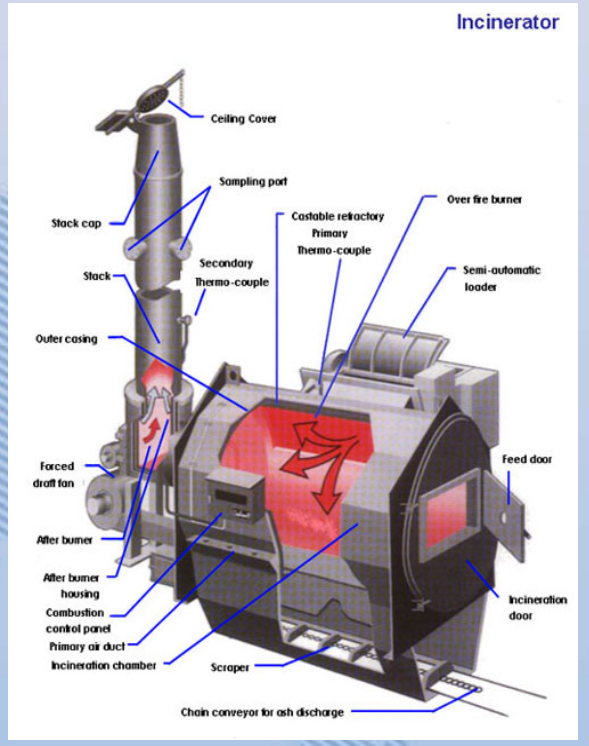
- Do not place sealed containers into autoclave
- Do not autoclave items containing solvents, volatiles, radioactive or corrosive chemicals
- Use shallow metal pans for best results and heat transfer
- Check drain and seals

Incineration

- Treatment of choice for animal bedding, carcasses and pathological wastes; but not plastics!
- Reduces volume of waste by up to 95%
- May allow energy generated to be recovered
- Operation parameters:
  - Primary chamber: 1400°F-1800 °F (760 °C-982 °C)
  - Secondary chamber: >2000 ° F (1093 °C)

Incineration Concerns

- Can generate smoke, residues with heavy metals, gases (e.g., HCl, CO, PCBs, etc.)
- May require pollution control devices, e.g., wet/dry air scrubbers, electrostatic precipitators
- Loading needs to be controlled
- May require permits



Incinerator?


BREAK

Validation Methods

- Work with your group to discuss methods or ways in which you can assure that the following procedures actually result in decontamination:
  - Chemical disinfection – surfaces
  - Chemical disinfection - liquids
  - Autoclave sterilization
  - Incinerator run

Review of decontamination

Review

To wrap-up, let's discuss what we learned about decontamination in a biological laboratory setting.

What did we learn?	What does it mean?	Where do we go from here?

Action Plan(pg X)

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Thank You!

Don't forget to complete your evaluation!

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