

# Decontamination, Sterilization and Disinfection

SAND2012-6686P

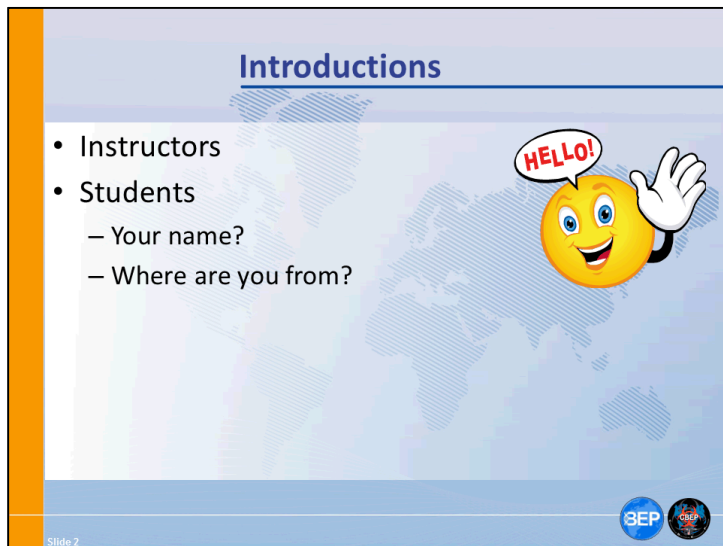
## *Student Guide*



## *Decontamination*

## *Welcome & Introductions*





**Introductions**

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

HELLO!

BEP CSP

Slide 2

---

---

---

---

---

# Action Plan

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



### Key Messages

- Disinfection and decontamination have similar meanings. Both are less rigorous than sterilization which is the complete removal of all life.
- No disinfectant is ideal, they all have strengths and limitations. Understanding the strengths and limitations is key to their use.
- There are a number of factors that determines how effective a particular disinfectant is.
- Micro-organisms have various innate resistance to disinfectants.



Page 4

### Key Messages – Continued

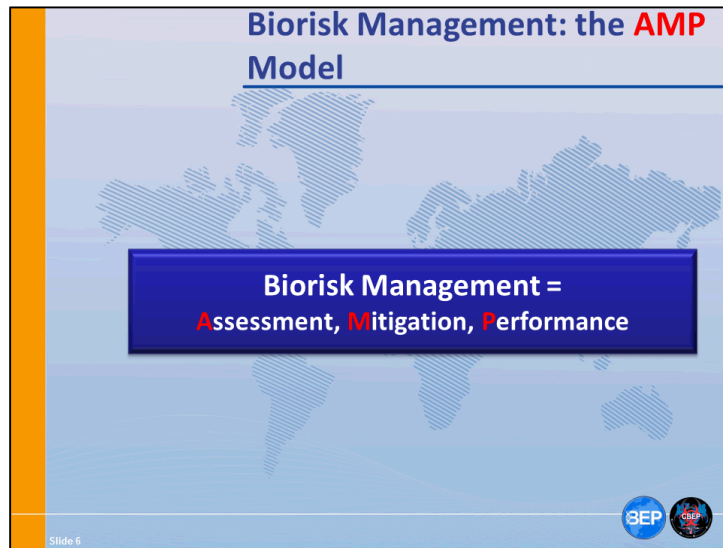
- Autoclaves can be used to sterilize things through wet heat and the application of appropriate time, pressure and temperature.
- Wet heat is much more effective than dry heat.
- Validation is a process to ensure that the decontamination, disinfection or sterilization process used was complete and achieved its requirements.



Page 5


## *Decontamination*

## *Biorisk Management*







### Key Components of Biorisk Management

 **Biorisk Assessment**

- Process of identifying the hazards and evaluating the risks associated with biological agents and toxins, taking into account the adequacy of any existing controls, and deciding whether or not the risks are acceptable



Slide 7

Define Biorisk Assessment:

---


---

---




---

---

### Key Components of Biorisk Management

 **Biorisk Mitigation**

- Actions and control measures that are put into place to reduce or eliminate the risks associated with biological agents and toxins



Slide 8

Define Biorisk Mitigation:

---

---


---

---




---



### Key Components of Biorisk Management

 **Performance**

- The implementation of the entire biorisk management system, including evaluating and ensuring that the system is working the way it was designed. Another aspect of performance is the process of continually improving the system.



Slide 9

Define Performance:

---

---

---



---

---

## Decontamination

### 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 & Methods of Decontamination


### 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)



Slide 12

BEP

## *Decontamination*


## *Chemical Disinfection*

### Classes of Chemical Disinfectants

**Chemical Disinfectants**

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

Slide 13




**The Ideal Chemical Disinfectant**

**Group Exercise:**

You are looking for the perfect chemical disinfectant.

**In your groups**, please spend **5 minutes** to **list all of the properties of the ideal chemical disinfectant**. Write one property per **sticky note** and post them on your **flip chart**.

Slide 14



### Factors Affecting Disinfection

#### **Group Exercise - Continued:**

Now consider the conditions and **factors** that might affect how well a chemical disinfectant will work.

**In your groups**, please spend **5 minutes** to **list all of the factors**. Write one factor per **sticky note** and post them on your **flip chart**.




Slide 15




### Factors Affecting Disinfection

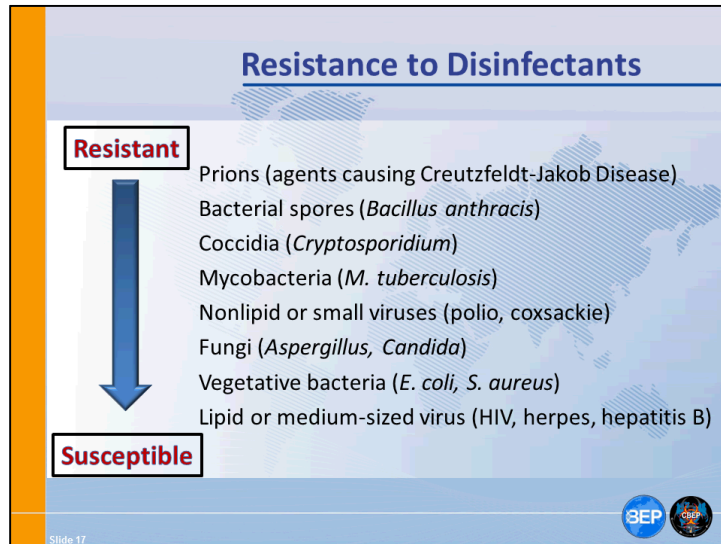
- Number of microorganisms
- Location of microorganisms
- Innate resistance to the disinfectant
- Concentration and potency of the disinfectant
- Physical and chemical factors
- Presence of organic matter
- Duration of exposure
- Biofilms



Slide 16



Notes:






Notes:

DRAFT

### Environmental Factors

**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.






Notes:

**Product Factors**

**Product Factors**

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



How do each of these affect the effectiveness of the chemical disinfectant?

- Age:
- Method:
- Rate:
- Storage:

---

---

---

---

---

## Decontamination

## Properties and Selection of Chemical Disinfectants

### Properties of Chemical Disinfectants


**Group Exercise:**

Your group will be assigned a chemical disinfectant to **research** including the following information:

- Mode of action
- Typical concentration used
- Uses in the laboratory
- Advantages
- Limitations/Disadvantages

**In your groups**, please spend **10 minutes** to **review the resource material** provided. **Complete the table** in your **student guide** and be prepared to report to the class.

Slide 20




## Properties of Chemical Disinfectants

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




**Choosing a Chemical Disinfectant**

**Group Exercise:**

**In your groups**, please spend **5 minutes** to **read the scenario** provided. **Discuss and select** an appropriate disinfectant. Using the template in your workbook, spend **15 minutes** to **write an SOP** for using the disinfectant in the scenario.

Slide 22



## 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**

**Group Exercise - Continued:**


Give your SOP to another small group for evaluation.

**In your groups**, spend **15 minutes** to read the SOP you’ve been asked to evaluate and **answer the following questions**:

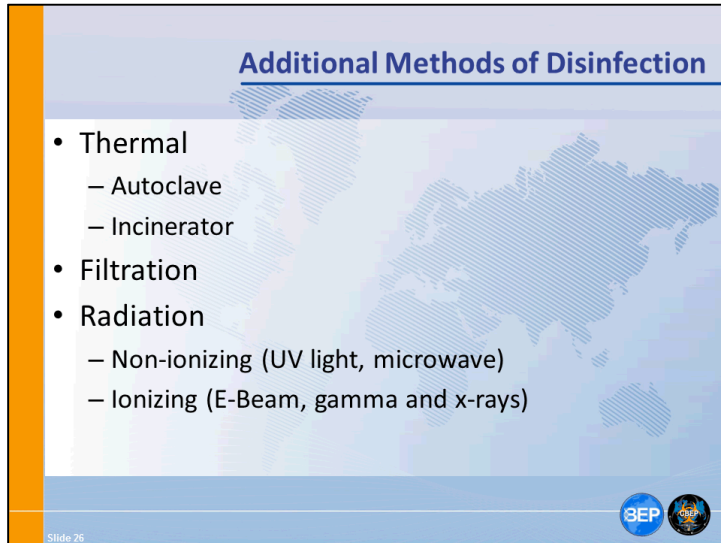
- Did you understand the SOP?
- Is it physically possible to follow the SOP?
- What questions do you have?
- What suggestions might make the SOP easier to understand and follow?

**If time allows**, come to a class-wide consensus on the SOP to be used.

Slide 25



## *Decontamination*



**Additional Methods of Disinfection**

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

Slide 26

BEP

## *Additional Methods of Disinfection*

Notes:

DRAFT

---

---

---

---

---

## Decontamination

## Additional Methods of Disinfection

### Autoclaves



Slide 27



### 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

Slide 28





### 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/cm<sup>2</sup>
- Time required depends on the nature of the material to be **sterilized**. (Generally 1 hr for waste)



Slide 29

For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

- 3 minutes holding time at 134°C
- 10 minutes holding time at 126°C
- 15 minutes holding time at 121°C
- 25 minutes holding time at 115°C

### Steam Penetration

- Steam **must** directly contact **all** areas of the load (bags should be loosely gathered)
- If the steam cannot 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



Slide 30

Notes:



---

---

---

---

---


### When to Autoclave?

**Group Exercise:**

In your groups, spend **10 minutes** 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?

Slide 31



- Advantages:

- Disadvantages:

---

---

---

---

---

### 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



Slide 32

### 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**



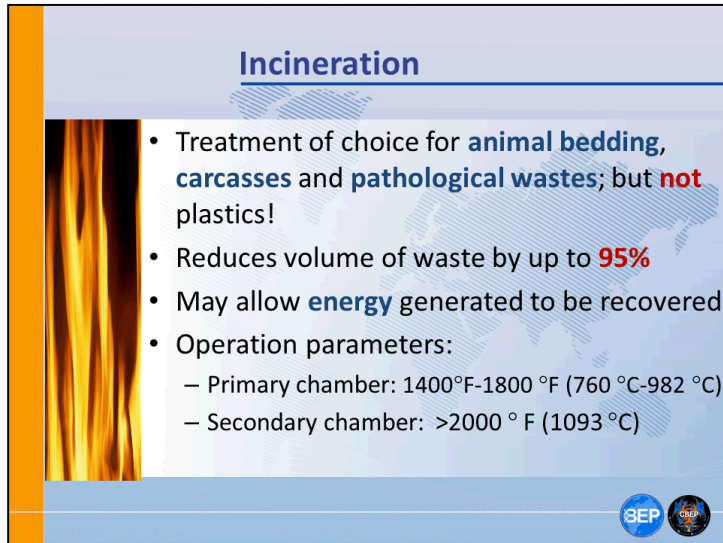
Slide 33

## *Decontamination*

## *Additional Methods of Disinfection*





- What do you notice about this picture?
- Are there any practices or procedures you would modify? Anything with the autoclave itself?



### 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)



- What is the advantage of an incinerator over an autoclave or chemical disinfectants?

---

---

---

---

---

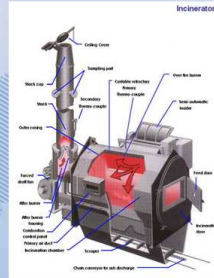


## Decontamination

### *Additional Methods of Disinfection*

## 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**



- What are the disadvantages of an incinerator over an autoclave or chemical disinfectants?



- What is required to make a fire an incinerator?
- When is fire okay?
- When does fire need to be an incinerator?

## Decontamination

## Validation of Decontamination



### Validation Methods

**Group Exercise:**

In your groups, spend **10 minutes** 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

38



## Decontamination

## Review & Wrap Up

### Review of decontamination



#### Review

For **10 minutes**, 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?



---

---

---

---

---

### Review Key Messages

- Disinfection and decontamination have similar meanings. Both are less rigorous than sterilization which is the complete removal of all life.
- No disinfectant is ideal, they all have strengths and limitations. Understanding the strengths and limitations is key to their use.
- There are a number of factors that determines how effective a particular disinfectant is.
- Micro-organisms have various innate resistance to disinfectants.



Page 40

### Review Key Messages – Cont.

- Autoclaves can be used to sterilize things through wet heat and the application of appropriate time, pressure and temperature.
- Wet heat is much more effective than dry heat.
- Validation is a process to ensure that the decontamination, disinfection or sterilization process used was complete and achieved its requirements.



Page 41