

Decontamination, Sterilization and Disinfection

SAND2012-6686P

Student Guide





Decontamination



Welcome & Introductions





Decontamination

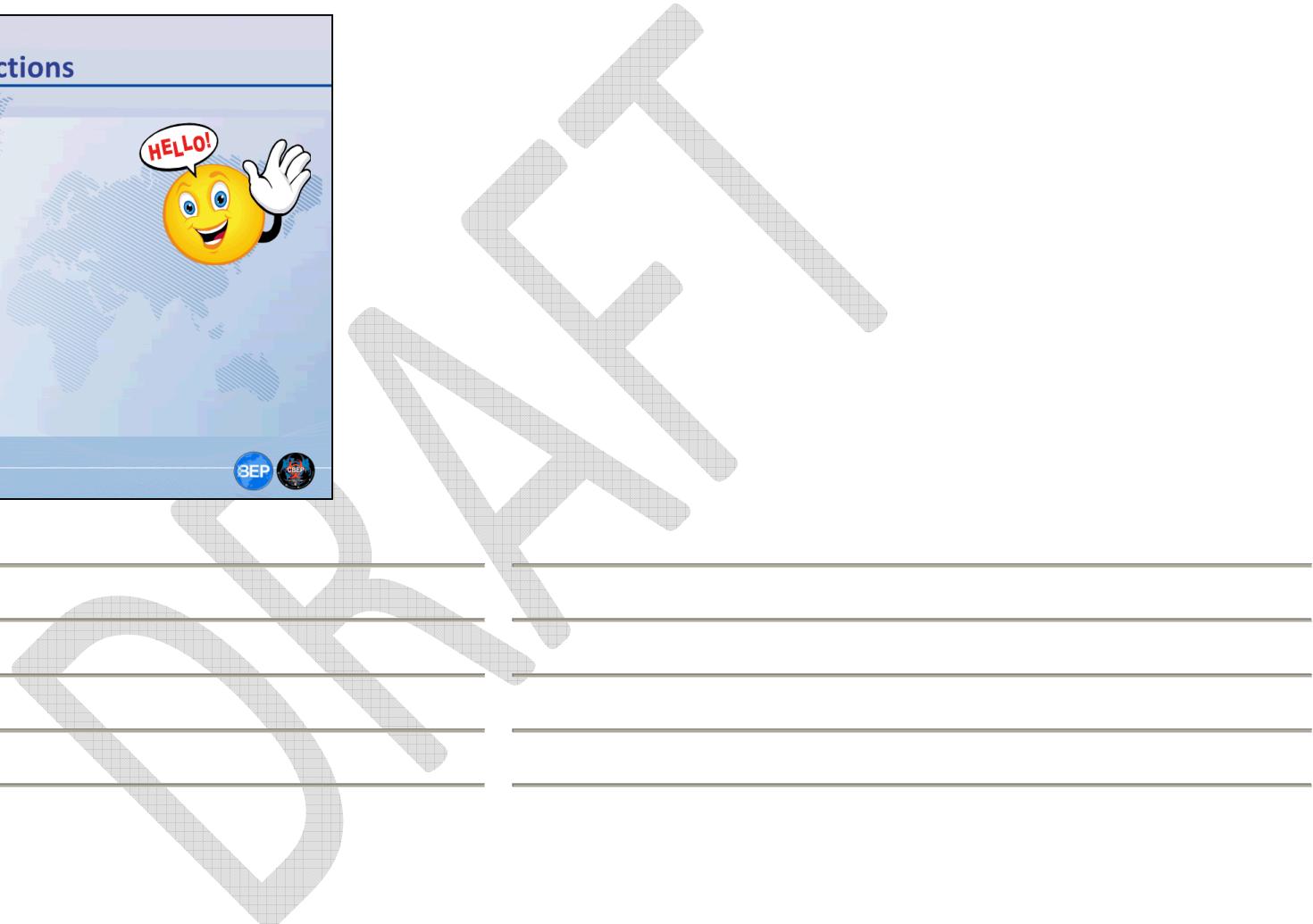
Welcome & Introductions

Introductions

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

Slide 2

BEPI





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





Decontamination

Welcome & Introductions

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.

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Key Messages – Continued

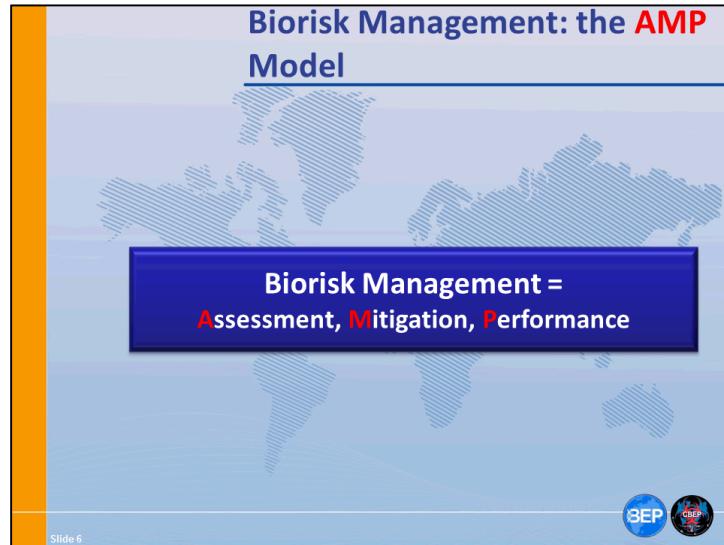
- Autoclaves can be used to sterilize things through wet heat and the application of appropriate time, pressure and temperature.
- Wet heat is much more effective than dry heat.
- Validation is a process to ensure that the decontamination, disinfection or sterilization process used was complete and achieved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

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Decontamination



Biorisk Management





Decontamination

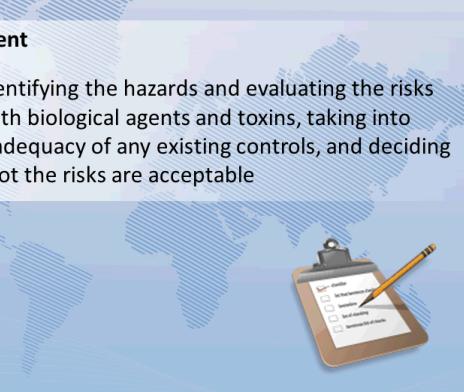
Biorisk Management

Key Components of Biorisk Management

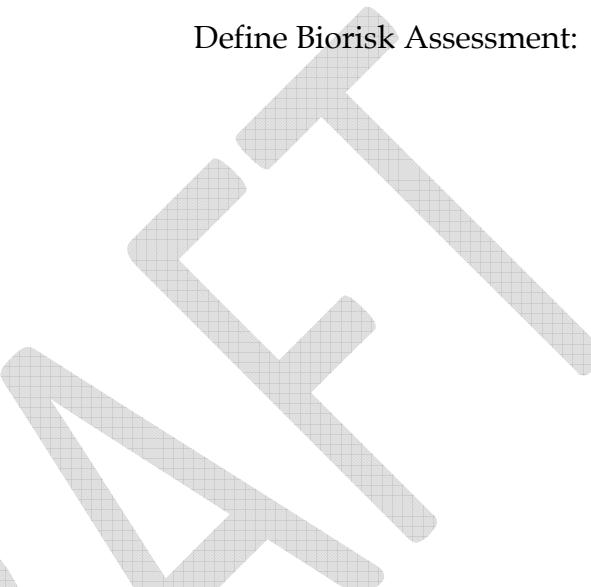
⌚ Biorisk Assessment

- 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:





Decontamination

Biorisk Management

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

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Define Biorisk Mitigation:



Decontamination

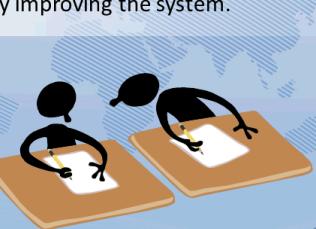
Biorisk Management

Key Components of Biorisk Management

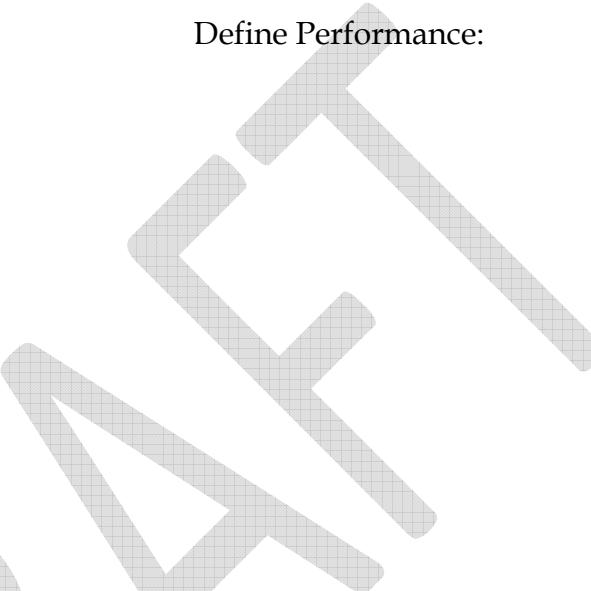
Performance

- 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).

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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.

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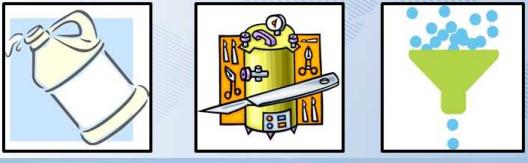


Decontamination

Definitions & Methods of Decontamination

Methods of Decontamination

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



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Decontamination

Classes of Chemical Disinfectants

Chemical Disinfectants

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

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Chemical Disinfection





Decontamination

Chemical Disinfection

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**.

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Decontamination

Chemical Disinfection

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**.

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Decontamination

Chemical Disinfection

Factors Affecting Disinfection

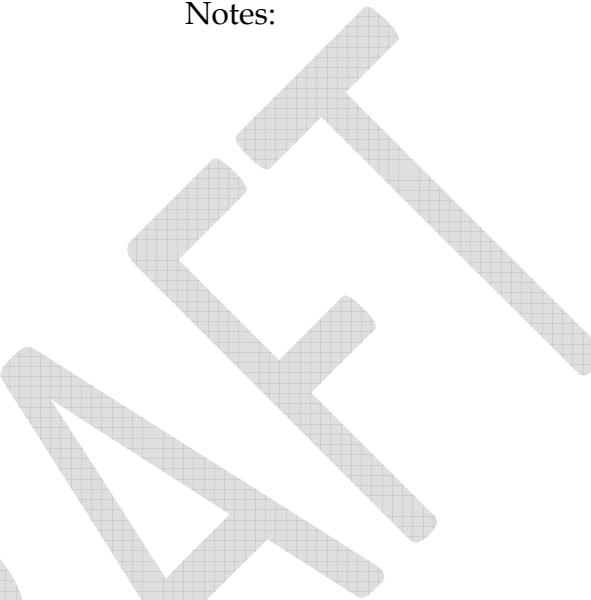
- Number of microorganisms
- Location of microorganisms
- Innate resistance to the disinfectant
- Concentration and potency of the disinfectant
- Physical and chemical factors
- Presence of organic matter
- Duration of exposure
- Biofilms



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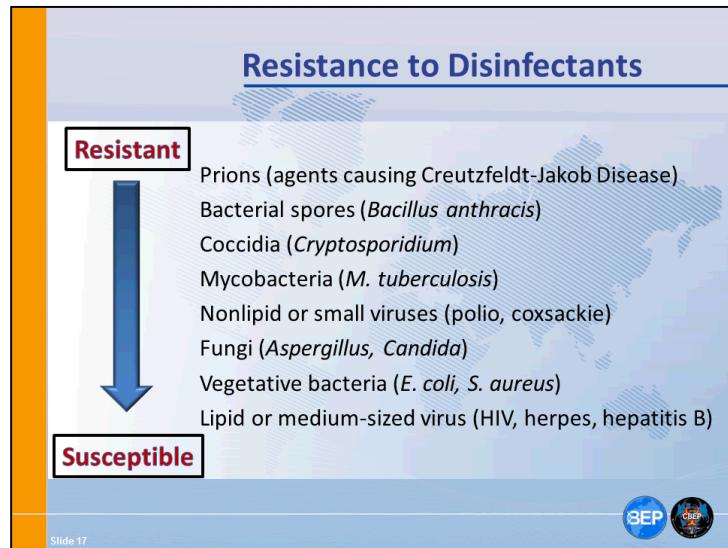
Notes:



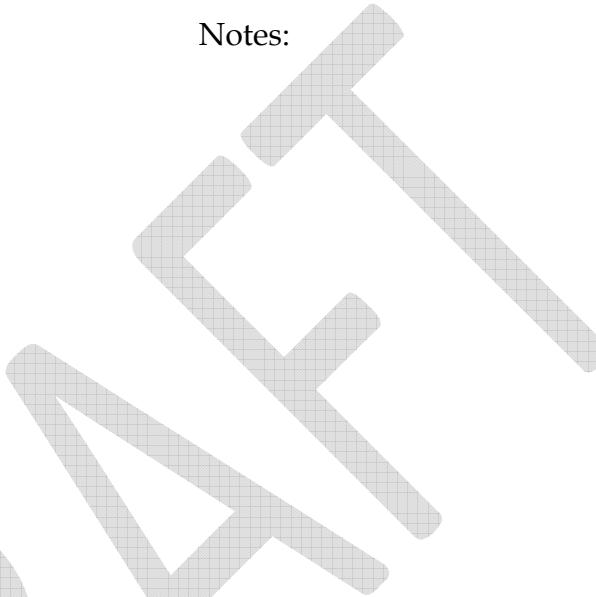


Decontamination

Chemical Disinfection



Notes:





Decontamination

Chemical Disinfection

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.



BEP CBEP

Notes:



Decontamination

Chemical Disinfection

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 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.

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BEP CDP

Properties and Selection of Chemical Disinfectants





Properties of Chemical Disinfectants

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



Decontamination

Properties and Selection of Chemical Disinfectants

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.

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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	



Decontamination

Properties and Selection of Chemical Disinfectants

“Evaluating” your SOP

Group Exercise - Continued:

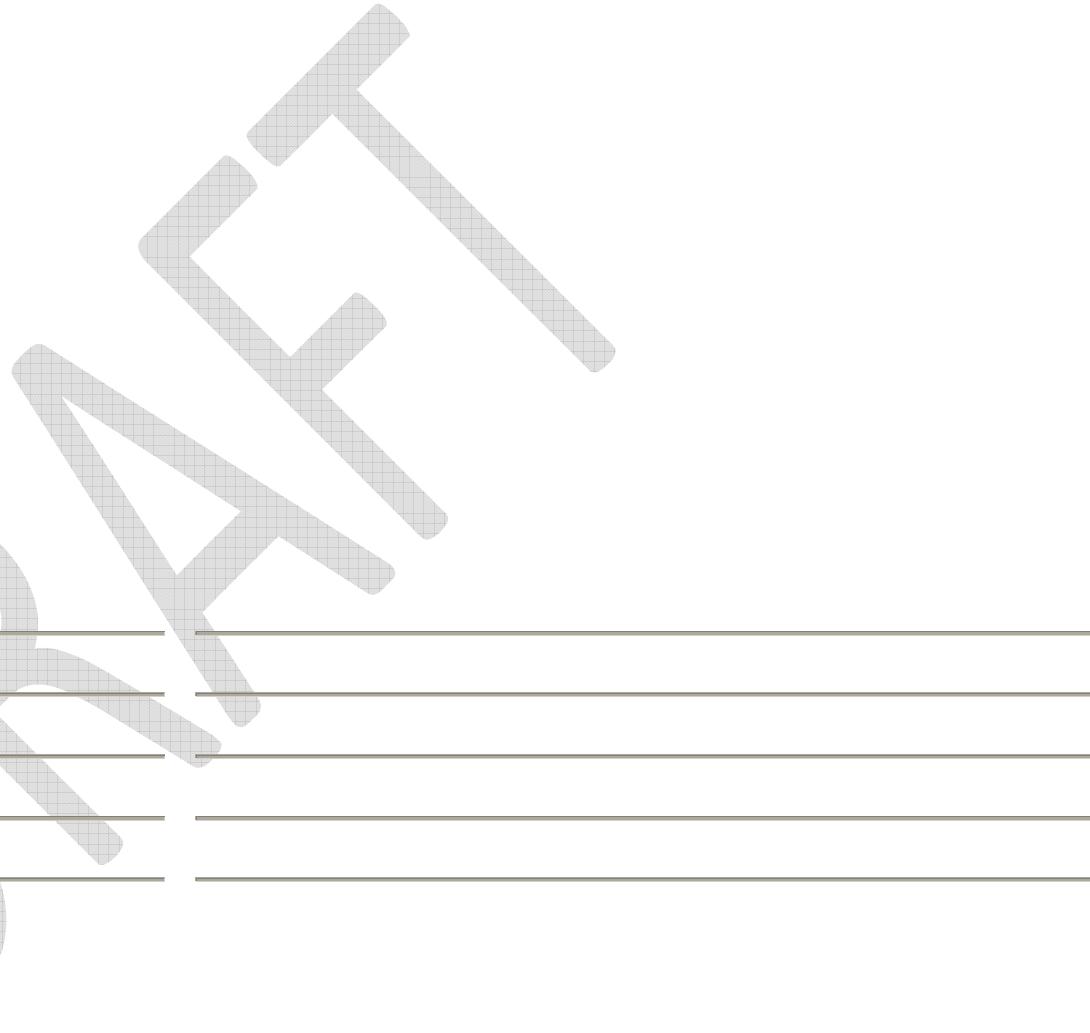
Give your SOP to another small group for evaluation.

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.


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Decontamination

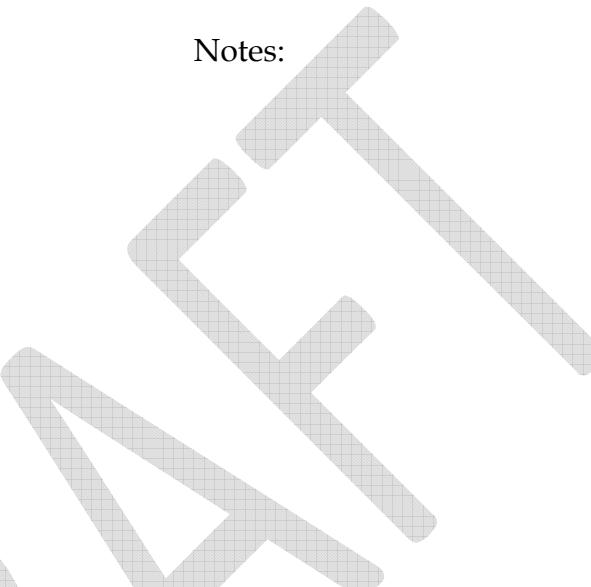
Additional Methods of Disinfection

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

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Additional Methods of Disinfection

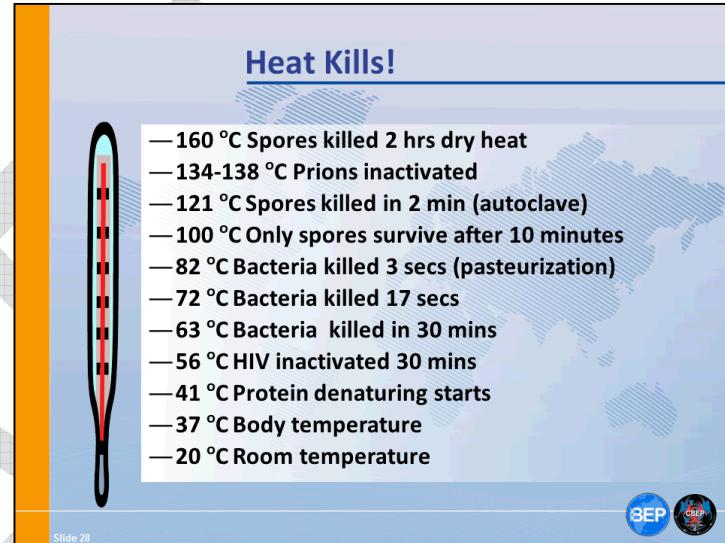
Notes:





Decontamination

Additional Methods of Disinfection





Decontamination

Additional Methods of Disinfection

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)

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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



Decontamination

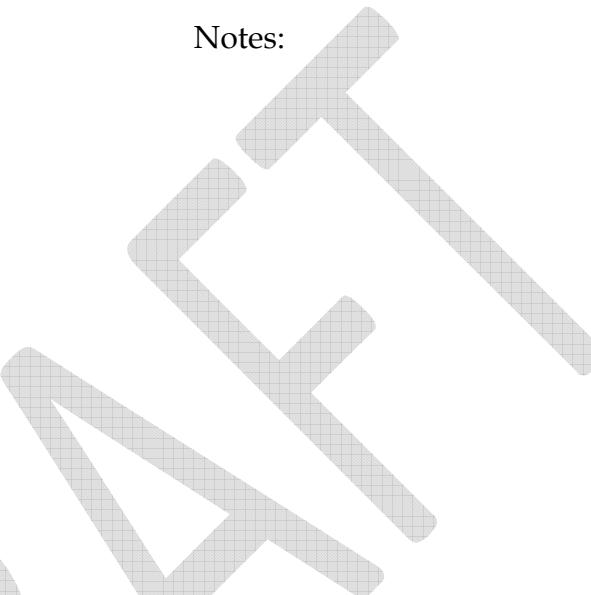
Additional Methods of Disinfection

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

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Notes:





Decontamination

Additional Methods of Disinfection

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?

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- Advantages:

- Disadvantages:



Decontamination

Additional Methods of Disinfection

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

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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**

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Decontamination



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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?



Decontamination

Additional Methods of Disinfection

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)

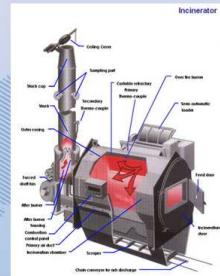
- What is the advantage of an incinerator over an autoclave or chemical disinfectants?

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?



Decontamination

Chemical Disinfection



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- What is required to make a fire an incinerator?

- When is fire okay?

- When does fire need to be an incinerator?



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

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Validation of Decontamination





Decontamination

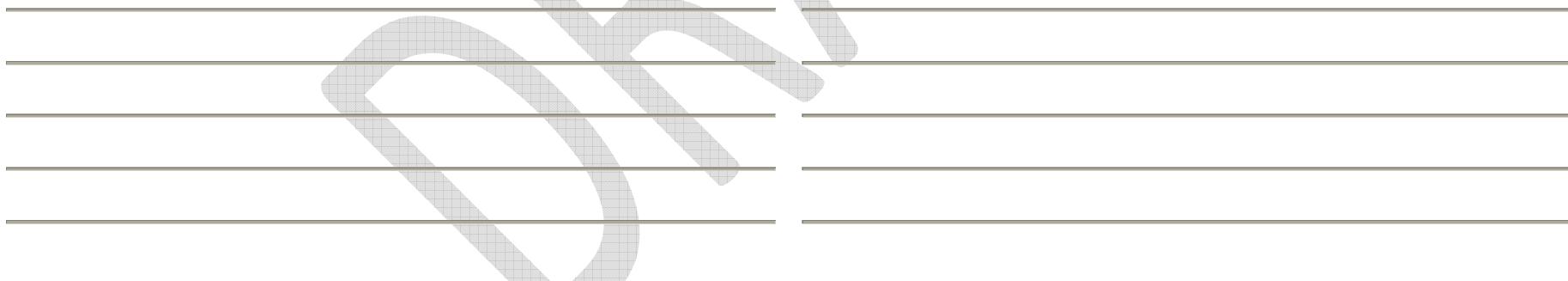
Review of decontamination

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 & Wrap Up





Decontamination

Review & Wrap Up

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.

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Review Key Messages – Cont.

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- 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.

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