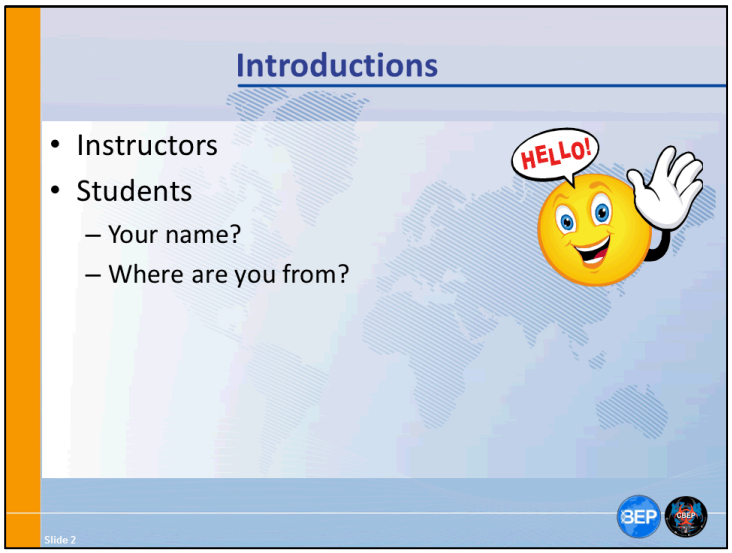
SAND2012-9977P

Decontamination, Sterilization, and Disinfection

Student Guide

Welcome & Introductions


Introductions
• Instructors
• Students
– Your name?
– Where are you from?
HELLO!
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.

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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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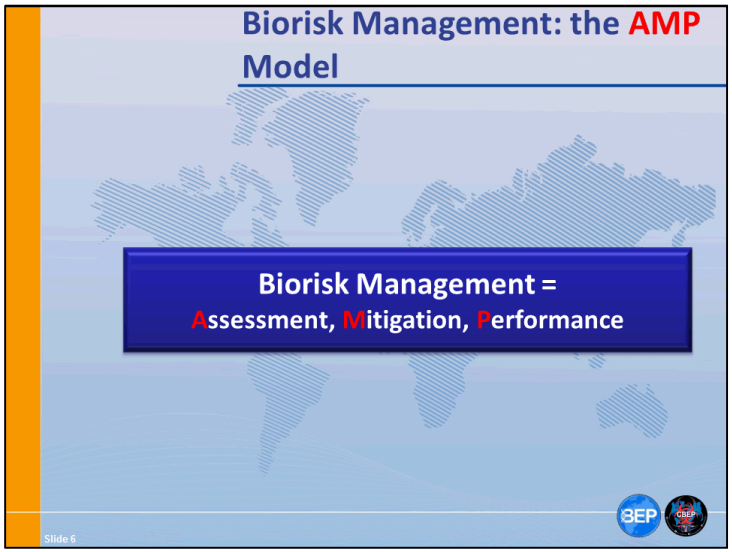
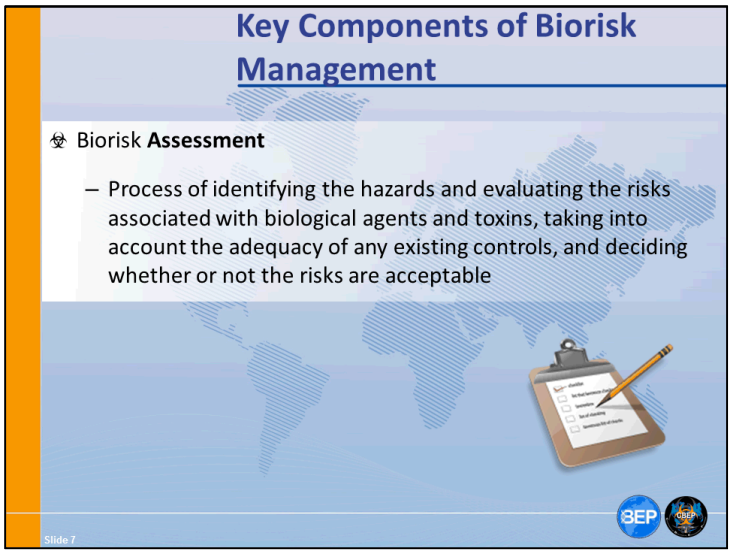
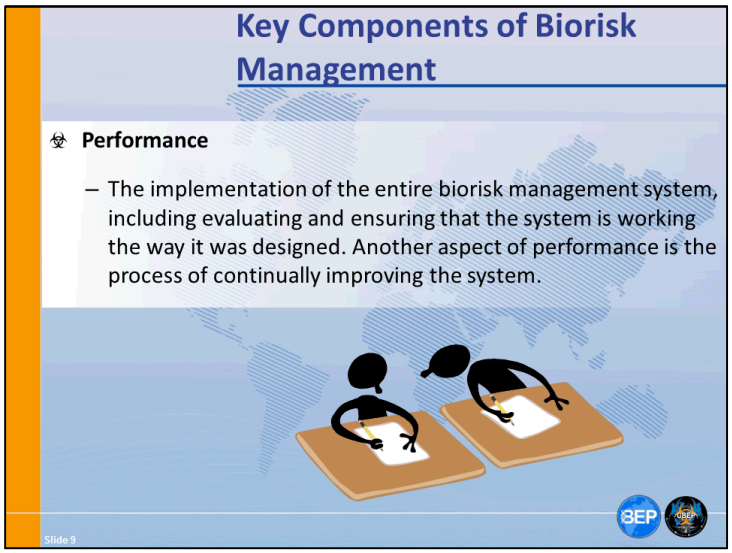
Biorisk Management





Define Biorisk Assessment:

Define Biorisk Mitigation:



Define Performance:

Definitions & Methods of Decontamination

Definitions

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.

Different Methods of Decontamination:

- ***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.

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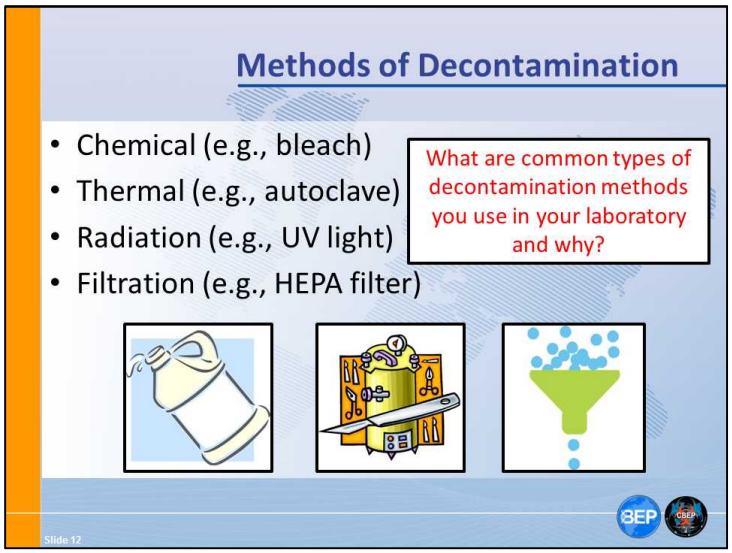
Definitions, continued

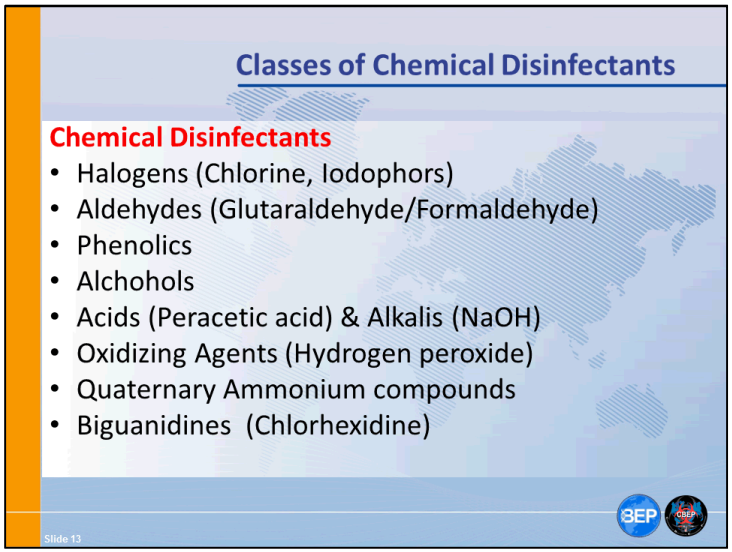
- ***Disinfection*** - Generally less lethal process than sterilization. It is the elimination of nearly all recognized pathogenic micro-organisms but not necessarily all microbial forms (e.g., bacterial spores, generally used on nonliving things).

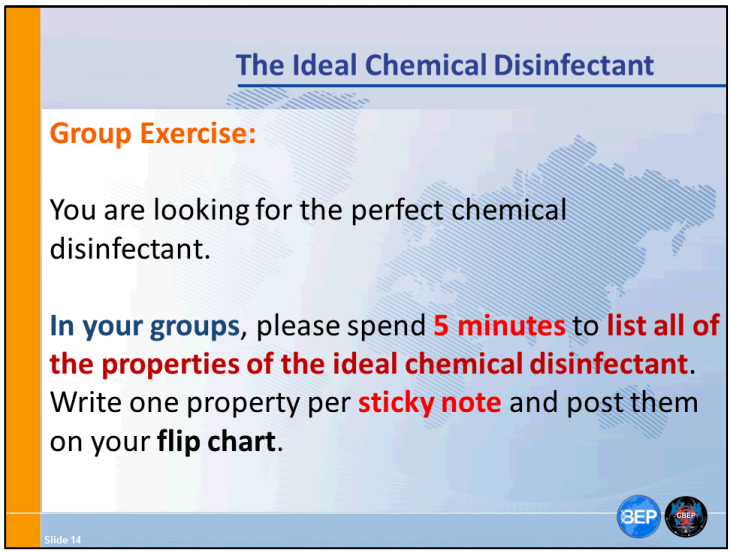
- ***Antiseptic*** - a substance that prevents or arrests the growth or action of microbes, either by inhibiting their activity or by destroying them (e.g. used on living things).
  - "septic" – containing disease causing organism, anti - remove

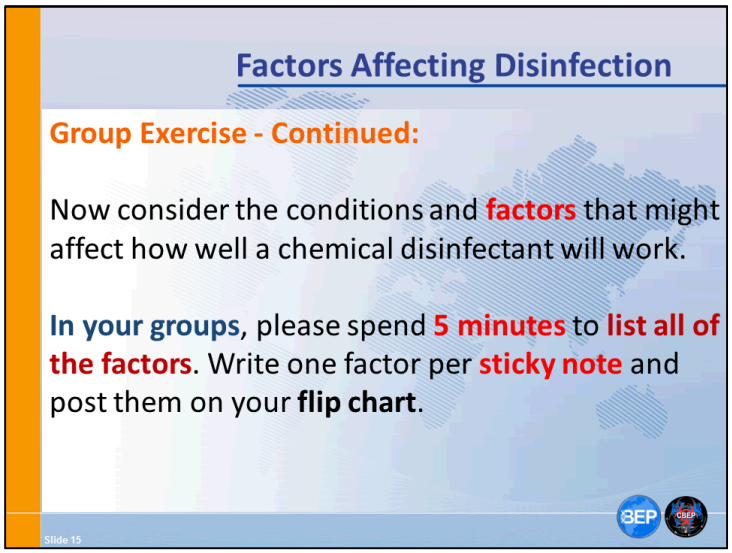
Slide 11

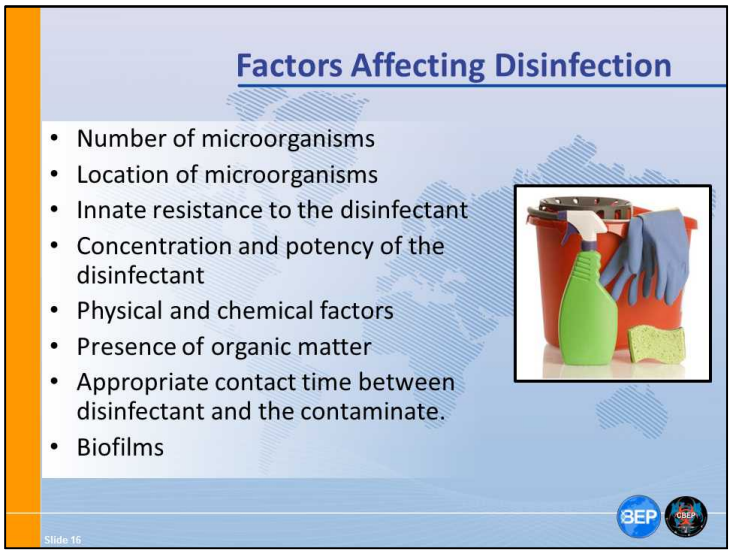

Methods of Decontamination
• Chemical (e.g., bleach)
• Thermal (e.g., autoclave)
• Radiation (e.g., UV light)
• Filtration (e.g., HEPA filter)
What are common types of decontamination methods you use in your laboratory and why?
Slide 12

Chemical Disinfection

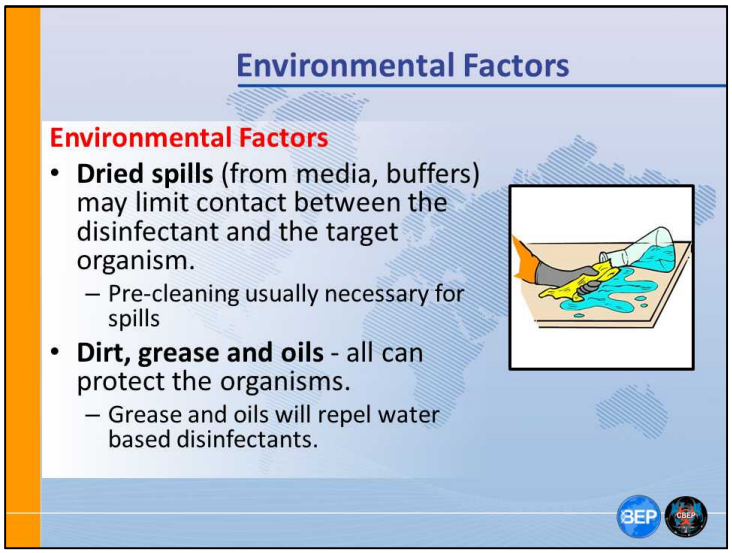



The Ideal Chemical Disinfectant
Group Exercise:
You are looking for the perfect chemical disinfectant.
In your groups, please spend 5 minutes to list all of the properties of the ideal chemical disinfectant. Write one property per sticky note and post them on your flip chart.
Slide 14

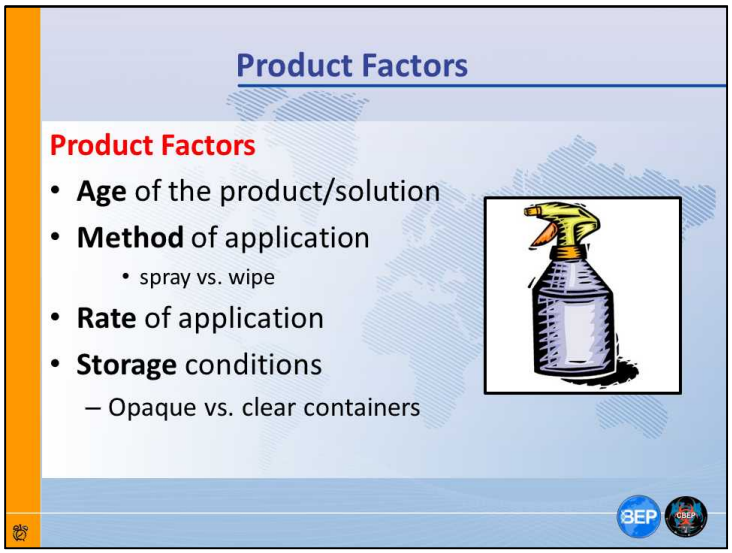

Factors Affecting Disinfection
Group Exercise - Continued:
Now consider the conditions and factors that might affect how well a chemical disinfectant will work.
In your groups, please spend 5 minutes to list all of the factors. Write one factor per sticky note and post them on your flip chart.
Slide 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
• Appropriate contact time between disinfectant and the contaminate.
• Biofilms
Slide 16

Notes:

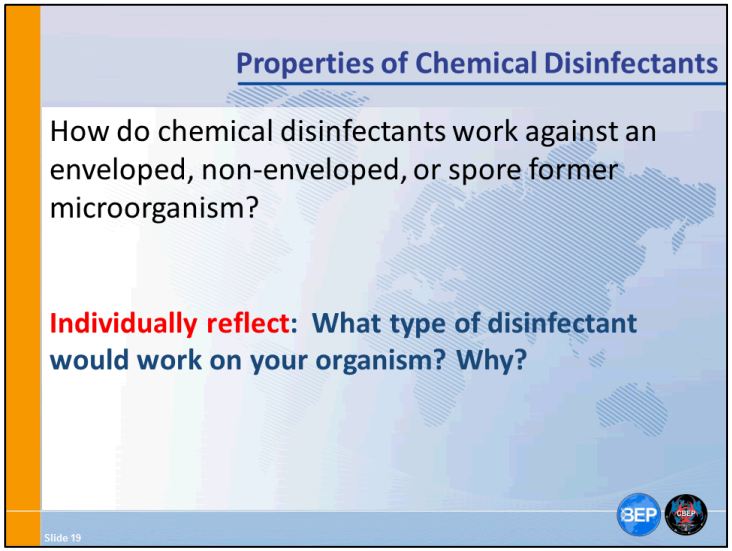

Environmental Factors
Environmental Factors
• Dried spills (from media, buffers) may limit contact between the disinfectant and the target organism.
– Pre-cleaning usually necessary for spills
• Dirt, grease and oils - all can protect the organisms.
– Grease and oils will repel water based disinfectants.

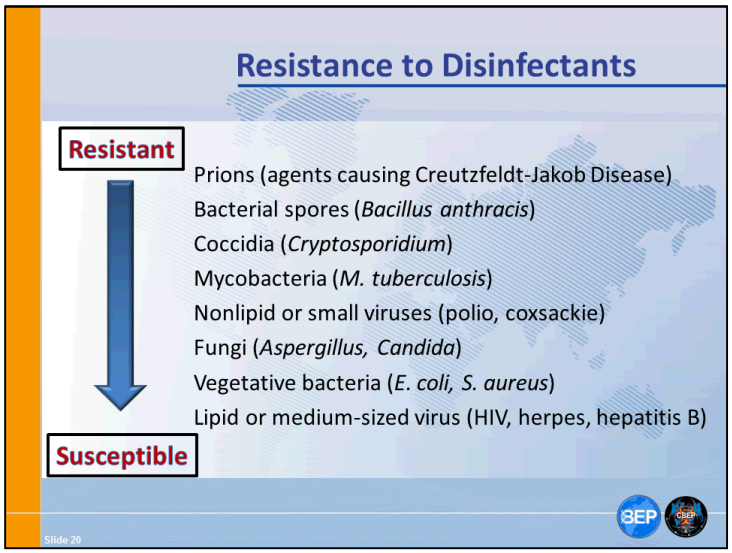
Notes:

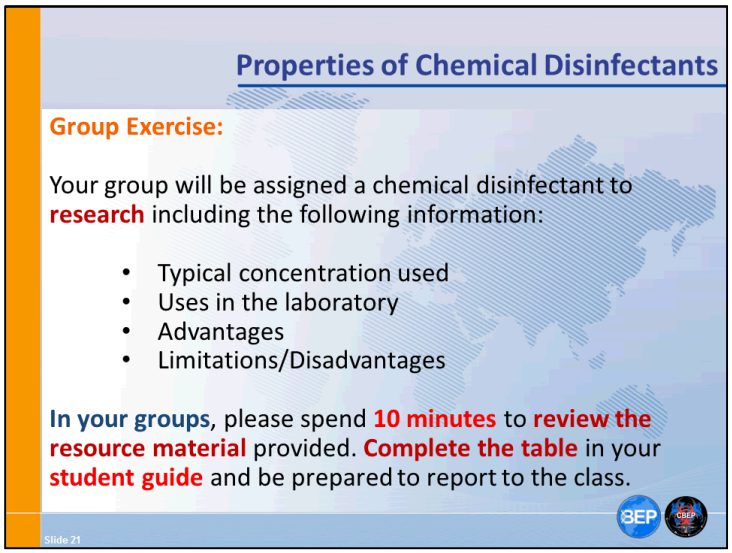


How do each of these affect the effectiveness of the chemical disinfectant?

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


Properties of Chemical Disinfectants
How do chemical disinfectants work against an enveloped, non-enveloped, or spore former microorganism?
Individually reflect: What type of disinfectant would work on your organism? Why?
Slide 19


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
Slide 20


Properties of Chemical Disinfectants
Group Exercise:
Your group will be assigned a chemical disinfectant to research including the following information:
• 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 21

Properties of Chemical Disinfectants

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

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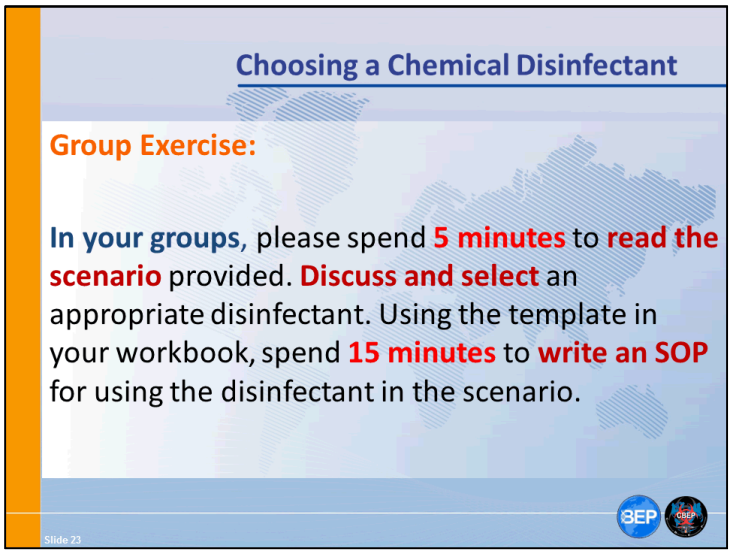
Choosing a Chemical Disinfectant
Group Exercise:
In your groups, please spend 5 minutes to read the scenario provided. Discuss and select an appropriate disinfectant. Using the template in your workbook, spend 15 minutes to write an SOP for using the disinfectant in the scenario.
Slide 23

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?***

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Standard Operating Procedure for: Chemical Disinfection	
Conditions	
Who should use the SOP?	Everyone in the laboratory
When should it be used?	Before and after the laboratory procedure
Why should the SOP be used?	To disinfect the surfaces and reusable materials
Where should it be used?	In the laboratory
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	

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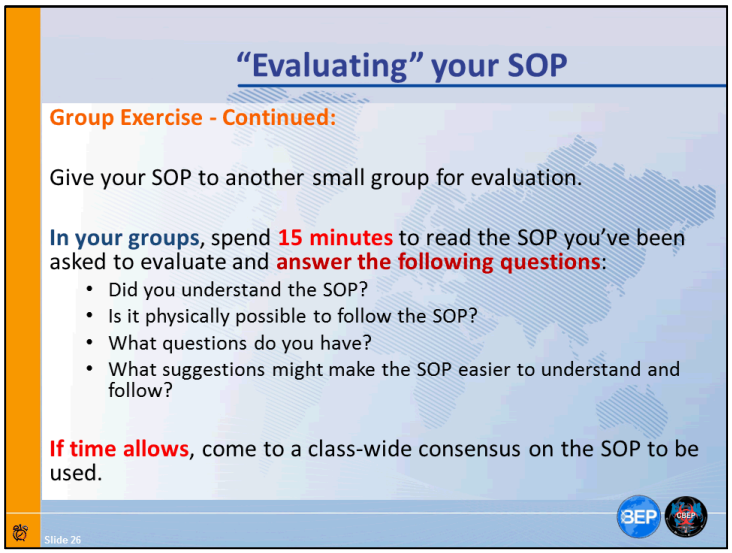
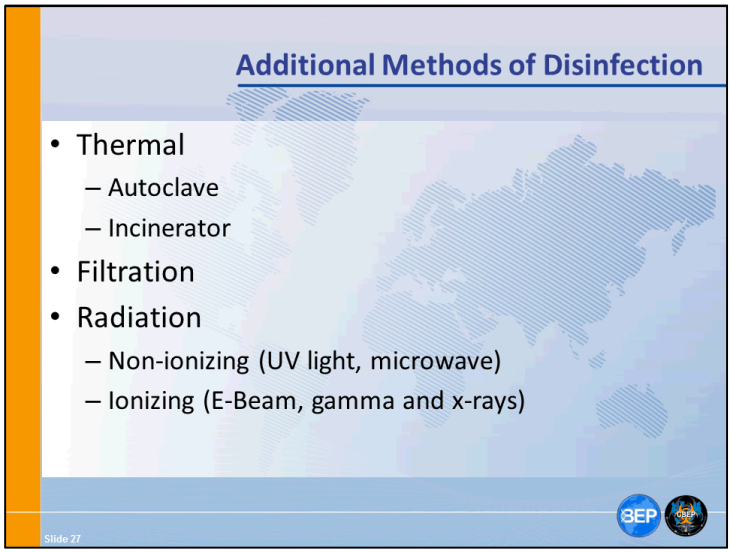

"Evaluating" your SOP
Group Exercise - Continued:
Give your SOP to another small group for evaluation.
In your groups, spend 15 minutes to read the SOP you've been asked to evaluate and answer the following questions:
• Did you understand the SOP?
• Is it physically possible to follow the SOP?
• What 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 26

Additional Methods of Disinfection



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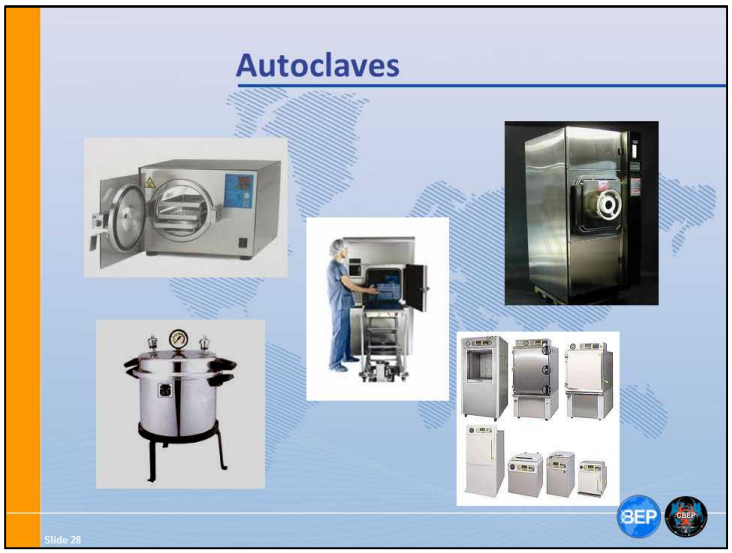
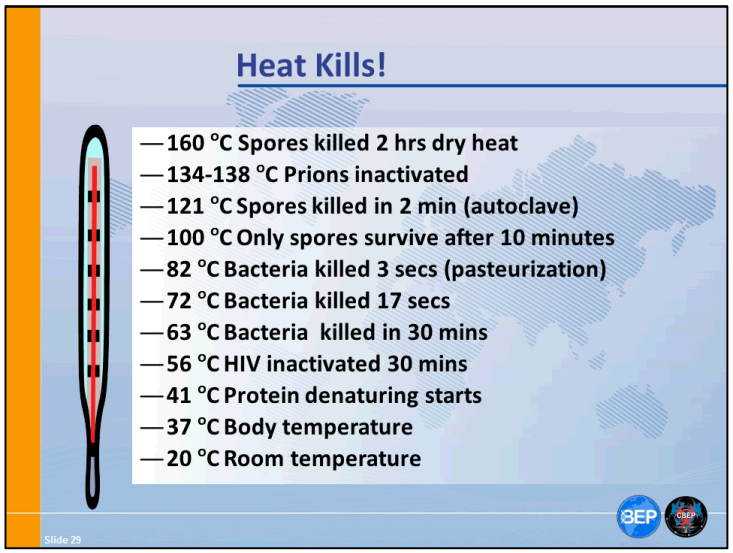
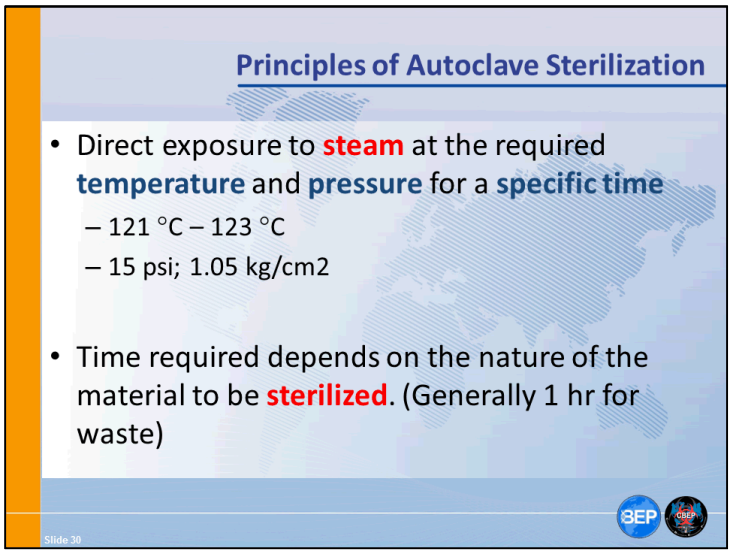
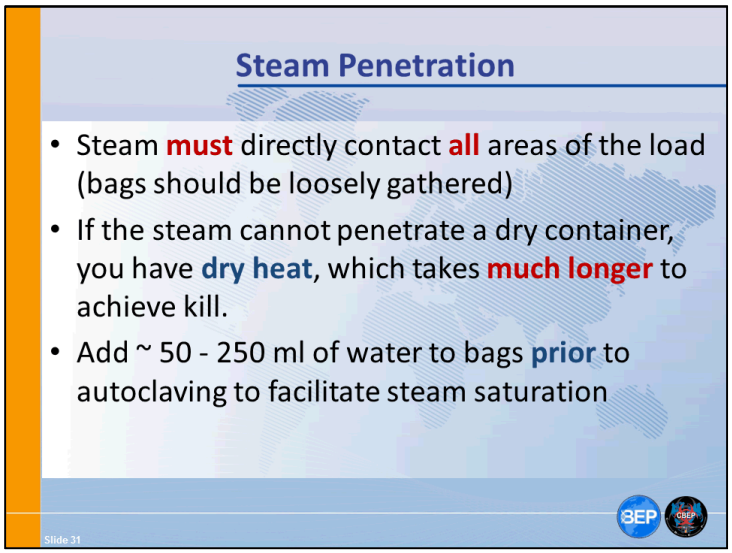

Autoclaves
Slide 28


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 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
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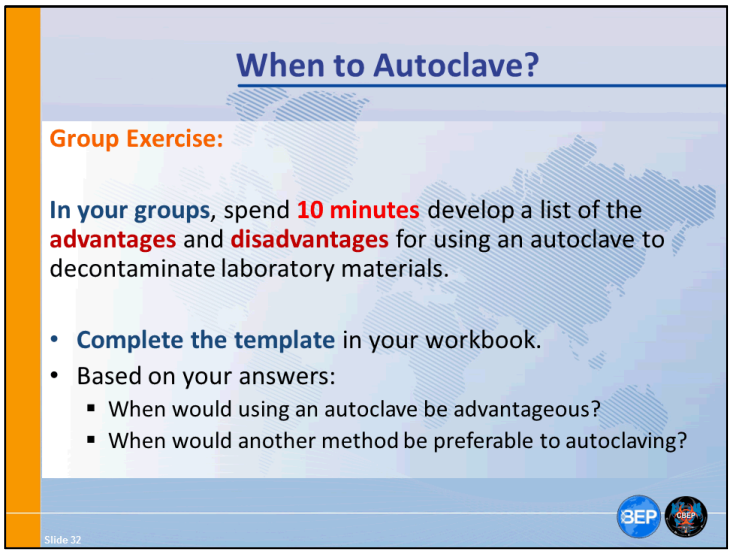
Notes:



- 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

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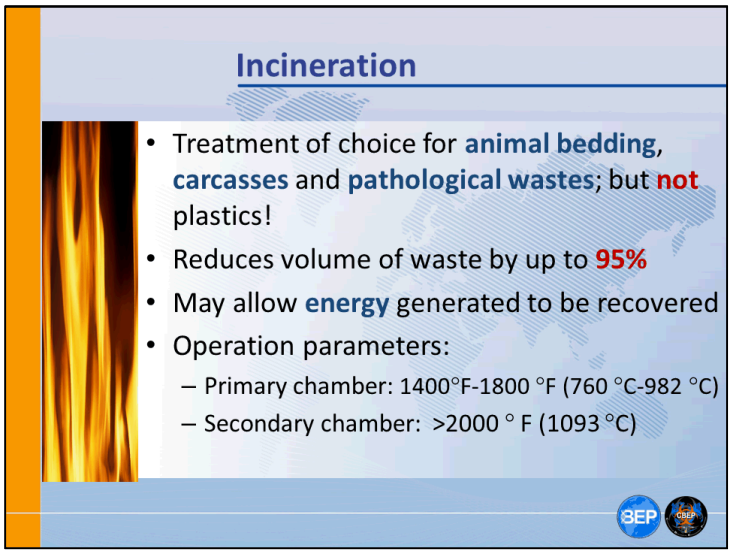
Autoclave safety

- 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**

Slide 34

- What do you notice about this picture?

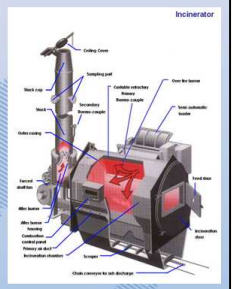
- Are there any practices or procedures you would modify? Anything with the autoclave itself?



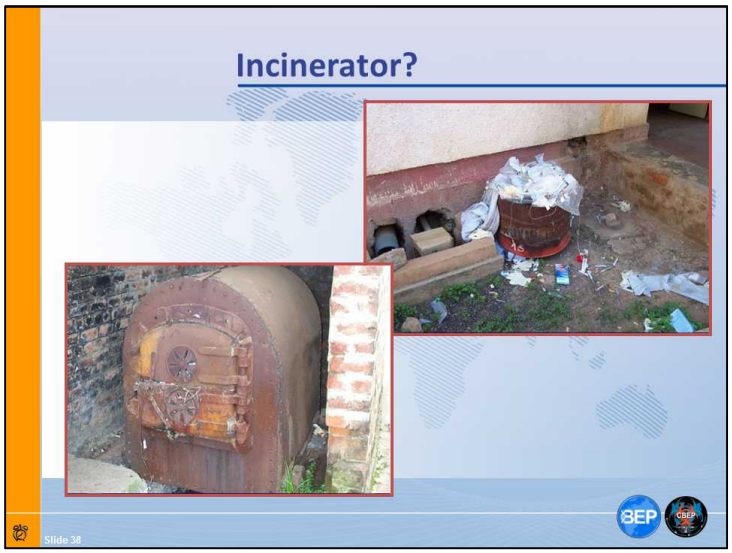
- What is the advantage of an incinerator over an autoclave or chemical disinfectants?

Incineration Concerns

- Can generate **smoke**, residues with **heavy metals**, **gases** (e.g., HCl, CO, PCBs)
- 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?

Validation of Decontamination

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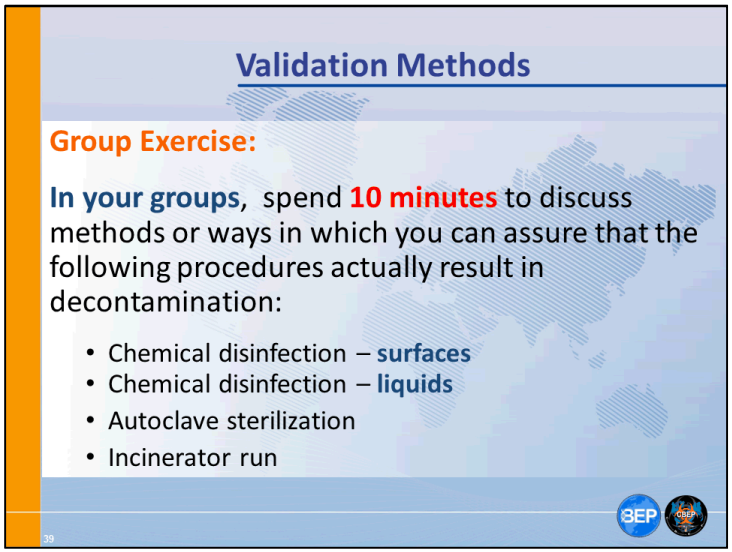
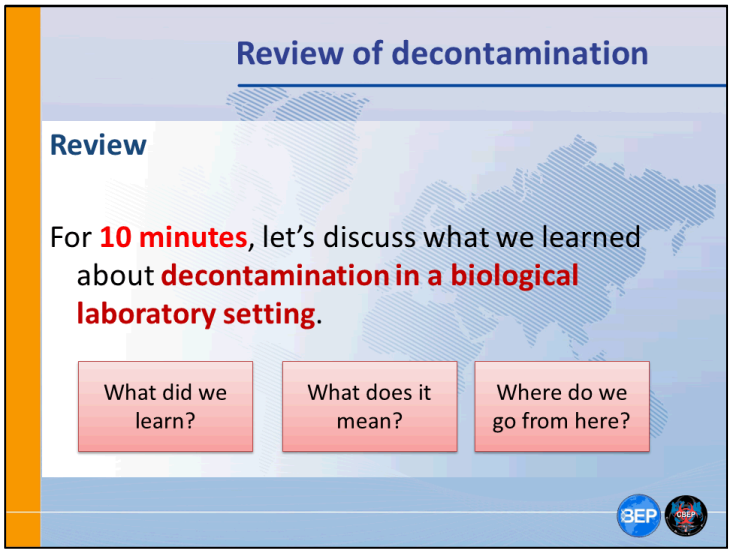


Review & Wrap Up



Review Key Messages

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- Micro-organisms have various innate resistance to disinfectants.

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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.

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