

# Decontamination, Sterilization and Disinfection

## *Instructor Guide*



 Sandia National Laboratories



# Welcome & Introductions

## Slide 1



### Introduce Instructor(s):

[Introduce others associated with the training, as appropriate]

Name

Affiliation

Representation (I'm here on behalf of. . .)

Quick Experience Glimpse

Relevancy of the Course to your experience

# Welcome & Introductions

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**Before you introduce yourselves, I'd like to provide some reminders about this facility and the training:**

1. Restrooms are . . .
2. Exits are . . .
3. Evacuation procedures are . . .
4. [any escort or restricted access procedures]
5. We will have intermittent breaks during the course, but please feel free (or not) to take a quick break if you need to at other times during the course
6. Beverages and snacks will be available at (time) and at (location). You may/may not eat and drink in this room
7. Please silence any cell phones or other noise-making devices.
8. Others . . .

## Slide 2



**Introductions**

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

HELLO!

BEP

Slide 2

# Welcome & Introductions



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Let's go around the room and let each of you introduce yourself. Please tell us your name, where you work (organization and/or title, as appropriate), and what you hope to gain from the course.

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## Ground rules

This will be a very interactive session and you will learn the most if you participate fully. We will not intentionally force any one to speak or to do an activity that embarrasses them – if you are uncomfortable, please speak to one of the leaders. For those of you who like to talk, please share your expertise but be aware of those around you who may be quieter and give them time to share their opinion as well. We ask that everyone respect the break times and report back promptly when asked to do so. But most of all, we want to make this a fun time to learn, so remember to smile and enjoy yourself!

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## Transition to Objectives



## Goal

To review the Action Plan and Learning Objectives for the course and to solicit any additional learning goals from the participants.

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## Time

20 minutes

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# Welcome & Introductions

## Slide 3



Action Plan			
By the end of this lesson, I would like to:			
KNOW	FEEL	BE ABLE TO DO	
<i>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.</i>			
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



### Instructions for the Action Plan handout:

- The Action Plan handout is on page \_\_ of the student guide.
- It is designed to help you assess your learning of the material as we go through the course. It is also referred to as a learning contract.
- Go over each section of the Action Plan. . .
- The sections KNOW, FEEL and DO are designed to help outline personal learning objectives for this course.
- Ask each participant to think about what they would like to be able to KNOW, FEEL, and DO once this course is completed
- Tell the students that this is their own Action Plan. It does not need to be shared with anyone. It can be used during the course and after the course to help continually reach learning goals.
- Allow 5 minutes

# Welcome & Introductions



## Slide 4



### 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





## Slide 5



### 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





# Welcome & Introductions



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## Key Messages for Instructor

1. Disinfection and decontamination have similar meanings. Both are less rigorous than sterilization which is the complete removal of all life.
2. No disinfectant is ideal, they all have strengths and limitations. Understanding the strengths and limitations is key to their use.
3. There are a number of factors that determines how effective a particular disinfectant is.
4. Micro-organisms have various innate resistance to disinfectants
5. Autoclaves can be used to sterilize things through wet heat and the application of appropriate time, pressure and temperature.
6. Wet heat is much more effective than dry heat.
7. Validation is a process to ensure that the decontamination, disinfection or sterilization process used was complete and achieved its requirements



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## Background Information for Instructor

Review the course objectives, these can be read from the slide. Check for understanding and verify that these objectives are consistent with student expectations.



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## Capture any additional KNOW, FEEL, or DO or other learning goals

Capture any learning goals that will supplement course objectives and address any that are outside the scope of the course.

This course is flexible in nature. If there is a learning goal that is easily incorporated into the course, feel free to add it. Please note successful additions and consistently requested learning goals in the evaluation portion of this course and/or to GBRMC administrators.



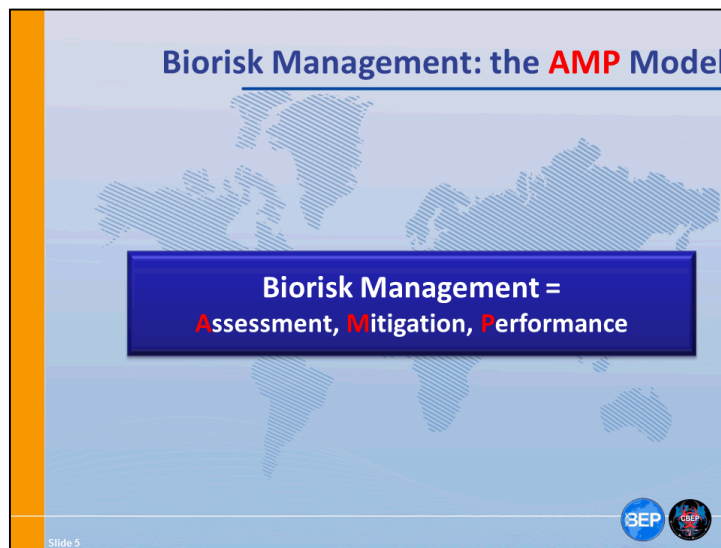
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## Transition to Biorisk Management Touchstone

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# Biorisk Management

## Slide 6



## Background Information for Instructor

- Review the AMP model of Biorisk Management with the participants.
- The following three slides provide specific definitions for A, M, and P.
- Integration of laboratory biosafety (protect people from pathogens) and laboratory biosecurity (protect pathogens from people)




# Biorisk Management




## Slide 7



### 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 6



## Background Information for Instructor

The instructor uses the following three slides: Biorisk Assessment; Biorisk Mitigation; and Performance to define key components of biorisk management


## Slide 8



### 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 7

# Biorisk Management



## Background Information for Instructor

The instructor uses this slide and following slide (Performance) to define key components of biorisk management

### Slide 9



**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.

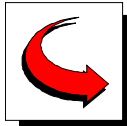
Slide 8

The slide features a blue header with the title 'Key Components of Biorisk Management'. Below the title, there is a section titled 'Performance' with a biohazard icon. A bulleted list describes performance as the implementation and evaluation of the biorisk management system. At the bottom, there is an illustration of two people sitting on the floor, writing on papers. Logos for BEP and another organization are in the bottom right corner.



## Lecture

Taken together, the three elements of AMP constitute a complete biorisk management system. The elements of the AMP model also underpin CWA 15793:2011 – Laboratory Biorisk Management Standard



## Transition to Definitions & Methods of Decontamination

# Definitions & Methods of Decontamination

## Slide 10





### Definitions

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

**Different Methods of Decontamination:**

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





## Background Information for Instructor

These definitions are essential for the students to KNOW. Note that the first three can all describe “decontamination”. Other examples of decontamination could include soap and water or physically removing particles.

Spend some time here.

If you have time, you could give half the students a card with the words (one per card): sterilization, disinfection, antiseptic, decontamination – on them (if you have 16 students; eight will receive word cards – there will be two sets of the four cards).

The other half of the class would have a card that had the definition of each of those words (if you have 16 students, the remaining eight would divide two sets of definition cards among themselves).

Give the students 1 minute to find a match between the word and the definition. If you do this, make sure that the slides are not visible and ask the students to close their books. Give a prize to the first four to match.

# Definitions & Methods of Decontamination



## Slide 11



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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



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## Background Information for Instructor

The most commonly thought-of decontamination methods for biological contamination are chemical and thermal. But filtration and radiation methods are also often used every day in the lab in the forms of HEPA filters and UV lights. Even though they are important, we won't spend time on the filtration and radiation methods in this course but rather we will focus on the methods that require choice to be made to use them properly. So the majority of the course will focus on chemical decontamination and a small portion on thermal decontamination.

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# Definitions & Methods of Decontamination



## Slide 12



### Methods of Decontamination

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





Change slide to decontamination



## Background Information for Instructor

The most commonly thought-of decontamination methods for biological contamination are chemical and thermal. But filtration and radiation methods are also often used every day in the lab in the forms of HEPA filters and UV lights.

Even though they are important, we won't spend time on the filtration and radiation methods in this course but rather we will focus on the methods that require choices to be made to use them properly. So the majority of the course will focus on chemical decontamination and a small portion on thermal decontamination.

# Definitions & Methods of Decontamination



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## Plenary Discussion (5 minutes).

Question to consider:

What are common types of decontamination methods you use in your laboratory and why?

Expected Response:

Chemical disinfectants: 5% bleach, 70% alcohol and hydrogen peroxide (dependent on the organism and contact time).

Thermal Sterilization: autoclaves to sterilize glassware, dissecting instruments, decontaminating waste products (different waste requires different autoclave programs-contact time).

Antiseptics: using iodine on surgical incision site.



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## Transition to Chemical Disinfection

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# Chemical Disinfection



## Lecture

There are a variety of chemical disinfectants – and nearly all chemicals are disinfectants – there are very few sterilants in the chemical category. We will discuss the properties of many of these classes later.

## Slide 13



**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 12

## Slide 14



**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 13

# Chemical Disinfection



**Small group activity (5 minutes).**



## **Activity Instructions (to students)**

- You have 5 minutes, in your groups, to list all of the properties of the ideal chemical disinfectant.
- To help with this task list all properties on sticky-notes and place them on your flip chart.
- Be prepared to report your definition and rationale to the class.



**You have 5 minutes to complete this activity**

## ***Directions for Instructor:***

- Allow 5 minutes – this is a brainstorming session and does not require detailed, complete answers.
- Lead a 5-minute plenary discussion. Begin by asking for one group of students to report the properties they came up with.
- Ask one group to post one sticky note on a flip chart or surface (wall, floor, or table) located in a space that all students can see. Ask that group why this property would make a disinfectant ideal.
- Ask the other groups if they have a match for that note. If so, post their notes on top of the first note.
- Repeat this process until all the properties are used up.
- If you want to provide a reward, reward unique answers (answers that only one group came up with) – it's easier to use different colored sticky notes to help keep track of this.
- Emphasize the many factors that must be considered for a good disinfectant. Tell the students that most chemical disinfectants have some of these properties, but not all of them and that is why it is important to know which properties are associated with different chemical disinfectants.



# Chemical Disinfection

## Expected Responses

- 
- Broad spectrum (kills almost everything)
  - High efficiency (kills rapidly)
  - Unaffected by organic matter, soaps & detergents, water hardness, pH
  - Nontoxic
  - Noncorrosive
  - Nonflammable
  - Odorless
  - Cheap
  - Stable (Stores for a long time)
  - Environmentally friendly
  - Penetrates readily
  - Easily monitored

New Responses from Students:

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# Chemical Disinfection

## Slide 15



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### 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 14





## Small group activity (5 minutes).



### Activity Instructions (to students)

- You have 5 minutes, in your groups, to think of the things that might get in the way of whether a chemical disinfectant works well or not.
  - To help with this task write each factor on sticky-notes and place them on your flip chart.
  - Be prepared to report your factors and rationale to the class.
-

# Chemical Disinfection

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**You have 5 minutes to complete this activity**

***Directions for Instructor:***

- Allow 5 minutes – this is a brainstorming session and does not require detailed, complete answers.
  - To report out, use the next slide which has animations that will reveal one factor at a time (per mouse click or down arrow). Reveal the first factor (Number of microorganisms) and ask the groups who had a match. Ask the matching groups to post their sticky notes together on a flip chart titled, Factors Affecting Disinfection. Ask the first group to respond why the factor affects disinfection. Repeat throughout the list on the slide. Then ask what other factors remain and work with the class to determine if they are new factors or if they belong with one of the other factors.
  - Allow a good 10 to 15 minutes for the reporting-out phase of this exercise.
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# Chemical Disinfection

## Expected Responses

- 
- 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

New Responses from Students:

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# Chemical Disinfection


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## Slide 16



### 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

An illustration of disinfection supplies including a red bucket, a green spray bottle, a pair of blue gloves, and a yellow sponge, set against a white background with a black border.



Slide 15



## Background Information for Instructor

Information below on Expected Results is derived from CDC Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

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# Chemical Disinfection

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## Expected Results:

### Number and Location of Microorganisms:

All other conditions remaining constant, the larger the number of microbes, the more time a germicide needs to destroy all of them. Spaulding illustrated this relation when he employed identical test conditions and demonstrated that it took 30 minutes to kill 10 *B. atrophaeus* (formerly *Bacillus subtilis*) spores but 3 hours to kill 100,000 *Bacillus atrophaeus* spores. Researchers also have shown that aggregated or clumped cells are more difficult to inactivate than monodispersed cells.

The location of microorganisms also must be considered when factors affecting the efficacy of germicides are assessed. Surfaces that have crevices, joints, and channels are more difficult to disinfect than are flat-surfaces because penetration of the disinfectant of all areas is more difficult. Porous, uneven surfaces (such as untreated wood) can be particularly difficult to chemically disinfect. Only surfaces that directly contact the germicide will be disinfected, so there must be no air pockets and the area must be completely immersed for the entire exposure period. Proper design of the work surfaces in a bioresearch laboratory is very important.

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# Chemical Disinfection

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## Expected Results:

### Innate Resistance:

Microorganisms vary greatly in their resistance to chemical germicides and sterilization processes (see the next slide). Intrinsic resistance mechanisms in microorganisms to disinfectants vary. For example, spores are resistant to disinfectants because the spore coat and cortex act as a barrier, mycobacteria have a waxy cell wall that prevents disinfectant entry, and gram-negative bacteria possess an outer membrane that acts as a barrier to the uptake of disinfectants. Implicit in all disinfection strategies is the consideration that the most resistant microbial subpopulation controls the sterilization or disinfection time. That is, to destroy the most resistant types of microorganisms (i.e., bacterial spores), the user needs to employ exposure times and a concentration of germicide needed to achieve complete destruction. **Show the next slide to review order of resistance.** Except for prions, bacterial spores possess the highest innate resistance to chemical germicides, followed by coccidia (e.g., *Cryptosporidium*), mycobacteria (e.g., *M. tuberculosis*), nonlipid or small viruses (e.g., poliovirus, and coxsackievirus), fungi (e.g., *Aspergillus*, and *Candida*), vegetative bacteria (e.g., *Staphylococcus*, and *Pseudomonas*) and lipid or medium-size viruses (e.g., herpes, and HIV). The germicidal resistance exhibited by the gram-positive and gram-negative bacteria is similar with some exceptions (e.g., *P. aeruginosa* which shows greater resistance to some disinfectants). *P. aeruginosa* also is significantly more resistant to a variety of disinfectants in its "naturally occurring" state than are cells subcultured on laboratory media. *Rickettsiae*, *Chlamydiae*, and mycoplasma cannot be placed in this scale of relative resistance because information about the efficacy of germicides against these agents is limited. Because these microorganisms contain lipid and are similar in structure and composition to other bacteria, they can be predicted to be inactivated by the same germicides that destroy lipid viruses and vegetative bacteria. A known exception to this supposition is *Coxiella burnetti*, which has demonstrated resistance to disinfectants.

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# Chemical Disinfection

## Expected Results:

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### Concentration and Potency of Disinfectants:

With other variables constant, and with two exceptions (70% ethanol; 1% povidone-iodine), the more concentrated the disinfectant, the greater its efficacy and the shorter the time necessary to achieve microbial kill. Generally not recognized, however, is that all disinfectants are not similarly affected by concentration adjustments. For example, quaternary ammonium compounds and phenol have a concentration exponent of 1 and 6, respectively; thus, halving the concentration of a quaternary ammonium compound requires doubling its disinfecting time, but halving the concentration of a phenol solution requires a 64-fold (i.e.,  $2^6$ ) increase in its disinfecting time.

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## Expected Results:

### Physical and Chemical Factors:

Several physical and chemical factors also influence disinfectant procedures: temperature, pH, relative humidity, and water hardness. For example, the activity of most disinfectants increases as the temperature increases, but some exceptions exist. Furthermore, too great an increase in temperature causes the disinfectant to degrade and weakens its germicidal activity.

An increase in pH improves the antimicrobial activity of some disinfectants (e.g., glutaraldehyde, quaternary ammonium compounds) but decreases the antimicrobial activity of others (e.g., phenols, hypochlorites, and iodine). The pH influences the antimicrobial activity by altering the disinfectant molecule or the cell surface.

Relative humidity is the single most important factor influencing the activity of gaseous disinfectants/sterilants, such as EtO, chlorine dioxide, and formaldehyde.

Water hardness (i.e., high concentration of divalent cations) reduces the rate of kill of certain disinfectants because divalent cations (e.g., magnesium, calcium) in the hard water interact with the disinfectant to form insoluble precipitates.

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# Chemical Disinfection

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**Expected  
Results:**  
**Presence of  
Organic Matter**

## **Presence of Organic Matter:**

Organic matter in the form of serum, blood, pus, or fecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or nongermicidal, leaving less of the active germicide available for attacking microorganisms. Chlorine and iodine disinfectants, in particular, are prone to such interaction. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier.

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**Expected  
Results:**  
**Duration of  
Exposure**

## **Duration of Exposure:**

No disinfectant works immediately. Surfaces and items must be exposed to the germicide for an appropriate minimum contact time (which varies with the disinfectant used and concentration).

Air pockets interfere with the disinfection process, and items that float on the disinfectant will not be disinfected. In general, longer contact times are more effective than shorter contact times.

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# Chemical Disinfection

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## Expected Results: Biofilms

### Biofilms:

Microorganisms may be protected from disinfectants by production of thick masses of cells and extracellular materials, or biofilms. Biofilms are microbial communities that are tightly attached to surfaces and cannot be easily removed. Once these masses form, microbes within them can be resistant to disinfectants by multiple mechanisms, including physical characteristics of older biofilms, genotypic variation of the bacteria, microbial production of neutralizing enzymes, and physiologic gradients within the biofilm (e.g., pH). Bacteria within biofilms are up to 1,000 times more resistant to antimicrobials than are the same bacteria in suspension. Although new decontamination methods are being investigated for removing biofilms, chlorine and monochloramines can effectively inactivate biofilm bacteria. Investigators have hypothesized that the glycocalyx-like cellular masses on the interior walls of polyvinyl chloride pipe would protect embedded organisms from some disinfectants and be a reservoir for continuous contamination. Biofilms have been found in whirlpools, dental unit waterlines, and numerous medical devices (e.g., contact lenses, pacemakers, hemodialysis systems, urinary catheters, central venous catheters, endoscopes). Their presence can have serious implications for immunocompromised patients and patients who have indwelling medical devices. Some enzymes and detergents can degrade biofilms or reduce numbers of viable bacteria within a biofilm.

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# Chemical Disinfection



## Slide 17



### 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.







## Lecture


Organic matter in the form of serum, blood, pus, or fecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or nongermicidal, leaving less of the active germicide available for attacking microorganisms. Chlorine and iodine disinfectants, in particular, are prone to such interaction. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier.



## Slide 18



### Product Factors

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





# Chemical Disinfection



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## Plenary Discussion (2 minutes).

Question to consider:

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

Expected Response:

Age – The older the product, possibly the less stable it is, and the less effective it can become.

Method – How the disinfectant is applied can affect how effective it is depending on the coverage area. Some application methods will have a better coverage area than others, sometimes this is necessary and others it is not.

Rate – Some disinfectants take time to work. Depending on the disinfectant used this could be anywhere from 1-2 minutes or up to an hour. Disinfection time will also depend on the agent characteristics. For example bacterial spores will take longer to kill compared to an unstable enveloped virus.

Storage – Many disinfectants have different rates of decay that can be accelerated by light, air, etc., It is important to determine the optimal storage conditions for a particular disinfectant as it could become ineffective if these considerations are not met.



Ask: Any questions on Chemical Disinfection?



## Take a Break (10 minutes)

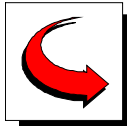


## Time Check

You should be approximately 1 hour and 20 minutes into the course. You have 2 hours and 20 minutes of the course remaining.

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# Chemical Disinfection



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**Transition to Properties and Selection of Chemical Disinfectants**

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# Properties and Selection of Chemical Disinfectants

## Slide 19



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**Properties of Chemical Disinfectants**

How do chemical disinfectants work?

Consider the mode of action of a disinfectant on an enveloped, non-enveloped, or spore former microorganism.

**Individually reflect: What type of disinfectant would work on your organism?**

Slide 20



## Plenary (5 minutes).



### Activity Instructions (to students)

- You have 5 minutes, to think about and discuss in your group in you'd like, to come up with the mode of actions for chemical disinfectants.
- Then individually reflect on what disinfectants would work on your organism, considering whether it is an enveloped or non-enveloped organism and why (using the factors we just learned in class)

# Properties and Selection of Chemical Disinfectants



**You have 10 minutes to complete this activity**

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**Directions for Instructor:**

- Allow 5 minutes – this is a brainstorming session and does not require detailed, complete answers.
  - After 5 minutes, ask volunteers from the group to report on mode of actions for chemical disinfectants.
  - Allow a good 5 minutes for the reporting-out phase of this exercise.
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# Properties and Selection of Chemical Disinfectants



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## Background Information for Instructor

Go over and explain mode of action (mechanism of action):

- All disinfectants have different mechanisms of action by harming microorganism by protein denaturation, membrane disruption, nucleic acid damages or metabolic inhibition. This mode of action is the effect of the chemical formulation (i.e. chelators, oxidizers, acids, alkalines, lipophilic, surfactants, alcohols, halogens, corrosives and anti-corrosives agents) and efficacy of the decontaminating agent on the microorganism's structure and functions.
  - Ex: ionized disinfectant like detergents will change the charges of the phospholipids and lipopolysaccharide cellular wall of the organism at contact which can cause the cell wall to dissolve. Oxidizing agents (i.e. chlorine) can destabilize spore structures (i.e. doesn't kill it but damages the membrane enough to prevent germination). Denaturing agent (i.e. 70% alcohol) is an effective disinfectant for cleaning BSC and materials.
  - Reiterate previous slides in regards to choosing the proper disinfectant:
    - What type of microorganism is the target
    - Which disinfectant is known to inactivate that microorganism and the degree of inactivation
    - How much organic matter is involved
    - Concentration of the microorganism
    - Is the disinfectant able to be in contact with the microorganism and how long
    - Is the disinfectant compatible to the material that microorganism is in (i.e. to the liquid, surface, material)
    - Is the disinfectant stable (i.e. flammable) and safe (i.e. carcinogenic, toxicity, irritant, vapor or odor etc.)
    - Does the disinfectant leave a residue on the material





# Properties and Selection of Chemical Disinfectants

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## Expected Responses

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For an enveloped – Alcohol, chlorine

Non- enveloped – chlorine

Spore former – chlorine

### Why

Bleach – oxidant to poke holes in

Alcohol - is a desiccant (sucks water out), solubilizes lipids of the

Oxidizing agents (i.e. chlorine) can destabilize spore structures (i.e. doesn't kill it but damages the membrane enough to prevent germination).

New Responses from Students:

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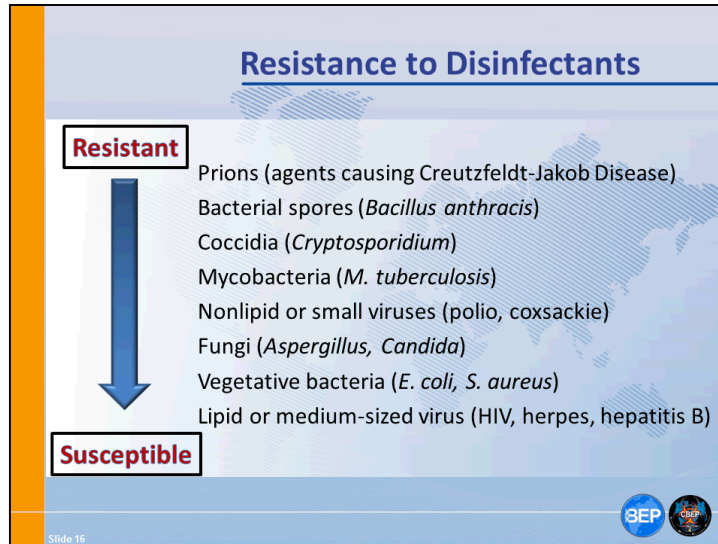
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# Properties and Selection of Chemical Disinfectants

Slide 20



# Properties and Selection of Chemical Disinfectants



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## Lecture:

Microorganisms vary greatly in their resistance to chemical germicides and sterilization processes (see the next slide). Intrinsic resistance mechanisms in microorganisms to disinfectants vary. For example, spores are resistant to disinfectants because the spore coat and cortex act as a barrier, mycobacteria have a waxy cell wall that prevents disinfectant entry, and gram-negative bacteria possess an outer membrane that acts as a barrier to the uptake of disinfectants. Implicit in all disinfection strategies is the consideration that the most resistant microbial subpopulation controls the sterilization or disinfection time. That is, to destroy the most resistant types of microorganisms (i.e., bacterial spores), the user needs to employ exposure times and a concentration of germicide needed to achieve complete destruction. **Show the next slide to review order of resistance.** Except for prions, bacterial spores possess the highest innate resistance to chemical germicides, followed by coccidia (e.g., *Cryptosporidium*), mycobacteria (e.g., *M. tuberculosis*), nonlipid or small viruses (e.g., poliovirus, and coxsackievirus), fungi (e.g., *Aspergillus*, and *Candida*), vegetative bacteria (e.g., *Staphylococcus*, and *Pseudomonas*) and lipid or medium-size viruses (e.g., herpes, and HIV). The germicidal resistance exhibited by the gram-positive and gram-negative bacteria is similar with some exceptions (e.g., *P. aeruginosa* which shows greater resistance to some disinfectants). *P. aeruginosa* also is significantly more resistant to a variety of disinfectants in its "naturally occurring" state than are cells subcultured on laboratory media. *Rickettsiae*, *Chlamydiae*, and mycoplasma cannot be placed in this scale of relative resistance because information about the efficacy of germicides against these agents is limited. Because these microorganisms contain lipid and are similar in structure and composition to other bacteria, they can be predicted to be inactivated by the same germicides that destroy lipid viruses and vegetative bacteria. A known exception to this supposition is *Coxiella burnetii*, which has demonstrated resistance to disinfectants.

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# Properties and Selection of Chemical Disinfectants

## Slide 21



**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



## Small group activity (20 minutes).



### Activity Instructions (to students)

- You have 10 minutes, in your groups, review the resource material provided.
- Complete the table in your student guide.
- Be prepared to discuss with the rest of the class.

# Properties and Selection of Chemical Disinfectants



**You have 20 minutes to complete this activity**

## ***Directions for Instructor:***

- Assign one group with bleach. (i.e. this information is needed for the scenario (spore forming) on slide 24 in the next group exercise)
- Participants will need a copy of the WHO Laboratory Biosafety Manual (or at least a copy of Chapter 14 on disinfectants). Chapter 14 of the third edition has information for each type/class of disinfectant. Chapter 14 is in the additional course materials included with this course and is also included in the Student Guide.
- Assign each table one or two of the disinfectants on the list below.
- Participants should be directed to read and discuss their assigned disinfectant class in small groups and be prepared to report their findings to the whole group. They can record their answers in the template in their workbook. And on their group's flip-chart.

Classes of disinfectants to be assigned:

- Bleach (Chlorine)
  - Iodophor
  - Glutaraldehyde
  - Formaldehyde
  - Phenolic compounds
  - Quaternary Ammonium
  - Alcohols
  - Hydrogen peroxide and peracids
- 
- After 10 minutes, ask each group to report their findings and also to place a copy of their template and findings on a flipchart labeled, Properties of Chemical Disinfectants.
  - Be sure to highlight any similarities, differences or unique answers.

# Properties and Selection of Chemical Disinfectants

Slide 22



Properties of Chemical Disinfectants				
Criteria				
Name of Chemical Disinfectant:				
Typical Concentration used				
Uses in the Laboratory				
Advantages				
Limitations/Disadvantages				

**Expected Responses**

Chapter 14 – LBM will guide the expected responses.

New Responses from Students:

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# Properties and Selection of Chemical Disinfectants

## Slide 23





### 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 21





## Slide 24



### 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?**

Slide 22



# Properties and Selection of Chemical Disinfectants



**Small group activity (25 minutes).**



## **Activity Instructions (to students)**

- 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).



**You have 25 minutes to complete this activity**

## ***Directions for Instructor:***

- The report-out phase is covered in the next slides.
- Expected Responses:
- For surfaces and reusable materials – use bleach
- Note: if the students bring up how to decon a large spill, using vaporized hydrogen peroxide or paraformaldehyde would be a better opt



# Properties and Selection of Chemical Disinfectants

## Slide 25



Standard Operating Procedure for:	
<b>Conditions</b>	
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
<b>Context</b>	
Input(s):	Contaminated surfaces and reusable materials
Output:	Disinfected surfaces and reusable materials
Preparation required:	
<b>Actions</b> (steps required to move from the input to the output)	
Step 1	
Step 2	
Step 3	
Step 4	
Step 5	

Slide 25



## Slide 26



### "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



# Properties and Selection of Chemical Disinfectants



**Small group activity (15 minutes).**



## Activity Instructions (to students)

- Trade SOPs with another group.
- Read the SOP you have been given (5 minutes)
- Answer the questions on the slide (10 minutes).
- Be prepared to share your evaluation.



**You have 15 minutes to complete this activity**

## Directions for Instructor:

- Direct the groups to exchange SOPs for evaluation.
- If time allows, guide class through the selection of the best SOP presented.

## Expected Responses

For this disinfectant SOP the questions that should be addressed is the preparation required, and the actions, which are the steps that must be taken to move from the input to the output. Try to encourage students to use these questions to guide them through the review.



**Take a Break (10 minutes)**



## Time Check

You should be approximately 2 hours and 30 minutes into the course. You have 1 hours and 30 minutes of the course remaining.

# Properties and Selection of Chemical Disinfectants



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**Transition to Additional Methods of Disinfection**

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# Additional Methods of Disinfection


## Slide 27



**Additional Methods of Disinfection**

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

Slide 25



## Lecture

As mentioned at the beginning, we will discuss primarily chemical and thermal means of decontamination. Before we move to thermal decontamination, let's discuss, briefly, filtration and radiation.

How does a HEPA filter work as a decontamination procedure?

By removing potentially infectious particles from the air it filters

Other examples of using filtration as a decontamination method?

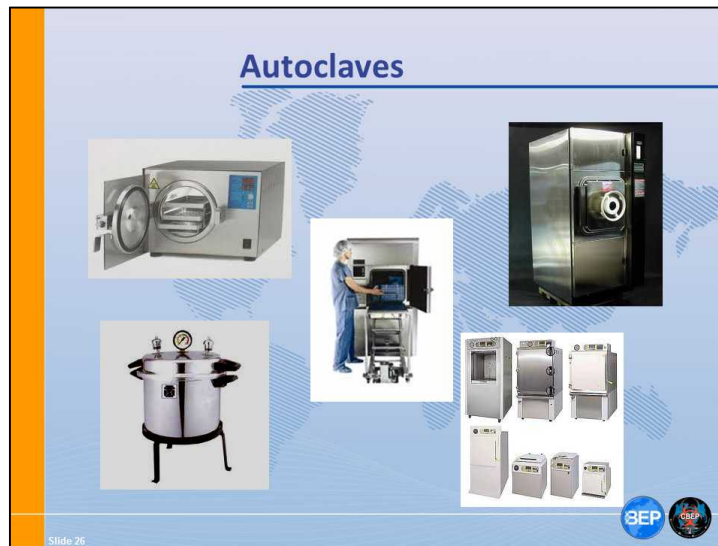
Using Millipore filters to filter liquid media or other heat sensitive products – same principle – filters out particulates

How does radiation work as a disinfectant?

The ultraviolet light kills microorganisms by penetrating the cell wall and reaching directly inside the cell where it causes alterations in the DNA strands, thereby leading to disruption of the genetic material. As a result, the cell becomes inactivated and is no longer able to reproduce. All that the ultraviolet radiation does is damage the cellular DNA and prevent multiplication of cells.

# Additional Methods of Disinfection

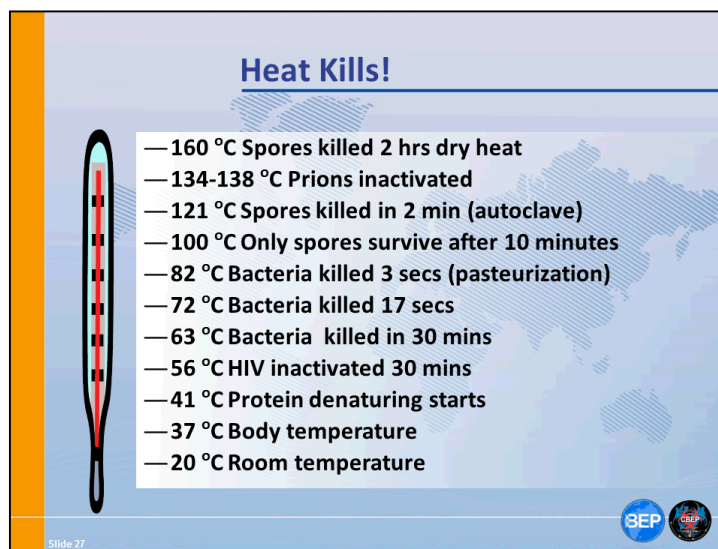
Slide 28



## Lecture

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. Autoclaves come in a variety of shapes and sizes.

Slide 29



# Additional Methods of Disinfection

## Slide 30




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### 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 22



## Background Information for Instructor

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

1. 3 min holding time at 134 °C
2. 10 min holding time at 126 °C
3. 15 min holding time at 121 °C
4. 25 min holding time at 115 °C.

# Additional Methods of Disinfection



## Slide 31



### 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 28



## Background Information for Instructor

Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents.

## Slide 32





### 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 29



# Additional Methods of Disinfection



**Small group activity (15 minutes).**



## **Activity Instructions (to students)**

- List the advantages and disadvantages of using an autoclave. (5 minutes)
- Complete the template in your workbook (5 minutes).
- Be prepared to discuss your answers.



**You have 15 minutes to complete this activity**

## ***Directions for Instructor:***

- Use 5 minutes to have each group report their responses.



# Additional Methods of Disinfection

## Expected Answers

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### Pros:

- readily available
- well-tested technology
- generally low maintenance
- cycle parameters easily verified
- Testing relatively simple

### Cons:

- Requires operator training, written SOPs, and use of PPE
- cannot be used for heat-sensitive materials
- may cause corrosion, dulling of sharp edges
- hazards :
  - high-temperature
  - pressure vessel
  - immediate environment can be wet, slippery
  - processing plastics can produce toxic components in exhaust steam
  - nitrocellulose can ignite
  - Cost
  -

### When to autoclave:

- Materials that will withstand the heat, moisture, and pressure
- Require high level disinfection or sterilization

### When to NOT autoclave

- Materials that CANNOT withstand the heat, moisture, and pressure
  - Materials with significant odor during autoclaving (still might be best option but consideration for the smell needs to be addressed)
  - Volatile chemicals, especially those that are corrosive
-


# Additional Methods of Disinfection

Slide 33





### 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

An icon showing a pair of orange nitrile gloves, representing personal protective equipment (PPE).

Slide 33




Slide 34





### 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**

An icon of a shallow metal tray with a perforated bottom, used for autoclaving items.

Slide 34



Ask students to relate any “horror” stories about autoclave use. Almost everyone has experienced something related to the high heat or high pressure of an autoclave. However, along with these stories, ask students to consider how many times they were able to use the autoclave safely – proving that it is a very valuable tool in a biological laboratory

# Additional Methods of Disinfection

## Slide 35



### Plenary Discussion (2 minutes).

What do you notice about this picture of an autoclave? Any practices or procedures that you would modify? Anything with the autoclave itself?

Expected Responses:

The rubber sealing doesn't seem to be functional as it is taped.

The tape could inhibit a continual seal, which could inhibit the autoclave from reaching temperature correctly so sterilization may not occur as desired.

Electrical cords are not organized and are a hazard.

Not clean.

Technician is not wearing any PPE.

Storage boxes are too close to the autoclaves.

Room is cluttered.




# Additional Methods of Disinfection

## Slide 36



### 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)



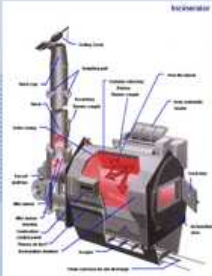


## Slide 5



### 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**



# Additional Methods of Disinfection

Slide 38



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

Expected Responses:

Advantage:

It reduces the volume of overall waste that would need to be taken care of.

Disadvantage:

Harmful to environment depending on what is incinerated.

Efficient operation depends on the right mix of materials in the waste being treated.

Require permits

# Additional Methods of Disinfection



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## Plenary Discussion (3 minutes).

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

The level of heat is the key factor in incineration – the two chambers also provide an additional opportunity to burn materials that are released from the heat in the first chamber.



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## Take a Break (10 minutes)



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## Time Check

You should be approximately 3 hours and 20 minutes into the course. You have 40 minutes of the course remaining.



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## Transition to Validation of Decontamination

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# Validation of Decontamination

Slide 39




## 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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**Small group activity (10 minutes).**



### Activity Instructions (to students)

- Each group should discuss one of these processes and decide what you can do to validate the effectiveness of the methods.
- Take about 5 minutes to discuss and gather ideas.
- Be prepared to share your ideas with the rest of the class.



# Validation of Decontamination

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**You have 10 minutes to complete this activity**

***Directions for Instructor:***

- Each of these processes should be assigned to different groups.
  - After 5 minutes, ask the students to stop working on the exercise
  - Ask groups to report out their ideas and why they think that their validation methods would work. Ask the other groups for any comments.
  - Discuss in plenary – Why is validation important? When is validation important?
  - Be sure to highlight any similarities, differences or unique answers.
-



# Validation of Decontamination

## Expected responses

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### Chemical disinfection – surfaces

- Rodac plates can collect organisms from surface – compare before and after (will not demonstrate effectiveness on specific organism)
- Total ATP before and after – again won't show effectiveness on specific organisms but will demonstrate 'decontamination' of viable organisms.
- Read label for organisms tested – follow label precisely.

### Chemical disinfection – liquid

- Note that labels very rarely talk about effectiveness in liquid solutions.
- Before and after plating of liquid (serial dilutions) after specified contact time.

### Autoclave sterilization

- Temperature and pressure records
- Autoclave tape – is heat sensitive – only registers high temp, not pressure
- Chemical Indicators that change colors under appropriate temp and pressure
- Biological indicators – measure actual reduction in heat resistant spores – must be placed in representative autoclave load in hard-to-decontaminate place

### Incinerator run

- Temperature records
- Ash should be sterile – hard to sample using sterile techniques
- Biological indicator placed in burn-proof container – should show 6-log (at least reduction) for sterilization

### Why is validation important?

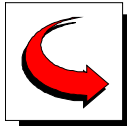
Validation, at its best, allows for all the factors that can impact the effectiveness of a decontamination method to be tested, real-time. If the decontamination goal is reached, then those factors can be included. If not, factors may need to be eliminated before the technique will work. For mechanical systems, also indicates that the system is functioning.

### When is validation important?

Because of the highly dynamic nature of the factors that impact effectiveness, validation should be performed frequently. After a baseline is established for a reasonably stable procedure, sometimes surrogate measures can be used daily with more rigorous procedures performed weekly or less frequently. For example, using a chemical or heat indicator for the autoclave on a daily basis, with biological indicators used weekly (depending on the risk assessment).

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# Validation of Decontamination



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**Transition to Review**

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# Review & Wrap Up



## Goal

The purpose and goal of this section is to recap the key messages of the course and to conduct a “What? So What? Now What?” review of the course and key messages.



## Time

Allow 10 minutes to get through the Review section.

## Slide 40





### 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?
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# Review & Wrap Up



Hold a contest among the class to see who can answer each question first. Once someone has gotten the right answer first, they cannot answer another question.

**What are three types of decontamination?** (chemical, thermal, filtration, radiation)

**What are three types of chemical disinfectants?**

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

**What is the definition of 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.

**What is the definition of 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.

**Name three factors that impact chemical disinfectant effectiveness.**

- 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

Other questions can also be used depending on time.

# Review & Wrap Up

## Review, continued

Ask these questions in plenary (not part of the contest): Why is decontamination important in biorisk management? What type of containment does decontamination help provide – primary or secondary? (both)

What next steps will you take to increase your knowledge or practices around decontamination? Make sure to note these on your action plan.

### Slide 6



### 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

### Slide 7



### 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

# Review & Wrap Up



## Review Key Messages

Include discussion on how activities/examples relate to the Key Messages of the course and how the messages can be applied.

1. Disinfection and decontamination have similar meanings. Both are less rigorous than sterilization which is the complete removal of all life.
2. No disinfectant is ideal, they all have strengths and limitations. Understanding the strengths and limitations is key to their use.
3. There are a number of factors that determines how effective a particular disinfectant is.
4. Micro-organisms have various innate resistance to disinfectants.
5. Autoclaves can be used to sterilize things through wet heat and the application of appropriate time, pressure and temperature.
6. Wet heat is much more effective than dry heat.
7. Validation is a process to ensure that the decontamination, disinfection or sterilization process used was complete and achieved its requirements.

## Slide 8



Action Plan			
By the end of this lesson, I would like to:			
KNOW	FEEL	BE ABLE TO DO	
<i>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.</i>			
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

BEP



Remember to complete your action plan – it is helpful to give yourself a due date for next steps.

# Review & Wrap Up

## Slide 9



## Level 1 Evaluation

- Ask students to complete the course evaluation and to put it in the evaluation box (alternately, give students instructions for completing the evaluation on-line).