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INTRODUCTION TO BIOSAFETY
INSTRUCTOR WORKBOOK

Biosafety Curriculum for Undergraduate and Graduate Students

This workbook to be meant as a companion to the didactic portion of the class. It is written to facilitate group work in-class. Many problem solving scenarios are presented; they are heavy on compare/contrast work and then ask students to choose the best course of action and to then justify their reasoning. These scenarios are designed to be as close to realistic scientific processes as possible. The students should keep in mind the following tenants:

- There are multiple ways to successfully accomplish a goal
- Everything you do in a lab (or in life) will have some element of risk. Successfully dealing with this risk and preparing for potential outcomes requires a risk assessment.

This workbook will prepare students to work safely in the lab - they will be able to:

1. plan procedures ahead of time
2. identify hazards early
3. mitigate hazards before or as they occur
4. communicate and keep good records
5. report exposures
6. develop a healthy work environment

Week 1 Questions - Biosafety history, LAIs and BSLs: (answers in *red italics*)

1. The profession of biosafety has roots in which of the following programs (select one):
   a. Ecology and environmental research efforts by the USDA
   b. The Biological Weapons program operated during the Cold War
   c. Industrial food production and food science pioneered during WWII
   d. The Occupational Safety and Health Administration
2. True or False, human research has never been performed with fully virulent Risk Group 3 pathogens
   a. *False* - Operation Whitecoats among other examples
3. True or False, scientists imposed a moratorium on their own research to first set up a system to ensure worker, community, and environmental safety in response to the first recombinant DNA experiments
   a. *True* - the Asilomar Conferences
4. True or False, laboratory workers have become infected with their research subjects in the past
   a. *True* - multiple examples, Richard Din and Malcolm Casadaban
5. True or False, bystanders near research laboratories have become infected with the organism(s) used inside
   a. *True* - Janet Parker, for one

Week 1 Activity - LAIs and BSLs:

1. **Janet Parker**

On August 11th 1978, in Birmingham, UK, Janet Parker began to feel unwell. She reported a headache and muscle pain. Her condition fluctuated over the next several days, however it ultimately deteriorated to the point that she sought medical attention. This included the development of a rash in addition to the other symptoms. . On Wednesday, August 16 she was visited by her doctor who prescribed her an antibiotic. Two days later, on the 18th she was visited by her doctor's medical partner who diagnosed her rash as a drug rash and discontinued the antibiotic. Both doctors were very wrong about what Mrs. Parker was ill with. She continued to develop spots on her face, limbs, and trunk. On Monday the 21st she was transferred to her parents' home where on Thursday the 24th she was visited by her parent's physician. He referred her to the hospital and Mrs. Parker was admitted to an isolation cubicle at East Birmingham Hospital at 3:00 pm on that same day. Hospital staff suspected she was infected with Smallpox and that evening sent specimens of vesicle fluid to the Regional Diagnostic Smallpox Laboratory. Electron microscopy revealed brick-shaped particles in her vesicular fluid, providing a positive diagnosis for the viral infection. However the accurate diagnosis of Smallpox even on the night of her admission to the hospital did not prevent her tragic fate. Janet Parker was transferred to Catherine-de-Barnes Isolation Hospital in Birmingham where she succumbed to her illness on the September 11th 1978. She was the last person on planet earth to die of Smallpox.

How she came to be infected has been something of a mystery. An investigation was launched, under the direction of Professor R.A. Shooter, to determine the source and cause of her infection. Several site inspections, tests, and interviews later, their conclusion was that the source of her infection was the Smallpox laboratory located directly beneath her office at the University of Birmingham. The route of transmission from the laboratory to Mrs. Parker could not be unequivocally defined, however, evidence suggested that Mrs. Parker most likely contracted the virus while using a telephone located in a room into which air from the Smallpox laboratory leaked. They suspect this occurred on July 25th as she had been using the telephone heavily that day to place a large number of purchase orders coinciding with the end of her work group's fiscal year. Investigation of the nearby Smallpox lab found that although Biosafety Cabinets were available and functioning properly, much of the work in the lab was performed on the open bench. In addition to this, samples were frequently moved from room to room without decontaminating the exteriors of containers. Furthermore, only individuals working directly with the virus were required to be vaccinated against the disease - Mrs. Parker's most current vaccination against Smallpox had occurred 14-years prior. Protection against Smallpox via the vaccination wanes after 5-years and standard policy for laboratory workers was vaccination every other year. The impact of Mrs. Parker's infection did not end with her: the director of the laboratory committed suicide over guilt that he had allowed this to happen and Mrs. Parker's father died of shock over the incident. In addition, several dozens of people were screened and monitored for symptoms of Smallpox until their window of risk for presentation had passed.

In the case of Mrs. Parker's fatal infection, please answer the following questions:

- What factors contributed to her exposure, disease, and death?
  - *Exposure - leaking air from the laboratory, work done on the open bench and samples moved between rooms*
  - *Disease - vaccination policy should have covered everyone in the building (and was a finding of the investigation)*
  - *Death - giving a proper history to her doctor could have helped with accurate initial diagnosis and resulted in better, earlier treatment*
- What might be done to prevent such a thing from happening again?
  - *Locate the laboratory away from office space*
  - *Seal ALL laboratory penetrations and keep them sealed (i.e. if maintenance work or construction performed, ensure the facility is intact before reopening)*

- *Perform ALL work in the BSC*
- *Vaccinate EVERYONE in the building*
- *Provide everyone in the building training and information on the pathogens used there and their signs and symptoms*
- *Work in a BSL-3 or -4 (or equivalent thereof since the concept wasn't concrete at the time)*

2. Richard Din

Richard Din was 25 years old. On a Friday evening - the night of April 27th 2012 he developed a headache, fever, neck pain, and stiffness. The next morning, his roommate drove him to the hospital. He lost consciousness in route. Despite the herculean efforts of the doctors and nurses at San Francisco Veterans Administration Medical Center to treat what they diagnosed as meningitis, he never woke up. He was declared dead approximately 3-hours after his arrival. Analysis of his blood and tissue samples showed he had been infected with *Neisseria meningitidis*, serogroup B.

Mr. Din had recently graduated from the University of California at Berkeley with a degree in Microbiology. He worked in a BSL-2 laboratory at the Veterans Affairs administration in the North California Institute for Research and Education which studied *Neisseria meningitides*—the same strain that took his life. It was concluded that Mr. Din was exposed during his work in the lab. An investigation of the laboratory in the aftermath of his death revealed that, despite his reputation as a fastidious researcher who always followed proper safety procedures, he had been working with the bacteria on the open bench and likely inhaled the organism. The investigation placed the blame for the incident upon the institution, finding that Mr. Din did not have sufficient supervision or information regarding the risks he was working with, and that there was no policy in place to conduct work with the pathogen in the Biosafety Cabinet.

In the case of Mr. Din's fatal infection, please answer the following questions:

- What factors contributed to his exposure, disease, and death?
  - *Exposure - Working on the open bench, his lack of training on the proper work practices and signs/symptoms of infection (presuming he would take proper precautions if informed)*
  - *Disease and death - virulence of the organism and route of exposure, improper reaction to initial symptoms (should have called ambulance to ER immediately upon headache and neck ache)*

- What might be done to prevent such a thing from happening again?
  - *improved training/information for new lab workers*
    - *on practices/procedures*
    - *risks present in the lab and their potential consequences*
    - *how to recognize those risks and how to respond*
  - *new policy of work with live organism only being performed inside BSC*
  - *vaccination? (no Group B Vx currently licensed in US, but experimental one in Europe and another Vx for non-Group B. N. meningitidis exists)*

3. Malcolm Casadaban

Dr. Malcolm Casadaban was a bacterial geneticist at the University of Chicago who was well-known for his insightful and useful contributions to the field. He had recently been preforming research on an attenuated strain (*pgm⁻) of Yersinia pestis* when he developed fever, body aches, and cough. After three days of these symptoms, Dr. Casadaban sought medical treatment at an outpatient clinic where, on September 10th 2009, he was referred to an emergency department by a clinical physician on suspicion of influenza or another acute respiratory infection; he did not seek that treatment. After three more days of worsening symptoms, including a new symptom: shortness of breath, he called for an ambulance and was transported to a Chicago hospital emergency department. His medical evaluation there led to an initial diagnosis of congestive heart failure, however it was amended once extracellular bacteria were seen in a peripheral blood smear. Upon a new diagnosis of bacterial infection, his medical team administered vancomycin and piperacillin/azobactam intravenously. It was not enough to save his life. He died approximately 13 hours later on September 13th 2009. An accurate diagnosis of his infection was finally made on September 18th after diagnostic test were completed and numerous misdiagnoses were discarded: Dr. Casadaban died of septicemic plague. However, the *Yersinia pestis* Dr. Casadaban was infected with was not a fully virulent strain; Dr. Casadaban had been infected with the *pgm⁻* strain from his laboratory. A post-mortem examination of Dr. Casadaban revealed that, in addition to the bacterial infection, he also had a condition known as hereditary hemochromatosis. Hemochromatosis is the medical term for a human body that is overloaded with iron; hereditary hemochromatosis is typically due to a mutation in the iron uptake transporter which causes too much iron to be taken up from an individual's diet. It is the suspicion of those who investigated this case that his high iron levels complemented the attenuating mutations in *Y. pestis*, allowing for fulminant disease.

Even given the unique features of this case, investigators sought to understand how he became exposed to the bacteria in the first place. In investigating the laboratory and interviewing his colleagues and lab mates, no serious deficiencies with the facility were discovered. Furthermore, no major reported injuries or known exposure events were on file for the laboratory. They did learn, however, that Dr. Casadaban used gloves only infrequently in the laboratory, suggesting a transdermal or mucosal route of infection from contaminated hands. It is also possible that he inhaled a small number of microbes which established a persistent colonization of his lung vasculature which subsequently disseminated, causing his septicemic plague. To further complicate the case, investigators discovered that Dr. Casadaban had failed on two counts in regards to his search for medical care: (1) to report to the University's Occupational Health clinic, and (2) to report that he worked with *Yersinia pestis* **-** both requirements of existing laboratory policy. Finally, neither did the outpatient clinic or the Emergency Department he sought care at record the fact that he worked with the causative agent of plague.

It is possible that if Dr. Casadaban had prevented exposure to his research subject, or sought medical care at his University's Occupational Health Clinic, or his doctors had become informed of Dr. Casadaban's research subject, he may have been able to receive effective antibiotic therapy in time to save his life. Tragically, those things did not happen.

In the case of Dr. Casadaban's fatal infection, please answer the following questions:

- What factors contributed to his exposure, disease, and death?
    - *Exposure - inconsistent glove use and work on open bench*
    - *Disease and death - unforeseen complementation of bacterial attenuation, inaccurate diagnosis and delayed treatment*

- What might be done to prevent such a thing from happening again?
    - *Improve consistency in PPE use in the lab*
    - *Improve handwashing practices in the lab*
    - *Educate/train researchers on signs and symptoms of the organism*
    - *Encourage individuals to discover any conditions they might have that could compromise their immune system and self-report to Occupational Health*
    - *Require work to be performed inside BSC? Perhaps not appropriate for all personnel, but is it worthwhile to err on the side of caution?*

4. Elizabeth R. Griffin

On October 29th 1997, Elizabeth Griffin was transporting a macaque from an outdoor pen to an indoor facility for its annual medical exam. She peered into the cage to check on the primate and when she did, it splashed her with a bodily fluid. Ms. Griffin wiped her eye, shrugged the incident off, and proceeded with her work. Once she finished with her work, she rinsed her eye briefly, and then went home.

Elizabeth, or Beth, was a warm, talented, artistic, outgoing young woman who was trying to choose between a career as a dancer or as a primate researcher. She did not get to make that decision. Her symptoms (an irritated eye) began approximately 10-days after her exposure on the 29th and she sought medical care at an emergency room in a nearby hospital. The doctors there were not aware of Beth's work with primates and diagnosed her with conjunctivitis ("pinkeye") then sent her home with eye drops. She did not have pinkeye. Her condition worsened over the next two days: her eye became more painful and she developed a headache and a low-grade fever. She sought medical care again, this time from her private eye doctor who was also not aware of Beth's work with primates or the diseases they could carry. He sent a sample to a managed care testing facility and sent her home with antibiotics. Things did not improve for Beth; over the next two days, her eye became red and even more painful; she had also developed a full-grade fever, a stiff neck, and vomiting. By this point, her occupational exposure had finally been reported and she was referred to the Infectious Disease doctors at Emory University Hospital who recognized her infection likely to be Herpes B (a.k.a. macacine herpesvirus 1, a.k.a. cercopithecine herpesvirus 1. They sent samples to a reference lab that could perform the test and began treatment with antivirals.

Initially, Beth seemed to worsen on the treatment, but over the course of the next ten days, her symptoms began to improve. She could walk, she was eating well, and she felt much better. The hospital decided to release her with the necessary equipment for home care.
However by 8:00 am the next day, she was readmitted to the hospital with ascending encephalomyelitis, resulting in paralysis. Beth was no longer able to walk on her own, or even able to breathe. She never left the hospital; eventually developing seizures, severe lung and nerve damage, a blood infection, and bacterial pneumonia. She died at 3:00 pm on December 10th. She was 22 years old.

Elizabeth R. Griffin had died of a completely preventable disease and had missed a golden window for treatment, resulting in her death. Yet according to her knowledge and training, she had done nothing wrong. The common understanding at the time was that individuals were not at severe risk from Herpes B exposure if exposed via a mucous membrane route. Beth had never received any training to the contrary or any advice that she should have protected her eyes and face while working with the primates. This is unfortunate since a 1993 study in a similar research facility had observed that roughly half of their monkeys tested positive for the virus (1). It is possible that if Beth Griffin had protected herself with the appropriate equipment, she would not have passed away at such a young age.

1. J Infect Dis. 1993 Feb;167(2):257-63. Epidemiology of cercopithecine herpesvirus 1 (B virus) infection and shedding in a large breeding cohort of rhesus macaques. Weigler BJ, Hird DW, Hilliard JK, Lerche NW, Roberts JA, Scott LM.

In the case of Ms. Griffin's fatal infection, please answer the following questions:

- What factors contributed to her exposure, disease, and death?
  - *Exposure - no use of eye and mucous membrane (face) protection*
  - *Disease and death - incorrect initial and early diagnoses and inaccurate/incomplete patient history*

- What might be done to prevent such a thing from happening again?
  - *Require the use of eye and face protection when working with primates*
  - *Develop Herpes B-negative primate colonies*
  - *Require all exposure to non-human primate blood and bodily fluids be reported and*

followed up on by occupational medicine professionals

- *Develop a closer relationship between the primate center and the infectious disease group at the local hospital*

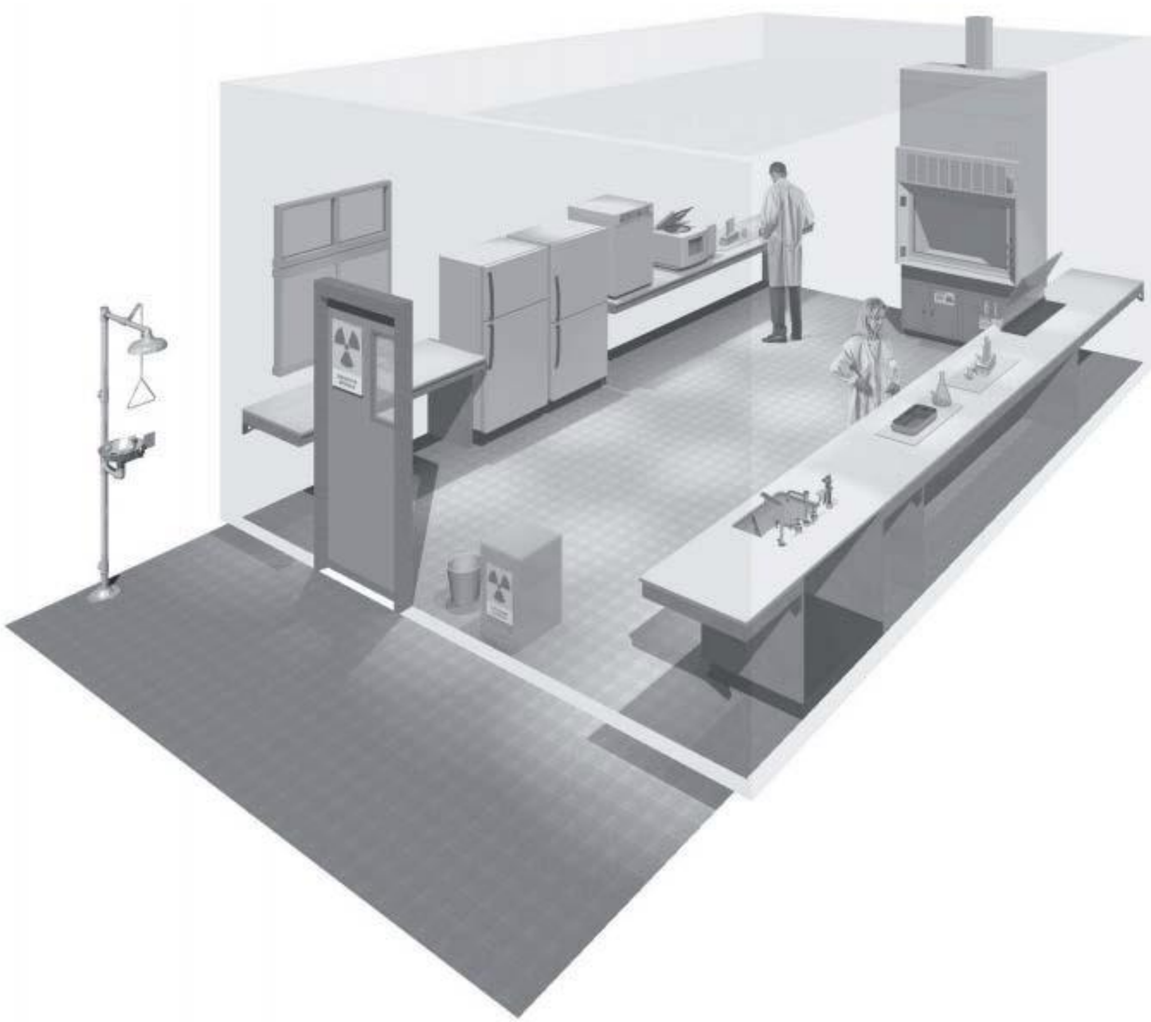
Week 1 - Exercise Biosafety Level

Biological Safety Level

Definition: Describes the **minimum** set of standard practices, safety equipment, and facility requirements that provides protection to personnel, the environment, and community when working with biological hazards.

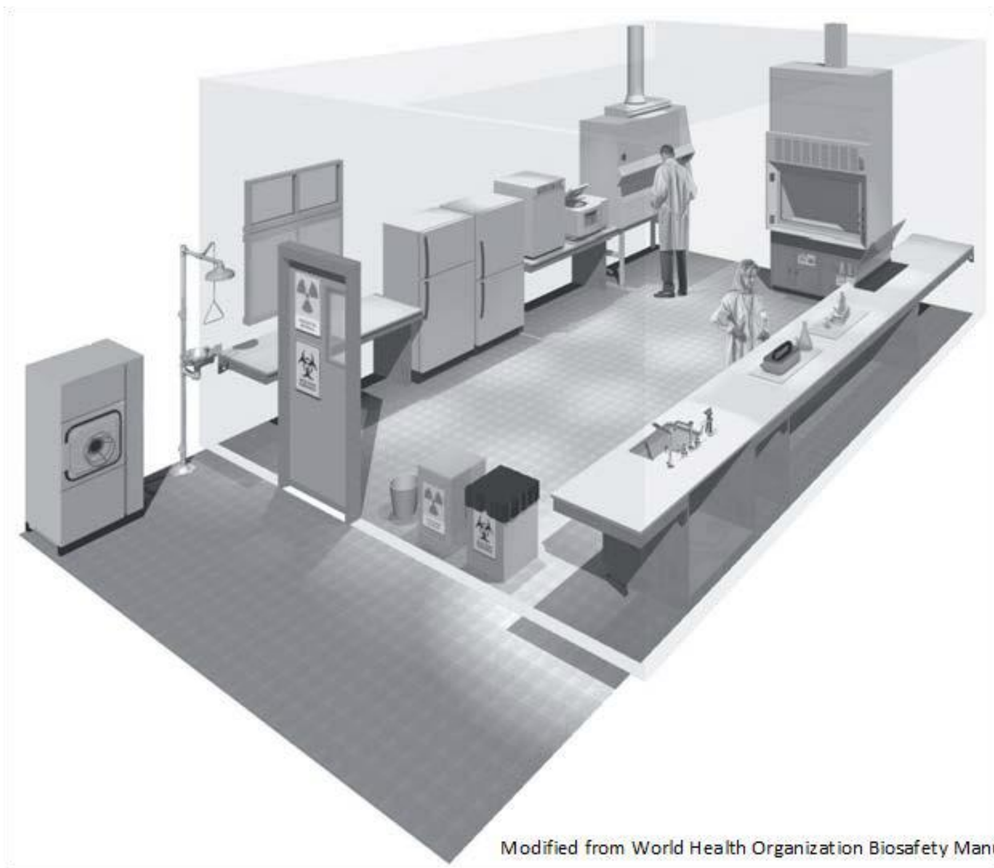
There are 4 biosafety levels which are described as follows:

•**Biosafety Level 1** (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. Work in a BSL-1 should be conducted using standard microbiological practices with the appropriate protective laboratory coat, gowns, gloves, and eyewear.



•**Biosafety Level 2** (BSL-2) builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures. Access to the laboratory is restricted when work is being conducted. All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Critical Components for BSL-2

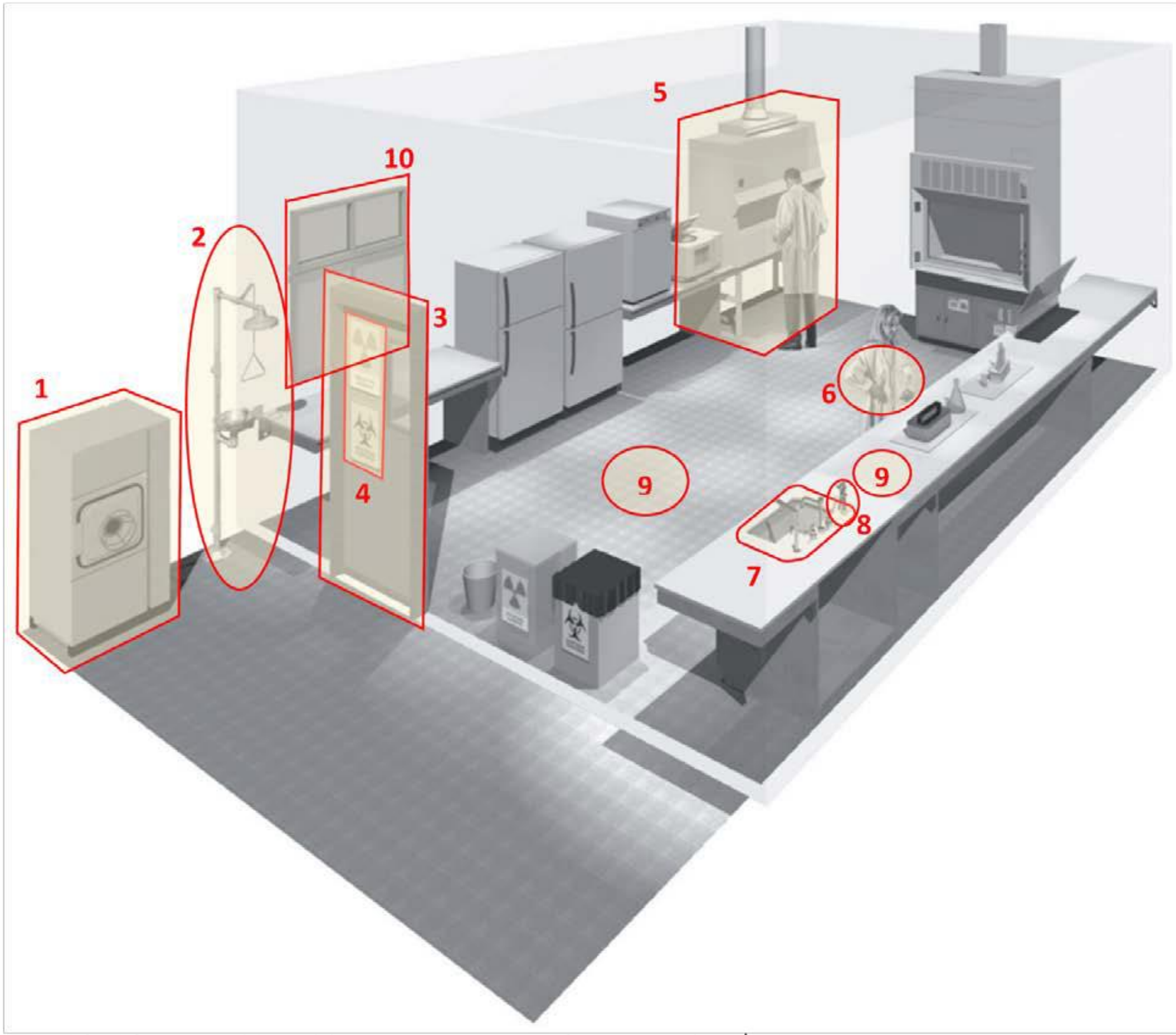


Modified from World Health Organization Biosafety Manual, 3rd ed. 2004

Circle and label the following features on the above diagram

1. Autoclave accessible
2. Emergency shower
3. Controlled access
    a. self-closing door
    b. lock
4. Proper signage
5. Biosafety Cabinet when necessary
6. Proper PPE
7. Handwashing sink available
8. Eyewash station available
9. Appropriate laboratory surfaces
10. Any unsealed windows fitted with screens

BSL-2



Modified from World Health Organization Biosafety Manual , 3rd ed. 2004

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2. Emergency shower
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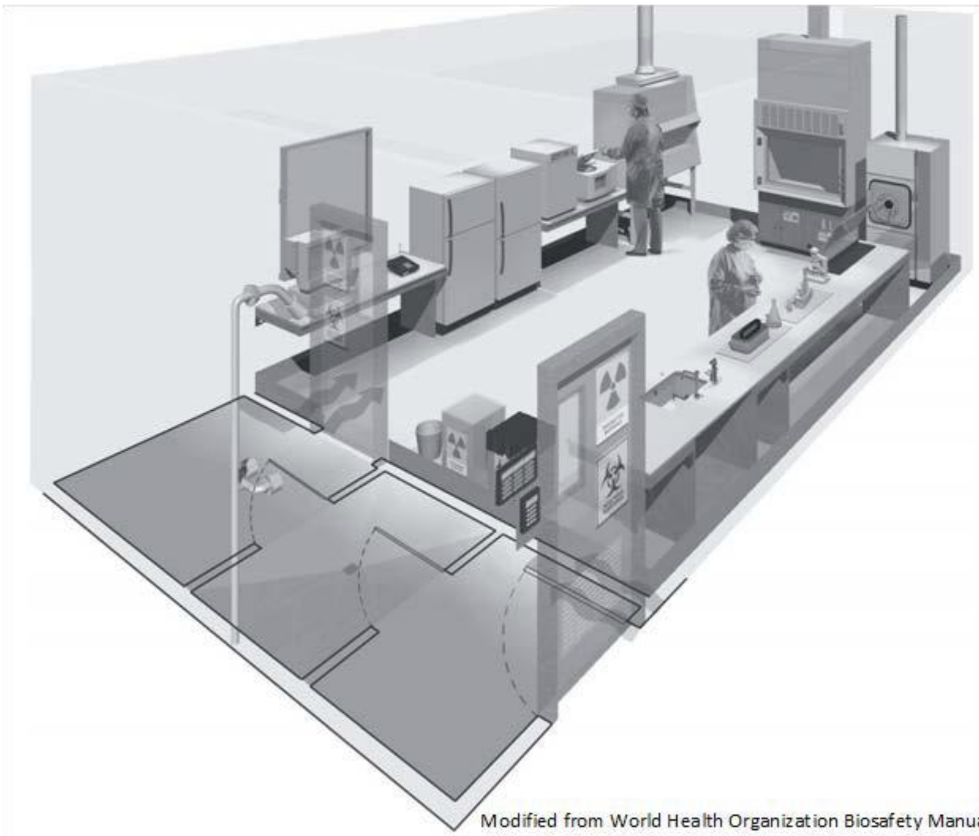
5. Biosafety Cabinet when necessary
6. Proper PPE
7. Handwashing sink available
8. Eyewash station available
9. Appropriate laboratory surfaces
10. Any unsealed windows fitted with screens

Biosafety Level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Many of the components of a BSL-3 are designed to minimize or eliminate hazards from aerosol exposure.

Critical Components for BSL-3

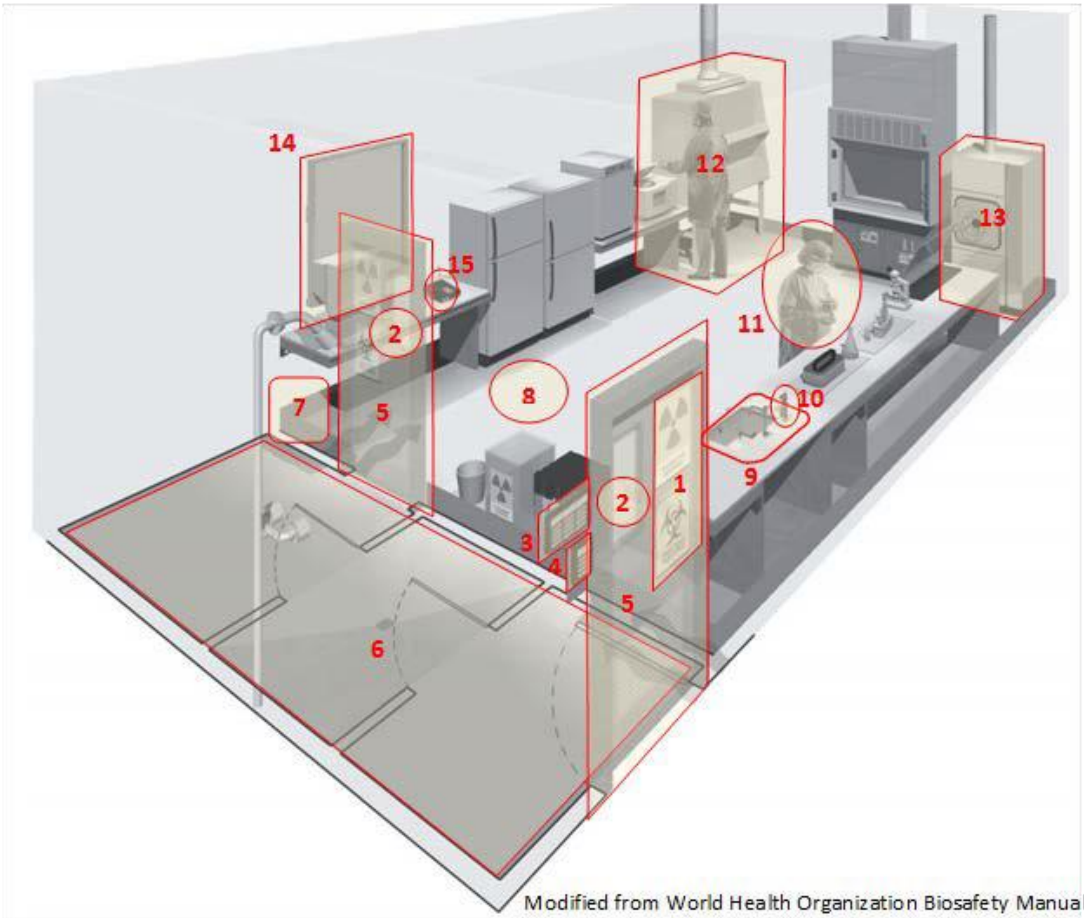


Modified from World Health Organization Biosafety Manual, 3rd ed. 2004

Circle and label the following features on the diagram above:

1. Sign with:
    a. Biohazard symbol
    b. Agent(s) present
    c. Contact information
    d. Entry requirements
2. Posted communication
    a. Spill notice, etc.
3. Directional airflow indicator
4. Restricted access
5. Interlocking double-door entry with self-closing doors
6. Anteroom
7. Integral, cove-molded floors
8. Seamless, chemical-resistant floors, walls, and ceilings
9. **Hands-Free** sink
10. Eyewash station
11. PPE (respiratory protection common)
12. BSC used for **all** manipulations
13. Autoclave within facility (preferred inside containment)
14. Any windows sealed
15. Biosafety plan

Critical Components for BSL-3



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5. Interlocking double-door entry with self-closing doors
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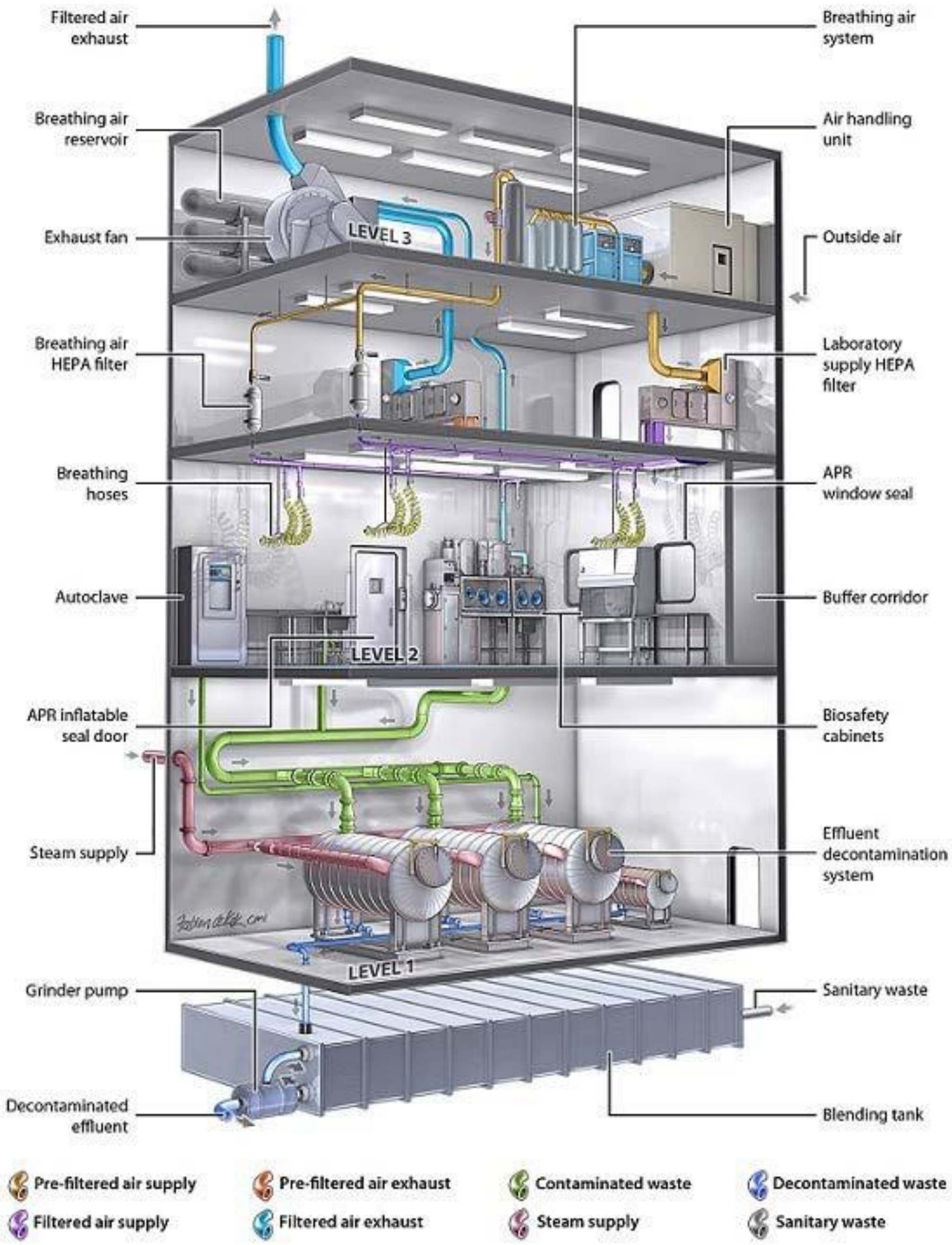
7. Integral, cove-molded floors
8. Seamless, chemical-resistant floors, walls, and ceilings
9. **Hands-Free** sink
10. Eyewash station
11. PPE (respiratory protection common)
12. BSC used for **all** manipulations
13. Autoclave within facility (preferred inside containment)
14. Any windows sealed
15. Biosafety plan

•**Biosafety Level 4 (BSL-4)** is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

BSL-4 Diagram



http://www.niaid.nih.gov/about/organization/dir/irf/Pages/facilityOverview.aspx

Outbreak movie:

Week 1 Take home assignment (Outbreak the movie)

1. Name 2 things wrong in the biosafety level 1 lab as presented in the beginning of the movie Outbreak.
   a. *No gloves*
   b. *Agents listed as biosafety 1 are not biosafety 1. Most pneumococcal species (streptococcus pneumoniae) and Salmonella species (salmonella enterica) are risk group 2 agents that requires BSL-2 containment*

2. Identify the clean air bench in the BSL-1 lab.
   a. *Explain it is ok to have a clean air bench in a BSL-1 laboratory*

3. In the BSL-2 what equipment are the lab personnel using that is typically not found in a BSL-2? What would you substitute instead?
   a. *Lab personnel are using N-95s*
   b. *I would substitute a biosafety cabinet (where is their biosafety cabinet???)*

4. In the biosafety level 3 laboratory can you identify something wrong with the PPE?
   a. *Not all lab members are wearing the same PPE, some have on Tyveks and other scrubs*

5. What is wrong with this BSL-3 from a facility standpoint?
   a. *Guy entering the BSL-3 through a door from the hallway. There is no anteroom.*

6. List 3 unusual biosafety observations
   a. *None of the doors are closed in any of the laboratories.*
   b. *Researchers are wearing lab coats into the hallways.*
   c. *Non-lab personnel operating the autoclave in the hallway (army guy)*
   d. *First biosafety cabinet spotted in the BSL-3 lab.*
   e. *Researcher takes off respiratory protection before exiting the BSL-3*

Week 2 Exercises - Basic Microbiology and Epidemiology

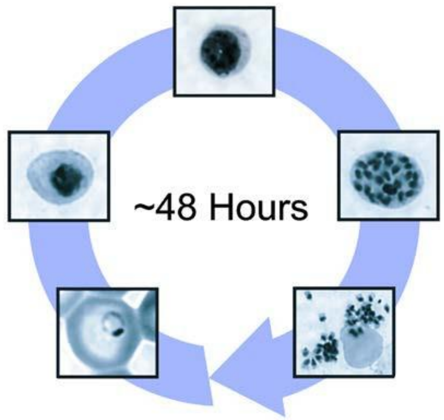
The purpose of this section is to expand upon the learning objectives in the course outline by providing the students with questions, scenarios, and exercises that cover specific tenets of basic microbiology and epidemiology. It should be understood that any institution that has their own microbiology program should use that program in place of what is outlined here in week 2. Furthermore, if the instructor wishes to omit week 2 entirely because of prerequisites for the class it is encouraged for them to do so, you wouldn't want the information to be completely redundant.

Basic microbiology (discussion questions)

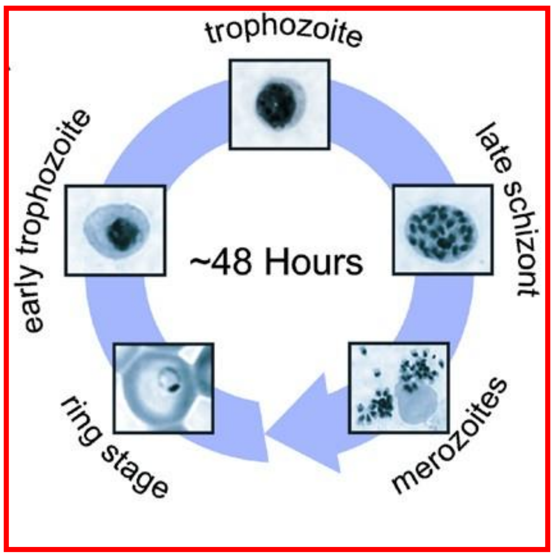
1. List several characteristics of microorganisms that make them particularly easy to transmit? Your focus is on the organism and not host pathogenesis.
    a. *Environmental stability*
    b. *Size and relative abundance*
    c. *Environmental availability*
2. What are some features that may enhance the above characteristics? Remember the focus is on enhancing transmission.
    a. Possible answer: *Environmental stability: Some organism have stages of life that enable them to survive in the environment (capsule or spore coat). Moisture, the availability of a suitable carbon source, lack of direct sunlight, and moderate temperatures are all conditions that can increase an organism's stability in the environment.*
    b. *Possible answer: Size and relative abundance: Because microorganisms by their very definition (micro) are not detectable by sight and are found everywhere in/on earth it makes them impossible to avoid. Therefore, unless a person is actively sick, they are unaware of when they acquire or transmit organisms. Furthermore, their small size can allow them to be distributed easily via aerosol*
    c. *Possible answer: Environmental availability/abundance: Many pathogenic microorganisms can be found in the soil, waterways, or carried by unassuming animals. The ability of microorganisms to move around by their own means (motility), with weather, or be carried by an animal/insect (vectors) means that they are always available in our environment.*
3. What are some difference between bacteria in a vegetative state vs spore state when it comes to biosafety? Are there any additional hazard associated with spores?
    a. *Possible answer: Bacteria in a vegetative state are actively consuming nutrients, sensing their environment, and readily reproducing. They also have a high water content. Bacteria in a spore state go into a stage of dormancy where reproduction is nonexistent as well as nutrient consumption. The spore itself is typically a complex matrix of proteins and sugars that is extremely resistant to temperature, chemicals treatments (disinfectants), and other environmental factors. They also have limited water content and decreased surface area as well as volume. Bacteria in a vegetative state are easier to handle from a biosafety perspective because they are actively dividing and taking up nutrients which allows opportunity for a disinfectant/antibiotic to penetrate and destroy them.*
4. Based on what you know about *Aspergillus fumigatus* what stage of its life cycle is the most transmissible and what biosafety equipment/gear would you use to protect yourself?
    a. *Possible answer: Aspergillus fumigatus is typically transmitted when it is in its conidial form, where it can be easily breathe into the lungs. Because Aspergillus fumigatus conidia pose an aerosol risk any equipment that provide a barrier to the airways will be effective against its spread (i.e. BSC, N95, PAPR)*

5. Why are enveloped viruses less resilient to disinfectant than non-enveloped viruses?
    a. *Possible answer: Enveloped viruses are covered in a lipid bilayer that that is sensitive to environmental factors like desiccation and heat as well as detergents. This makes enveloped viruses easier to disinfect compared to non- enveloped viruses that don't need an envelope for cellular entry.*
6. List at least one reason why it is difficult to develop new and novel antifungals? List one antifungal.
    a. *Possible answer: Because fungi are eukaryotic organism, like humans, many of the antifungals developed are often toxic to our cells as well as the fungi.*
7. What are the differences between a pathogen, an opportunistic pathogen, a colonizer, and a commensal microorganism? Can an organism fit into one or more of these categories, explain?
    a. Definitions
        i. *Pathogen: an infectious agent that can cause disease or illness to its host*
        ii. *Opportunistic pathogen: is a pathogen that can cause disease or illness if it host immune system is in some type of weakened or altered state.*
        iii. *Colonizer: a microorganism that can infect an individual and cause no apparent symptoms of infection.*
        iv. *Commensal microorganism: organisms that typically live on/in the host's body and develop a symbiotic relationship with the host.*
    b. Can an organism fit into one or more of these categories?
        i. *Possible answer: Yes a microorganism can fit into multiple categories. What categories an organism fits into has lot to do with the host, site of infection, and strain/serotype. An organism that would normally colonize its host can be pathogenic depending on the host (elderly vs. teenagers), some organisms have strains that behave differently and are more/less pathogenic, and infection can progress differently depending on the site of initial infection (the eye vs. big toe vs. lung vs. gut).*
8. Compare and contrast some of the difference/similarities of toxins and chemicals in regards to biosafety?
    a. Compare: *Possible answer: Like chemicals toxins can be harmful in certain concentrations. Toxins and chemicals do not proliferate like other microorganisms. Thus, dilution of a chemical or toxin can inhibit harmful effects*
    b. Contrast: *Possible answer: Toxins differ from chemicals because they can be generated from organisms with little refinement. Thus, an organism that can continuously produce a toxin until it reaches a harmful concentration.*
9. Are microorganisms malicious? In most cases is it in the best interest of the organism to kill the host? Describe situations where it may be beneficial for the organism to kill the host.
    a. *Possible answer: Microorganisms are not malicious. They typically want nothing more than to reproduce and continue their existence. A lot of the harmful effects from infection are perpetrated by the host immune system trying to clear the infection. Many microorganisms rely on the host to provide nutrients or an environment that enable them to proliferate. So in many cases the death of the host leads to the eventual death of the organism.*
    b. *Possible answer: A case where it may be beneficial for a microorganism to kill the host is if the host's death enhances its dissemination.*

10. Label the major life stages of *plasmodium falciparum*



Source Wikimedia Commons



Source Wikimedia Commons

Answer

Week 2 Scenario

You are an epidemiologist working for the CDC. You have been tasked to complete an investigation into the recent increase in the number of deaths from Sudden Human Death Virus (SHDV). Using your knowledge of disease transmission and epidemiology answer the questions below.

You know that SHDV is a hemorrhagic virus similar to Ebola with an incubation period of 24-hours (thus why it is called "sudden") before the onset of symptoms. Unlike Ebola, SHDV can be spread via aerosol droplets similar to Influenza virus. Cases of the disease are occurring in the rural part of NoManhstan. SHDV has not been reported in NoManhstan in almost a hundred years, however this year there are currently 10 cases.

1. Your supervisor has sent you to interview patient zero and ask you to use contact tracing to find other potentially infected people. Describe the methods used:

Possible answer: interview patient zero and determine who s/he has had contact with and who s/he has spent time in close quarters with - especially those in his/her family and those s/he may have worked with. Find those people if possible, explain the disease they may have been exposed to, the symptoms of the disease and what they can do about it. Take their vital signs and monitor them for at least 24 hours. Ask who they have been in contact with or been physically close to. You may also interview other villagers to corroborate the information from the individuals you interviewed.

2. Based on the information provided your boss is asking you to classify the disease as either endemic occurrences, an outbreak, or an epidemic and give your reasoning why:

Possible answer: this is an outbreak because it is a larger number of cases than usual, located in a specific location, and not yet sustained across a large region which would make it an epidemic.

3. The Czar of NoManhstan has asked to make recommendations for him to protect his people. Based on what you know about the route of infection what would you recommend?

Possible answer: suggest that individuals who present with illness be cared for by trained medical professionals equipped with respiratory protection and that their loved ones and close workers be vigilant for signs of infection in themselves. Indicate that people may wear respiratory protection but it is not effective unless it fits properly and is worn properly. Suggest the country mount a campaign to promote handwashing, staying home when sick, and reporting illnesses to a doctor. Promote confidence in the government and the medical professionals in the country. Suggest that sick individuals should not leave their care facility (home or hospital) until 24 hours after symptoms have resolved. Suggest investing in a governmental diagnostic laboratory or number of laboratories to help achieve accurate and timely diagnoses. Suggest collaborating with other doctors and scientists to provide care for the sick and work to develop potential cures for the disease. Suggest mounting a field expedition to identify the source of the infection for patient zero so that future contact with the hazard can be avoided.

Week 2 Lab Exercise (If laboratory is available)
Handwashing Exercise

1. Give all students GloGerm gel or lotion, and have students rub it in well all over their hands.
2. Then take students to a sink, and have them wash their hands as they normally would.
3. Once they have washed their hands, take the black light and show them how much GloGerm residue is left in their hands.
4. Once done, cover the importance of handwashing in preventing contamination.
5. Now that they have seen how much can be left behind, ask how they might change their hand-washing technique?

Biosafety cabinet smoke demonstration

The students in the class should be walked through a smoke demonstration to show them how airflow affects the biosafety cabinet. Below are several links that demonstrate how this procedures should be done.
https://www.youtube.com/watch?v=KqaWM5Dd15c
https://www.youtube.com/watch?v=DihUzswUkQ8

The videos cover how to properly set up a biosafety cabinet and concepts on cleaning biosafety cabinet

Week 3 Questions - Molecular Biology

1. Order the following steps according to the Central Dogma of Molecular Biology:
   _*6*_ mRNA is bound by ribosomes

   _*1*_ DNA is replicated by a semiconservative mechanism

   _*8*_ amino acid chains are folded by chaperones

   _*2*_ Transcription initiation complex binds DNA

   _*5*_ mRNA is exported from the nucleus

   _*9*_ amino acid chains are modified as necessary and released to the appropriate cellular compartment

   _*4*_ pre-mRNA is processed by splicing

   _*7*_ ribosomes translate mRNA into chains of amino acids

   _*3*_ Transcription generates pre-mRNA from DNA
2. What is the definition of Molecular Biology?
   a. *The study of biological molecules and how they interact to produce the properties and behaviors of cells, tissues, and organisms*
3. What are two common viruses that have been modified to be used as tools in molecular biology? (pick two)
   a. Adenovirus (AdV)
   b. Lymphocytic Choriomeningitis Virus (LCMV)
   c. *Human Immunodeficiency Virus (HIV)*
   d. Ebola virus
   e. Human Papillomavirus (HPV)
4. What was the name of the conference of scientists convened to determine the safest way to proceed with recombinant nucleic acid research?
   a. *The Asilomar Conference*
5. True or False, altering the genetic content of an organism can change its properties or behaviors
   a. *True*
6. True or False, modifying the genetic content of an organism can change the risks that that organism poses to people, plants, animals, or other living things
   a. *True*
7. True or False, recombinant nucleic acid research performed using funds from the Department of Health and Human Services (e.g. The National Institutes of Health) is regulated
   a. *True*
8. What is the name of the process used to identify risks and mitigate them?
   a. *Risk Assessment*
9. What is the name of the Institutional body that reviews research and determines what Biosafety Level the research should be performed at?
   a. *Institutional Biosafety Committee*
10. True or False, biological toxins require a Risk Assessment and may require higher Biosafety Level containment for work
    a. *True*

Week 3 Exercises - Molecular Biology

1. Case Studies
   a. Students will read the following papers and discuss in class how recombinant technology resulting in unexpected changes in a microorganism's behavior and properties should have changed a risk assessment
      i. Jackson, R. J., et al. "Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox." J Virol 75(3): 1205-1210. PubMed ID: 11152493
      ii. Smulian, A. G., et al. "Expression of hygromycin phosphotransferase alters virulence of Histoplasma capsulatum." Eukaryot Cell 6(11): 2066- 2071 PubMed ID: 17873086
      iii. Fu, Y., et al. "High-frequency off-target mutagenesis induced by CRISPR- Cas nucleases in human cells." Nat Biotech 31(9): 822-826. PubMed ID: 23792628
      iv. Tumpey, T. M., et al. "Characterization of the reconstructed 1918 Spanish influenza pandemic virus." Science 310(5745): 77-80. PubMed ID: 16210530
      v. Cello, J., et al. "Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template." Science 297(5583): 1016-1018. PubMed ID: 12114528
2. Job Safety Analysis
   a. Students will select a common molecular biology procedure from the handout [Molecular Biology Tools], develop a protocol using the selected procedure, and identify the risks and hazards present in the process.

Week 4 - Micro

Investigational Scenario 1 (Influenza Laboratory)

Instructions:

This can be ran as an in class discussion, breaking the students into groups, allowing them to discuss each question, and present it back to the class.

Scenario:

A researcher wants to start using Influenza A virus in his/her laboratory. She/He calls you to discuss some of the possible hazards or risk associated with introducing Influenza virus into the laboratory. She/He tells you that they want to work with a non-pandemic/low-pathogenic avian influenza virus (either H9N2 or H7N7).

1. New sample into lab
    a. What are the hazards of performing influenza research? (Risk of exposure (LAI), risk of containment breach, etc...)
    b. Does the risk change if it is highly pathogenic avian influenza versus low pathogenic? (There are additional things to consider when working with highly pathogenic avian influenza)
        i. USDA quarantine procedures
        ii. Risk group of the organism
        iii. Federal Select Agent requirements
            1. BSL3 facility requirements
            2. Administrative practices (shower out)
            3. Biosecurity (agricultural definition)
    c. What are some mitigation measures that can be taken to prevent illness?
        i. Vaccination of everyone in the laboratory
        ii. Disposable PPE
        iii. Dedicated facility clothing (scrubs, shoes)
        iv. Strict adherence to aseptic microbiological procedures

Investigational Scenario 2 (Influenza Laboratory)

Some time has passed since the researcher has started working with the low path influenza. You get a call to respond to a possible exposure in their lab. Someone in the laboratory has come down with flu-like symptoms and a fever and think it could be linked to research they were conducting in the laboratory. You are charged with investigating and determining what happened.

Researcher (via the telephone) explains to you that they were centrifuging influenza samples (not using a safety cup) when the samples break. Unsure about what to do the researcher opens the failed centrifuge

2. <excellent break point for risk assessment> (clarifying point: What are the potential hazards of opening the centrifuge)
    a. What are the hazards present?
        i. Possible aerosol exposure from opening a broken centrifuge.
        ii. Physical hazard from the centrifuge
    b. What is the potential outcome?
        i. Possible outcome could be the aerosolization of the influenza virus.

c. What is the probability and severity?
    i. Probability that this would happen in the absence of safety caps is moderate to high. Centrifuges fail regularly and because of the high speed at which they operate or due to user error they can easily aerosolized a sample. Severity is pretty low because this is low pathogenic avian influenza the chances of causing a secondary infection is pretty low.

d. Is this risk acceptable?
    i. This can only be determined by your institution or biosafety program. (maybe useful to discuss what is meant by institutional risk)
        1. Institutional risk tolerance is the amount of risk your organization is comfortable with assuming for a given experiment or activity
            a. It can vary based on the activity or experiment
            b. It is influenced by multiple of factors including recent and past history, state and federal regulations, institutional culture, community perception, and personal bias

e. How do you mitigate it in the future?
    i. Retrain laboratory personnel how to respond to a failed centrifuge.
    ii. Make the addition of safety caps on centrifuges mandatory.
    iii. Retrain on how to properly use a centrifuge or reset centrifuge parameters (run it at a lower speed).

3. Patient 1 is diagnosed with influenza by clinic (you get the results from the clinic that the person in the laboratory indeed has the flu from a lab strain. They have been self- isolating at home for the last few days. The problem is that this person came into the laboratory while presenting symptoms.

4. <excellent break point for basic microbiology of Influenza>
    a. How is influenza transmitted? (Low pathogenic avian influenza requires direct transmission through mucous membrane after close contact with birds. Which include oral ingestion and conjunctivitis. Not typically spread from human to human.)
    b. What are the routes of exposure in this scenario? Ocular exposure, inhalation exposure, direct transmission to mucous membrane, or contact with fomites
    c. How long can influenza survive on a surface? (Influenza A virus can surviveon hard, nonporous surfaces (e.g., stainless steel, hard plastic) for 24 – 48 hours and on porous materials (e.g., cloth, paper) for < 8 – 12 hours in ambient temperatures. Virus persistence on surfaces increases up to 72 hours when those surfaces are moist or wet) .http://www.flu.gov/planning- preparedness/hospital/influenzaguidance.html
    d. Name several ways that a secondary infection may acquire?
        i. direct transmission to mucous membrane or contact with fomites

Investigational Scenario 3 (Influenza Laboratory)

You are in the final stages of your investigation when you get a report that a second laboratory worker becomes ill with fever and flu like symptoms. You are sent to determine if this is related to the initial exposure and if so find out how.

5. <break point for incident investigation with Patient 2> - have students list useful questions for Patient 2 - some examples:
    a. Was she present during the centrifuge exposure?
        i. Patient 2 answer (No, was not in the room but was at lab that day.)
    b. Did s/he have contact with Patient 1?
        i. Patient 2 answer (maybe. can't remember)
    c. What work does s/he do in the lab?
        i. Patient 2 answer (reagent prep and lab inventory management)
    d. Could she have had an influenza exposure outside of lab?
        i. Patient 2 answer (no)
    e. Is the BSC used properly?
        i. Patient 2 answer (varies, depending on the person)
    f. What procedures are common in the lab?
        i. Patient 2 answer (patient sample processing - cell culture inoculation, storage, and harvest, ELISA, PCR, sequencing, etc.)

6. When you investigate the lab, you find the wrong disinfectant in the lab (or out of date bleach, or low % alcohol)

7. What is the likelihood that this is another laboratory acquired infection?
    a. Diagnosis for Patient 2 comes back as reovirus, considered community-acquired and therefore different event from Patient 1 and therefore not lab-associated.

8. Debrief Section for exercise
    a. What lab practices contributed to Patient 1 getting sick?
    b. If the influenza virus was drug-resistant, would that change your risk assessment or your interactions with the lab or occupational health?
    c. Would the response to Patient 1 require contact tracing? Or other public health measures?

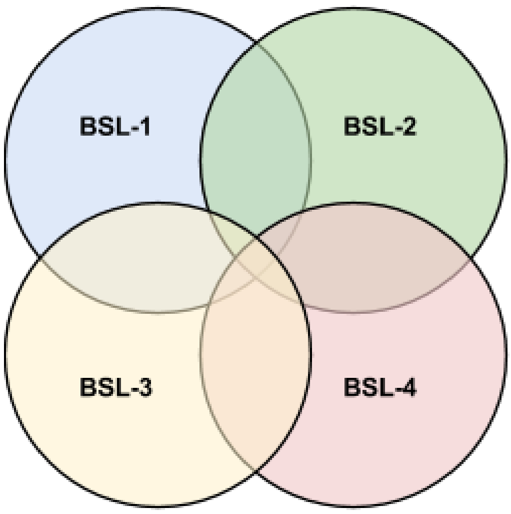
Week 5 Questions - BSLs

1. Choose an effective and appropriate risk mitigation strategy from the following options for a research proposal that calls for a Risk Group II organism to be aerosolized:
    a. Don't perform the work
    b. *Perform the work at BSL-3 with respiratory protection*
    c. Perform the work at BSL-2 with respiratory protection
    d. Perform the work in a separate tissue culture room at BSL-2
2. True or False, a BSL-1 requires a Biosafety Plan
    a. *False*
3. True or False, a Biosafety plan is recommended but not required for a BSL-2
    a. *True*
4. True or False, a BSL-3 is required to have its exhaust HEPA-filtered
    a. *False*
5. True or False, a biometrics scanner is required for a BSL-3
    a. *False*
6. According to the BMBL (5th edition), a BSL-3 requires all of the following except (pick one):
    a. An autoclave within the facility
    b. An anteroom
    c. A Biosafety Plan
    d. *An effluent decontamination system*
    e. Permanently sealed windows
    f. Sealed floors, walls, and ceilings
7. According to the BMBL (5th edition), the following are requirements of all biomedical and microbiological laboratories (all BSLs), except:
    a. Easily-cleaned laboratory furniture
    b. Chemically resistant work surfaces (benchtops)
    c. No carpets, rugs, or otherwise absorbent floor coverings
    d. *Restricted access (not required for BSL-1)*
    e. A sink for handwashing
    f. A door
    g. Supervisor of the laboratory enforces all institutional policies
    h. Supervisor of the laboratory ensures all individuals entering it receive proper training
8. True or False, a BSL-4 incorporates and builds upon the requirements of all lower containment levels
    a. *True*
9. Identify two unique features of BSL-3 relative to BSL-2
    a. *Sustained, directional airflow is required in BSL-3 but only suggested for BSL-2*
    b. *An autoclave is required within the facility for a BSL-3 but only access to one (potentially off-site) is required for a BSL-2*
    c. *Integral cove-molded floors are required for BSL-3, not BSL-2*
    d. *An anteroom is required for BSL-3, not BSL-2*
10. *(other answers possible)*

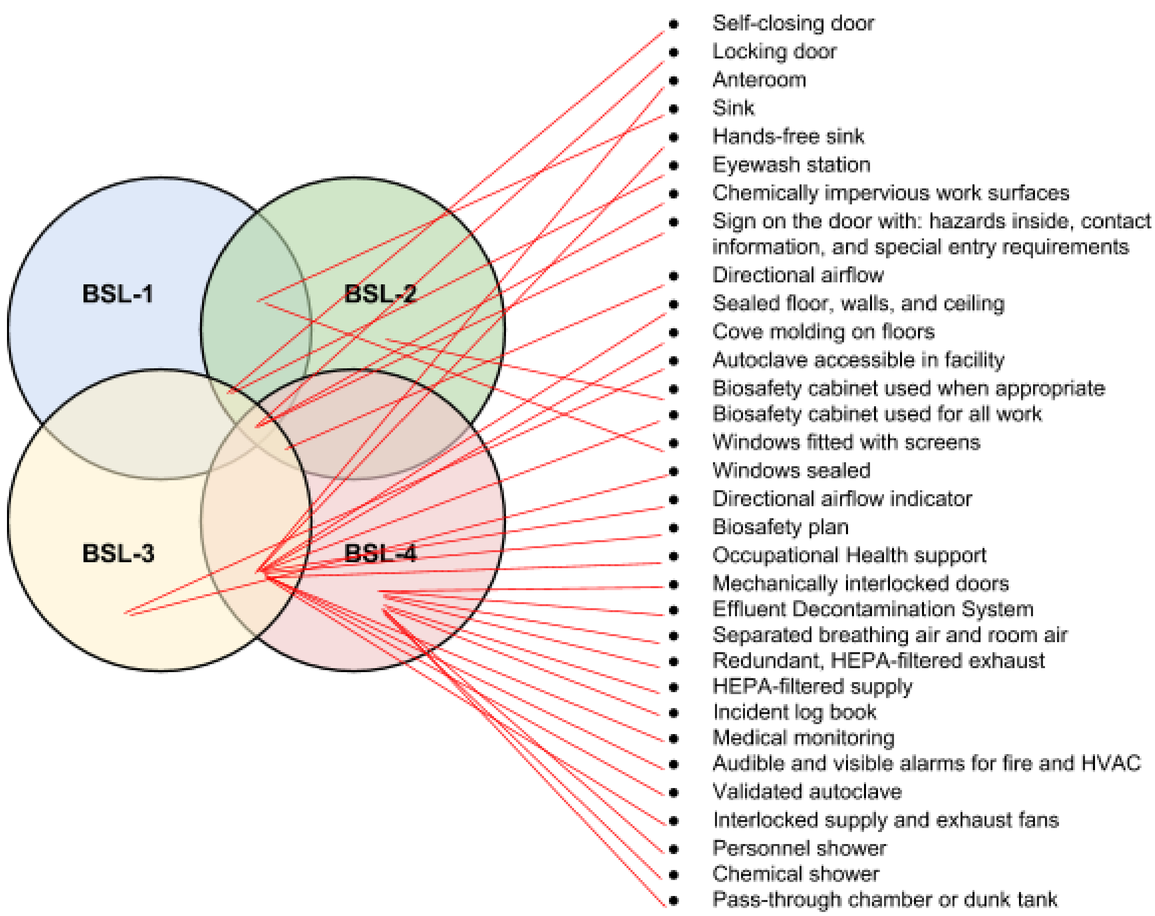
11. Identify two unique features of BSL-4 relative to BSL-3
    a. *A validated autoclave is required within the lab at BSL-4*
    b. *A BSL-4 must pass a pressure-decay test*
    c. *A BSL-4 must have two redundant HEPA filters in series on laboratory exhaust and one HEPA filter on supply*
    d. *A BSL-4 lab can be either a Class III cabinet lab or a positive pressure suit lab but workers in BSL-4 labs must not share air with the research subjects (animals, cell culture, pure virus, or any other component of the research)*
    e. *Lab workers must take a personal body shower upon exit from a BSL-4, that is optional at BSL-3*
    f. *A BSL-4 must have an Effluent Decontamination System to treat all effluent from the lab*
    g. *(other answers possible)*
12. List at least two types of specialized containment research facility
    a. *BSL-3 Ag (or Agricultural)*
    b. *Greenhouse*
    c. *ACL-3 (or Arthropod Containment Level 3)*
    d. *Aquatics facilities*

Week 5 Exercise - BSLs

1. Using the BMBL as guidance, connect the BSL component listed below on the rightto the appropriate Biosafety Level on the left:



- Self-closing door
- Locking door
- Anteroom
- Sink
- Hands-free sink
- Eyewash station
- Chemically impervious work surfaces
- Sign on the door with: hazards inside, contact information, and special entry requirements
- Directional airflow
- Sealed floor, walls, and ceiling
- Cove molding on floors
- Autoclave accessible in facility
- Biosafety cabinet used when appropriate
- Biosafety cabinet used for all work
- Windows fitted with screens
- Windows sealed
- Directional airflow indicator
- Biosafety plan
- Occupational Health support
- Mechanically interlocked doors
- Effluent Decontamination System
- Separated breathing air and room air
- Redundant, HEPA-filtered exhaust
- HEPA-filtered supply
- Incident log book
- Medical monitoring
- Audible and visible alarms for fire and HVAC
- Validated autoclave
- Interlocked supply and exhaust fans
- Personnel shower
- Chemical shower
- Pass-through chamber or dunk tank



2. As a more involved exercise, the instructor may choose to have the students design a containment facility. This has been a popular course component with other undergraduates in the past. This should be a multi-week project and could perhaps serve as a portion of the final exam. For this exercise we recommend that the instructor either be proficient at design review or recruit the help of someone who is (possibly a high containment engineer or a Biosafety professional). If the instructor chooses to include this project in the coursework, we recommend the following steps:

   a. In groups, students will develop a “Program of Requirements” (POR) aka a list of features and functionalities they desire for this facility. We recommend the instructor guide these students to keep this as simple as possible. Some program elements they may wish to consider are: what pathogen will be used, what Biosafety Level they will need, the presence of animal work, animal imaging, cell sorting in high containment, in vitro work, microscopy, and any aerosolization studies

   b. The instructor will receive the POR from the student groups and suggest changes as appropriate. Once the instructor approves of the POR, the students can begin work on sketching their facility.

c. A rough draft of the facility (corresponding to a 50% submission) should be submitted to the instructor. Elements that should be included are:
    i. Structural and Architectural features: walls, doors, windows, sinks, casework/shelving,
        1. do not concern the students with plumbing or structural load/integrity issues

    ii. Equipment: autoclave, Biosafety cabinet, cage racks (if appropriate), cage-change stations (if appropriate), down-draft necropsy tables, aerosolization devices, and other primary containment systems, centrifuges

    iii. HVAC: where the containment boundary is, how many pressure drops there are, where the differential pressure monitors/airflow monitors are located
        1. do not concern the students with fan numbers or capacity, ductwork, isolation dampers or valves, static pressure, heat loads, air balancing, or HEPA filters

    iv. Electrical: the locations and types of alarms, the locations and types of data/voice connections, the locations of video cameras (if appropriate)
        1. do not concern the students with wiring, electrical load issues, information security, and Building Automation Systems or measurement points

d. Some tools that the students may make use of to develop their designs are:
    i. Paper and pencil or pen
    ii. The free video game, Minecraft
    iii. FreeCAD
    iv. LibreCAD
    v. Microsoft PowerPoint
    vi. Google Draw
    vii. Google SketchUp (if the project falls within the license)
    viii. AutoCAD (if they have access to a licensed copy)

3. Once the design is approved by the instructor, the students should work on a “Final” (99%) submission. Elements that should be included are:
    a. Pathways and flow for the following:
        i. Personnel
            1. Researchers
            2. Animal caretakers
            3. Housekeeping/janitorial (if appropriate)
            4. Maintenance

ii. Animals
    1. naive animals
    2. infected animals
    3. bedding waste
    4. dirty cages
    5. carcass waste

iii. Waste
    1. Solid waste
    2. Liquid waste
        a. Biohazard only
        b. Mixed waste

iv. Equipment

v. Disposables

vi. Biosafety plan

vii. Security plan

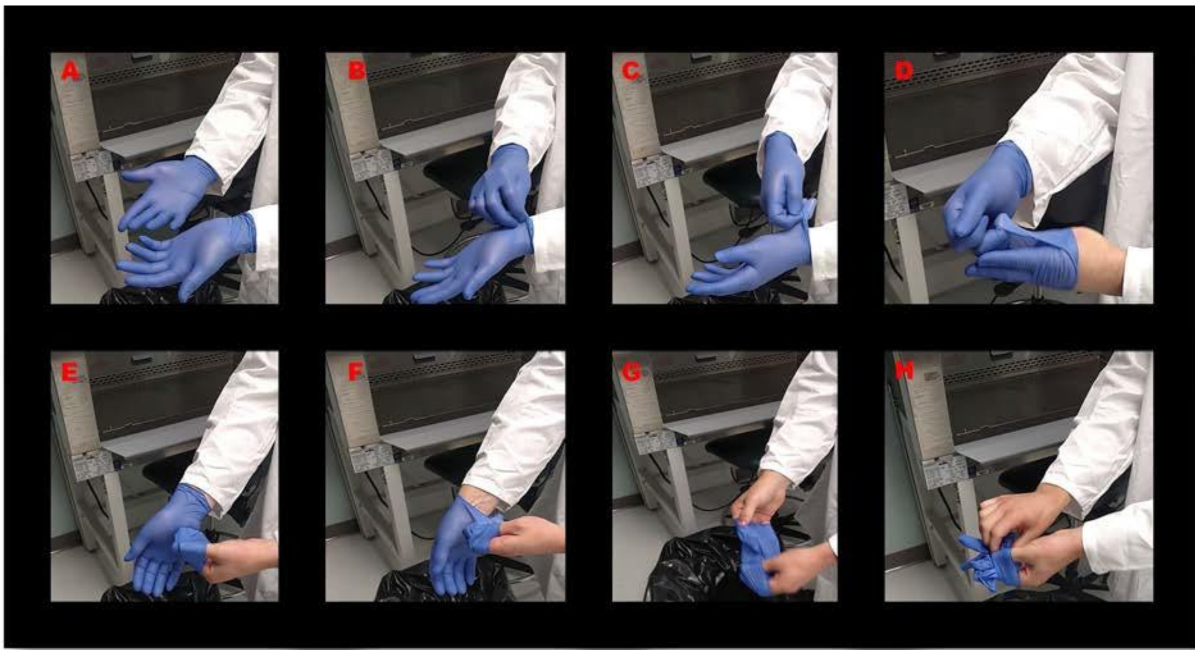
Week 6 Questions/Exercise - PPE

Don/Doff Gloves PPE exercise

The purpose of this exercise is for the students to get practices trying on different types of PPE. In order for this exercise the work the hosting institution must have some PPE on hand.

Students will practices donning and doffing Gloves. Gloves are typically the most contaminated form of PPE. Learning how to properly don and doff gloves is a vital skill for any researcher and biosafety professional.

1. Give all students a pair of latex or nitrile gloves to wear. Then put GloGerm (gel or lotion on the student's gloves. Have them smear the GloGerm all over their hands. Then tell them to take off gloves (without showing them how). All students to check their hands with a black light.
2. Repeat the exercise but show students how to properly remove gloves.



A. Before removal inspect gloves for contamination **B.** Pinch outside cuff of the glove **C.** Invert the cuff exposing the inside **D.** As you pull the glove down invert the cuff making sure the inside of the cuff is exposed **E.** Using the inside of your the glove (clean side) pinch the outside cuff of the other glove **F.** Pull down your other glove inverting the cuff taking care not to let the outside of the glove touch **G.** Pull down the other glove making sure to only touch the clean inside of the glove **H.** remove both gloves into the waste basket.

Exercise: Order of operations for PPE removal

Purpose of this exercise is to test the ability of the students to think critically about how they remove their PPE. In this exercise the students will be given a list of PPE and are told to put them in the order they would remove the PPE. Students are asked to explain why they made their choices. A. Lab coat B. Safety glasses C. Shoe cover D. Gloves

A

B

C

D

C>A>C>D (possible answer)

When taking off PPE you want to remove the most contaminated surfaces first and work towards the least contaminated surfaces. You leave the gloves on to provide a barrier between you and any residual contamination on your PPE

Exercise: PPE removal with GloGerm

1. Students will be able to demonstrate proper doffing procedure with a functional assessment using GloGerm or equivalent. The exercise will focus on cross-contamination, proper doffing, and disposal of PPE.
    a. Students will don a disposable closed front gown, surgical mask, safety glasses, gloves, and shoe covers.
    b. The instructor will smear GloGerm on the student's gloves, gown, glasses, and shoe covers.
    c. Students will then remove PPE without contaminating themselves
    d. Instructor will take a black light around students to check efficiency of removal.

Circle All that Apply

2. You are entering in to a BSL-1 laboratory to conduct recombinant research with a K12 *E.coli* strain (Risk Group I). You will extract the genetic material on the open bench. From the list below select the minimum amount of PPE that you should wear.

a. *safety glasses*	b. N95 respirator
c. *latex or nitrile gloves*	d. PAPR
e. puncture resistant leather gloves	f. chemical resistant gloves
g. face shield	h. medical scrubs
i. *lab coat (washable)*	j. *street clothes*
k. disposable lab coat	l. shoe covers
m. closed front gown	n. *close-toed shoes*
o. surgical mask	p. double gloves

3. You are working in an ABSL-2 laboratory to conduct research with mice infected with *streptococcus pneumoniae* (Risk Group II). You are conducting a necropsy in a biosafety cabinet to harvest the lungs. What PPE would you recommend wearing?

a. *safety glasses*	b. N95 respirator
c. *latex or nitrile gloves*	d. PAPR
e. *puncture resistant leather gloves*	f. chemical resistant gloves
g. face shield	h. medical scrubs
i. *lab coat (washable)*	j. *street clothes*
k. disposable lab coat	l. shoe covers
m. *closed front gown*	n. *close-toed shoes*
o. surgical mask	p. double gloves

4. You are a researcher working in an ABSL-3. You have to go into the ABSL-3 to do a quick visual inspection of the mice. You will not be actively manipulating the animals. What PPE would you wear?

a. safety glasses	b. N95 respirator
c. latex or nitrile gloves	d. PAPR
e. puncture resistant leather gloves	f. chemical resistant gloves
g. face shield	h. *medical scrubs*
i. lab coat (washable)	j. street clothes
k. disposable lab coat	l. *shoe covers*
m. closed front gown	n. *close-toed shoes*
o. surgical mask	p. *double gloves*

5. True or False: PPE should be your first option when considering how to mitigate a hazard.
   a. *False*
6. True or False: If you change gloves before you leave the lab it is ok to wear them in the common halfway or corridor.
   a. *False*
7. True or False: Surgical mask do not provide respiratory protection from aerosols generated during procedures.
   a. *True*
8. List 3 scenarios when you would change your gloves in a BSL-2 laboratory
   a. *Gloves become contaminated*
   b. *Glove integrity is compromised*
   c. *Changing experiments*
9. Select and justify the appropriate PPE for the following scenarios. Answering this question will require research about the agent listed, the typical BSL that handles that agent, and risk assessment of the procedures being performed. Answers should be approximately 100 words or less and mention at least 2 types of PPE. (Note: The scenarios are written very generally to allow the students to fill in the gaps. It is perfectly ok for students to assume different agents strains or situations (i.e. bench top or BSC) the importance is discussing the appropriate PPE.)
   a. A laboratory that uses botulinum neurotoxin in tissue cultures assays to study cytotoxicity
      i. *Possible answer: the primary hazards are the generation of aerosols from manipulations and needles sticks. I would recommend working in a BSC/CFH with a disposable lab coat (minimize residual accumulation) and gloves. If working with powdered/ lyophilized it is recommended that you use respiratory protection N95 or PAPR.*
   b. A laboratory that uses *mycobacterium tuberculosis* in aerosol challenges of guinea pigs
      i. *Possible answer: because the intention is to perform aerosol challenges, I would recommend PAPRs or N95 to provide some respiratory protection. A Tyvek suit/shoe covers to protect the clothing and prevent the agent from being carried out of the laboratory.*

c. A laboratory that studies innate immune response to west nile virus using a primary cell line

   i. *Possible answer: I would recommend changing out of street cloth and into scrubs to minimize the potential of contaminating clothing. When working with agent I would wear a disposable closed front gown (minimize change of splashes getting on clothing), safety glasses (protect my eyes from splashes), gloves, and a N95 (for respiratory protection). The major hazards are sharps and mucous membrane exposure.*

d. A laboratory doing a necropsy on a mouse infected with *streptococcus pneumoniae.*

   i. *Possible answer: Necropsy in the BSC would require a disposable gown (to prevent splashes from blood), and puncture resistant gloves (agent can cause sepsis may want to protect hands when handling mice).*

Week 7 Questions - Engineering Controls

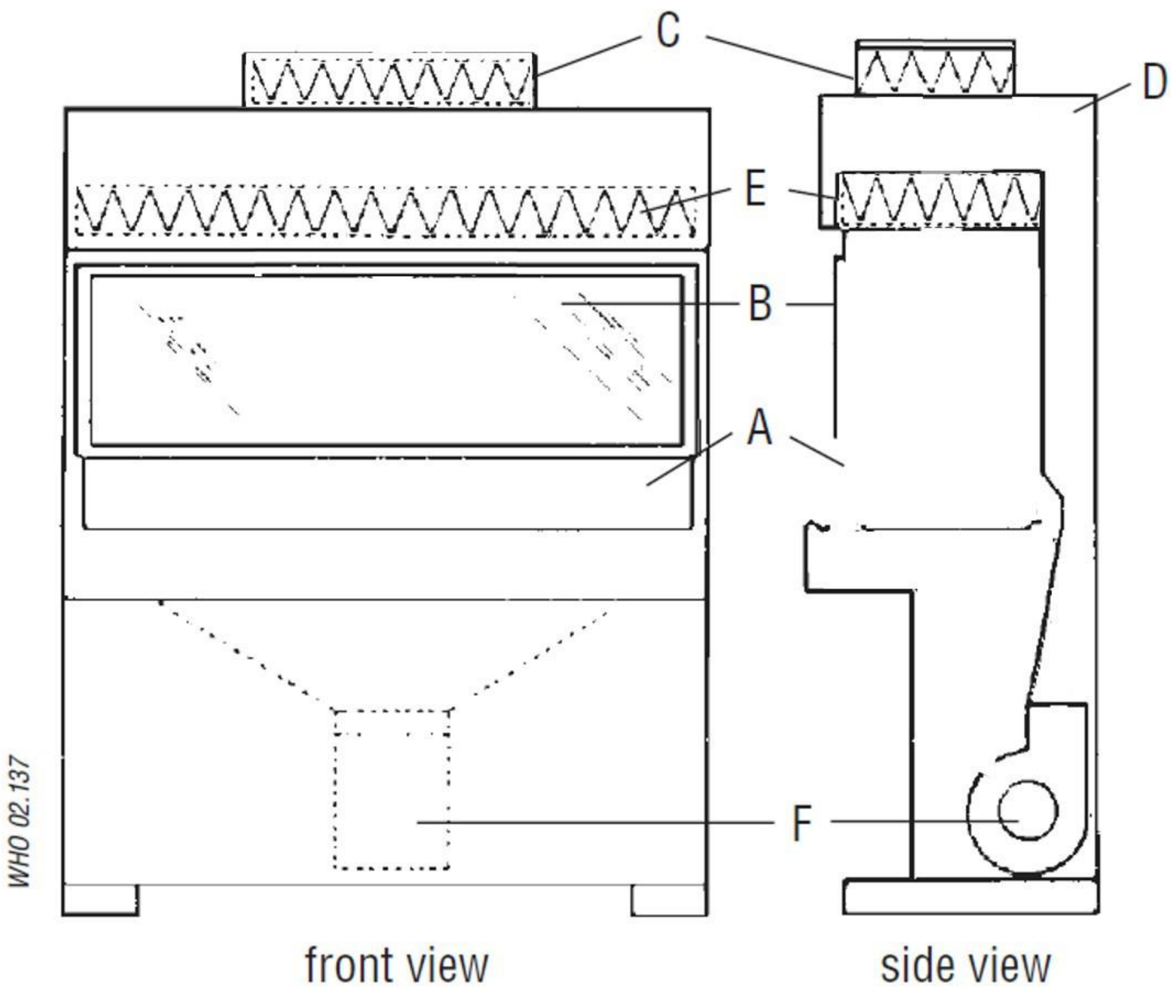
1. Directional airflow should be configured to pull air:
    a. *From areas with least potential for contamination to areas with greatest potential for contamination*
    b. From areas with greatest potential for contamination to areas with least potential for contamination
    c. From laboratories to office space and non-laboratory space
2. True or False, air from a BSL-2 can be recirculated to the laboratory
    a. *True*
3. True or False, air from a BSL-3 can be recirculated to the laboratory
    a. *False*
4. Which of the following can potentially be integrated into a Building Automation System for monitoring and/or control:
    a. Biosafety Cabinets
    b. Ultra Low Temp (ULT) Freezers (-80°C freezers)
    c. Other refrigerators and freezers
    d. Incubators
    e. Autoclaves
    f. Interior doors
    g. Exterior doors
    h. Video cameras
    i. Motion sensors
    j. Proximity card (badge) readers
    k. Biometrics scanners
    l. Airflow control valves (Venturi valves or Phoenix valves)
    m. Airflow dampers
    n. Plumbing dampers
    o. Plumbing control valves
    p. Fire alarms
    q. Fire detection systems
    r. Fire suppression systems
    s. Exhaust fans
    t. Air handling units (supply fans)
    u. Differential pressure monitors
    v. Effluent decontamination systems (EDSs)
    w. Temperature and humidity controls
    x. Overhead lighting
    y. *all of the above*
5. What does the acronym NFPA stand for?
    a. National Fire Prevention Association
    b. *National Fire Protection Association*
    c. National First Aid Provider Association
    d. National Fire Policy Association
6. Directional airflow is typically achieved by:
    a. Pushing more into a lab than is pulled out of it
    b. *Pulling more air out of a lab than is pushed into it*
    c. A complex system of compressed gas cylinders and thermal exchange units
    d. Having everyone take shallower breaths inside a laboratory

7. Typically a__________will respond to problems with Heating, Ventilation, and Air Conditioning (HVAC) systems:
    a. Laboratory manager
    b. Principal Investigator
    c. Building engineer
    d. Janitor
8. An effluent decontamination system can use chemical or thermal inactivation methods, but it must be:
    a. certified by the manufacturer
    b. certified by an independent validation body (e.g. Underwriter Laboratories)
    c. validated empirically under normal operating conditions
    d. showing an “all systems operational” message on the control panel
9. True or False, spaces in buildings are rated for flammability based on the bulk volumes of flammable material expected to be kept there
    a. True

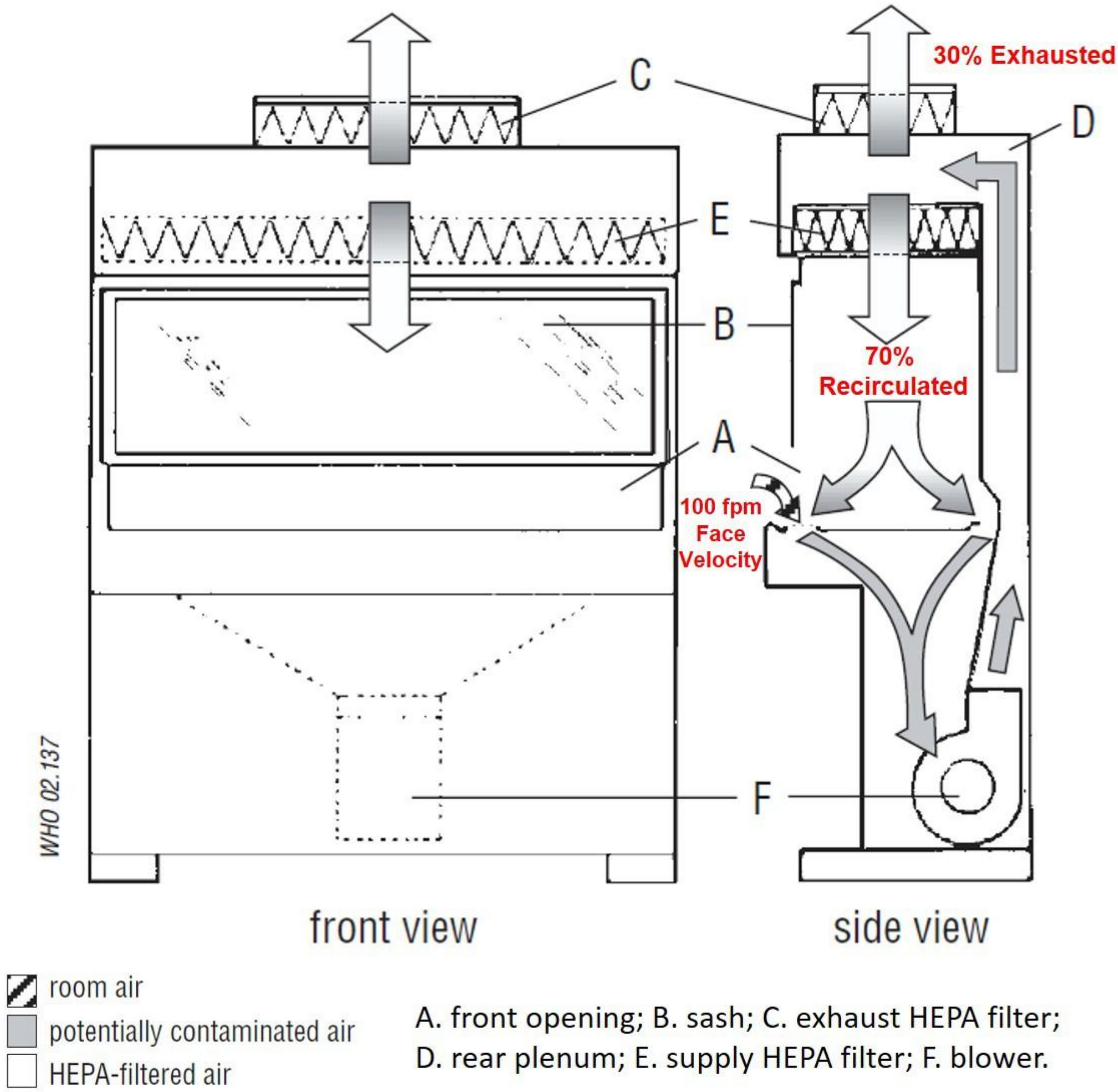
Week 7 Exercises - Engineering Controls

Diagram airflow in a biosafety cabinet (Type II A1)

1. The students must label the diagram of the class II A1 biosafety cabinet below. 1) Indicate the direction of airflow as well as show contaminated and clean air, 2) list the face velocity of the cabinet and percent recirculated/exhausted, and 3) name all of the indicated components on the diagram below (A-F).



Modified from the world health organization Biosafety Manual 3rd ed. 2004

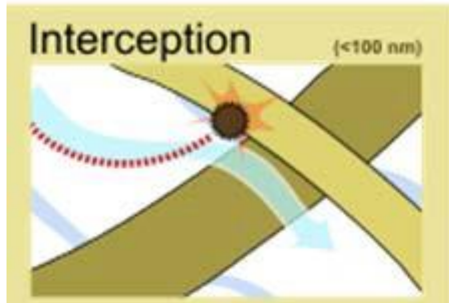


Modified from the world health organization Biosafety Manual 3rd ed. 2004

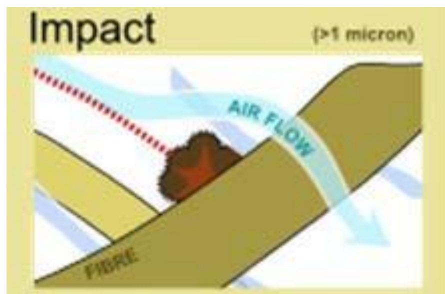
(Possible answer)

2. Diagram of explain the 3 ways that a HEPA filter traps particulates

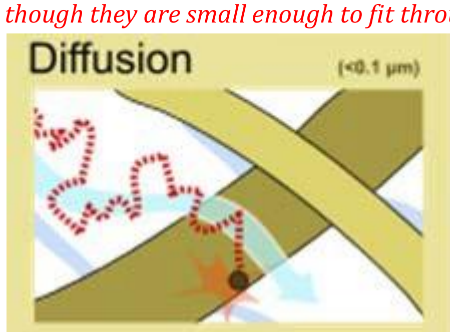
   a. *Possible answer: Inception traps particles when they try to follow the air current around the fibers but get to close and get trapped. This is typically the behavior of particles greater than 100 nm in diameter.*



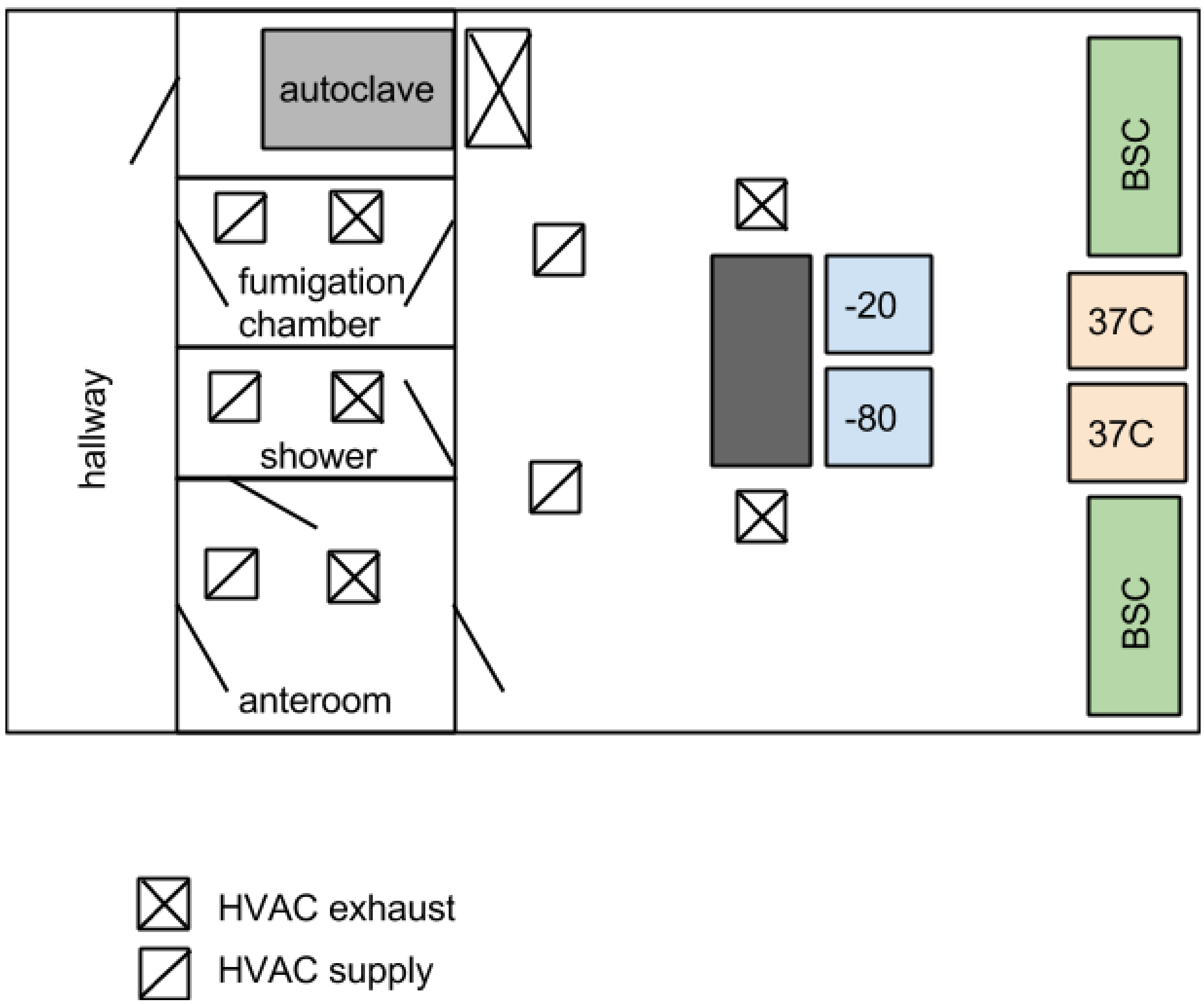
   b. *Possible answer: Impaction traps particles that are too large to avoid the fibers by following the air current and run directly into them. This is typically behavior of particles greater than 1 micron.*



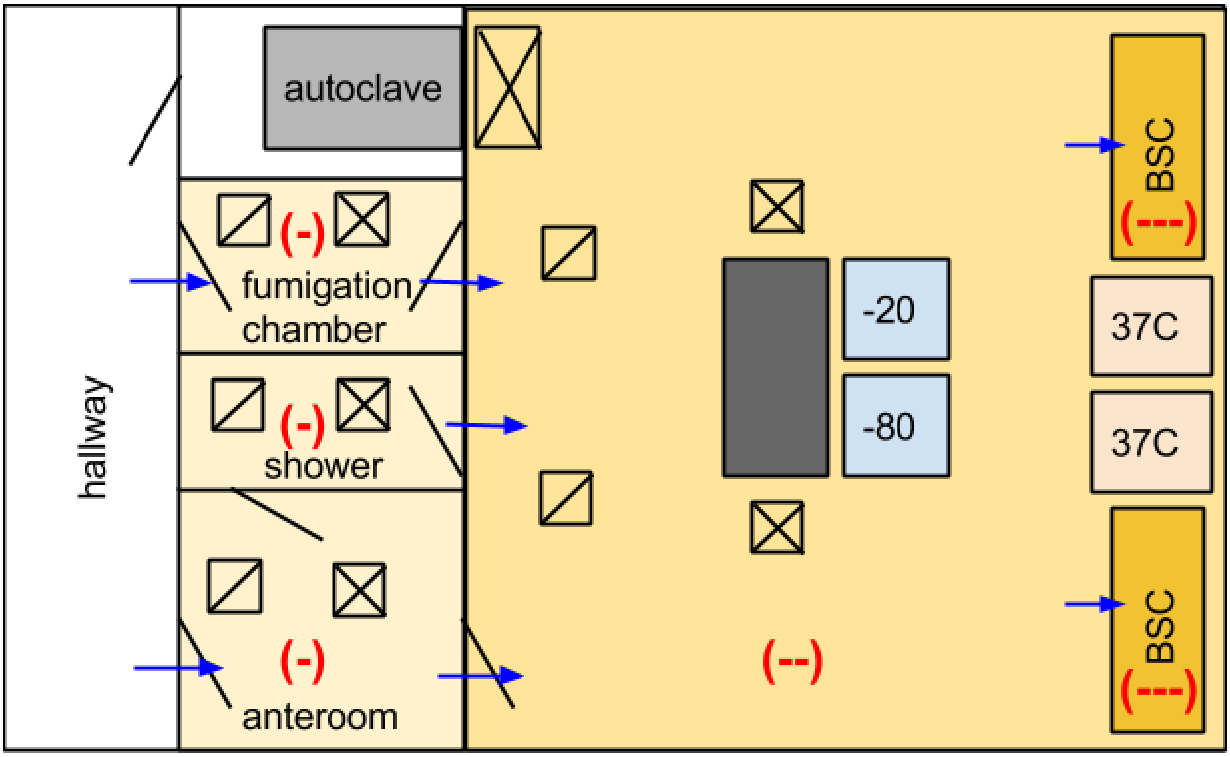
   c. *Possible answer: Diffusion traps particles that are extremely small typically less than 0.1 micrometers. Particles of this size have an erratic flight path because they are disturbed very easily by the molecules in the air (Brownian motion). This flight pattern means that they easily collide with the fibers even though they are small enough to fit through.*



3. Identify zones of negative pressure in the following diagram and their relative magnitude. Draw arrows at doorways and boundaries between pressure zones to indicate the direction of airflow. Zones can be shaded or hatched to indicate the different boundaries. Indicate relative pressure drop using a minus symbol “(-)”



Pressure zones in deepening shades of yellow and relative pressure indicated by number of negative signs (-) in a space



Week 8 Questions - Disinfection, Decontamination, and Sterilization

1. True or False, spraying a spill can cause contamination to spread
    a. *True*
2. What is considered the best method for cleaning up a spill on an otherwise clean surface?
    a. *Surround the spill and cover it with absorbent material such as paper towel, then flood the absorbent material with fresh disinfectant starting from the outside in. The decontaminant should be chosen specifically for the circumstances and should be known to be active under those circumstances. Allow for the appropriate contact time; while waiting, wipe down any vertical surfaces that were splashed on and then collect the paper towel into a biohazard bag and transport it to an autoclave for sterilization. Wipe the affected area(s) a second time with fresh disinfectant and then clean the disinfectant off of the surfaces if necessary. During the spill cleanup, change gloves after handling anything that is potentially contaminated and before handling anything else; likewise if other PPE has been contaminated by the spill or during spill cleanup it may be wise to leave the area and change to clean PPE before returning to complete the spill cleanup. If the spill occurs outside primary containment and the organism is known to be a respiratory pathogen, it may be wise to leave the area and wait for directional airflow to clear the air of airborne microbes, in that case it would be good to also notify nearby co-workers of the spill cleanup in progress.*
3. All of the following factors except what can play into a decision on what decontaminant to use:
    a. Activity against target contaminant (microorganism or toxin or chemical hazard)
    b. Materials compatibility with surfaces it will be used on
    c. Contact time required
    d. Volatility (rate of evaporation)
    e. *Activity against all microorganisms*
    f. Compatibility with secondary methods of decontamination or sterilization (e.g. autoclaving)
    g. Dependence on environmental conditions (temperature, humidity, etc.)
    h. Activity in the presence of organic load
    i. Environmental impact
    j. Toxicity to humans

4. Which federal agency maintains a list of registered disinfectants? (choose all correct answers)
    a. *The Environmental Protection Agency (EPA)*
    b. The Centers for Disease Control and Prevention (CDC)
    c. The National Institutes of Health (NIH)
    d. The National Institute of Standards and Technology (NIST)
    e. *The Food and Drug Administration (FDA)*
    f. The United States Department of Agriculture (USDA)

5. True or False, waste disposal laws are standardized across the country and do not vary from state to state or city to city
    a. *False*
    6. Give an example of an oxidizer frequently used as a disinfectant/decontaminant
    a. *Bleach aka Sodium hypochlorite*

7. Give an example of a class of decontaminants that can disrupt membranes
    a. *Quaternary ammonium compounds*
    b. *Phenolics*
    c. *Alcohols*
    d. *Formaldehyde*
8. What is a mixed waste?
    a. *A mixed waste is a waste product that has more than one categorization or hazard contained in it - e.g. biohazard and radioactive, or biohazard and chemical hazard*
9. True or False, all microbes have the same sensitivity to a given decontaminating agent regardless of growth phase or environment
    a. *False*
10. True or False, decontamination removes all forms of life from a target object or surface
    a. *False*

Week 8 Exercise - spill response

If a spill occurs in a laboratory, the following procedures are a good procedure to follow to clean up the spill. It would be most useful for students to perform this procedure in a functional facility with liquid GloGerm and a black light or another visual indicator so they can see splatter and splashes as well as the efficacy of their cleaning measures. However, if a facility is not available to use, asking the students to come up with a good spill response can be a useful activity to get them thinking critically about the process. Once they have developed their own procedure, compare it to the procedures below and discuss similarities and differences between the two and their relative strengths and weaknesses

Spill Clean-up Inside BSC

1. Remove outer gloves
2. Don fresh, clean gloves
3. Ensure a point-of-use biohazard bag is prepared with at least 10mL disinfectant in the bottom
4. Place paper towel around and over spill, being careful to avoid cross-contamination
5. Flood paper towel with disinfectant (do not spray)
6. Apply disinfectant to any contaminated equipment
7. Allow contact time (10 min)
8. Gather paper towel and place in point-of-use biohazard bag
9. Change outer gloves
10. Cover spill area with fresh paper towel
11. Flood paper towel with disinfectant (do not spray)
12. Apply disinfectant to equipment a second time
13. Allow contact time (10min)
14. Gather paper towel and place in point-of-use biohazard bag
15. Wipe down work surface and equipment with cleaning solution if necessary
16. Seal biohazard bag
17. Disinfect the exterior of the biohazard bag and place in a second biohazard bag
18. Stage biohazard bag for autoclaving
19. Resume work
20. Report incident to supervisor and occupational medicine

Clean-up of Large Spill of a Respiratory Pathogen outside BSC

1. Leave the area, notify co-workers and post on the door that a spill has occurred and cleanup is in progress
2. Wait at least 30 minutes, change any contaminated PPE (lab coat, eye protection, gloves, respirator, etc.), being careful to dispose of contaminated PPE appropriately. While waiting, contact your biosafety representative and prepare fresh disinfectant/decontaminant
3. Don fresh, clean PPE including two pairs of gloves and re-enter space
4. Cleaning a path to the spill, once at the spill, surround it with paper towels or other absorbent material
5. Flood absorbent material with disinfectant (do not spray)
6. Apply disinfectant to any contaminated equipment or vertical surfaces that were splashed on
7. Allow contact time (30 min) (dependent on specific decon agent used)
8. Gather paper towel and place in a biohazard bag
9. Change outer gloves
10. Wipe down contaminated surfaces a second time with fresh disinfectant
11. Gather paper towel and place in biohazard bag
12. Change gloves
13. Wipe down surfaces and equipment with cleaning solution if necessary
14. Place any soiled PPE in biohazard bag
15. Seal biohazard bag
16. Disinfect the exterior of the biohazard bag and place in a second biohazard bag
17. Stage biohazard bag for autoclaving
18. Resume work
19. Report incident to supervisor and occupational medicine

Week 9 Exercise - mock lab inspection (plus questions)

Overview

The purpose of this section is for the student to exercise the knowledge that they have learned over the last few weeks in a comprehensive assessment. The assessment should be a hands on laboratory inspection.
The students will be sent into a BSL-2 laboratory and told to catch all of the safety deficiencies and evaluate other researchers (staged actors) work.

Equipment list

It should be understood in order to take full advantage of this exercise that a full laboratory should be available along with several actors to portray the researchers. It is up to the institution to determine what competencies they wish to test the students on, but we provide a list of helpful equipment below.

Assortment of laboratory equipment: Biosafety cabinet (preferable Type II A2), fume hood, incubators, microscope, serological pipettes, pipette aids, pipettor (P1000 or P200), pipette tips, razor blades (or other sharps), tissue culture flasks, beakers, and other standard laboratory equipment.

Assortment of PPE and clothing: Gloves, laboratory coat, safety glasses, jeans, shorts, flip-flops (opened toed shoes), and other clothing typical for the area.

Exercise Instructions:

Students will be given paper and pencil and ask to perform a "mock annual inspection on the laboratory. For large classes students should be allowed to work in pairs. Each students should be given 30-45 minutes to complete the assessment depending on the size of the laboratory.
After the assessment is completed the students will turn over their findings and discuss what they saw with the instructor. Scoring is based on the number of deficiencies identified as well as the explanation of corrective actions.

Staging: The area

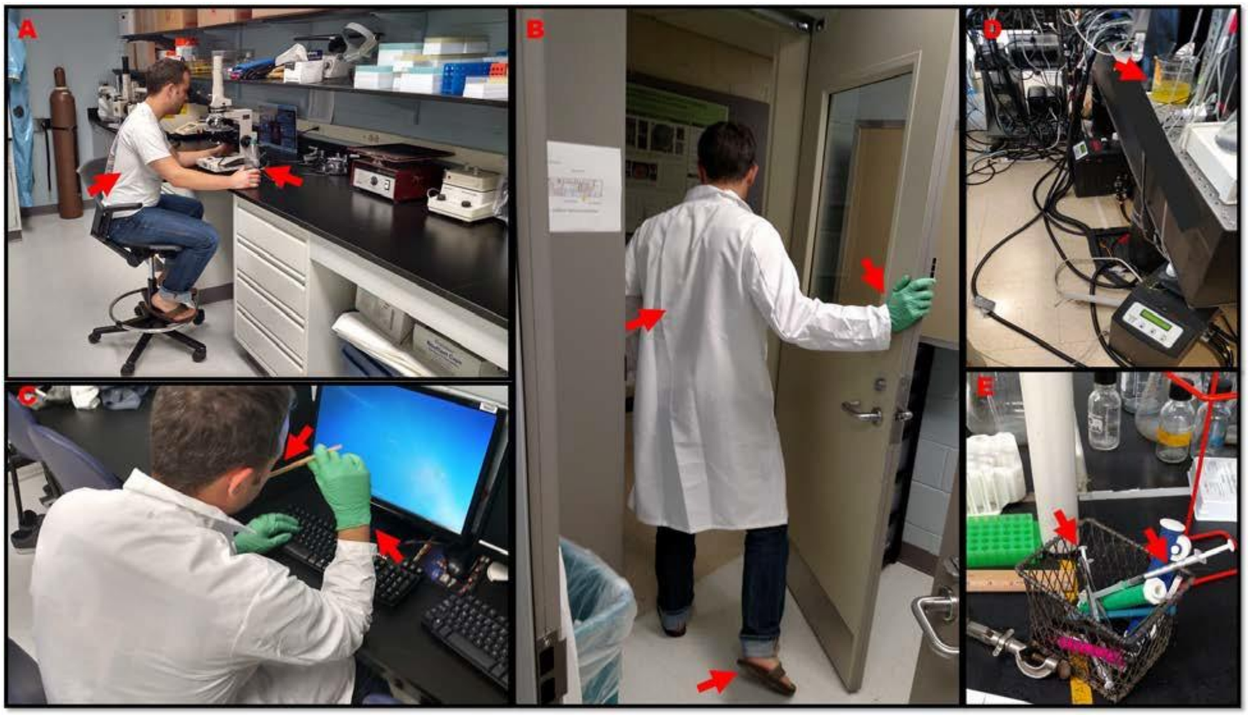
The best case scenario there are two actors in the room prepared to demonstrate routine procedures for the students. An example would be if the researcher had to use the biosafety cabinet to infect a 6 well plate. The actors should deliberately make mistakes while demonstrating the procedure and wear various levels of PPE while performing the tasks. An example would be if one actor has on a lab coat but open toed shoes and no gloves. Scenarios are based on the expertise and availability of the hosting institutions. Below are some pictures that can serve as an examples.

Picture review

If a BSL-2 laboratory is not available you can use the pictures below (or pictures you stage yourself) to check the students' biosafety knowledge. Have them review the pictures and identify areas of concern.
Look at the pictures below of a researcher working in a BSL-2 laboratory and identify all biosafety/laboratory safety issues in each picture.

A. Researcher looking in microscope at unfixed cells
B. Researcher leaving the laboratory
C. Researcher using the laboratory computer
D. Equipment setup for the laboratory
E. Bench top in a BSL-2 laboratory

(Possible answers)

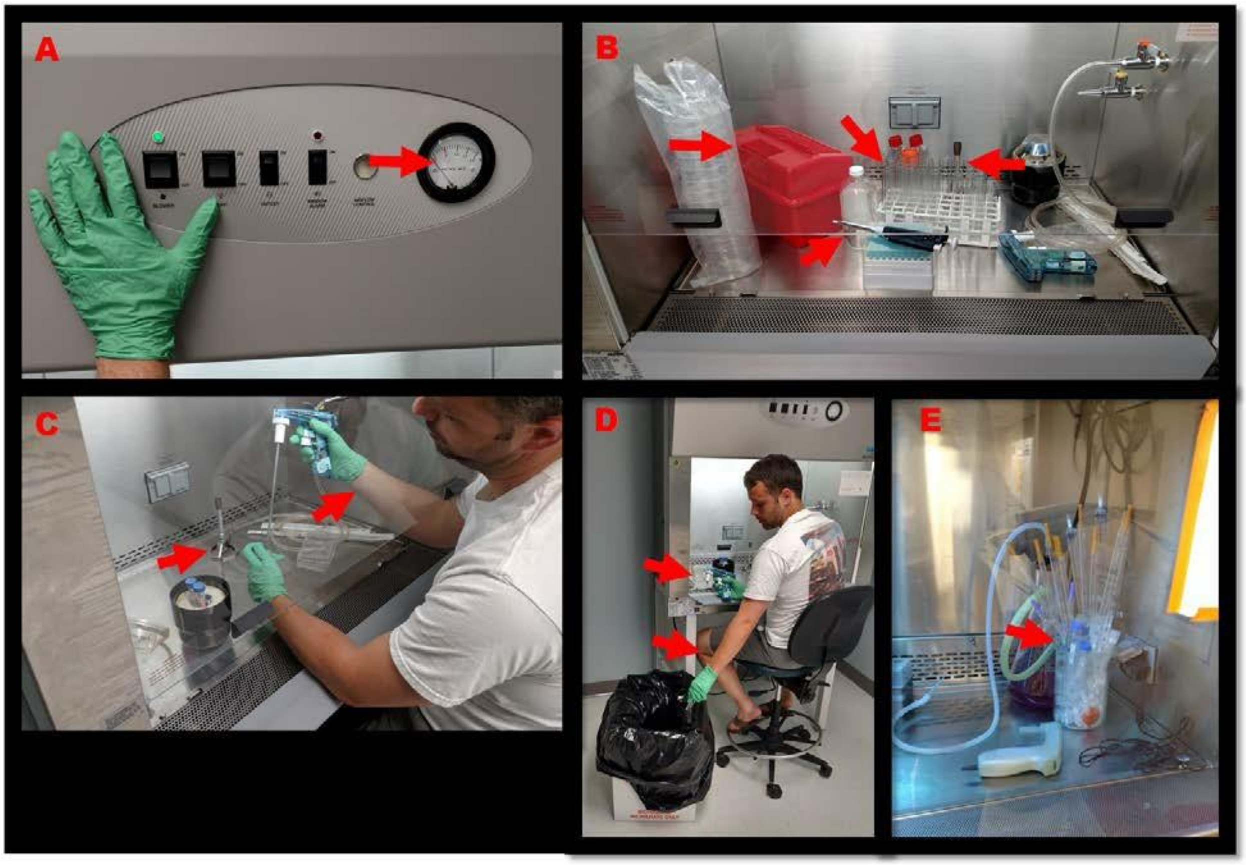


A. Researcher working with unfixed cells with no laboratory coat, opened toe shoes, no gloves, while drinking from a water bottle
B. Researcher has on PPE while exiting the laboratory space, researcher also has on open-toe shoes
C. Researcher has on gloves at laboratory computer (this maybe allowed check laboratory policy, researcher is chewing on a pencil in lab
D. mystery liquid is unlabeled and sitting next to a lot of equipment on the edge of a table
E. Sharps being held in the same container as pens and other laboratory equipment

Look at the pictures below of a researcher working in a BSL-2 laboratory and identify all biosafety/laboratory safety issues in each picture.

A. The front panel of a biosafety cabinet.
B. Biosafety cabinet set up
C. researcher pipetting in biosafety cabinet
D. Researcher disposing of used serological pipette
E. Biosafety cabinet after use

(Possible answers)



A. The BSC is on but the gauge reads off even though the light on the cabinet is on

B. BSC is overcrowded, there is a Bunsen burning, there are unnecessary things in the BSC

C. Researcher are not wearing the required PPE, Bunsen burner in BSC

D. Researcher disposing of serological pipettes outside of the BSC, again researcher NOT wearing proper PPE

E. BSC is overcrowded, waste container is full

Week 10 - Laboratory Security & Emergency Response

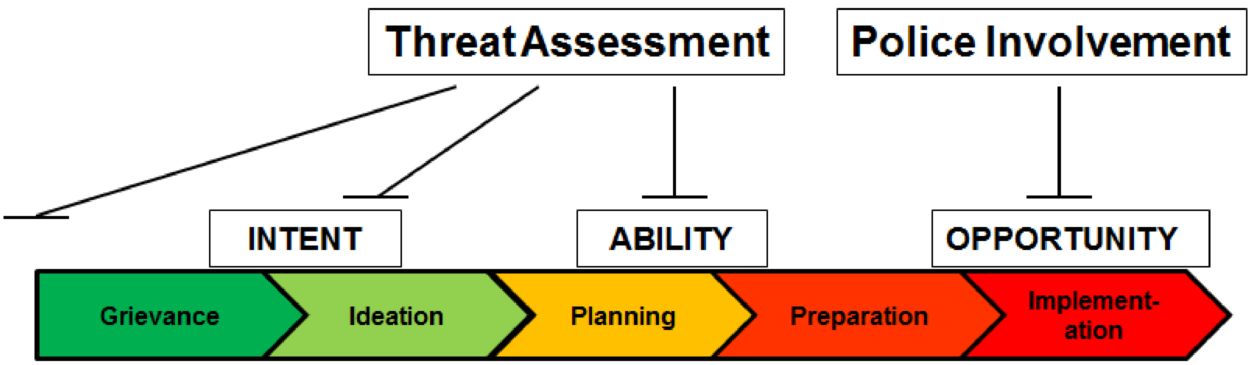
Question

1. In your own words define the following terms: Insider Threat, Outsider Threat, Targeted Violence

- *Possible answer Insider Threat: a person in the workplace that wishes to steal, vandalize, or harm people or property at the institution. Other possible answer: a person in the workplace who presents a security risk through ignorance or complacency.*
- *Possible answer Outsider Threat: a person outside the workplace that wishes to steal, vandalize, or harm people or property at the institution.*
- *Possible answer Targeted Violence process: violent incidents where both the perpetrator and target (s) are identified or identifiable prior to the incident (Fein et al., 1995). This process uses a threat assessment to analyze 3 stages of violence **Intent, Ability, and Opportunity/implementation** of violence.*

2. Diagram and define all of the steps in the targeted violence process. (*This is a detailed questions that should be worth more points than any of the other questions in this section)

(Possible answer 1)



(Possible answer 2)

- **Threat Assessment**: *The process used to identify "potential" violent offenders. It is during this process where it is still possible to peacefully mitigate a "potential" violent offender.*
- **Police involvement**: *Is where the institution has to summon law enforcement to deal with a violent offender. The offender at this is in the final stages of preparing their act.*
- **There are three developmental categories**
  - **Intent**
    - Grievance: *an individual experiences what they view as unjust or unfair treatment at the hands of another person or an institution*
    - Ideation: *the "wronged" individual begins thinking about how to exact revenge upon that person or people or institution*
  - **Ability**
    - Planning: *the wronged individual starts conceptualizing the attack and gathering information*

- ■ Preparation: *the wronged individual gathers equipment/weapons needed for the attack*
- ○ **Opportunity**
  - ■ Implementation: *the aggressor commences the attack*

3. Please circle all of the following that are associated with **pre-access suitability.**

a. *background investigation*
b. peer reporting
c. guards
d. cameras
e. *one-on-one interviews*
f. counseling
g. information security
h. *mental health screening*
i. whistleblower protection
j. access control
k. *financial records*

4. Please circle all of the following that are associated with **personnel reliability.**

a. background investigation
b. *peer reporting*
c. guards
d. cameras
e. one-on-one interviews
f. *counseling*
g. information security
h. *mental health screening*
i. whistleblower protection
j. access control
k. financial records

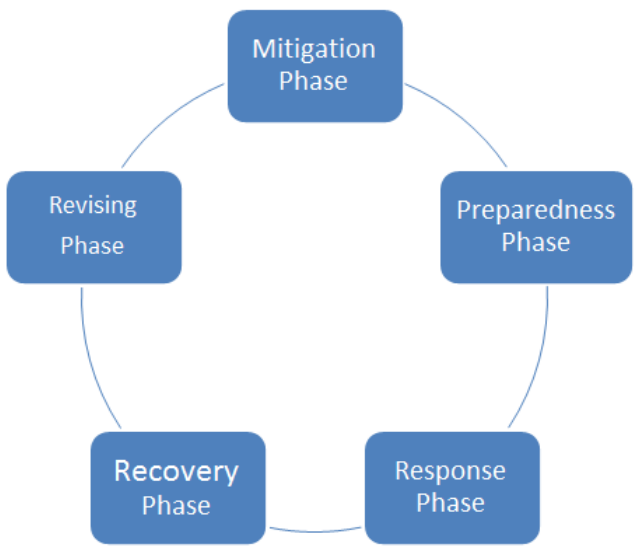
5. Please circle all of the following that are associated with **personal security.**

a. background investigation
b. peer reporting
c. *guards*
d. *cameras*
e. one-on-one interviews
f. counseling
g. *information security*
h. mental health screening
i. whistleblower protection
j. *Access control*
k. financial records

6. Outline (using a diagram or just short summaries) the steps in developing an effective emergency response.

(Potential answers)

a. ***Mitigation phase***- *This utilizes preemptive strategies to keep emergencies from happening. An examples would be a proactive maintenance schedule.*
b. ***Preparedness phase***- *This is when you access your facility and prepare for the most common emergencies and write contingencies for the least common.*
c. ***Response phase***- *This phase is utilized when an emergency is happening, it involves managing the emergency, and implementing the response plan.*
d. ***Recovery phase***- *This part of your emergency response plan is dedicated to restoring your facility to its normal operating capacity.*
e. ***Revise phase***- *Here is when you review the events that took place during the emergency and evaluate your plan and everyone's response. During this phase do a gap analysis and make improvements to the plan.*



Week 10 - Laboratory Security & Emergency Response (Exercise)

Overview

Students will be tasked with designing their own emergency response plan to the listed emergency. The student should use the categories of an effective emergency response plan to guide them. The students should think through this plan holistically including listing the stakeholders (or people involved) and possible problems they may encounter. The write up should have these few key elements. List:

- stakeholder
- preventive measures (preparedness phase)
- a plan to implement during the emergency (response phase)
- a plan to get back to prior conditions (recovery phase)
- a list of potential problems and solutions (revising phase).

Note* (The instructor can provide the scenario or use the one listed. Depending on the size of the class this exercise can be done individually or with groups).

Optional Assignment

The students may enjoy taking the plans they develop and acting out the scenarios. This project could be as simple as assigning roles and discussing the scenario during a mock table top. It also could turn into a major production where students are told to bring in props and act out their scenario. The goal of this optional assignment is to get everyone think of different perspectives. It also meant to encourage creativity and group participation.

Scenario

1. Water main break in a research building, multiple labs on the second floor are flooding, and power is knocked out. There is a worry that the samples will be lost.
2. Your laboratory is in a relatively isolated area in the northern part of the country. There is a small town that serves as housing for laboratory residence. A major snowstorm came through and effectively blocked the roads in and out of the town for days. Your animals are running out of food and your generators need more gas.

***Scenario 1** potential answers*

***Stakeholders-** principal investigators, laboratory staff, facility staff (electrician, general maintenance), local fire department, key administrative staff at your institutions (emergency response coordinator, biosafety officer).*

***Mitigation phase**- One mitigation practice could be the periodic inspection of the pipes and taking note of any leaks. For areas with leaks check the pipe's integrity and seal or replace pipe. Another mitigation strategy would be to check their generators in the building and make sure that the relevant equipment (e.g. -80 freezers) is connected to the emergency power.*

***Preparedness phase**- Develop a plan for handling the flood. This includes noting where the main water shut off valves are located and designating a person who is responsible for shutting off the valve. Preparing a site to relocate equipment if it cannot stay in the laboratory.*

***Response phase**-* Calling the fire department to have them deal with any potential fire hazards. Executing the previously prepared plan and mitigating problems as they arise. For example, shutting off the water to the area did not stop the flood, you had to call the city in to get them to shut the water off to the entire building. That took a few hours which led to more damage.

Recovery phase- Assessing the damage done to the facility. Calling the flood remediation company to help get rid of all of the water. Contracting out the removal of damaged title and equipment. Preparing a place for the researchers to work while their space is being repaired. Calling the mold assessment team to make sure you do not have a mold problem in the future. ***Revise phase***- You original plan did not account for the need to call the city and have them shut off the water. Your revised plan now includes the city and you now have the contact information of the person who can directly shut off the water. They have agreed to show up within a specified time. Furthermore, you are trying to work out a deal to get some of your maintenance group access to that area.

Week 11 - Administrative Controls Questions,

1. Choose the proper definition of an administrative control from the following options:
    a. The elimination of a hazard at its source by facility or equipment design
    b. The mitigation of a hazard by choosing not to do the work
    c. *The mitigation of a hazard to an acceptable level by institutional policy or workplace practices*
    d. The elimination of a hazard by institutional policy or workplace practices
2. True or False, an administrative control such as a policy or a Standard Operating Procedure (SOP) is effective regardless of the compliance by those performing the work
    a. *False*
3. Choose the option that best represents hazard mitigation by administrative control
    a. *Choosing to perform work originally planned for a fully virulent organism with a less virulent but related organism, instead*
    b. Purchasing a new containment device for a cell sorter to be used with unfixed Risk Group III pathogens
    c. Contracting out work to another institution with high containment capacity
    d. Applying for a grant to build a high containment facility for a high-risk research project
4. One way to communicate a standard practice to lab-mates is:
    a. Write down the procedure in your lab notebook, knowing that everyone will think to look there first for that information
    b. Tell one person the right way to perform a task and assume s/he will communicate that to everyone else
    c. Assume everyone thinks exactly as you do and will perform a task the same way
    d. *To write and post a Standard Operating Procedure (SOP)*
5. True or False, your institution will usually consider rules and regulations from multiple regulatory and legal bodies to set policy on a given issue
    a. *True*
6. True or False, when your institution puts a sign up, they do not want you to follow its directions
    a. *False*
7. List at least three common programs in place at several institutions to support laboratory safety
    a. *Protocol review and approval by IBC, IACUC, or other safety committee*
    b. *Occupational medicine*
    c. *Respiratory protection program (respirator medical clearance and fit test)*
    d. *Laboratory Safety Surveys (or inspections)*
    e. *Select Agents program*
    f. *Preventative Maintenance programs*
    g. *Hazardous Waste and Radiation safety programs*
    h. *HazMat or other emergency response teams*
    i. *Biosurety or other suitability assessments including background screening*
    j. *Mental health counseling or other resources*
8. Order the following types of controls according to their effectiveness as seen in the Hierarchy of Controls
    a. Personal Protective Equipment
    b. Engineering Controls
    c. Awareness Tools
    d. Training and Procedures

e. Elimination or Substitution

Answer:

Effectiveness	Type of control
Most effective	Elimination or substitution
	Engineering controls
	Awareness tools (administrative control)
	Training and procedures (administrative controls)
Least effective	Personal protective equipment

Week 11 – Standard Operating Procedure (SOP) Exercise

1. Instruct the students to select one of the following activities or brainstorm another activity and develop a SOP for it. The students should proceed with the guidance that the SOP should describe the proper place, circumstances, and way to perform a specific task. The SOP should be sufficiently specific but not overly wordy, includes pictures when helpful, and can (ideally) fit on a single page.

- entering a high containment laboratory
- entering a low containment laboratory
- preparing an animal for surgery
- performing animal health checks
- reporting sick or dead animals
- conducting a spill cleanup
- recording entries in an inventory
- performing a serial dilution
- setting up a Biosafety Cabinet
- Removing PPE
- Exiting a high containment laboratory
- Exiting a low containment laboratory
- Reporting an exposure to a microorganism
- Reporting a "close call" (aka "near miss) with a microorganism
- Preparing waste for the autoclave
- Autoclaving waste

2. Have the students identify an administrative control solution to the following challenging situations:
    a. Doors to a BSL-3 anteroom should be interlocking but mechanically interlocking them after constructing the facility is cost prohibitive.
        i. *Possible answer: post an SOP that shows the proper use of the anteroom is to only open one door at a time*
    b. People remove their PPE differently every time, potentially contaminating their hands and faces with their research subjects, inoculating themselves with those biohazards
        i. *Possible answer: post an SOP with pictures that shows the proper order for removing (doffing) PPE*
    c. Laboratory researchers often fail to wash their hands before leaving the laboratory, contaminating the door handle and various other surfaces throughout the institution with their research subjects
        i. *Possible answer: post a sign on the door reminding individuals to remove their gloves and wash their hands before leaving the laboratory*
    d. Campus wastewater facilities is receiving notification from the EPA that they are frequently out of compliance regarding the pH on the water leaving campus
        i. *Possible answer: enact a campus-wide policy that only pH-neutral liquids may be flushed down the drain, including dishwashers and cage washing machines*
    e. A PI on campus wishes to begin working with Select Agents; everything is in place except you don't know if the autoclaves he plans to use work properly or are even reliable
        i. *Possible answer: develop an autoclave validation program that tests the function of the autoclaves on a regular basis and empirically demonstrates their function*