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INTRODUCTION TO BIOSAFETY
STUDENT WORKBOOK

Biosafety Curriculum for Undergraduate and Graduate Students

This workbook is 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 and you must often choose the best course of action and then justify your reasoning. These scenarios are designed to be as close to realistic scientific processes as possible. As you work through the workbook 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 you to work safely in the lab - you 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:

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

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

4. True or False, laboratory workers have become infected with their research subjects in the past

5. True or False, bystanders near research laboratories have become infected with the organism(s) used inside

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 16th 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?
- What might be done to prevent such a thing from happening again?

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 meningitidi—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 BiosafetyCabinet.

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

- What factors contributed to his exposure, disease, anddeath?
- What might be done to prevent such a thing from happeningagain?

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 performing 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?
- What might be done to prevent such a thing from happening again?

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 occupational exposure to Herpes B was not unheard of: in 1987, four individuals required hospitalization after contact with macaques and it was published in the Morbidity and Mortality Weekly Report (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. Epidemiologic Notes and Reports B-Virus Infection in Humans -- Pensacola, Florida" from March 1987 (Griffin DG, Sutton EW, Goodman PL, et al. Epidemiologic notes and reports B-virus infection in humans -- Pensacola, Florida. *MMWR* 1987;36(19);289-90,295-6)

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

- What factors contributed to her exposure, disease, and death?
- What might be done to prevent such a thing from happening again?

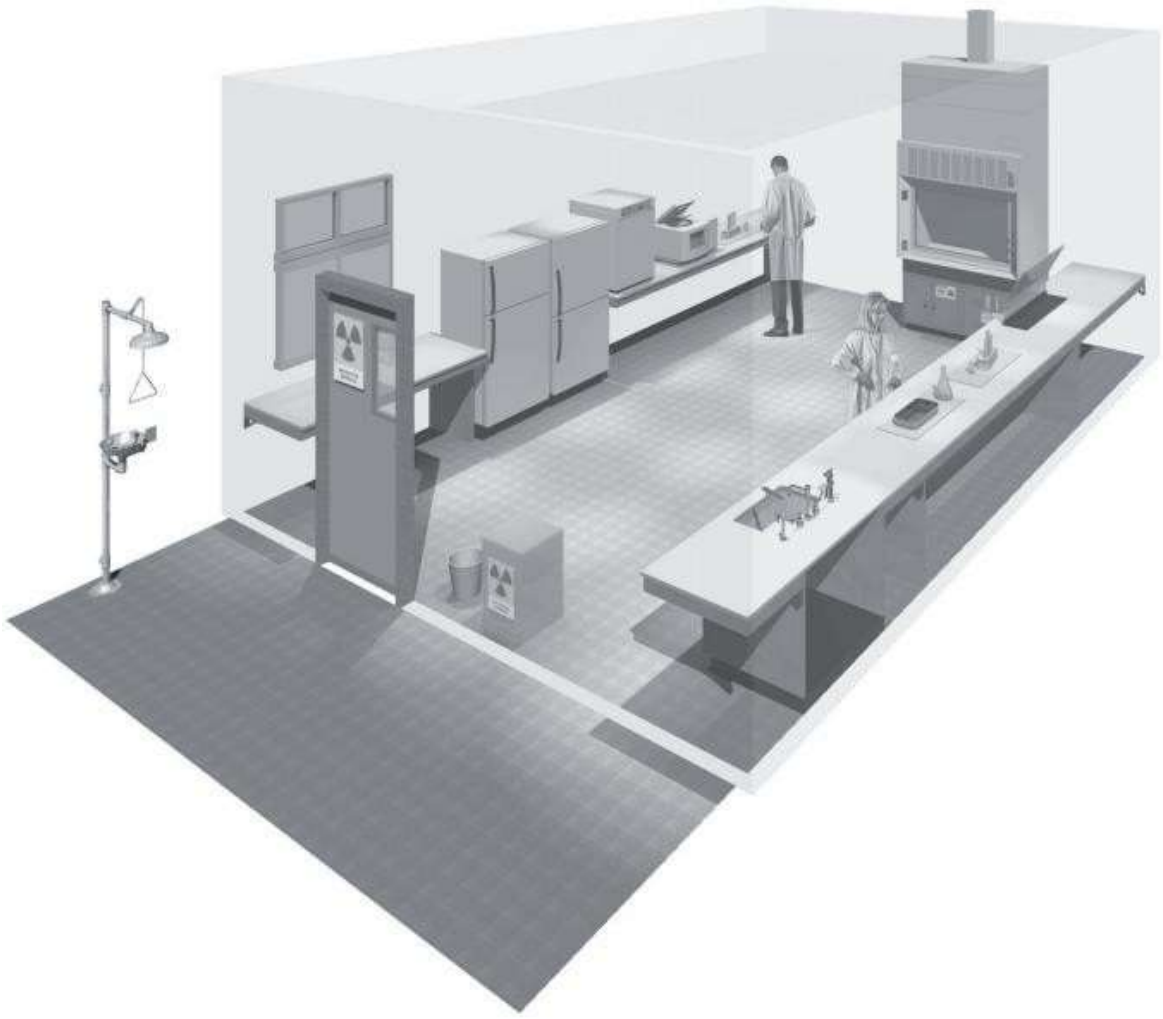
Week 1 - Exercise Biosafety Level

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.

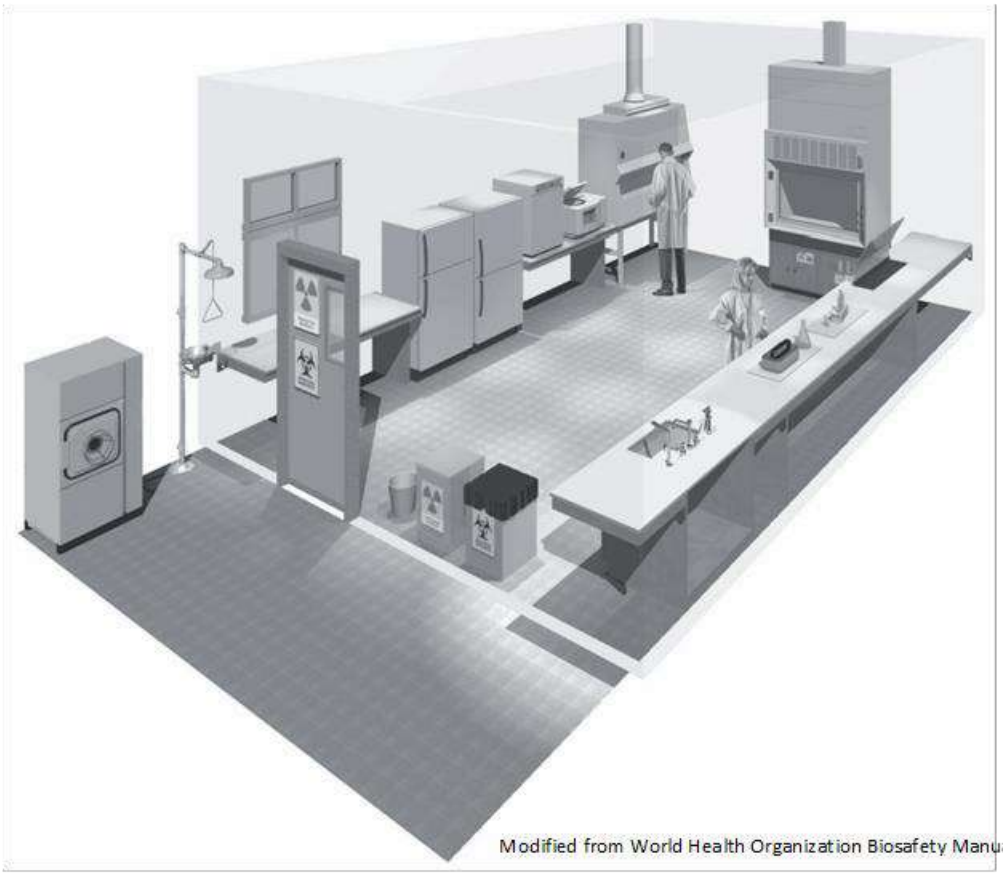
There are 4 biosafety levels which are described as follows:

•**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



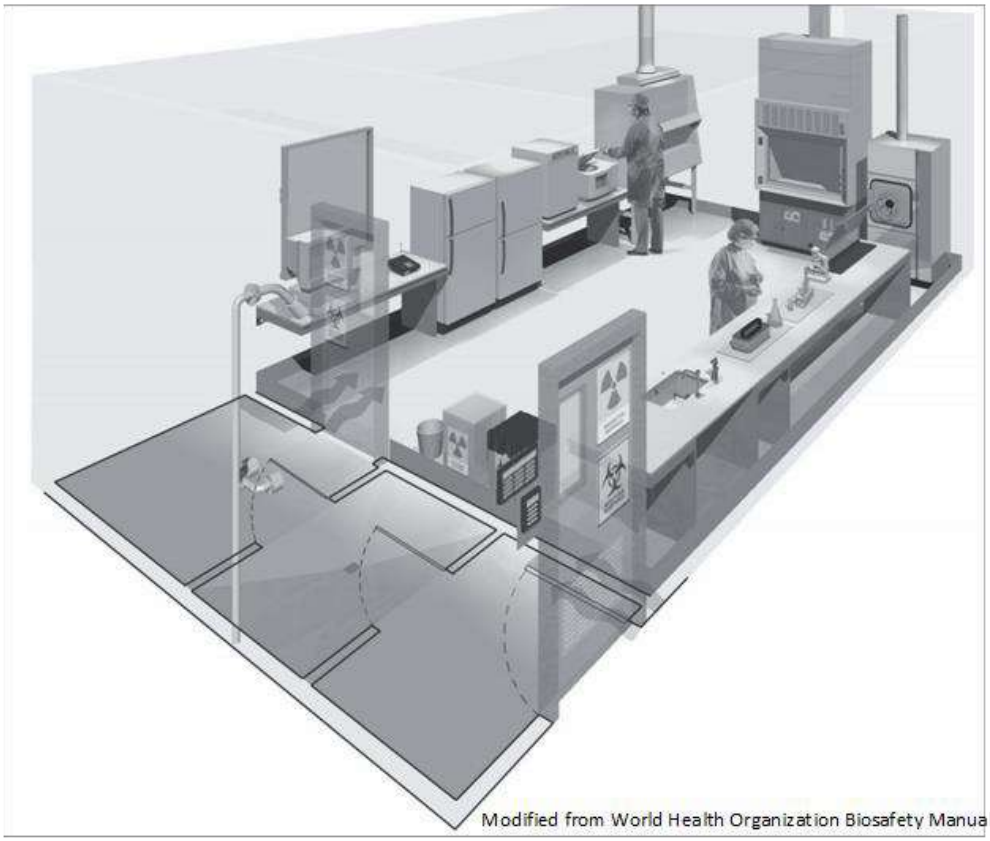
Modified from World Health Organization Biosafety Manual, 3rd ed. 2004

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

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



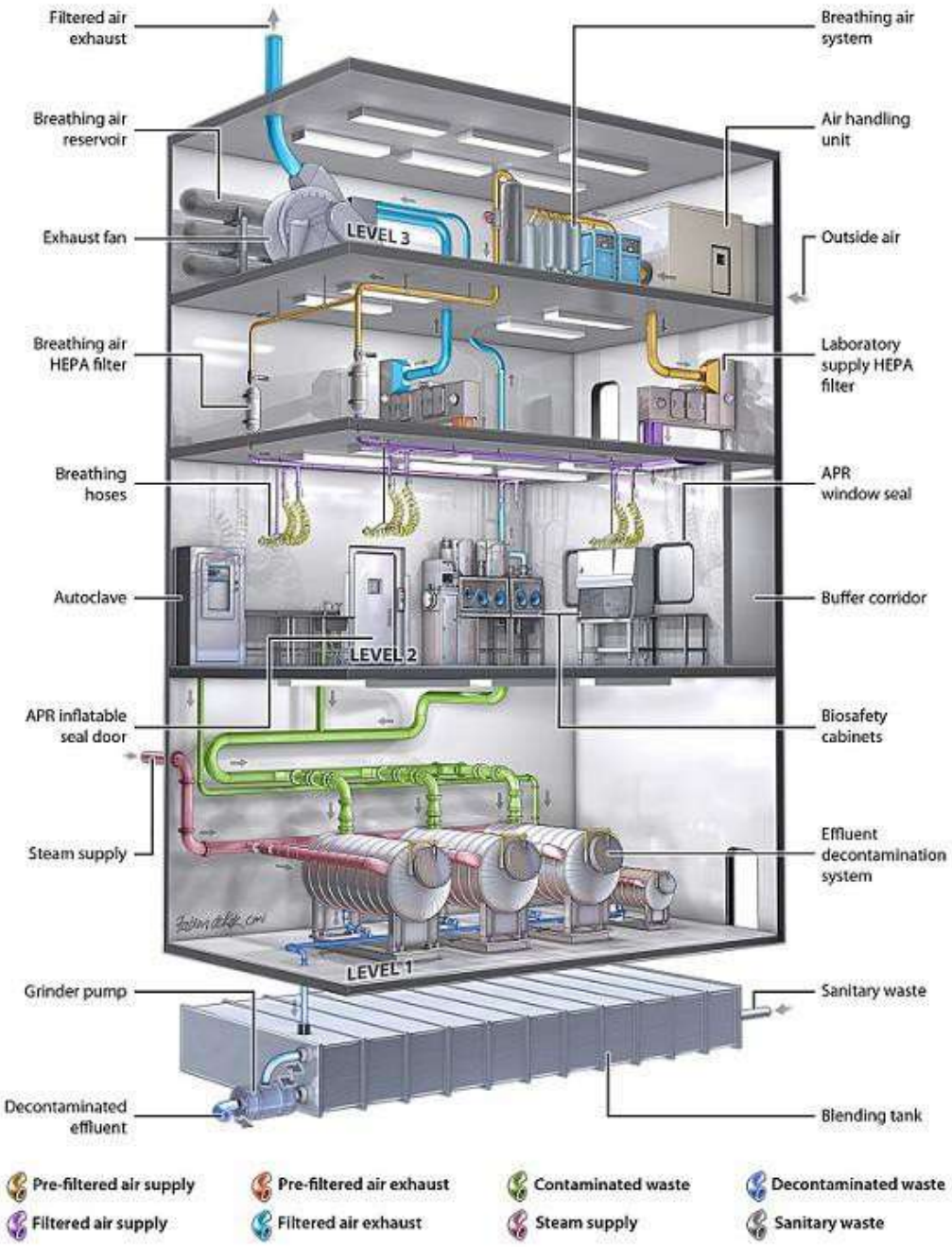
Modified from World Health Organization Biosafety Manual, 3rd ed. 2004

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

•**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.

2. Identify the clean air bench in the BSL-1 lab.

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?

4. In the biosafety level 3 laboratory can you identify something wrong with the PPE?

5. What is wrong with this BSL-3 from a facility standpoint?

6. List 3 unusual biosafety observations

Week 2 Exercises - Basic Microbiology and Epidemiology

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.

2. What are some features that may enhance the above characteristics? Remember the focus is on enhancing transmission.

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?

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?

5. Why are enveloped viruses less resilient to disinfectant than non-enveloped viruses?

6. List at least one reason why it is difficult to develop new and novel antifungals? List one antifungal.

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

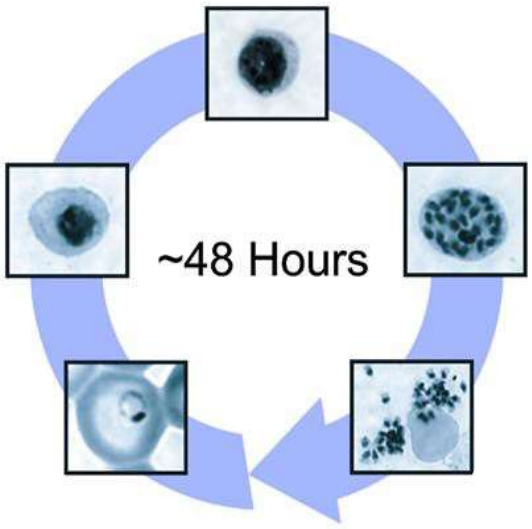
    b. Can an organism fit into one or more of thesecategories?

8. Compare and contrast some of the difference/similarities of toxins and chemicals in regards to biosafety?
    a. Compare:

    b. Contrast:

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.

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



Source Wikimedia Commons

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 methodsused:

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 yourreasoning why:

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

Week 2 Lab Exercise Handwashing

Exercise

1. You will be given GloGerm gel or lotion, and must rub it in all over your hands.
2. Then go to the sink, and have wash your hands as you normally would.
3. Once you have washed your hands, the instructor will take the black light and show you how much GloGerm residue is left on your hands.

Biosafety cabinet smoke demonstration

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:
   ____mRNA is bound by ribosomes

   ____DNA is replicated by a semiconservative mechanism

   ____amino acid chains are folded by chaperones

   ____Transcription initiation complex binds DNA

   ____mRNA is exported from the nucleus

   ____amino acid chains are modified as necessary and released to the appropriate cellular compartment

   ____pre-mRNA is processed by splicing

   ____ribosomes translate mRNA into chains of amino acids

   ____Transcription generates pre-mRNA from DNA

2. What is the definition of Molecular Biology?

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?

5. True or False, altering the genetic content of an organism can change its properties or behaviors

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

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

8. What is the name of the process used to identify risks and mitigate them?

9. What is the name of the Institutional body that reviews research and determines what Biosafety Level the research should be performed at?

10. True or False, biological toxins require a Risk Assessment and may require higher Biosafety Level containment for work

Week 3 Exercises - Molecular Biology

1. Case Studies
    a. 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. 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)

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)

    c. What are some mitigation measures that can be taken to preventillness?

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 thepotential hazards of opening the centrifuge)

    a. What are the hazards present?

b. What is the potential outcome?

c. What is the probability and severity?

d. Is this risk acceptable?

e. How do you mitigate it in the future?

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?

    b. What are the routes of exposure in this scenario?

    c. How long can influenza survive on a surface?

    d. Name several ways that a secondary infection may acquire?

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. List useful questions for Patient 2:

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?

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 beaerosolized:
    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
3. True or False, a Biosafety plan is recommended but not required for a BSL-2
4. True or False, a BSL-3 is required to have its exhaust HEPA-filtered
5. True or False, a biometrics scanner is required for a BSL-3
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 ensuresall individuals entering it receive proper training
8. True or False, a BSL-4 incorporates and builds upon the requirements of all lower containment levels

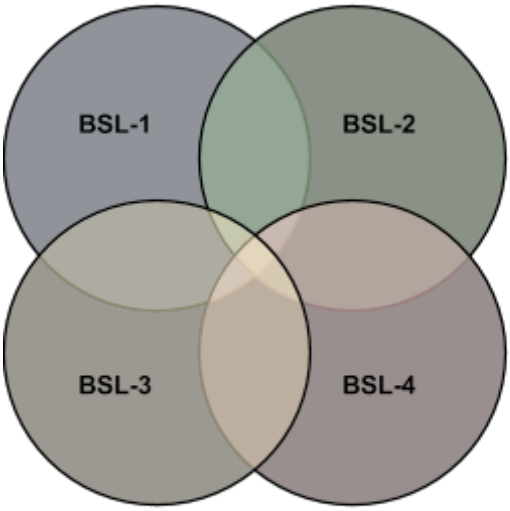
9. Identify two unique features of BSL-3 relative to BSL-2

10. Identify two unique features of BSL-4 relative to BSL-3

11. List at least two types of specialized containment research facility

Week 5 Exercise - BSLs

1. Using the BMBL as guidance, connect the BSL component listed below on the right to 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

Week 5 Exercise – Laboratory Design

2. In groups work to design your own containment facility. Complete the following steps:

    a. In groups, develop a "Program of Requirements" (POR) aka a list of features and functionalities you desire for this facility. Some program elements you 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. Once the instructor approves of the POR, you can begin work on sketching your 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 yourself 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 yourself 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 yoursefl with wiring, electrical load issues, information security, and Building Automation Systems or measurement points

    d. Some tools that you may want to use to develop your 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
viii. AutoCAD

3. Once the design is approved by the instructor, you 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

You 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. You will be given a pair of latex or nitrile gloves to wear. Once gloves are on smear them GloGerm. Then gloves and try not to let the GloGerm touch your skin.

Exercise: Order of operations for PPE removal

In this exercise you will be given a list of PPE and must put them in the order that you would remove them to minimize contamination. **Hint: Most contaminated to least*

A. Lab coat B. Safety glasses C. Shoe cover D. Gloves

A

B

C

D

Exercise: PPE removal with GloGerm

1. You will 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. You will don a disposable closed front gown, surgical mask, safety glasses, gloves, and shoe covers.
    b. The instructor will smear GloGerm on your gloves, gown, glasses, and shoe covers.
    c. You will then remove PPE without contaminating yourself
    d. Instructor will take a black light around you 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.

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.

7. True or False: Surgical mask do not provide respiratory protection from aerosols generated during procedures.

8. List 3 scenarios when you would change your gloves in a BSL-2 laboratory

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.
    a. A laboratory that uses botulinum neurotoxin in tissue cultures assays to study cytotoxicity

    b. A laboratory that uses *mycobacterium tuberculosis* in aerosol challenges of guinea pigs

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

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

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

3. True or False, air from a BSL-3 can be recirculated to the laboratory

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

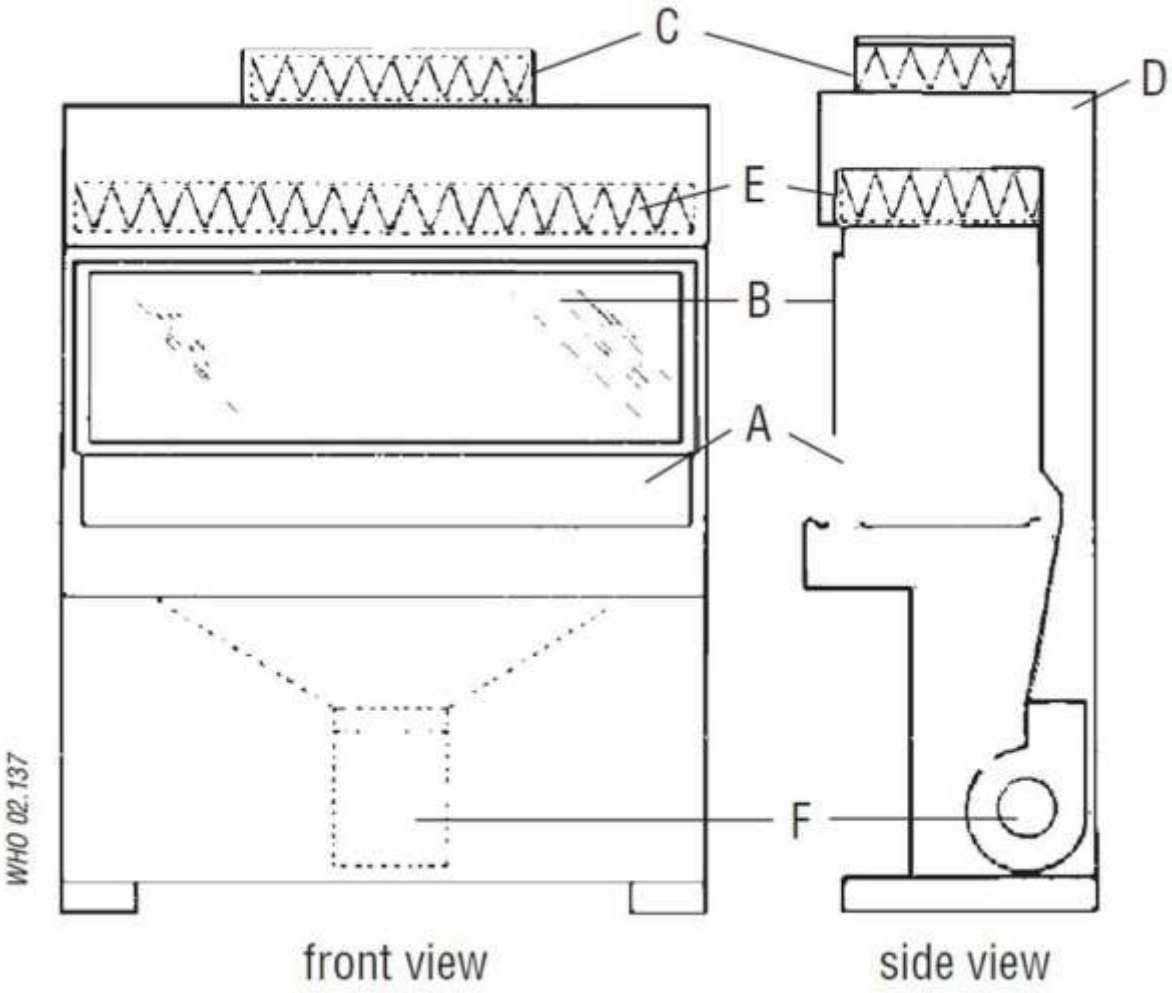
8. An effluent decontamination system can use chemical or thermal inactivation methods, but it must be:
    a. certified by the manufacturer
    b. certified by an independent validation body (e.g. Underwriter Laboratories)
    c. validated empirically under normal operating conditions
    d. showing 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

Week 7 Exercises - Engineering Controls

Diagram airflow in a biosafety cabinet (Type II A1)

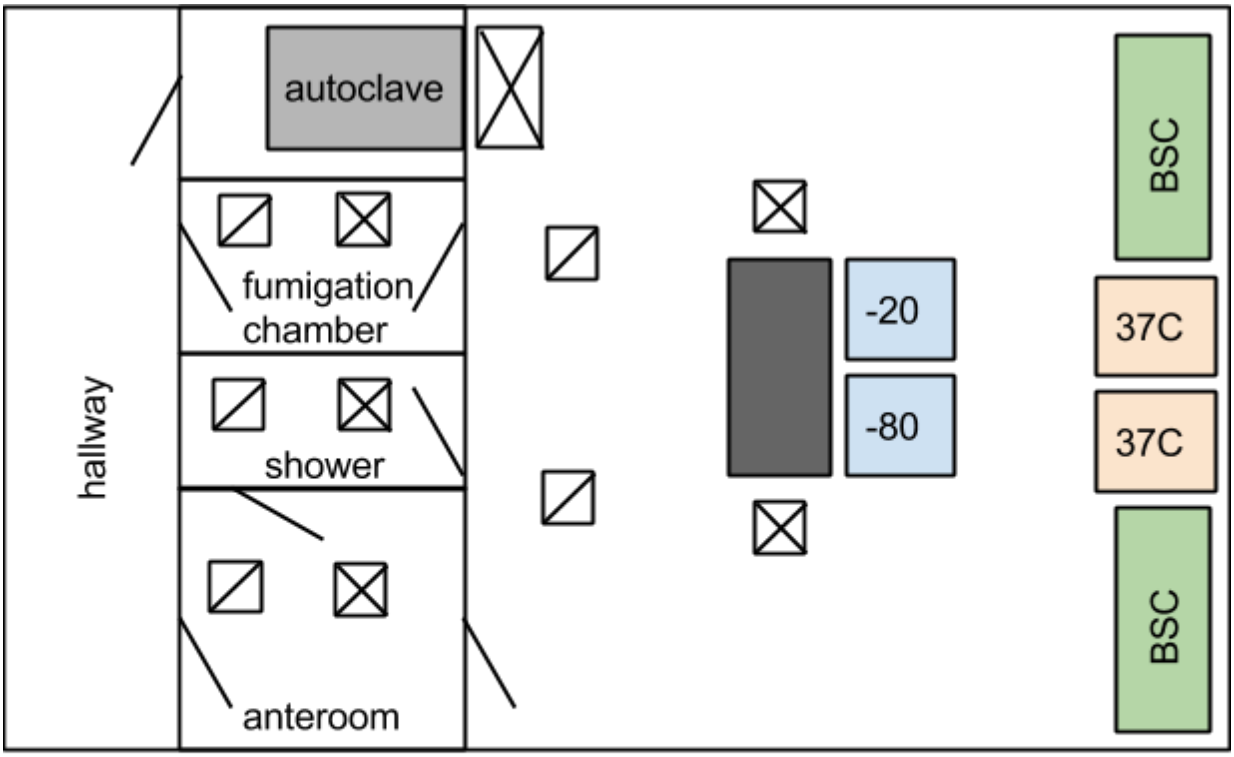
1. Label the diagram of the class II A1 biosafety cabinet below. 1) Indicate the direction of airflow as well as show contaminated and clean air, 2) list the face velocity of the cabinet and percent recirculated/exhausted, and 3) name all of the indicated components on the diagram below (A-F).



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

2. Diagram or explain the 3 ways that a HEPA filter trapsparticulates

4. 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 "(-)"



HVAC exhaust

HVAC supply

Week 8 Questions - Disinfection, Decontamination, and Sterilization

1. True or False, spraying a spill can cause contamination to spread

2. What is considered the best method for cleaning up a spill on an otherwise clean surface?

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.

6. Give an example of an oxidizer frequently used as a disinfectant/decontaminant

7. Give an example of a class of decontaminants that can disrupt membranes

8. What is a mixed waste?

9. True or False, all microbes have the same sensitivity to a given decontaminating agent regardless of growth phase or environment

10. True or False, decontamination removes all forms of life from a target object or surface

Week 8 Exercise - spill response

Develop your own spill response procedures for each scenario below. Once you have developed your own procedure, compare it to your institutions procedures and discuss similarities and differences between the two and their relative strengths and weaknesses

Spill Clean-up Inside BSC

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

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

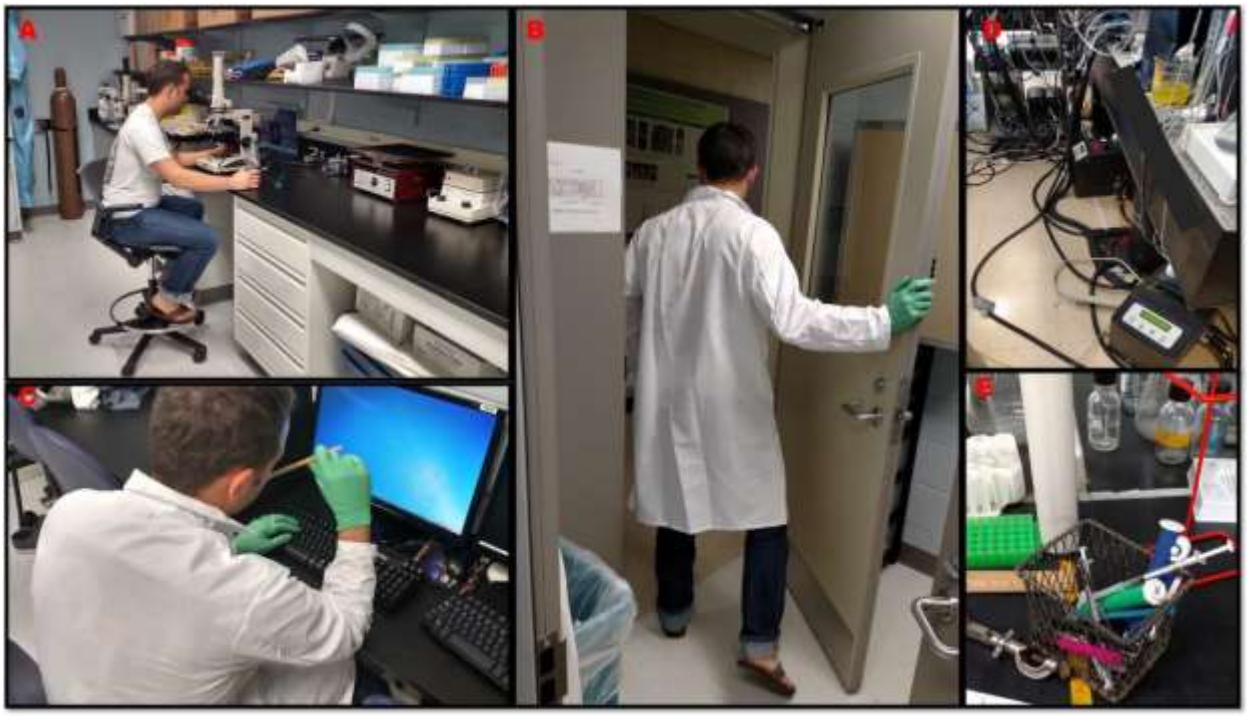
Clean-up of Large Spill of a Respiratory Pathogen outside BSC

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)

Exercise Instructions:
You are assigned to be a laboratory inspector. You will be given 30-45 minutes to complete the assessment depending on the size of the laboratory. After the assessment is completed you will turn over your findings and discuss them with the instructor. Scoring is based on the number of deficiencies identified as well as the explanation of corrective actions.

Picture review
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

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

Week 10 - Laboratory Security & Emergency Response

Question

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

- **Threat Assessment**:

- **Police involvement**:

- **There are three developmental categories**
    - **Intent**
        - Grievance:
        - Ideation:
    - **Ability**
        - Planning:
        - Preparation:
    - **Opportunity**
        - Implementation:

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 **personnelreliability.**

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 **personalsecurity.**

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 developingan effective emergency response.

Week 10 - Laboratory Security & Emergency Response (Exercise)

Overview

You are tasked with designing your own emergency response plan to the listed emergency. You should use the categories of an effective emergency response plan for a guide. 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).

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.

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

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

6. True or False, when your institution puts a sign up, they do not want you to follow its directions

7. List at least three common programs in place at several institutions to support laboratory safety

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

Week 11 – Standard Operating Procedure (SOP) Exercise

1. Select one of the following activities or brainstorm another activity and develop a SOP for it. You 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. 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.

    b. People remove their PPE differently every time, potentially contaminating their hands and faces with their research subjects, inoculating themselves with those biohazards

    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

    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

    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