This lecture provides an overview of some of the safety precautions you can practice in order to create a safe lab environment.
• Training is by far the most important aspect of safety.
• reduces illness/injury but also increases worker efficiency and awareness.
• Preventing hazards and increasing awareness is the goal
1. Emergency Plan
2. Hazards identification
3. Glassware safety
4. Fire Prevention
5. Personal Protective Equipment
6. General Lab rules/housekeeping
7. Vital lab information
8. Chemical safety
9. Sharps
10. Risk groups
11. Biological safety levels
12. Safety equipment
13. Decontamination
14. Spills
15. Waste management
PRINCIPAL 1
EMERGENCY PLAN

- THE FIRST STEP IS TO KNOW
- YOUR SURROUNDING.
  1. The exits and emergency escape routes
  2. The positioning of the fire extinguishers and the fire alarms
  3. the emergency security numbers
  4. The location of first aid boxes and the first aiders
  5. Do not wait any longer than necessary to call, time is of essence!
When you first walk into a lab you should always take note of the location of the:

- Safety Showers
- Safety Eye Washes
- Emergency Exits
- Fire Extinguishers
- Emergency Electrical cutoff Switch
Hazards are classified into two
1. Health hazards
2. Physical Hazards

A HEALTH HAZARD is classified as a hazard that can do harm to people, but from a health definition. Breathing vapors from a harmful chemical is a health hazard, or exposure of a harmful chemical through absorption of the skin into your blood stream is an example of a health hazard.

EXAMPLES INCLUDE:
- Carcinogen
- Irritants
- Corrosives
A PHYSICAL HAZARD is classified as a hazard that can do external harm to your body, your skin or other body parts. If you spill sulfuric acid on unprotected skin, the acid will burn your skin.

EXAMPLES INCLUDE:
- Explosive
- Flammable
The following guidelines should be checked every time you deal with glassware

1. Inspect glassware before and after each use.
2. Discard any cracked, broken, or damaged glassware.
3. Thoroughly clean and decontaminate glassware after each use.
4. When inserting glass tubing into rubber stoppers, corks, or tubing, follow these guidelines:
   - Use adequate hand protection.
   - Lubricate the tubing.
   - Hold hands close together to minimize movement if the glass breaks.
   - Never use laboratory glassware to serve food or drinks.
DISPOSING OF BROKEN GLASS

• Do not pick up broken glass with bare or unprotected hands.

• Use a brush and dust pan to clean up broken glass.

• Remove broken glass in sinks by using tongs for large pieces and cotton held by tongs for small pieces and slivers.

• Place all the recovered broken glass into the broken glass container.
The best method of fire fighting is preventing fire from occurring in the first place

- Have good housekeeping practices.
- Use the smallest amount of flammable solvents possible.
- Keep the flammable solvents away from ignition sources.
- Store flammables in a flammable storage cabinet.
This is a photo of a lab fire that occurred due to improperly stored chemicals.
FIRE PROCEDURE

- don’t panic, just simply:
- Alert the people around you to evacuate the lab
- activate the fire alarm.

- If the fire is large or you do not feel comfortable trying to extinguish it, GET OUT, and let the professionals do their job.

- If it is a small controllable fire, either smother the fire or use the correct fire extinguisher associated with the type of fire.

- If trying to extinguish the fire always stay on the exit side of the fire.

- Report the incident to the appropriate supervisors.
PRINCIPLE 5
PERSONAL PROTECTIVE EQUIPMENT
PPE is short for personal protective equipment.

This is the equipment that is necessary to protect yourself from hazardous materials.

PPE could be gloves, safety glasses, lab coat, shoe covers, respirator or any other item that could protect you from dangerous materials that you may encounter in the lab.
Knowing *what* to use and *when* to use it is the key to properly protecting yourself. There could be situations that would be more of a risk and require more PPE than others.

- The basic point to remember is that a risk assessment will help determine the PPE to be used.
Knowing how to properly use PPE can be the key to adequate protection.

If it is too big or too small, it is not right for you! Let your supervisor know if you need a different size.
In order to be able to rely on your equipment, you must take care of it.

Keep the PPE clean and disinfected.

It should be cleaned before and after use when possible.

Proper maintenance can not only extend the life of the equipment, but also keep cost down. Keeping the cost down on the Personal Protective Equipment can help to better allocate the money toward larger equipment needed for research.
The next portion of the training is simply review and is meant to remind you of responsible conduct in a laboratory setting. As the hazards increase, the risks increase, and the responsibility must increase.
LAB ACCEPTED ATTIRE

- You should remember the following
  - No open-toed shoes
  - No shorts unless a lab coat is used
  - Restrain hair when working with hazardous materials
  - Remove protective clothing right outside the workstation
  - Use the proper Personal Protective Equipment for the job
PERSONAL HABITS

- Personal habits play a large role in minimizing hazards. The following measures must be taken:
  - Do not eat, drink, smoke, chew gum or apply cosmetics, or remove/insert contact lenses while in the laboratory
  - Do not store food or beverages in the lab or in chemical refrigerator
  - Do not mouth pipette
  - Wash hands before leaving laboratory or after handling contaminated material
SAFE PRACTICES

These safe practices should be followed to ensure safe working conditions:

- Do not use chipped or cracked glassware
- Know emergency procedures
- Keep the laboratory neat and clean
- Use hazardous chemicals under a fume hood and biohazardous materials under a biosafety cabinet (BSC)
- Decontaminate as needed
- All procedures should be performed to minimize aerosol
At the end of lab: make sure
- Each workstation is cleaned up
- All common areas are cleaned up
- All benches are wiped down
LABORATORY HOUSEKEEPING

How many problems can you identify in this Picture?
PRINCIPLE 7
VITAL LABORATORY INFORMATION!
LABORATORY INFORMATION

- Emergency response plan
- Chemical hygiene plan
- Lab safety manual
- Hazardous waste management plan
- Material safety data sheet location
- Standard operating procedures for equipment and for the processes in the lab.
PRINCIPLE 8
CHEMICAL SAFETY

HAZARD RATING CHART

HAZARD INFORMATION
Chemical safety procedures are set to protect the students, employees, and the environment from possible harm. This is accomplished by:

- Proper labeling
- Proper storage
- MSDS locations

What really is in that bottle? DMF? Chloroform? Methanol?
BSL-1 Laboratory

BIOHAZARD

All Personal Protective Equipment shall be removed prior to leaving the work area.

Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in this work area.

Name of infectious agent(s):
________________________________________________________________
________________________________________________________________
________________________________________________________________

Transmission route(s):
________________________________________________________________
________________________________________________________________
________________________________________________________________

Special requirements for entering this area:
________________________________________________________________
________________________________________________________________
________________________________________________________________

Emergency contacts:
Name: ___________________________________________ Title: ______________
Phone: ____________________________

Emergency contacts:
Name: ___________________________________________ Title: ______________
Phone: ____________________________

Emergency contacts:
Name: ___________________________________________ Title: ______________
Phone: ____________________________

Emergency contacts:
Name: ___________________________________________ Title: ______________
Phone: ____________________________

*BSL-1 Laboratory is similar to BSL-2 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-2 in that:
1. Lab personnel have specific training in handling pathogenic agents and directed to competent scientist.
2. Access to the lab is limited when work is being conducted.
3. Extreme precautions are taken with contaminated sharp items and:
4. Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.
It is important to know as much about a chemical as possible. The most dangerous substance is the one that has no label. Communicating information is essential in the science field.

When labeling the secondary container the following information must be included:
- Identity of the hazardous chemical
- Appropriate hazard warnings
- Date of putting chemical in satellite container.
Chemical Storage

- Group chemicals according to their hazard category (i.e., acids, bases, flammables, etc.).
- Separate acids from bases. Store these chemicals near floor level.
- Separate highly toxic chemicals and carcinogens from all other chemicals. This storage location should have a warning label and should be locked.
- Separate acids from flammables.
In addition to labeling in a laboratory, the next most important type of communication regarding hazards is the MSDS. This is the acronym for Material Safety Data Sheet.

So what is an MSDS? An MSDS is a document that relays vital information about certain chemicals and biological agents.
Why is an MSDS important? When you know characteristics about a substance it can aid in precautionary measure to take when using it.

Also, if there is a spill either on a surface or on your skin, the MSDS can supply you with the information needed for first aid.

The MSDS to every chemical in your lab must be available to you. It may be in a notebook in your lab. Make sure you find the location of the MSDSs in your room.
A sharp is defined as any instrument, tool, or item that has rigid, acute edges, protuberances or corners capable of cutting, piercing, ripping or puncturing such as syringes, blades, and broken glass. Items that have the potential for shattering or breaking are also considered sharps.
When using a sharp there is a risk of being cut by the object and possible infection occurring depending on what the sharp was used for.

If hypodermic needles are used, special precautions must be taken to reduce the risk of a needlestick.

After use of the needle **do not recap**, place directly in the sharp container.
• All sharps must be placed into a rigid, puncture and leak-resistant container that is also impervious to moisture.
• The sharps container must be labeled either with “Biohazard” or “Infectious Waste”. Do not over fill the sharps container.

When the sharps container is 3/4 full it must be collected for disposal and incinerated.
There is a risk associated to certain research. This is targeting the laboratories that work with infectious agents.

A risk group (RG) is determined by the nature of the research.

The more hazards there are with the agent/material, the higher the risk group and therefore more stringent the containment and work practices become.
The risk groups are broken down by the following:

- **Risk Group 1 (RG1)** Agents are not associated with disease in healthy adult humans (low individual & community risk) Example: *Bacillus subtilis*

- **Risk Group 2 (RG2)**
  Agents are associated with human disease, which is rarely serious, and for which preventive or therapeutic interventions are often available (moderate individual risk, limited community risk) Example: *Streptococcus aureus*
**Risk Groups**

- **Risk Group 3 (RG3)**
  Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk). Example: Hantavirus

- **Risk Group 4 (RG4)**
  Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk & high community risk). Example: Ebola Virus
Along with the Risk Group there is also an associated Biological Safety Level (BSL). This has the same principle as the RG in that as the risk increases, the containment and practices become more stringent. For most cases, if your lab is a RG 1 then the BSL is 1.

The different BSLs have been established as preventative measures against human infection. Taking precautionary measures can help to minimize hazards associated with infectious agents.

The levels are 1, 2, 3, 4 with the PPE becoming more as you increase in the level.
Is the level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed facility.

- Levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4)
Biosafety Level 1

- Agents not known to cause diseases (*Bacillus subtilis*, infectious canine hepatitis)
- Standard microbiological procedures
- No special practices required
- Not separated from other areas
biosafety level 2

- work involving agents of moderate potential hazard to personnel and low risk to the environment
- personnel have specific training in handling pathogenic agents
- access controlled when work is being conducted
- extreme precautions are taken with contaminated sharp items
- procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets
For organisms that may be transmitted by airborne route
- Often have low infectious doses to cause effect
- Can cause serious or life threatening diseases
  - Yellow fever virus, TB
- Physically separate from access corridors
- Self closing double doors
- Negative airflow
- Exhausted airflow not recirculated
PRINCIPLE 12
SAFETY EQUIPMENT
Certain equipment is necessary to achieve compliance and most importantly to provide adequate protection.

The safety equipment that is needed is known as primary and secondary barriers. Let’s take a look at the difference in the barriers.

Primary barriers are referring to protective measures including engineering controls. This includes not only PPE that has already been covered, but it also includes safety cabinets, fume hoods, and autoclaves.
The fume hood is used with chemicals.

The main function is to exhaust the vapors and gases that are generated in the hood to the outside. The hood is designed to minimize your exposure to airborne contaminants.

This is not to be used with biohazardous materials.
PROPER FUME HOOD USE

When using the fume hood you first need to make sure the exhaust blower is operating and air is entering the hood.

Remember, do not put your face inside the hood!

Minimize storage of chemicals in the hood

Clean spills immediately

Work with the sash at the proper operating level as indicated by the arrows
FUME HOODS, CONTINUED

Sash all the way up

Less effective control

Airfoil sill

Vapors can spill over the sill and into the room air.

Cross-section of laboratory chemical hood

Sash lowered to proper operating position

More effective control

Vapors are trapped within the hood.
The biological safety cabinet (BSC) is used as a containment for infectious agents. The BSC has a HEPA filter in the exhaust system to protect the environment and yourself.

The high efficiency particle (HEPA) filter is a high efficiency particulate air filter. It is able to remove particles at a size of 0.3 µm with an efficiency of 99.97%. It is also able to remove both smaller and larger particles.
There are 3 classes of BSC that are used. The higher the risk group and biosafety level, the higher the class of cabinet that is used.

If there is an infectious agent being used, whether it is used in research animals or cultured, it must be manipulated inside the BSC.

When using this containment device, remember to also use the proper personal protective equipment.

Always make sure that the BSC has been decontaminated both before and after use. Decontamination methods vary depending on the infectious agent being used. Once the decontamination of the cabinet is complete, place the waste in a biohazard bag for autoclave.
**USE OF BSC**

- Do not bring contaminated materials out of the cabinet until they have been surface decontaminated. If you are unable to decontaminate, place the material into a closed container to transfer it to the autoclave.

Remember to follow the work practices:

- Work in such a way that your face is above the front opening.
- Wait for 1 minute after placing hands/arms inside the cabinet to stabilize the air flow.
- Work at least 4 inches from the inside edge of the front of the grille.
- Remember to place all materials as far back in the cabinet as possible.
- Limit the storage within the cabinet.
An autoclave is used to treat infectious/biological material. As a standard, all infectious material must be autoclaved as a safety precaution. The autoclave is able to render the material as non-infectious.
The autoclave is able to reach high temperatures to sterilize the agent.

It is important to know the standard operating procedures (SOP) for the autoclave.

The SOP is located next to each autoclave. If the temperature or pressure is inadequate, the bag is overfilled, or the peak time is not long enough the material will not be properly decontaminated.
The material being autoclaved must be placed inside an autoclave bag or in a pan (Do not overfill the bag).

Add ~250-500ml water to the bag or pan for steam to generate and properly circulate.

A spore strip or other approved tape must be placed on the outside of the bag or pan to verify that the heat treatment was successful. You will have to verify that a color change took place.

The autoclave must reach a temperature of 121°C (250 °F) for at least ½ hour at a pressure of 15psi.

After successfully being autoclaved, the material can be placed in regular trash.

Sharps must be incinerated. When the container is 3/4 full
Decontamination is the removal or neutralization of toxic agents or the use of physical or chemical means to remove, inactivate, or destroy living organisms. This includes both

**STERILIZATION** means to kill ALL microbes - whether harmful or not - and their spores present on a surface or object.

**DISINFECTION** To disinfect means to eliminate most harmful microorganisms (not including their spores) from surfaces or objects; inactivate viruses
Using Liquid Bleach

Example I

Chlorine in liquid bleach comes in different concentrations. Any concentration can be used to make a dilute chlorine solution by applying the following formula:

$$\left[ \frac{\% \text{ chlorine in liquid bleach}}{\% \text{ chlorine desired}} \right] - 1 = \text{Total parts of water for each part bleach}$$

Example: To make a 0.5% chlorine solution from 3.5% bleach:

$$\left[ \frac{3.5\%}{0.5\%} \right] - 1 = [7] - = 6 \text{ parts water for each part bleach}$$

Therefore, you must add 1 part bleach to 6 parts water to make a 0.5% chlorine solution.

† “Parts” can be used for any unit of measure (e.g. ounce, litre or gallon) or any container used for measuring, such as a pitcher.

‡ In countries where French products are available, the amount of active chlorine is usually expressed in degrees chlorum. One degree chlorum is equivalent to 0.3% active chlorine.
Autoclave

The autoclave is the most effective method to use for decontamination purposes. As a general rule of thumb, autoclave all materials that are considered infectious agent, or resemble components of this nature. When in doubt, AUTOCLAVE! If a material is not capable of autoclave because of its size, material, or it is stationary, then rely on chemical disinfectant as a second option.
INCINERATION

- There are other methods that can be used for decontamination such as incineration. This is the most reliable method, but it will destroy all materials placed in it and it creates environmental pollutants.
- Ensure you have a primary, secondary and tertiary chamber for a good incinerator
When a spill occurs, an aerosol can be created which can make the material several times more potent.

The first response to a spill should be to evacuate the immediate area until the scope of the hazard has been addressed.

Seek medical attention if necessary.

Allow sufficient time for the aerosol to settle before considering entering the room.

If you are responsible for clean up, proper training shall be addressed.

Report the spill and record on a log.
Waste can be classified as either hazardous or non-hazardous. Let’s take a closer look at the differences.

**Hazardous Waste** - This is a waste which contains the characteristics of being any of the following:

- Toxic
- Corrosive
- Flammable
- Oxidizer
- Infectious
- Radioactive
Waste Management Processes

1. Segregation
2. Packaging
3. Labeling
4. Handling, and storage of waste products
5. Transportation
6. Disposal
REMEMBER THE WAY TO BE SAFE IS NEVER TO FEEL SECURE!!!!