Lab 1

Lab Safety & The Ubiquity of Microorganisms

Part 1: Microbiology Lab Safety Briefing

Rule number one when it comes to lab safety is to "use your common sense"—*if it might get you or someone else contaminated, sick, injured, burned, or just generally worse off than you were when you arrived to lab—then don't do it.* **We will routinely deal with fire, hot objects, flammable liquids, broken glass and potentially infectious bacteria.** So, being a "klutz" is not a problem as long as you are a "responsible" klutz and clean up after yourself, keep a well organized work area, be aware of the dangers possible and **ask for help as needed**. The lab routine can be intimidating at first, but you will quickly get comfortable once you have been through 2 or 3 labs. Knowing what to do when an accident happens is helpful, but it is probably more important to **BE CALM** in order not to make things worse, and get my help right away to address the situation.

Know where the following items are in the lab (tip… this "easy" stuff is testable!):

1. **fire extinguisher & fire blanket**
2. **eyewash station & safety shower**
3. **gas shutoff valve**
4. **two exits** out of the lab
5. **red garbage cans** (for "biohazard" material, such as used plastic Petri dishes)
6. **black garbage cans** (for "regular" trash, such as paper)
7. broken/non-broken **glass waste box** (i.e. we routinely throw away used microscope slides here)
8. **10% Bleach solution** (used to clean spills [such as dyes and bacteria]; plus you use it to wipe down your table area before and after lab—a good lab habit to learn from day one).
9. **Distilled water solution** (the faucet color these bottles can be refilled from is "solid white").
10. **100% Ethanol solution** (for flame sterilization of certain equipment, and to remove dye stains).
11. **37 °C Incubator & Refrigerators**
12. At your station is *usually* the following items (stuff gets moved, cleaned and put away often so ask if you missing something you need—many things are shared, but others you should have your own of):
    - **Bunsen Burner with hose**
    - **striker (with flint and steel inside it—to light the Bunsen Burner)**
    - **white test tube tray**
    - **inoculation loop & inoculation stab**
    - **Wooden Clothes Pin** (to hold hot items—like slides during heat-fixation).
    - **Microscope Slides & Coverslips**
    - **Immersion Oil & Lens Wipes**
    - **Glass Hockey Stick**
    - **Permanent Black Marker** (very important for every lab—don't accidentally take it home!)
    - **Ruler**

13. **Microscope Cabinet** (where you get and return any microscope you use).
14. **Discard Area for used items to be washed** (glass test tubes, glass Petri dishes, etc…).
15. **Red Pipet Bucket** on table for the used glass pipets (which will be collected and washed later).
16. **Autoclave**.

Microbiology Lab Rules (these are testable items, so known them too.):

1. **Wash your hands with soap and water *before* & *after* lab.**
2. **Clean your lab table area with 10% bleach *before* & *after* lab.**
3. **NO food and NO beverages in lab** (even if you bring it in a sealed container and never open it), there are biohazard level 2 microorganisms used here and you do not want to accidentally contaminate yourself and become ill.
4. You don't need a lock, but it is fine to put personal belongings in the lab drawers to keep them out of the way during the lab—especially when available table space is at a premium during experiments.
5. Keep long hair (and beards) pulled or tied back so you don't catch them on fire.
6. Spills, broken glass, and anything on fire needs to be reported to me immediately so I can be sure no one is injured and the incident is properly handled—*you only get in trouble when it happens and you try to hide it*.
7. Don't wear "nice stuff" (if you can avoid it) as we use bleach twice or more in every lab, plus certain experiments will use difficult to remove biological stains. Lab coats are available if you need one.
8. Gloves and safety goggles are not required for any of the labs, but feel free to use them if you want the extra protection. The gloves are especially nice in preventing the various stains from getting on your skin that can take days to go away.
9. Most labs require a demo before you can officially begin, but feel free to work on other stuff till lab starts.
10. I start on time so most labs will normally end in about an hour, giving you extra time if you need it. Please don't be late or you will miss vital instructions at the beginning!

Notes:

NO biosafety level area (even Level 1) is "100% safe" from the potential of microbes to cause illnesses—given enough time and exposure, contamination will occur. The way to hopefully limit the likelihood of it occurring is to follow the protocols for safety closely and to do them *every time* in a very regimented manner. No biosafety level is safe for eating, drinking, handling contact lenses, applying cosmetics and storing food or beverages. **ALL** proper biosafety level areas have tables, floors and other furnishings with **easy to clean surfaces**.

Biosafety Level 1 (BSL-1):

- You are working with **well-characterized microbes *NOT known to consistently* cause disease** in healthy adults.
- Microbes represent a **minimal potential hazard** to laboratory personnel and to the environment.
- BSL-1 areas do not need to be separate from other areas in a building.
- Special equipment is not required and usually not even used as generally available materials will suffice.
- You can work on a table's surface for all lab activities.
- Lab personnel have specific training in the procedures conducted in the lab and are supervised by a scientist with general training in microbiology or a related science.
- Windows are okay, but they should have fly screens.
- No specific safety equipment is required.
- a basic anatomy lab which works with preserved specimens and no infectious material is one example.

Biosafety Level 2 (BSL-2):

- **Labs using microorganisms which are known to cause human diseases**, especially if exposure occurs to damaged skin, mucous membranes, or the material is ingested, or enters the eyes.
- all BSL-1 safety rules plus **limited access** (i.e., locks or keypad entry) is added to all BSL-2 labs.
- Room has **specific locations for certain kinds of waste** (regular, biohazard, glass, sharps, etc...).
- Room has **hoods** to be used as needed.
- Room has **gloves**, **coats**, **eye protection**, and **full face masks** as needed for certain procedures.
- **Autoclave** is present to sterilize items.
- Example is **this microbiology lab.**

Biosafety Level 3 (BSL-3):

- Areas with "indigenous" or "exotic" microbes with the potential for **aerosol transmission**, thus spreading the infection via breathing the air to humans.
- BSL-2 safety rules plus controlled access with **self-closing double-doors**, with **room being physically separated from the halls**.
- **Room exhaust is NOT recirculated**, but sent through HEPA filtration before going outside.
- **Negative airflow into the lab** (air is always sucked into the room, especially when doors are open).
- **Respiratory protection is required**.
- Example is a **respiratory isolation room in a hospital** such as in the case of an Active Tuberculosis patient.

Biosafety Level 4 (BSL-4):

- Areas with "dangerous" or "exotic" microbes with pose a **very high risk of illness** with a **high likelihood of death if infection occurs because vaccines or other treatments are not available or very useful:**
  - **Marburg Virus**
  - **Ebola Virus**
  - **Lassa Virus**
  - **Smallpox**
  - other hemorrhagic fever diseases
- BSL-3 safety rules plus street clothing is changed to special lab clothing before entering, with a shower on exit and decontamination of all lab materials and clothing on exit as well.
- **Separate building with highly restricted access**. Guards present.
- Dedicated supply systems, exhaust systems, vacuum systems, and decontamination systems.
- **Full-body, air-supplied, positive pressure personnel suit required**.
- Multiple airlocks are passed through before reaching work station.
- One such facility is at the **Center for Disease Control (CDC)** in Atlanta, Georgia. Another is the **US Army Medical Research Institute of Infectious Diseases** (**USAMRIID**) at **Fort Detrick** in Frederick, Maryland (this was the site of the American biological weapons program from 1943 to 1969).

Notes:

Part 2: Nutrient Agar & the Ubiquity of Microorganisms

Microorganisms need food—and **Nutrient Broth** provides a *liquid medium* while **Nutrient Agar** provides a *solid medium* on which many (but not all) microorganisms can grow. The main ingredient in each is **Beef Extract**, which when soaked in water releases soluble carbohydrates (monosaccharides & polysaccharides), amino acids, purines, pyrimidines, vitamins, minerals and lipids. All of these soluble extracts are concentrated into a powder form and additional nutrients are added—such as peptones (small chains of amino acids). The only difference between the Nutrient Broth and the Nutrient Agar is the addition of agar to solidify the medium. The **agar** ingredient is a **powderized polysaccharide, which is isolated from seaweed**, that becomes a **liquid when boiled** (100ºC) and becomes a **solid when cooled** (at about 40ºC). *The solid form of Nutrient Agar allows cultures to "grow on the surface" and the "isolation of colonies" to be possible. Because the precise composition of the ingredients is unknown, Nutrient Broth and Nutrient Agar are referred to as the* **"undefined media"** *type, also known as* **"complex media"**. This is because of the use of an animal or plant extract where one or more ingredients will be unknown in composition and/or amount. In subsequent lectures and labs we will discuss and/or use other growth media terms such as:

- **Selective Media:** which favors the growth of one group of microorganisms while inhibiting or preventing the growth of others.
- **Differential Media:** a growth medium that will usually contain an indicator chemical that will change color (usually with pH changes), thus detecting the presence or absence of a specific type of metabolic activity.
- **Enrichment Media** a growth medium that will contain a **rare or unique ingredient**(**s**) to favor the growth of unusual or difficult to grow microorganisms.

The ubiquity of microorganisms should be evident after the end of this lab (or at the very least, by the end of this course). The more places in the world that scientists look (even dangerous and unlikely locations) the longer the list of known microorganisms grows. Each discovery adds to the evidence that confirms over and over how unique microbes are in their capabilities to adapt to a wide variety of environmental and nutritional conditions that most larger organisms could not survive. In general, if a location on the planet Earth can be touched (by the naked hand or via a tool), then it likely will have microorganisms located there which can be detected by various means (even it that means we can only sequence its genome). A big challenge is that many microbes are quite difficult to grow in the lab and most have no known way to culture them reliably or at all. Most microorganisms are **"free-living"** in that they do not require a specific host (such as a plant or an animal) in order to survive. Despite the extremely large variety and number of microorganisms, **about 99% of microorganisms do not cause any *known* disease**, and are termed "**nonpathogenic**". *If a microorganism is associated with a specific host organism, then it that microorganism has a greater likelihood of also being classified as a* ***pathogen***. *This does not necessarily mean a particular pathogenic microorganism will always cause a certain disease, but the chances are greater that it could given the "right conditions".* For example, one way certain fungi in the digestive system and vagina are able to cause an infection is when a person takes an antibiotic that inadvertently kills other bacteria in those same locations which keep the normal flora fungal populations in check. The fungi are able to increase their populations and cause disease symptoms only after the bacteria have been killed by the antibiotic.

*Even **commensal** or **mutualistic** microorganism that normally benefit us or cause us no harm (in normal situations) can become what are known as an **opportunistic pathogens** if they get access to areas where they are not normally found.* For example, *E. coli* is a bacteria that is normally found in the digestive system, but it can cause a urinary tract infection if it gets into the urethra or bladder. Any area, even sites outside the host organism, *where a microbe resides* and serves as a *potential source of infection—these are known as **reservoirs**.*

Lab Procedure for Part 1 (Ubiquity of Microorganisms—sampling the environment):

1. Obtain **2** Petri dishes and **7** sterile Q-tips with nutrient agar per student.
2. Divide each Petri dish into ***4*** *equal sectors* by drawing 2 lines on the bottom (*there is usually no reason to ever write on the lid in microbiology lab—lids rotate and fall off*). See figure 1 for where to write on the petri dish's bottom surface. WRITE SMALL so you don't block your view later with lots of big writing.
3. For just one of these Petri dishes, label one of the sectors "Negative Control", and be sure not to touch it with anything. Also, write your name and the date in this sector (see the diagram below).
4. For the remaining three sectors I want you to **culture three random but specific locations** (in the lab, on your person, etc...) and write the name of that location in the appropriate sector (see the diagram below). *Be sure to use a new sterile Q-tip for each of the three separate sample locations*
6. Put your completed Petri dish in the area designated on the counter (it will be incubating at room temperature— about 21 ºC to 25 ºC for this lab).
7. **Take home** the other Petri dish and four sterile q-tips and repeat the same process with any surface you wish. **Store it at ROOM TEMPERATURE in a safe place** (NOT in the FRIDGE) and **bring it to lab** when we meet again. Be as creative and unusual with the areas you culture—just don't do any human body parts as that will be another lab later on.
8. Observe the findings of both Petri dishes at the next lab (DON'T forget to bring back the one you took home!!!)

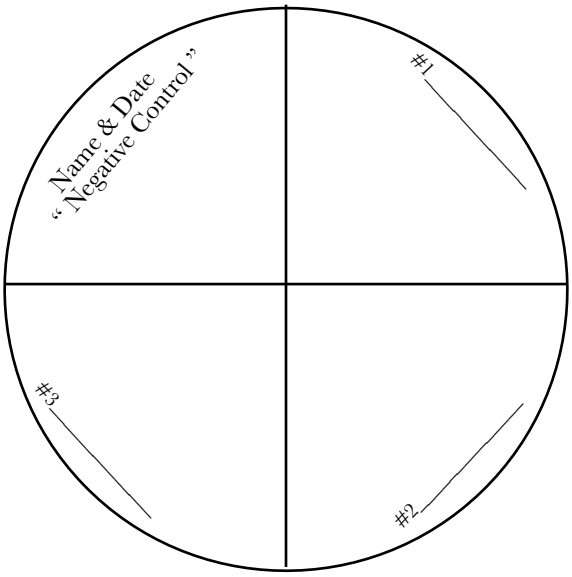

Figure 1: Labeling a Petri dish on the <u>bottom</u> side only. *Write small and around the periphery so your writing does not block your view of colony growth later on.* Drawing lines can help to visually mark where you need to do certain experiments like this one where each sector is for inoculating a sample from a different surface. *The dividing lines are not necessary for all labs.*

Lab 1 Questions (***Due at the end of lab***)

Name: _____ **Grade:** _____ **of 10 points**

1. (2 points) Organisms growing on a **solid surface** (Nutrient Agar) can tell you what kind of information that organisms growing in a liquid (such as Nutrient Broth) can't provide? List **2** things to get full credit.

- _____.
- _____.

2. (2 points) Assume something grows on the Negative Control sector of your Petri dish. Provide **2** reasons why that could happen.

- _____.
- _____.

3. (2 points) Describe the features or location of a surface which has a **statistically low chance** of having living microbes on it. List at least **4** features/locations to get full credit.

- _____.
- _____.
- _____.
- _____.

4. (2 points) Provide **2** or more reasons why some and not all microbes grow on the nutrient agar even if you pick up a bunch with the sterile Q-tip and put them on the Petri dish correctly?

- _____.
- _____.

5. (2 points) Look at the 3 areas you sampled in the lab. Hypothesize (educated guess) what you think the order of variety (*number of different species of microbes*) will be. *You can't really get this question wrong (it is a hypothesis).*

#1 location (highest variety of microbes): _____.

#2 location (middle variety of microbes): _____.

#3 location (least variety of microbes): _____.