Part 1: Epidemiology Lab Follow–Up

**Theory:**

Every disease-causing microorganism must come into direct contact with the host organism's cells and tissues in order to cause a disease. How the microorganism "spreads" to the host can be highly variable, with examples being **direct contact**, **droplet spread**, **blood-borne**, **fecal-oral**, and **airborne**. Even when the spread of a microorganism has been successful, *most are unable to cause a particular disease unless the host also has a compromised immune system, some other conditions are met to provide a successful portal of entry, and usually it is both*. Here are some common terms and their definitions frequently used when discussing the statistics of infectious diseases and epidemiology:

**Incidence**: This is the number of **NEW cases** of a specific disease, in a defined population of AT RISK people only and during a specific time period. This number is similar to the **morbidity rate** of a disease which is reported in the United States as a number per 100,000 people, rather than as a percentage. This allows one to easily compare the incidences of different diseases. It is a way of quantifying **how infectious a disease is and how easily it is spreading amongst at specific at risk population.** *Remember, only people who are still "at risk" and "currently free of of the specific disease" have the potential of getting the illness for the first time or getting a repeat infection.*

$$\textbf{\% Incidence Rate} = \frac{\textbf{\# of NEW cases}}{\textbf{\# of individuals in the "At Risk" Population}} \text{ X } 100\%$$

**Prevalence:** There are 2 types of Prevalence, but both refer to the **TOTAL** number of diseased people within a TOTAL population. The total population number even includes those who are not at risk of getting the disease (unlike the Incidence). The **Period Prevalence** looks at the total number of diseased individuals during a specific time period (a range of time such as last month, or 2012). The **Point Prevalence** looks at the total number of diseased people at a *specific moment* or *point in time* (such as today—i.e., "right now"). *Death or recovery from the disease removes that individual from the prevalence count*. As long as you are alive, you are counted in the total population. This is a way of quantifying **how "common" a disease is**.

$$\textbf{\% Prevalence} = \frac{\textbf{\# of TOTAL EXISTING cases}}{\textbf{\# of individuals in the "TOTAL" Population}} \text{ X } 100\%$$

**Mortality Rate:** This is the **death rate** from a specific cause, within a defined population, for a specific time period. In other words, if you get the disease, then what is the percent chance you will die from it?

**Sporadic Disease**: are "*rare*" (even 0% Prevalence) diseases that occurs only occasionally (sporadically) within a specific population. In the United States, these are diseases *usually kept under control by immunizations, and good sanitary conditions*. Examples include cholera, polio, plague, and botulism. Spikes in these diseases commonly occur when people do not get immunized in large numbers, or sanitary conditions are suddenly compromised.

**Endemic Disease:** are "*common*" diseases that are *nearly always present in some amount in a specific population*. The numbers will fluctuate over time, but *the disease never fully vanishes from the population*. Examples include tuberculosis, staphylococcal infections, streptococcal infections, syphilis, gonorrhea, the common cold, influenza, chickenpox and the mumps. Many of these diseases also have vaccination programs or sanitary methods to control their numbers, and spikes occur for the same basic reasons as sporadic diseases.

**Epidemic Disease:** are sporadic or endemic diseases that have a sudden or unusually high spike in their % Incidence Rate and % Prevalence in a specific period of time within a specific population. Simply put — *there are more cases that you would normally expect*. Examples include staphylococcal food poisoning, Legionnaires disease from contaminated air conditioners, Escherichia coli O157:H7 contaminated meat, the West Nile Virus after massive bird population deaths, and influenza viral illness.

**Pandemic Disease:** are epidemic diseases occurring **in many countries or populations** (possibly even worldwide) **at the same time**. In 1918, the Spanish flu pandemic killed over 20 million people worldwide.

**Reservoir:** *any living organism or inanimate object* that is the location where a microorganism can survive and multiply till it is transferred to a host. **Fomites** are inanimate objects capable of transmitting pathogens. Some microorganisms are limited by their biology to specific reservoirs which can even be down to a specific tissue or cell type in only one kind of species. A human who is currently colonizing a pathogenic organism, which is capable of being spread to another individual, is called a **carrier** — however, this organism is not currently causing a disease in the carrier. There are 4 basic types of carriers:

— **Passive Carrier:** carry the pathogens but do not have the disease.

— **Incubatory Carrier:** a person who is capable of transmitting the pathogen during the incubatory period of a particular disease.

— **Convalescent Carrier:** a person who is capable of transmitting the pathogen during the convalescent (recovery) period.

— **Active Carrier:** a person who has completely recovered from a disease, but continues to harbor the pathogen indefinitely. A famous historical example is Mary "Typhoid" Mallon, who recovered from *Salmonella typhi* (the causative agent of Typhoid Fever), but it was still living in her gallbladder and being secreted in her feces. She worked as a cook and rarely washed her hands after using the restroom—this resulted in many new cases of Typhoid Fever in her customers, but she never got ill again.

**Modes of Transmission:** There are 7 basic modes or categories of transmission for pathogens, and there can be some overlap in how a pathogen can be spread.

— **Direct skin–to–skin contact**: The most common method is handshaking. Frequent handwashing will reduce the chance of spread by this method.

— **Direct mucous membrane–to–mucous membrane contact**: Examples are kissing and sex. STDs are transmitted by this mode, and some can have sex transmission reduce by using condoms.

— **Indirect contact via airborne droplets of respiratory secretions**: Examples are sneezing or coughing. The droplets of a sneeze or cough can only project forward a few feet. Further distances can be achieved by settling on dust particles and getting blown by any kind of wind movement. Cold viruses, influenza, measles, mumps, pneumonia, and the chickenpox can be spread by this mode. Wearing a mask and blocking a cough or sneeze can reduce transmission.

— **Indirect contact via food and water contaminated with fecal material:** The most common method is the food preparer who fails to wash their hands after using the restroom.

— **Indirect contact via arthropod vectors**: Arthropods such as mosquitoes, flies, fleas, lice, ticks and mites can get a blood meal from an infected individual and transfer the infection to an uninfected individual by biting them as well. This is because such arthropods typically inject some digestive juices into the bite before sucking out a blood meal. Eliminating a vector can reduce the spread of these kinds of diseases: dog tapeworm, plague, Lyme disease, Leishmaniasis, and Chagas' disease.

— **Indirect contact via fomites that become contaminated by respiratory secretions, blood, urine, feces, vomitus, or wound exudates from ill individuals:** Uncleaned toilet seats, not changing gloves between patients, and not washing your stethoscope are common hospital modes of transmission.

— **Indirect contact via transfusion of contaminated blood or blood products from an ill person or by *parental injection* using non-sterile syringes and needles.** While controversial, needle exchange programs are one method to reduce transmission in the illegal IV drug use population.

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*** Complete the handout for Lab 3 on page 6 at this time. The formulas you need are on page 1.**

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Part 2: Aseptic Technique & The Streak-Plate Method of Isolation

## Theory:

The successful transfer of bacteria will require you to learn how to perform **aseptic technique** (also called **sterile technique**). This skill will allow the transfer of only the microorganism you are interested in and no additional microorganisms, which are collectively called **contaminants**. This lab will test your ability to not only avoid contamination but also how to separate two microorganisms that are growing together within a mixture. There are multiple ways of separating microorganisms, but today's lab will employ the "**Streak-Plate Method**".

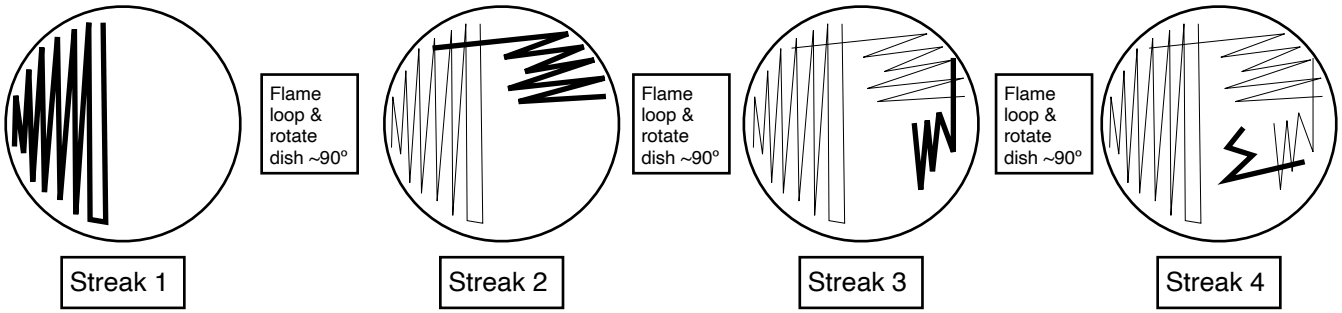
## Lab Materials:

| Amount per Student | Material | |
|---|---|---|
| 1 or 2 (as instructed) | Petri Dishes with Sterile Nutrient Agar | |
| 1 tube or Petri dish (as instructed) | Mixed Bacteria Sample | |
| 1 tube or Petri dish (as instructed) | Single Bacteria Sample | |
| 1 | Inoculating Loop | (at your station) |
| 1 | bunsen burner | (at your station) |
| 1 | Permanent Sharpie Marker | (at your station) |

## Procedure:

1. **Watch the Instructor DEMO the procedure before trying it on your own.** There are many safety concerns and procedural tips to take note of. Once I am done, follow the procedure and ask questions as needed.
2. In your dominant hand (right), pick up the inoculating loop and hold it like a pencil by using your thumb and index finger. You should be keeping your other three fingers free to hold the test tube cap (when needed at later steps).
3. **Sterilize the loop in the flame of the bunsen burner being sure to flame the loop and most of the stem till it is RED HOT. Then let it cool for about 10 to 15 seconds before using it.**
4. **Pick up the test tube with the single bacterial sample in it with your non-dominant hand (left) and remove the cap with the free fingers of your dominant hand (right). DO NOT PUT THE CAP ON THE TABLE — this will contaminate the cap** (which can transfer that contaminate to your test tube)**! Briefly (2 to 3 seconds) flame the lip of the open test tube.**
5. **Dip the loop in the broth of the open tube** till the loop is under the surface and remove it. You should see a film of liquid in the loop. THIS IS THE ONLY TIME YOU DIP THE LOOP INTO THE BACTERIAL SAMPLE BEFORE STREAKING ON THE PETRI DISH.
6. Set the tube back in your white test tube rack and replace the cap. Be sure not to "bump" the loop into anything before the next step or you will contaminate it.
7. **Lift the lid off the petri dish (but do not sit it on the table) and perform the 1st of the four streaks "Streak-Plate Method" as quickly as possible to avoid airborne contaminants.** Replace the lid after the streak is done. Refer to the following diagram below for the streak isolation sequence and general pattern. NOTE: there is an alternate way to do this, both will be demonstrated.
8. **Flame sterilize the loop and perform the 2nd streak.** Replace the lid after the streak is done.
9. **Flame sterilize the loop and perform the 3rd streak.** Replace the lid after the streak is done.
10. **Flame sterilize the loop and perform the 4th (and final) streak.** Replace the lid after the streak is done.
11. Flame the loop one last time and return it to the tool tray.
12. Label the plates with your name and date, then place them in the indicated area upside-down.

**Figure 4–1.** The sequence and pattern of streaks in performing the **streak–isolation technique**. The previous streaks will leave very light marks on the solid media. Only one of the marks for streaks 2, 3 and 4 should pass through the previously streaked area.




**Streak Isolation tips:**

1. Put the lid and bottom back together between streaks to avoid airborne contaminants.
2. Hold your breath or breath very shallow to avoid airborne contaminants.
3. Don't scribble randomly.
4. Avoid going back into the previous area more than 1 or 2 times. The more you enter a previous area, the less the streak isolation will result in isolated colonies.
5. Use the light in the room to see where the subtile marks from previous streaks are. It can be hard to see where you have been in previous streaks.
6. You will run out of room for the 4th streak sometimes. To have more room for it, use less space for streak 1.
7. One isolated colony is enough for success. You might find isolated colonies in streak 2, 3 and 4. The biggest and best ones tend to be in streak 3 or 4.
8. Don't worry too much about tiny gauges of the agar if you streak too roughly. It rarely matters for this lab and is a good learning experience to work on developing a delicate touch.
9. Memorize how to do this skill. It is a testable skill on either the mid-term exam, the final exam or both. We will be doing many more of these in future labs.

**Lab 4 Questions (Due at the end of lab)**

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

1. (10 points) Chart the Class Data from Lab 3: Epidemiology.
   - A "+" refers to an infected individual, and a "—" refers to an individual who is not infected or who has "recovered" from the illness.
   - Don't mark a "+" for the presence of any contaminants.
   - After the "," write the number of the person the handshake was with.
   - Write "INDEX CASE" next to the culture number it applies to.
   - *Assume at time "0" that all people were free of disease and were all equally potential candidates for infection.*
   - *Ignore rows if the class had less than 18 students.*
   - *We will fill out the data together in lab and then you can work on completing the chart like we did in the previous lab.*

| Culture # / Patient # | Negative Control | Positive Control (Contact 0) | Handshake 1 | Handshake 2 | Handshake 3 | Handshake 4 |
|---|---|---|---|---|---|---|
| 1 | | | , | , | , | , |
| 2 | | | , | , | , | , |
| 3 | | | , | , | , | , |
| 4 | | | , | , | , | , |
| 5 | | | , | , | , | , |
| 6 | | | , | , | , | , |
| 7 | | | , | , | , | , |
| 8 | | | , | , | , | , |
| 9 | | | , | , | , | , |
| 10 | | | , | , | , | , |
| 11 | | | , | , | , | , |
| 12 | | | , | , | , | , |
| 13 | | | , | , | , | , |
| 14 | | | , | , | , | , |
| 15 | | | , | , | , | , |
| 16 | | | , | , | , | , |
| 17 | | | , | , | , | , |
| 18 | | | , | , | , | , |
| # of TOTAL "+" Cases | | | | | | |
| TOTAL Population | | | | | | |
| # of NEW "+" Cases | | | | | | |
| Number in "At-Risk" Population* | | | | | | |
| % Prevalence | | | | | | |
| % Incidence Rate | | | | | | |