Making Microbiology

How we made a fun and interactive Microbiology Class

Sarah Mackie, CVT and Jennifer Yoon, CVT

About Us

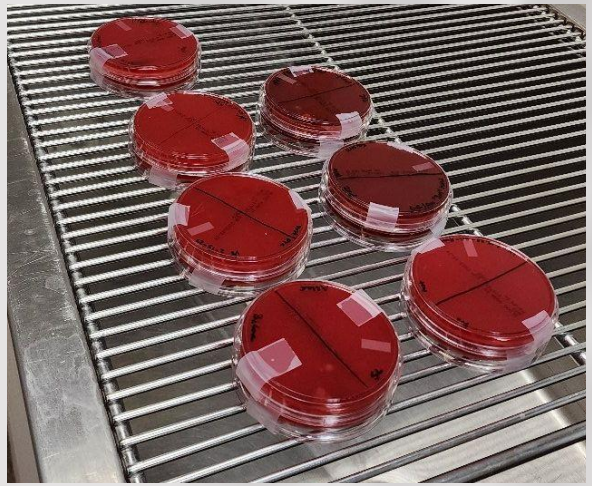
Sarah Mackie, CVT

- Started working as a Technician in 2007
- GP until 2014, ER 2015 to present
- Teaching since 2012 at Pima Community College
  - Clinical Pathology 1 & 2, Radiology
- Veterinary Technician Manager at Southern Arizona Veterinary Specialty & Emergency Center
- Awarded the AVTE Teaching Excellence Award 2022

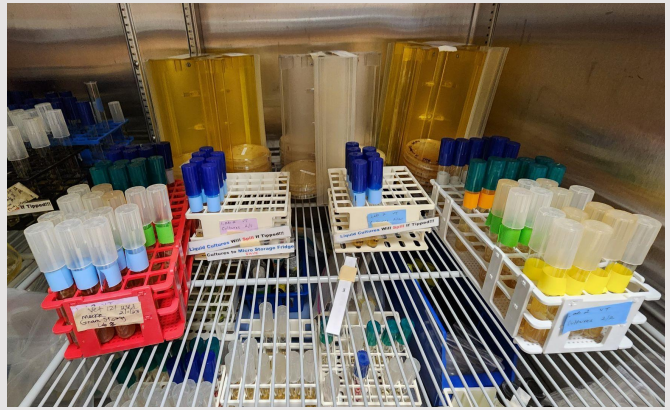
Jennifer Yoon, CVT

- 25 years in the veterinary field. Started out as a kennel assistant at 16.
- Spent 20 years in emergency medicine
- Worked in research at the University of Arizona
- Lab specialist/CVT at Pima Community College in Tucson, AZ

Clinical Pathology 2: Microbiology



- Focus
  - Students able to see what happens "Behind the Scenes" when they send a sample to the lab
  - Help students understand how and why tests are done
    - Benefits
  - Produce more well-rounded Technicians for our field
- Use AVMA Sign-offs as a guideline for the labs that we do in class (13)
- Work with the Lab that prepares for the General Microbiology class at Pima Community College



Collaboration with the Biology Department

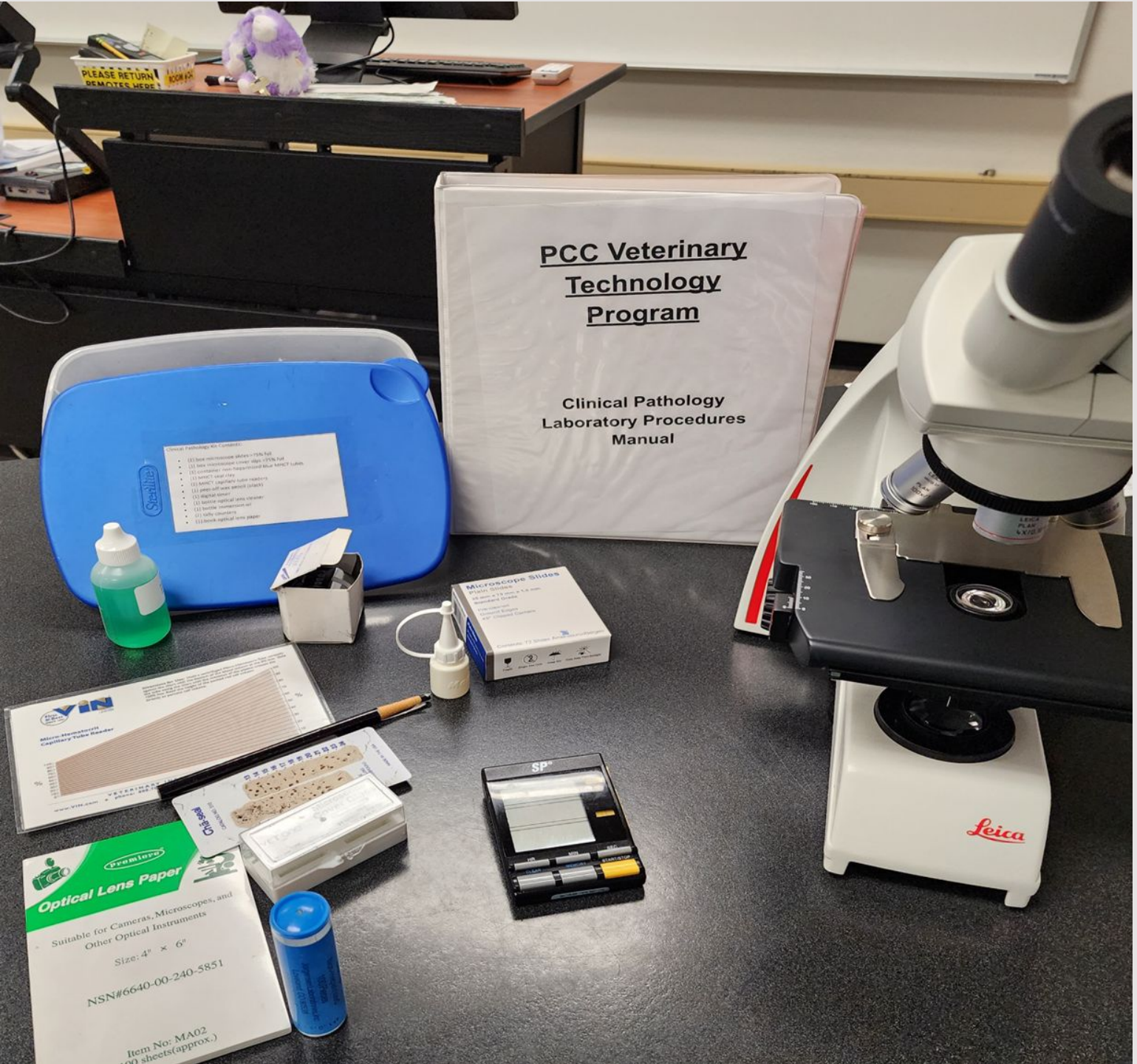
Microbiologist prepares and incubates the required bacterial cultures 48 hours prior to the scheduled lab

Vet tech lab specialist assembles the media, cultures, and other supplies using each lab session's preparation sheet and delivers them to the vet tech clinical pathology classroom shortly before session begins.

Students make their cultures on the provided media, and they are incubated for 48 hours. After incubation is complete the samples are refrigerated until the next class session

Culture Preparation

- CULTURES ARE MADE BY A MICROBIOLOGIST OR THE VET TECH LAB SPECIALIST 24 TO 48 HOURS PRIOR TO SCHEDULED LAB.
- PREPARED CULTURE SAMPLES ARE GROWN IN A 37 CELSIUS INCUBATOR FOR 24 TO 48 HOURS PRIOR TO LAB AND ARE THEN REFRIGERATED TO PREVENT OVERGROWTH

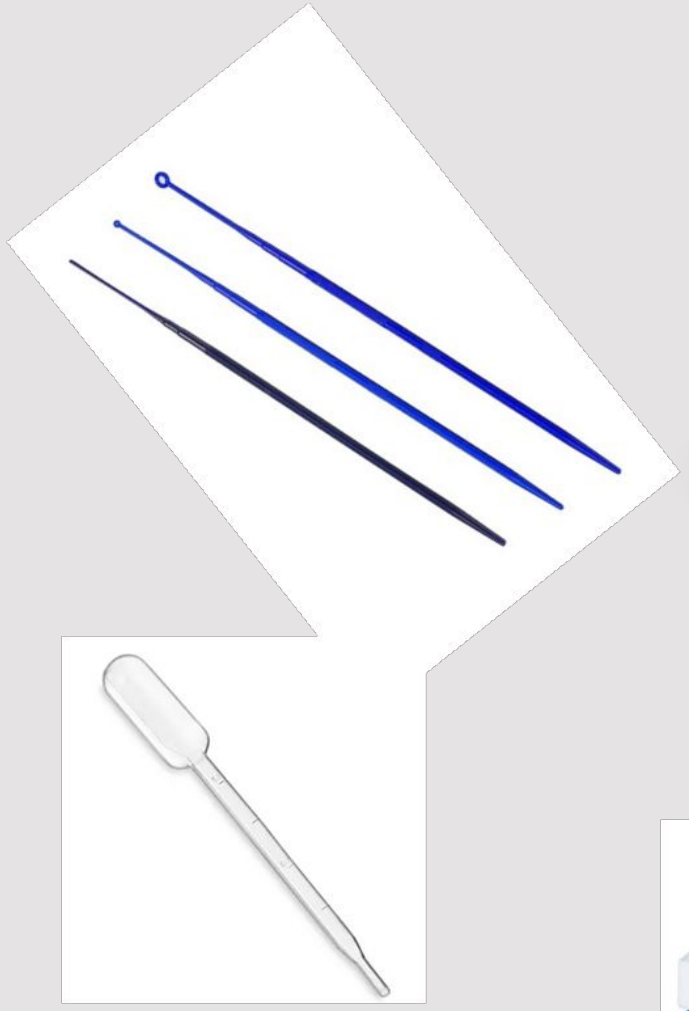


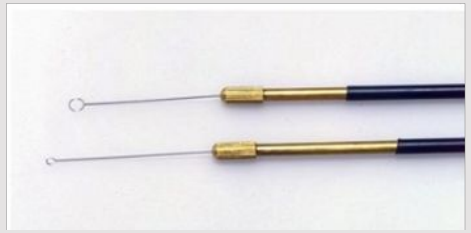
LABORATORY STATION SUPPLIES

- **EACH LABORATORY STATION IS STOCKED WITH**
  - MICROSCOPE
  - SLIDES AND COVER SLIPS
  - MHCT TUBES
  - MHCT SEALING CLAY
  - WAX PENCIL
  - DIGITAL TIMER
  - OPTICAL LENS CLEANER
  - IMMERSION OIL
  - TALLY COUNTERS
  - OPTICAL LENS PAPER
  - KIM WIPES
  - DISTILLED WATER BOTTLE
  - CLINICAL PATHOLOGY LABORATORY PROCEDURES MANUAL

COMMONLY USED LAB SUPPLIES

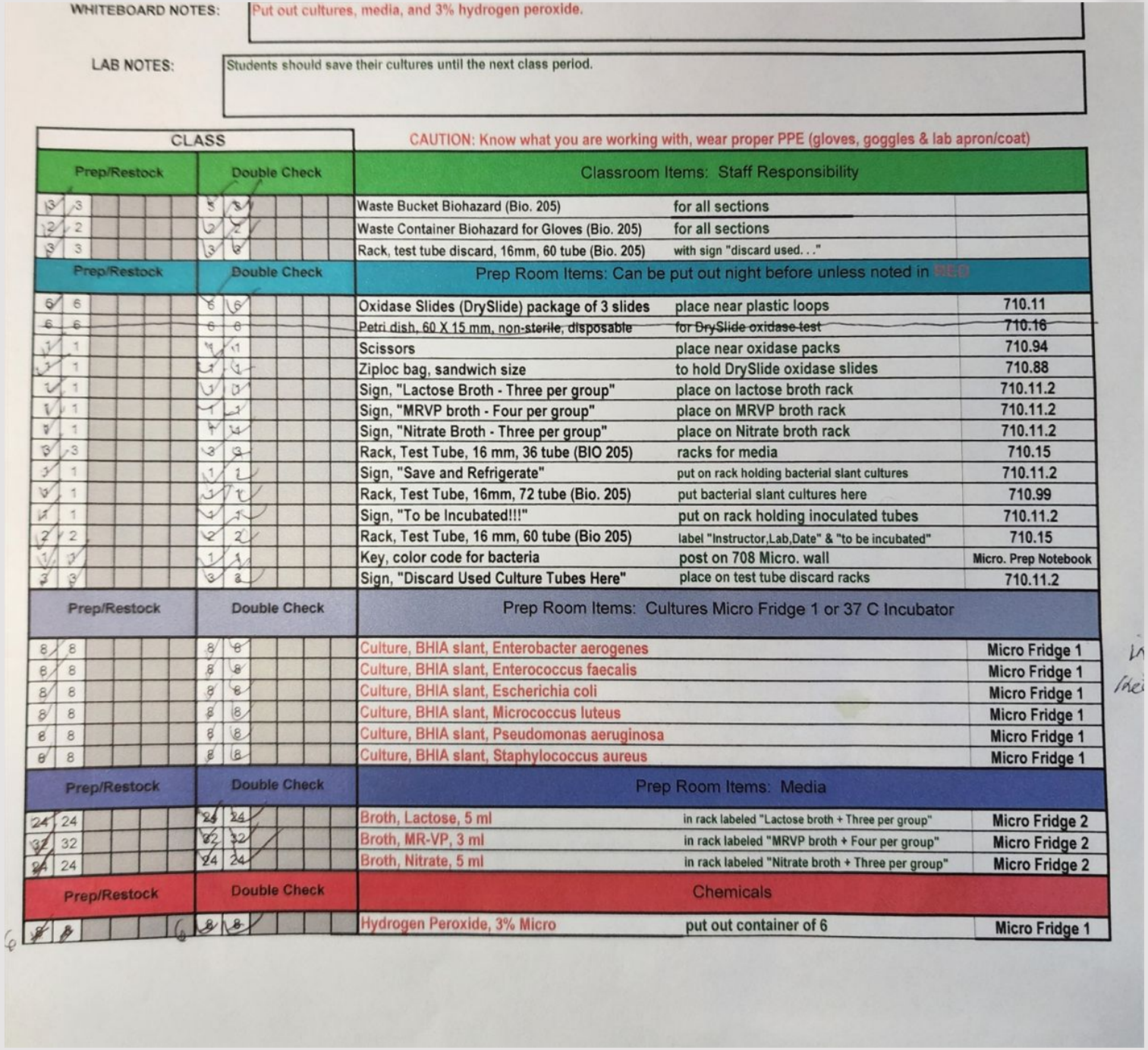
- CULTURE LOOPS
- STERILE COTTON TIP APPLICATORS
- GRAM STAIN KITS
- PIPETTES
- TEST TUBE RACKS
- PETRI PLATE RACKS
- GLOVES
- HYDROGEN PEROXIDE 3%
- ETHANOL 95%
- BACDOWN 1.6% SPRAY DISINFECTANT





PREPARATION SHEETS – HARD COPIES

- EACH LAB HAS A LIST OF REQUIRED ITEMS SUCH AS CULTURES, MEDIA, DEMO PLATES, AND OTHER PERTINENT LAB SPECIFIC SUPPLIES
- SUPPLY QUANTITIES TAILORED TO NUMBER OF GROUPS IN EACH CLASS
- VET TECH LAB SPECIALIST COMPILES SUPPLIES ON LAB CARTS 24 HOURS PRIOR AND DELIVERS SHORTLY BEFORE CLASS BEGINS



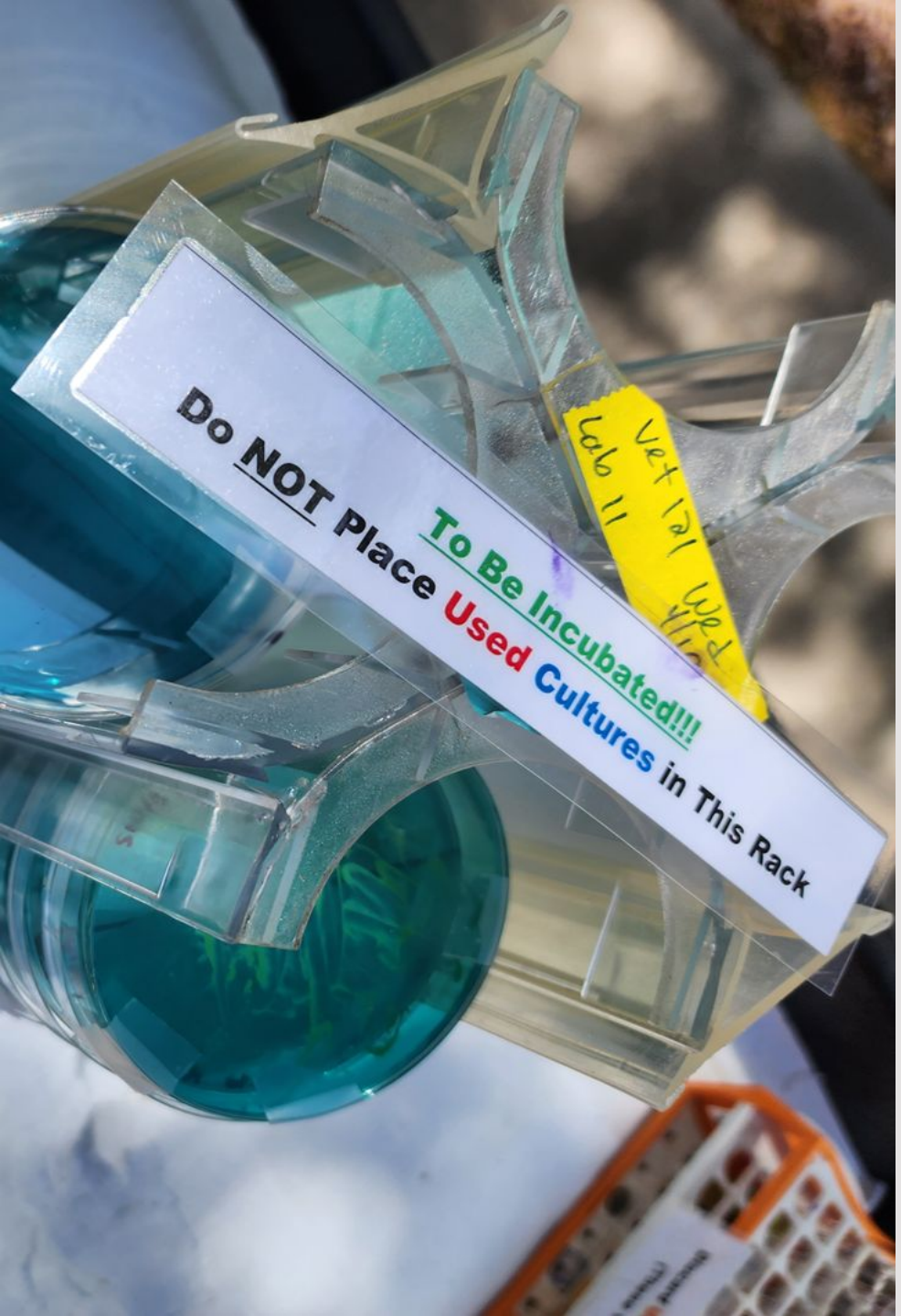
WHITEBOARD NOTES: Put out cultures, media, and 3% hydrogen peroxide.

LAB NOTES: Students should save their cultures until the next class period.

CLASS

CAUTION: Know what you are working with, wear proper PPE (gloves, goggles & lab apron/coat)

Prep/Restock	Double Check	Classroom Items: Staff Responsibility		
3	3	Waste Bucket Biohazard (Bio. 205)	for all sections	
2	2	Waste Container Biohazard for Gloves (Bio. 205)	for all sections	
3	3	Rack, test tube discard, 16mm, 60 tube (Bio. 205)	with sign "discard used. . ."	
Prep/Restock	**Double Check**	**Prep Room Items: Can be put out night before unless noted in RED**		
6	6	Oxidase Slides (DrySlide) package of 3 slides	place near plastic loops	710.11
~~6~~	~~6~~	~~Petri dish, 60 X 15 mm, non-sterile, disposable~~	~~for DrySlide oxidase test~~	~~710.16~~
1	1	Scissors	place near oxidase packs	710.94
1	1	Ziploc bag, sandwich size	to hold DrySlide oxidase slides	710.88
1	1	Sign, "Lactose Broth - Three per group"	place on lactose broth rack	710.11.2
1	1	Sign, "MRVP broth - Four per group"	place on MRVP broth rack	710.11.2
1	1	Sign, "Nitrate Broth - Three per group"	place on Nitrate broth rack	710.11.2
3	3	Rack, Test Tube, 16 mm, 36 tube (BIO 205)	racks for media	710.15
1	1	Sign, "Save and Refrigerate"	put on rack holding bacterial slant cultures	710.11.2
1	1	Rack, Test Tube, 16mm, 72 tube (Bio. 205)	put bacterial slant cultures here	710.99
1	1	Sign, "To be Incubated!!!"	put on rack holding inoculated tubes	710.11.2
2	2	Rack, Test Tube, 16 mm, 60 tube (Bio 205)	label "Instructor,Lab,Date" & "to be incubated"	710.15
1	1	Key, color code for bacteria	post on 708 Micro. wall	Micro. Prep Notebook
3	3	Sign, "Discard Used Culture Tubes Here"	place on test tube discard racks	710.11.2
Prep/Restock	**Double Check**	**Prep Room Items: Cultures Micro Fridge 1 or 37 C Incubator**		
8	8	Culture, BHIA slant, Enterobacter aerogenes		Micro Fridge 1
8	8	Culture, BHIA slant, Enterococcus faecalis		Micro Fridge 1
8	8	Culture, BHIA slant, Escherichia coli		Micro Fridge 1
8	8	Culture, BHIA slant, Micrococcus luteus		Micro Fridge 1
8	8	Culture, BHIA slant, Pseudomonas aeruginosa		Micro Fridge 1
8	8	Culture, BHIA slant, Staphylococcus aureus		Micro Fridge 1
Prep/Restock	**Double Check**	**Prep Room Items: Media**		
24	24	Broth, Lactose, 5 ml	in rack labeled "Lactose broth + Three per group"	Micro Fridge 2
32	32	Broth, MR-VP, 3 ml	in rack labeled "MRVP broth + Four per group"	Micro Fridge 2
24	24	Broth, Nitrate, 5 ml	in rack labeled "Nitrate broth + Three per group"	Micro Fridge 2
Prep/Restock	**Double Check**	**Chemicals**		
8	8	Hydrogen Peroxide, 3% Micro	put out container of 6	Micro Fridge 1



AFTER LAB: STUDENT SAMPLES

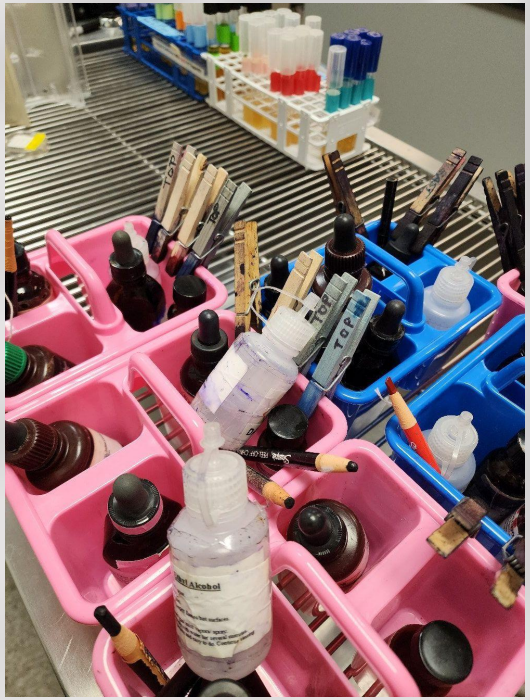
- STUDENTS ARE REQUIRED TO CAREFULLY LABEL THEIR SPECIMENS AND RETURN THEM TO A SPECIFIED RACK FOR INCUBATION
- VET TECH LAB SPECIALIST PLACES RACKS IN 37 CELSIUS INCUBATOR FOR 24 TO 48 HOURS AND THEN RETURNS THE RACKS TO REFRIGERATOR UNTIL NEXT CLASS. THIS PREVENTS OVERGROWTH ON THE PLATES

CLEANUP

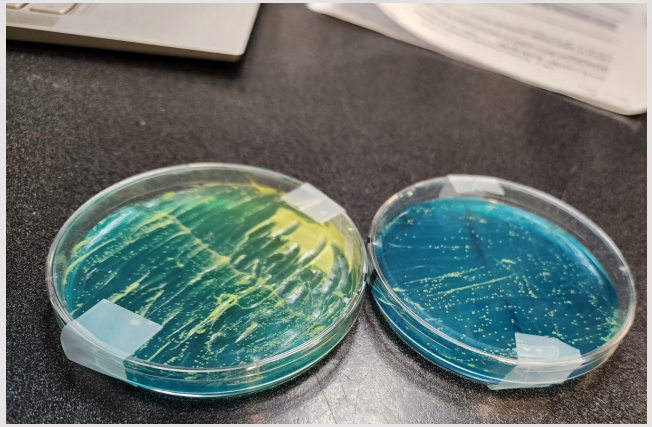
ITEMS TO BE DISCARDED ARE SEPARATED BY TYPE AND AUTOCLAVED

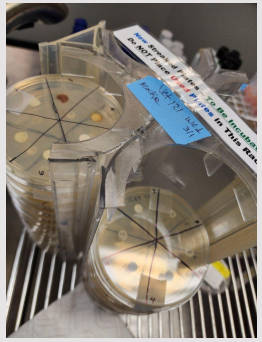
- **SHARPS:** INOCULATION LOOPS, PIPETTES, COTTON TIP APPLICATORS
- **SOLIDS:** USED PLATES, PAPER TOWELS, WIPES
- **GLOVES: NOT AUTOCLAVABLE! (PRODUCES CYANIDE GAS) DISPOSED OF SEPARATELY IN BIOHAZARD WASTE**

Class Flow

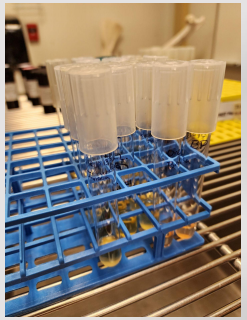


- Lab Manual
  - Focus of the Lab
  - Lab Supplies
  - Lab Steps
- Lectures Online
- In class – Review lab Instructions
- Students
  - Be able to follow instructions in Lab Manual
  - Use Critical Thinking to interpret and understand the results
  - Relate results to real life scenarios





AVMA
American Veterinary Medical Association



Sign-offs

- Perform microbiologic procedures/evaluations:
  - Collect Representative Samples
  - Culture Bacteria and Perform Sensitivity Tests
  - Identify Common Animal Pathogens using commercially available media and reagents
  - Perform Common Biochemical Tests
  - Perform Staining Procedures
- Prepare/Evaluate Ear Cytology, Reproductive Cytology, Dermatophytes
- Prepare diagnostic specimens for shipment
- Prepare, label, package, and store specimens for laboratory analysis

Labs

- Bacterial Smear Preparation from Liquid and Solid Growth Media
- Gram Staining
- Isolation of Pure Cultures of Bacteria
- Isolation of Cultures from Natural Sources
- Handwashing Lab
- Oxygen and the Growth of Bacertia
- Effects of Chemical Agents on Bacteria
- Biochemical Reactions
- Urinary Tract Pathogens
- Identification of Microbial Unknowns
- Evolution of Antibiotic Resistance in Bacteria
- Reproductive Cytology
- Tissue Cytology

Labs

- Bacterial Smear Preparation from Liquid and Solid Growth Media, Gram Staining, Isolation of Pure Cultures of Bacteria (2 parts), Isolation of Cultures from Natural Sources (3 parts)
  - Learn the Basics to use for Future Labs
- Handwashing Lab (2 parts), Effects of Chemical Agents on Bacteria (2 parts)
  - Understand how well chemical agents and antibiotics kill bacteria
- Oxygen and the Growth of Bacteria (2 parts)
  - Understanding why/how we can determine some bacterial O2 requirements
- Biochemical Reactions (4 parts), Identification of Microbial Unknowns (3+ parts)
  - Understand how we can determine bacterial properties
  - Use this to identify unknown bacteria
- Urinary Tract Pathogens (3 parts)
  - Understand how we run a Urine Culture and Sensitivity Test and what the results mean
- Evolution of Antibiotic Resistance in Bacteria (3+ parts)
  - Understand how a resistant bacteria is created
- Reproductive Cytology
  - Understand how to run a Vaginal Cytology and Sperm Evaluation
- Tissue Cytology (2 parts)
  - Understand what we are looking for on various types of cytology samples

Handwashing Lab

LAB 5 HANDWASHING LAB

INTRODUCTION:

WHY IS HAND WASHING IMPORTANT?

- ✓ Over 200 viruses can cause a common cold. Annually there are 61 million people who contract a cold. Most colds are acquired from touching contaminated surfaces, not from people sneezing on you.
- ✓ The CDC estimates that over 2,000,000 hospital patients acquire some type of nosocomial (hospital acquired) infection each year at an annual cost of over $45 billion.
- ✓ In 1993, 11 health-care workers became ill with hepatitis A because they didn't wash their hands after treating one of two patients with hepatitis A.
- ✓ In the food-service industry, studies indicate that inadequate hand-washing and cross-contamination is responsible for up to 40% of food-borne illnesses, Some of these illness are caused by bacteria (*Salmonella, Shigella, Staphylococcus, Streptococcus, Listeria, E. coli),* parasites (*Giardia),* and viruses (*Hepatitis A*).
- ✓ It is estimated that there are over 80 million cases of food poisoning in the United States each year and 325,000 hospitalizations resulting in as many as 9,000 deaths per year.

In the late 1840's, Dr. Ignaz Semmelweis, an assistant in the maternity wards of a Vienna hospital, was the first to observe the benefits of hand washing. There he observed that the mortality rate from puerperal fever (childbed fever) in a delivery room staffed by medical students was up to three times higher than in a second delivery room staffed by midwives. Dr. Semmelweis observed that the students were coming straight from their lessons in the autopsy room to the delivery room. He hypothesized that the students might be carrying the infection from their dissections to birthing mothers. He ordered doctors and medical students to wash their hands with a chlorinated solution before examining women in labor. The mortality rate in his maternity wards eventually dropped to less than one percent.

In the 1870's in France, one hospital was called the House of Crime because of the vast number of new mothers dying of childbed fever. In 1879, at a seminar at the Academy of Medicine in Paris, a noted speaker stood at the podium and voiced doubt that diseases could be spread by the hands. Louis Pasteur was in the audience that day. He shouted at the speaker: "The thing that kills women with [childbirth fever]...is you doctors that carry deadly microbes from sick women to healthy ones." Louis Pasteur contributed to the germ theory of disease and developed the concept of pasteurization.

Our hands are the source of many infections. The number of microorganisms we pick up from door knobs, counter tops, phones and other people are countless. You carry millions of microbes on your hands. Our hands are usually warm and moist, thus the perfect growth conditions for bacteria! Most microbes are harmless, but a small minority of microorganisms are opportunistic pathogens that can cause illnesses such as colds, flu, diarrhea, meningitis, hepatitis A and other diseases.

The unwelcome fact is that most routes of contamination are usually passed on from fecal matter to hands and toilet seats, and then passed onto flush levers, taps, soap bars, towels and door knobs. Only about two-thirds of American adults wash after using the toilet - women significantly more often than men.

One of the most common ways people catch colds is by rubbing their nose or their eyes after their hands have been contaminated with the cold virus. Just one in three people wash after sneezing or coughing. One in three *E. coli* outbreaks is caused by poor personal hygiene (hand washing) by food handlers.

One of four adults does not wash after changing a baby's diaper - creating a high risk of giving the care giver and other children infectious diarrhea and other diseases.
When we forget to wash our hands, or don't wash them properly, we can spread these microorganisms to other people, or give them to ourselves by touching our eyes, mouth, nose or a cut on the body. Think about the things you touch every day and how many people may have touched those objects before you.

Hand washing, when done correctly, is the single most effective way to prevent the spread of communicable diseases. Good hand washing technique is easy to learn and can significantly reduce the spread of infectious diseases among both children and adults. The bacterium that is carried on our hands does not usually pose a problem for a healthy person, but to anyone whose immune system is compromised, the result can be overwhelming infection - and sometimes death.

HOW SHOULD WE WASH OUR HANDS?
Good hand washing requires soap, warm running water. A quick rinse is not enough. You should rub hands together to make a lather. Do this away from the water so you don't wash the lather off. You need to wash palms, back of hands, fingers and under fingernails. Wash for 10-15 seconds then rinse under running water. When you wash your hands in public places place a paper towel over the taps before turning them off. When drying your hands use clean or disposable towels. There is more to hand washing than you think! By rubbing your hands vigorously with soapy water, you pull the dirt and the oily soils free from your skin. The soap lather suspends both the dirt and microorganisms trapped inside and are then quickly washed away. To prevent chapping use a mild soap with warm water; pat rather than rub hands dry; and apply lotion liberally and frequently.

WHEN SHOULD YOU WASH YOUR HANDS?
Wash your hands often and always: before and after eating; before and after handling food, especially raw meats; after using the bathroom; before and after changing a diaper or helping a child in the bathroom; after looking after a sick person; after sneezing, coughing or blowing your nose; before and after treating sores; before putting in or removing contact lenses; after any animal contact or after changing a litter box, and after gardening.

DOES ANTIBACTERIAL SOAP HELP?
Regular soap loosens skin oils that microorganisms live on. Friction and running water wash microorganisms off. Using antibacterial soaps has the same end result - microorganisms are washed away. Washing hands properly is more important than the type of soap used. Any type of soap may be used. However, bar soap should be kept in a self draining holder that is cleaned thoroughly before new bars are put out and liquid soap containers should be used until empty and cleaned before refilling. And if you use refillable liquid soap containers, wash them THOROUGHLY before refilling. Many studies have shown that bacteria grow in these refillable containers.

DO HAND WASHING GELS/WIPES WORK?
Alcohol based hand washing gels and hand wipes are an effective way of removing microorganisms; however, when hands are visibly dirty, they need to be washed with soap and water instead of gels or hand wipes. Alcohol rubs/gels/rinses are excellent hand disinfectants that contain more than 60% alcohol. They are widely used in the health care setting after washing hands or in situations when water is not available. Alcohol based hand disinfectants work best on clean skin, so if your hands are dirty, wash your hands with soap and warm water. Carefully dry your hands, as water on wet hands will dilute the alcohol content of the disinfectant product, decreasing its effectiveness. Apply enough disinfectant to make about the size of a dime onto your hands, enough when you rub your hands together to cover all areas of your hands, including under the nails. Use a rubbing motion to evenly distribute the disinfectant product for about 15 seconds, or until your hands feel dry whichever is longest.

WHAT ARE SOME MISTAKES I SHOULD AVOID REGARDING HAND WASHING?
◊ **DON'T** use a single damp cloth to wash a group of children's hands.
◊ **DON'T** use a standing basin of water to rinse hands.
◊ **DON'T** use a common hand towel. Always use disposable towels in day care or food preparation settings.
◊ **DON'T** use sponges or non-disposable cleaning cloths unless you launder them on a regular basis, adding chlorine bleach to the wash water. Remember that microorganisms thrive on moist surfaces. Microwaving **wet** sponges also gets rid of many bacteria.

Lab Period #1

MATERIALS:

MEDIA: One blood agar plate per student. You will work in pairs.

PROCEDURES:

In this experiment, you and your lab partner are going to choose two products to test or two procedures to test to determine which product or procedure removes the most bacteria from your hands. For example, you and your partner may decide to compare a regular soap with an antibacterial soap, a gel with soap, or compare drying hands with paper towel vs. air-drying. There are a number of options. Be creative. Once you have a plan, **check with your instructor before beginning the experiment** to make sure your plan will give you good results.

You are going to determine the effectiveness of the product or procedure by rubbing your fingers across the surface of an agar plate before and after using the product or procedure. The plates will then be incubated for 48 hours and then the number of colonies on the "before" and "after" sides of the plates will be counted. You will then determine the percent reduction in colonies on each plate to find out which product or procedure was more effective.

1. Each person will draw a line down the bottom of the dish using a Sharpie so that you have two halves. Label your plates, including which product or procedure you are testing with that plate. Be sure to write the active ingredients down in your worksheet for your report.
2. Also, mark one half "before" and the other half "after".

READ THE REMAINING INSTRUCTIONS THROUGH CAREFULLY BEFORE PROCEEDING ONTO THE NEXT STEP.

3. On the half that you marked "before" gently rub three fingers across the agar several times.
4. Then, each lab partner will wash their hands using the product or procedure agreed upon by the partners and the instructor. Do not touch any surfaces after you have washed your hands.
5. Once your hands have dried, gently rub the same fingers across the agar on the half marked "after" several times.
6. Place the lid on the Petri dish.
7. Place your plate into the incubator box agar side up for incubation at 37 degrees.

1. Gather supplies
2. Label each plate
3. Innoculate "Before" section
4. Wash hands according to individual test parameters
    a. Soap vs soap
    b. Drying variations
    c. Hand Sanitizer vs Soap
5. Innoculate "After" section
6. Seal and incubate

Lab Period #2

PROCEDURES:

1. Observe your hand washing plates. **Count the number of colonies on each side of the two plates and record yours <u>and your partner's results</u> on your worksheet**. To compare the two plates you will need to look at a percent reduction on your two plates. For example, assume plate A had 150 colonies on the "before" side and 70 colonies on the "after" side. Subtract the "after" from the "before" side and divide by the "before" side. Multiply by 100 to give you the percent reduction in colonies. This example gives you a 53.3% decrease in number of bacteria. Assume plate B had 34 colonies on the "before" side and 3 colonies on the "after" side. This gives you a 91.2% decrease in number of bacteria. Clearly plate 2 had the better result.

Report your data as % reduction in your report. NOTE: Some of you will have more colonies on the "after" side of the plate, so your % reduction will be a negative number.

Report (40 pts)

1. **Title page (2 pts)**
   a. Make a title page: This page should be the entire page and include a title of the experiment (be **<u>specific</u>** rather than general. DO NOT TITLE YOUR REPORT SOMETHING "CUTESY" AND NON-DESCRIPTIVE LIKE "HANDWASHING EXPERIMENT" for example. The title should be complete as regards the purpose of the experiment performed. A reader should be able to tell from the title what the purpose of the experiment was.). Underneath the title, put your name; and underneath your name, the name of your partner; then the date the report is due, and the class period. Everything must be there on the one page to get full points. Do not put this information at the start of the written report sections – this is a separate page. It is OK to add RELEVANT pictures.

   b. **Good titles**: "Variations of time spent hand washing and its effectiveness at removal of microbes from hands"; "Comparison of Ivory Soap and Citrus II Soap in their effectiveness at removing microbes from hands during handwashing"

   c. **Bad titles**: *"Does the type of soap you use really matter?"* (Matter for what? Not at all helpful to a potential reader as to the contents); *"Reduction of Microbes using Ivory Soap vs. Hibiclens Soap"* (Reduction where? Reduction of NUMBER OF microbes, not reduction of microbes – and state reduction from hands; be specific); *"What lives on your hands?"* (We were not determining which microbes inhabit your hands – you were trying to see if a product or procedure removed more microbes from your hands, regardless of who they were.)

2. **Abstract (2 pts)**: a **brief** explanation of experiment. Think of telling a friend what you are going to do in this experiment.
   a. What are you trying to show? Briefly describe your purpose.
   b. How are you going to show it? How are you going to execute the experiment?
   c. What products or procedures are you comparing?

3. **Introduction and purpose of the experiment (2 pts):** What question are you trying to answer?
   a. Do NOT just say you are trying to prove one product is "better" than another; define what "better" means.
   b. What products or procedures are you comparing?

4. **What is your hypothesis? (3 pts)**
   a. Tell me what you believe your results will show
   b. **WHY** do you think that??
      i. There should be a statement telling me which product or procedure you think will be more effective at removing microbes, but then tell me WHY you think that. **(There should be a "because" in the statement.)**

5. **What are the variables** in the experiment: **(2 pts)**
   a. **Independent (experimental) variable**. An experimental variable is what **differs** between the two tests. For example, the experimental variable may be a comparison of two products, one "regular" vs. an antibacterial soap; or between two methods, air-drying hands vs. towel-drying hands. The two products or procedures are not themselves <u>two</u> experimental variables – the variable is what is different between the tests.
   b. **Controlled variables**: Define as many controlled variables as possible – do not just give one or two. This includes the supplies, the equipment, the conditions and time of incubation, water temperature, etc. The list is quite long.

6. **List the supplies and equipment** you are using **(2 pts)** – for example, brand name of products **(INCLUDING ACTIVE INGREDIENTS)**, paper towels, blood agar plate, incubator, etc. There are more things used than you may think of initially. **Be complete**.

7. **List the steps <u>in detail (4 pts)</u>** that will be used. This should be complete enough that a person in a different research lab could replicate exactly what you did.
   a. Make sure you list the steps used by **<u>both</u>** students.
   b. Don't forget to discuss what you did on the second lab day.

8. **Results**: Report results for **both partners**, not just yours. You are trying to compare products or procedures so both sets of data are needed to make a comparison and decide which product or procedure you will recommend.
   a. **Make a brief written statement of your results. (2 pts)** This is NOT the place to draw conclusions about your results – just report the results here. Conclusions belong in the Discussion section.
      i. Which product or procedure showed the **greatest percent reduction**?
      ii. Describe how the **number of colony types** might have changed from the before and after sides of both plates.
   b. Show your calculations for percent reduction (2 pts) in t. Remember, if you had an increase in number on the "after" side, the number will be negative.

   c. To support your statement, **make a table ((1 pt)** showing the actual number of colonies you counted on both plates and sides of both plates, and the % reductions. See the examples below for how the table should look. USE THE NAMES OF THE PRODUCTS OR PROCEDURES IN YOUR DATA REPORTING – DO NOT USE "PLATE A" OR "PLATE B", OR THE NAMES OF YOU AND YOUR PARTNER. Examples:

Good example

Product	# of colonies	
Avagard D gel	100	25
Softcide	100	25

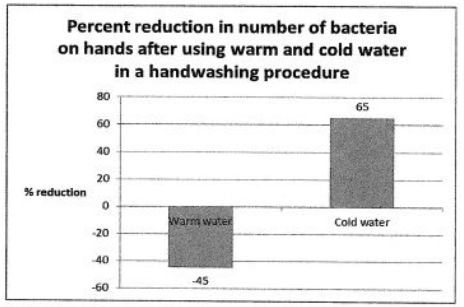
Bad examples

Product	# of colonies	
Plate A	100	25
Plate B	100	25

Product	# of colonies	
Mary	100	25
George	100	25

   d. Also, to support your statement, record differences in types of colonies (1 pt) you might have seen on each side of both plates
      i. Colonial morphology, color, size, etc.
      ii. Record the **change in the number** of different **types** of colonies on the two sides (before and after?

   e. Then graph the % reduction for both plates (3 pts).
      i. Use a bar graph. (See below for an example).
      ii. **<u>Include a title</u>** that is descriptive of your experiment, **<u>put "percent reduction" on the Y axis</u>**, and **label the two bars with either the name of the product or procedure**.
      iii. **Do not use your name or your partner's name as labels, or just "A" or "B"** to describe which bar goes with the product or procedure. I do not want to have to hunt around to figure out which data goes with which procedure or product.



9. **Discussion: (4 pts)**. Discuss your results.
   a. Did the results support your hypothesis or not? **Explain**.
      i. If they did, why do you think your product or procedure really **is** better at reducing microbes from hands?
      ii. If the results were **not** what you expected, **what do you think this means? What possible explanation might there be for these results?**
      iii. If you saw an increase in numbers of bacteria after the procedure, explain why that might have happened.
      iv. How did the number of types of organisms change on before and after plates? How does this relate to how effective the product or procedure was?

10. **Flaws and improvements: (3 pts)**
    a. Were there flaws in your experimental design or execution of the experiment? What were they? No experiment is ever without flaws. Did you forget to use a paper towel to turn off the water and that contaminated your hand? Did you wash your hands just before the start of the experiment so many microbes were already washed off? Did you use warm water and your partner use cold? Did you time washing and drying so they were the same? There are lots of things to think of here.

    b. What would you do next time to improve the quality of your data? **Hint: this is just one experiment you and your partner are doing – can you claim the results as FACT? How would you improve the believability of the results?** How would you redesign the experiment to get better data?

11. **Recommendation: (2 pts)** Based on **YOURS AND YOUR PARTNER'S DATA**, which product or procedure would you recommend to others? In other words, which product or procedure actually showed the greatest percent reduction **in your experiment**? **Do not** tell me that you recommend a product or procedure that did not perform as well in your experiment – this recommendation is based on **your actual data only**.
    a. However, if your data are contrary to common sense, you might explain why you might give an opinion as to why you might not actually recommend this product or procedure, and **tell me why**. This is your chance to give an opinion, but don't forget the first part – the data.

12. **Worksheet: (5 pts)** Attach the completed worksheet to the back of the report.

Lab 5 Hand washing

1. What independent (experimental) variable did you use in your experiment? For example, air drying vs. towel drying, or antibacterial soap vs. sanitizer.

_____

a. If you used different products as your variable, be sure to list the active ingredients.

A. Name of product (and active ingredients) or procedure:_____

B. Name of product (and active ingredients) or procedure:_____

2. Indicate the actual number of colonies you counted on the before and after sides of each plate in the table below. **Calculate the percent reduction for each plate**. Remember that if you had an increase in numbers on the after side, this means you will have a negative percent reduction.

Calculations here and in report (show your work). (Name product or procedure for

each calculation)

A.

B.

	Before	After	% reduction
Product or procedure A			
Product or procedure B			

2. Describe the appearance of yours and your partner's plates after they have been incubated (i.e. – do the colonies look the same on both sides of the plate? Do you see an increase or decrease in the type of colonies on the two sides? Did you see any fungal growth on either side?) Determine how many types you see on all plates and sides of plates. Did you get fewer types on the "after" sides?

_____

_____

_____

_____

_____

3 Could any of your colonies be from "normal flora"? Are these bacteria likely to cause disease?

_____

_____

_____

4 Why do surgeons wash their hands for many minutes instead of just 15 seconds?

_____

_____

_____

Chemical Agents Lab

LAB 7 EFFECTS OF CHEMICAL AGENTS ON BACTERIA – DISINFECTANTS, ANTISEPTICS, NATURAL PRODUCTS AND CHEMOTHERAPY AGENTS (ANTIMICROBIAL AGENTS) TESTING USING THE KIRBY-BAUER METHOD

INTRODUCTION:

Microbes are nearly everywhere; they are found in the water we drink, the air we breathe, and the earth we walk on. They live in and on our bodies. Microbes occupy ecological niches on all forms of life and in most environments. In most situations, the ubiquitous microorganisms are harmless.

Disinfectants are used on inanimate surfaces, antiseptics mainly on skin and mucous membrane surfaces of the body, and chemotherapeutic agents control multiplication of bacteria within the body. Disinfectants are usually strong inorganic or organic chemical compounds while antiseptics are weaker and less toxic to living tissues. Disinfectant solutions (germicides) are evaluated by determining their phenol coefficient.

Chemotherapeutic agents which are ingested (as pills) or injected are evaluated by determining their chemotherapeutic index which is their toxic dose versus their effective dose. In a clinical setting, choosing a treatment regimen for a bacterial infection has several components. First, the pathogen must be isolated from the normal flora organisms present in the specimen. Once isolated, the organism must be identified. Then the pathogen will be tested for its sensitivity to a number of antimicrobial agents to help the clinician choose the one that will be best for the patient. This choice is based not only on the organism's sensitivities, but also upon the effects the antimicrobials may have on the patient. For example, the aminoglycoside antibiotics are ototoxic, that is, they cause permanent hearing loss by damaging the vestibulocochlear nerve, and nephrotoxic, causing kidney damage. The Kirby-Bauer method of antibiotic sensitivity testing provides the clinician with a number of possible antimicrobials from which to choose the best.

Since microbial groups, bacterial genera, species and even strains vary widely in their sensitivity to disinfectants, antiseptics and especially to chemotherapy agents; it is a routine procedure to test a given isolated pathogen for its sensitivity to selected chemotherapy agents. The Kirby-Bauer method involves swabbing the bacteria onto an agar plate to obtain a lawn. A bacterial lawn is confluent growth of bacteria that covers the entire surface of an agar plate. Small paper disks that are impregnated with an antibiotic agent are placed on the inoculated agar. The disks are saturated with a known concentration of the agent and allowed to dry. As soon as the disk comes in contact with the agar the agent diffuses away from the disk. The plate is incubated for 24-48 hours. During this time, the bacteria divide and the antimicrobial agents diffuse into the agar. If the organism is susceptible to the agent, the agent will inhibit or kill the organism as long as the concentration of the agent is high enough. In other words, immediately surrounding the disk the concentration of the agent is the highest.

If an antimicrobial agent has an effect on the microbes, a zone of growth inhibition will appear around the disk. For a given antimicrobial, the diameter of the **zone of inhibition** of growth correlates with the sensitivity of the organism to clinically achievable levels of the drug. Generally, the larger the zone, the more sensitive the organism is to that agent. The size of the zone is also determined by the ability of the drug to diffuse into the agar. If the zone of inhibition for one antimicrobial is larger than that of another, it does not necessarily mean that the microbe is more sensitive to the drug producing the larger zone. It could simply mean that one drug is able to diffuse farther than the other. Because of this, tables have been constructed that correlate the zone size with sensitivity to the antimicrobial agent tested. The organism may be described as **sensitive, resistant, or of intermediate sensitivity** to the drug.

- **Sensitive** – the zone of inhibition is greater than or equal to the diameter for susceptibility on the standard table.
- **Resistant** – the zone of inhibition either does not exist (the organisms were not affected by the agent and grew up to the disk) or is smaller than the diameter for resistance on the standard table.
- **Intermediate sensitivity** to the drug – the zone of inhibition is in between the diameter for susceptibility and resistance. The drug is probably not effective.

These sensitivity tables are continually being revised and updated as new resistant strains of bacteria develop. Antibiotic sensitivity testing cannot be used to identify a particular species of microbe. One strain of a microbial species may show sensitivity to an antibiotic while another strain of the same species may be resistant to it. (The genes that code for antibiotic resistance can be exchanged between bacteria of different genera on extrachromosomal pieces of DNA called plasmids.)

It must always be kept in mind that the interpretation of the effectiveness of an antibiotic *in vitro* (in the laboratory) does not necessarily correlate with what will happen *in vivo* (in the body). The interaction of drug-host-microorganism is much more complicated than the situation in the laboratory. The ability of the drug to reach the site of infection and the ability of the drug to exceed the minimum levels needed to be effective are both factors (the minimal inhibitory concentration, or MIC). This MIC can be affected by the route of administration of the drug, the amount administered, and how quickly the drug is removed by the body. Or the microbe may have developed resistance in the body after the sample was taken.

PURPOSE:

The purpose of this exercise is to demonstrate the variation in bacterial response to different disinfectants, antiseptics, natural products and chemotherapy agents (antimicrobial agents). This is accomplished by measuring the zones of inhibition surrounding the disks.

Lab Period #1

Groups of 2 students

MATERIALS:

Microbiology kit
Sterile cotton swabs
Spot plates – 1 per group
Sterile blank discs – 6 disks per group
Antibiotic sensitivity discs (ampicillin, cefazolin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, penicillin, streptomycin, tetracycline, and vancomycin)

MEDIA:

Mueller-Hinton agar (MHA) plates – 3 per group

CULTURES:

In broth:
- *Escherichia coli*
- *Staphylococcus aureus*
- *Bacillus subtilis*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Proteus vulgaris*

REAGENTS/CHEMICALS/NATURAL PRODUCTS:

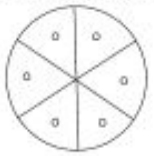
3% hydrogen peroxide
Vinegar
10% bleach
Simple Green d Pro 5
95% EtOH
Oregano oil
Betadine
Tea tree oil
Green tea extract
Grapefruit seed extract
Fresh garlic

PRECAUTIONS:

95% EtOH: Avoid contact with eyes. Wash thoroughly after handling. **DANGEROUS FIRE RISK! POISONOUS!**

IN CASE OF CONTACT WITH ANY OF THE ABOVE CHEMICALS, FLUSH EYES WITH WATER FOR AT LEAST FIFTEEN MINUTES.

1. Each group will be assigned one bacterial culture to be swabbed on three MHA plates.
2. Dip the sterile swab into the broth culture. Swab the entire surface of the three MHA plates with the culture, replenishing the inoculums between plates from the tube. Swab in three directions. Properly dispose of used swab in biohazard bag. DO NOT PUT IT BACK IN WRAPPER. Place wrapper in trash.
3. Each group will use one swabbed plate for products on blank disks to be prepared by the group (see # 5 below). Use the remaining two swabbed plates for commercially prepared antibiotic disks.
4. Label the bottom of each plate with name, date, medium, and your organism. There is no need to write the names of the antibiotics on the plate as the disks are labeled. Using the disk dispenser, have the instructor place 12 antibiotic disks on two plates (6 per plate). Sterilize your forceps by dipping them in 95% ethanol. Set them aflame briefly to burn off the alcohol. Lightly tap down the disks with the sterile forceps.



5. Divide the third plate into 6 sectors by drawing lines with your Sharpie on the bottom of the plate as above. Label each sector with the name of the 6 products you selected on the bottom of the plate. On your worksheet, also list the products you used in this experiment. For product testing, place **1-2 drops** of each product selected by your group in a well on the spot plate. (NOTE: It helps to place a piece of tape along the sides of the spot plate and write the names of the products on the tape near the well so you know which is which.) After sterilizing the forceps, pick up a dry, sterile disk and dip one end into the product. Allow product to move up the disk by capillary action. Drain off excess solution and carefully place the disk on the pre-marked, pre-swabbed plate. Tap it gently into place so it will not fall off when the plate is inverted for incubation. **DO NOT PLACE A SOAKING WET DISK ON THE PLATE! IF YOU DO NOT DRAIN OFF EXCESS SOLUTION, YOU WILL NOT GET READABLE RESULTS.**
6. Clean and resterilize the forceps between each product. Repeat the procedure for the remaining product solutions.
7. Invert all three plates and incubate at 37°C for 48 hours.

Lab Period #2

MATERIALS: Rulers

PROCEDURES:
Measure the diameter of the zone of inhibition of each disk in millimeters. Record the information on the worksheet, and compare the zone of inhibition with Table 1: Significance of Diameters of Zone of Inhibition. Determine whether your diameter signifies resistance, intermediate sensitivity, or sensitivity of the organism to the antibiotic. For the third plate with various products you selected, there is no table to indicate if the zone of inhibition indicates effectiveness of the product. Use your judgment, but as a guideline, if the zone is 10 mm or smaller, it is probably not effective.

- When using the chart below, first determine if you have a G+ or G- organism.
- Then look for the line that matches the appropriate Gram reaction. NOTE: The *Enterobacteriaceae* are all G- rods that live in the colon.
  - So if your organism is G-, line 1 or 2 will contain the data you use.
  - If your organism is a Gram- organism, some antibiotics are not shown to work for those, so there is no line to use – "none" in the table below indicates that that antibiotic should not be effective against that organism.
    - That indicated the antibiotic should not inhibit the organism.
  - So, any zone of inhibition in those situations would indicate sensitivity. For example, erythromycin, penicillin and vancomycin only show data for Gram+ organisms. These drugs are not supposed to inhibit G- organisms. **If you had a zone of inhibition of any size with your Gram- organism, consider the antibiotic effective (organism is sensitive to it) and place an "S" on the worksheet.**

To assist you further, here is a list of the organisms and line of data in the table you should use for that organism. If "none" is listed below, no lines apply, so use the guidelines above.

	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*S. marcescens*	*P. vulgaris*
Amp	1	2	2	1	1	1
Cef	1	1	1	1	1	1
Chlor	1	2	2	1	1	1
Eryth	none	1	1	none	none	none
Gent	1	2	1	1	1	1
Kan	1	1	1	1	1	1
Neo	1	1	1	1	1	1
Novo	1	1	1	1	1	1
Pen	none	1	2	none	none	none
Strep	1	none	1	1	1	1
Tet	1	2	2	1	1	1
Van	none	1	2	none	none	none

Record the antibiotic and other product information on the sheets provided for the class to share. Once all data is on the sheet, I will give you all a copy of the class data.

TABLE 1: SIGNIFICANCE OF DIAMETERS OF ZONE OF INHIBITION

Antimicrobial Agent	Resistant	Intermediate	Sensitive
Ampicillin (AM10) 10 mcg			
Gram negatives, *Enterococcus* sp.,	≤12	12 – 13	≥13
Staphylococcus	≤28	-----	≥29
Cefazolin (CZ-30) 30µg			
Enterobacteriaceae and *Staphylococcus*	≤14	15 – 17	≥18
Chloramphenicol (C-30) 30µg			
Enterobacteriaceae, P. aeruginosa, Staphylococcus, Enterococcus	≤12	13 – 17	≥18
Streptococcus pneumoniae	≤20	-----	≥21
Streptococcus (non *S. pneumoniae*)	≤17	18 – 20	≥21
Erythromycin (E-15) 15µg			
Staphylococcus, Enterococcus	≤13	14 – 22	≥23
S. pneumoniae & other streptococci	≤15	16 – 20	≥21
Gentamicin (GM-10) 10 µg			
Enterobacteriaceae, P. aeruginosa, Acinetobacter and *Staphylococcus*	≤12	13 – 14	≥15
Kanamycin (K-30) 30µg			
Enterobacteriaceae & Staphylococcus	≤13	14 – 22	≥23
Neomycin (N-30) 30µg	≤12	13 – 16	≥17
Novobiocin (NB-30) 30µg	≤17	18 – 21	≥22
Penicillin (P-10) 10 units			
Staphylococcus	≤28	-----	≥29
Enterococcus	≤14	-----	≥15
Streptococcus (non *S. pneumoniae*)	-----	-----	≥24
Streptomycin (S-10) 10µg			
Enterobacteriaceae	≤11	12 – 14	≥15
Tetracycline (Te-30) 30µg			
Enterobacteriaceae, P. aeruginosa, Staphylococcus, Enterococcus	≤14	15 – 18	≥19
S. pneumoniae & other streptococci	≤18	19 – 22	≥23
Vancomycin (Va-30) 30µg			
Staphylococcus	-----	-----	≥15
Enterococcus	≤14	15 - 16	≥17
S. pneumoniae and other streptococci	-----	-----	≥17

1. Create plates with bacteria and antimicrobial disks
2. Incubate
3. Review the Zones of Inhibition
4. Identify Resistant vs Intermediate vs Sensitive

Lab 7 Effects of Chemical Agents on Bacteria

Disinfectants, Antiseptics and Chemotherapy Agents (Antibiotic Sensitivity) Testing Using the Kirby-Bauer Method

Name_____Date _____

Name of organism tested by your group: _____

	Zone (mm)
Ampicillin	
Cefazolin	
Chloramphenicol	
Erythromycin	
Gentamicin	
Kanamycin	
Neomycin	
Novobiocin	
Penicillin	
Streptomycin	
Tetracycline	
Vancomycin	

1. Get class data on all organisms and antibiotics. When 2 groups had data on the same organism, take the average and enter below. Examine the data to determine whether or not the organism was resistant (R), sensitive (S) or intermediate (I) in sensitivity.

	E. coli		*S. aureus*		*B. subtilis*		*P. aeruginosa*		*S marcescens*		*P. vulgaris*	
	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S
Ampicillin												
Cefazolin												
Chloramphenicol												
Erythromycin												
Gentamicin												
Kanamycin												
Neomycin												
Novobiocin												
Penicillin												
Streptomycin												
Tetracycline												
Vancomycin												

2. Using the above data, determine which of the tested **antibiotics** would be suitable for control of each organism: (**List all** antibiotics useful for each organism, using abbreviations for the antibiotics, if desired)

E. coli _____

S. aureus _____

B. subtilis _____

P. aeruginosa _____

S. marcescens _____

P. vulgaris _____

3. Evaluation of the NON-ANTIBIOTIC products. Write down the zone of inhibition for each product you tested.

	Zone (mm)
Hydrogen peroxide	
Vinegar	
10% Bleach	
Simple Green	
Ethanol	
Oregano	
Betadine	
Tea tree oil	
Green tea extract	
Grapefruit seed extract	
Garlic	

4. Get class data on all organisms and products tested (other than antibiotics). When 2 groups had data on the same organism, take the average and enter below. Examine the data to determine whether or not the organism was resistant (R), sensitive (S) or intermediate (I) in sensitivity to each product. (Assume <10mm = resistant, 10-12mm = intermediate, and >12mm = sensitive.)

	E. coli		*S. aureus*		*B. subtilis*		*P. aeruginosa*		*S marcescens*		*P. vulgaris*	
	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S	Zone	R,I,S
Hydrogen peroxide												
Vinegar												
10% Bleach												
Simple Green												
Ethanol												
Oregano												
Betadine												
Tea tree oil												
Green tea extract												
Grapefruit seed extract												
Garlic												

5. Using the above data, determine which products might be useful in control of these organisms?

E. coli _____

S. aureus _____

B. subtilis _____

P. aeruginosa _____

S. marcescens _____

P. vulgaris _____

6. Why was this set of organisms chosen for this experiment? In other words, what is it about these organisms that we chose to test them in this lab? (Think carefully about this. If you understand the purpose of the lab and what we discussed in lecture about differences in organisms, you will get it.)

_____

_____

_____

_____

7. What is the significance of MRSA in the hospital environment and the community? What is MRSA? What qualities does MRSA have that makes it so important to us? How prevalent is it?

_____

_____

_____

_____

8. Distinguish between disinfectants and antiseptics.

_____

_____

_____

9. Why would a doctor want to know the results of antibiotic susceptibility testing on organisms from his/her patient?

_____

_____

_____

Urinary Tract Pathogens Lab

LAB 9 URINARY TRACT PATHOGENS LAB

INTRODUCTION:

Urinary Tract Infections (UTI) are the most common types of nosocomial infections. As health care workers, you will encounter many patients with UTI's. Lower UTI's must be treated promptly and aggressively, since they tend to travel upwards within the urinary tract and even beyond it into the bloodstream.

A bladder infection (cystitis) in an otherwise healthy adult is a painful annoyance. Pyelonephritis, though, is potentially life threatening, as it can lead to acute renal failure. Women are more likely than men to get UTI's due to the shorter length of their urethras. Catheterized patients are prime targets for UTI's; approximately 20% of catheterized patients get them. Older patients, chronically debilitated patients, and immunosuppressed patients are all potentially victims. In the nursery, a nosocomial UTI in a neonate can progress into neonatal sepsis.

You, as a health care worker, can help prevent UTI's in your patients by doing scrupulous hand washing, doing catheter care routinely, and maintaining sterile technique during urinary catheterization. It is also important that you monitor your patients for early symptoms of UTI, since prompt diagnosis and treatment improve prognosis. Urinary frequency and urgency and burning pain on urination are classic symptoms of UTI. These are not always present, or your patient may not be able to describe them to you. So you must also examine the urine itself for indications of UTI. These are cloudiness, foul odor, and alkaline pH. Normal urine is clear yellow with no foul odor. Normal pH of urine is 4.8 to 8.0.

PURPOSE:

The purpose of this exercise is to learn what the clinical medical technologist does to aid in the diagnosis and treatment of UTI's. You and your lab partners will receive a sample of urine contaminated with one of the following urinary tract pathogens:

Escherichia coli	*Enterococcus faecalis*	*Citrobacter freundii*
Staphylococcus aureus	*Pseudomonas aeruginosa*	*Proteus vulgaris*

You will determine the extent of bacterial contamination in the urine. This information is useful to the clinician in deciding how serious the infection is. You will also determine the scientific name of your contaminant, as well as to which antimicrobial agents it is sensitive. This is the information that appears on a culture and sensitivity (C & S) report.

Lab Period #1

MATERIALS:

Groups of 4 students

Pipettor - blue
Bacterial spreader and alcohol jar
1-ml sterile pipettes – 12 per group
Vortexer

MEDIA:

4.5 ml sterile 0.9% saline blank – 6 per group
PEA plate – 1 per group
Brain- heart infusion broth (BHIB) – 1 per group
NA plate – 6 per group
DLA plate – 1 per group

CULTURES:

Urine sample inoculated with bacteria – 1 per group

REAGENTS/CHEMICALS:

95% EtOH (ethyl alcohol)

PRECAUTIONS:

95% EtOH: Avoid contact with eyes. Wash thoroughly after handling. **DANGEROUS FIRE RISK! POISONOUS!**

IN CASE OF CONTACT WITH ANY OF THE ABOVE CHEMICALS, FLUSH EYES WITH WATER FOR AT LEAST FIFTEEN MINUTES.

1. Urine Gross Exam
2. Inoculate BHIB & Selective Media
   a. PEA G+, DLA G-
3. Create Dilutions of Urine Sample
4. Inoculate NA Plates
5. Incubate

PROCEDURES:

You and your lab partners will receive a sample of contaminated urine. (The urine is artificial urine, made from chemicals in the microbiology prep area.) During this lab period, you will perform a serial dilution and inoculate a streak plate on differential and selective growth media (refer to the handout on - Culture Media, Growth Characteristics, And Culture Techniques, if you need to refresh your memory regarding selective and differential growth media) to isolate the pathogen for later identification.

Step A: Examination of Urine Specimen.

1. Examine the urine specimen. Look at its color. Is it cloudy or clear? Is there sediment? Does it have a strong or unpleasant odor?

Step B: Isolation on Selective and Differential Growth Media.

1. Label your DLA and PEA plates with the patient's name, medium, date and your names.
2. Tighten the cap on the specimen cup and shake it 25 times to re-suspend the organism.
3. Using a sterile inoculating loop, make streak plates for isolation on PEA and DLA.
4. Incubate the plates, agar side up, at 37°C for 48 hours.

Step C: Serial Dilution.

1. Obtain six 4.5-ml sterile saline blank tubes and label them 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Also, get six nutrient agar (NA) plates for the enumeration of bacteria per millimeter of urine sample, and 12 sterile 1.0 ml pipettes. Label the NA plates to match the tubes. Get a pipettor, too.
2. Using a sterile 1.0 ml pipette and a pipettor, transfer 0.5 ml of urine into the tube marked 10^{-1}. Now you have a 1:10 dilution. Mix the microbes and saline together using the vortexer. Place the pipette in the bleach container for disposal.
3. Using a fresh pipette, transfer 0.5 ml of suspension from tube 10-1 to tube 10-2. This is a 1:100 dilution. Mix as in step 3.
4. Using a fresh pipette each time, repeat step four, pipetting from tube 10-2 to tube 10-3, from 10-3 to tube 10-4, from 10-4 to tube 10-5, and from 10-5 to tube 10-6.

Step D: Plate Counts.

1. Using a fresh pipette each time, transfer 0.1 ml of fluid from each tube into its matching plate of nutrient agar.
2. You will be spreading the bacteria over the entire surface of the plate using a bacterial spreader. Dip the spreader into the 95% EtOH, ignite the alcohol on the spreader in the Bunsen burner and let the alcohol burn off.

   DO NOT KEEP THE SPREADER IN THE FLAME – IT WILL GET TOO HOT! THE SPREADER MUST BE COOL BEFORE TOUCHING THE BACTERIA ON THE PLATE OTHERWISE YOU WILL KILL THE BACTERIA!

   Using the **sterile bacteria spreader** spread the 0.1 ml sample over the entire surface of the agar.
3. Dip the contaminated spreader into the alcohol then pass it through the flame and allow the alcohol to burn off. **THE SPREADER MUST BE COOL BEFORE GOING ON TO THE NEXT PLATE.**
4. Repeat until all plates have had the bacterial sample spread evenly over the plate count agar.
5. Incubate at 37°C for 48 hours.

Step E: Gram Stain and Subculture.

1. Inoculate a tube of BHIB from the urine sample for Gram staining next period.
2. Incubate at 37°C until next class period.

Lab Period #2

MATERIALS:

Hand tally counter

MEDIA:

Mueller-Hinton plate – 2 per group
Lactose broth – **1 per group, only if needed**
Simmon's citrate tube – **1 per group, only if needed**
MRVP tubes – **1 per group, only if needed**
Antibiotic sensitivity discs (ampicillin, cefazolin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, penicillin, streptomycin, tetracycline, and vancomycin)

REAGENTS/CHEMICALS:

95% EtOH (ethyl alcohol)
3% H_2O_2 (hydrogen peroxide)

IN CASE OF CONTACT WITH ANY OF THE ABOVE CHEMICALS, FLUSH EYES WITH WATER FOR AT LEAST FIFTEEN MINUTES.

PROCEDURES:

Step A: Quantitative Plate Count.

1. Choose one of your NA plates from Lab Period #1 that has more than 25 but less than 250 colonies on it.

2. Using a hand tally counter and a Sharpie, count all the colonies in the plates. Use a systematic approach so as not to skip any colonies.

   a. Now you can calculate the number of bacteria that were in the original sample. First, put the number of colonies you counted into scientific notation. For example, you counted 237 colonies, which would be 2.37×10^2. Then multiply this number by the reciprocal of the dilution factor. For example, if the dilution factor was 10^{-3}, the reciprocal is 10^3. Multiple by the reciprocal of the sample volume (10^1). Remember, when you multiply exponential numbers, you add the exponents together. So if, for example, you counted 237 colonies on your 10^{-3} plate, the calculation would look like this:

[# of colonies] [reciprocal of the dilution factor] [reciprocal of sample volume] = cfu per ml of original sample

$$(2.37 \times 10^2)(10^3)(10^1) = 2.37 \times 10^6 \text{ cfu/ml}$$

Step B: Gram Stain.

1. Perform a Gram stain from your subculture in the BHIB tube prepared in Lab Period #1.

Step C: Examination and Further Study of Streak Plates.

1. Examine your PEA and DLA plates from Lab Period #1. Correlate what you see with the results of your Gram stain. If they do not correlate (you see growth on both DLA and PEA plates), see the instructor.
   a. If you have a Gram-positive coccus, do a catalase test to differentiate between *Staphylococcus aureus* (catalase +) and *Enterococcus faecalis* (catalase -). **DO NOT DO THIS FROM A BROTH. USE A COLONY FROM ANY OF YOUR NA PLATES TO DO THE CATALASE TEST.** Just drop the hydrogen peroxide directly on the colonies and observe for bubbling.

   b. If you have a Gram-negative rod, inoculate a Simmons citrate tube, MRVP broth, and lactose broth.

Step D:

1. **EVERYONE WILL DO THIS STEP NO MATTER WHAT KIND OF MICROBE YOU HAVE.** From the tube of BHIB you inoculated in Lab Period #1, do a swab inoculation of two plates of Mueller-Hinton agar for the Kirby-Bauer antibiotic sensitivity test. Totally cover the plate so you will have a lawn of bacteria. There are twelve different antimicrobial discs; have the instructor place six different disks on each plate. Follow the procedure outlined from the Effects of Chemical Agents exercise you performed earlier in the semester.

Lab Period #3

MATERIALS:

Ruler

MEDIA:

CULTURES:

REAGENTS/CHEMICALS:

Methyl red reagent

PRECAUTIONS:

IN CASE OF CONTACT WITH ANY OF THE ABOVE CHEMICALS, FLUSH EYES WITH WATER FOR AT LEAST FIFTEEN MINUTES.

PROCEDURES:

Step A: Final Identification of Gram-negative Rods.

1. Determine if your organism ferments lactose by examining the lactose broth. If it does, go to Step 2 and look at the citrate tube. If it does not ferment lactose, go to Step 3 and perform the methyl red test on the MRVP tube.
2. If your organism fermented lactose and the citrate test is positive, you have ***Citrobacter freundii.***. If the citrate test is negative you have ***E. coli***.

3. If your organism did not ferment lactose, using your MRVP tube perform a methyl red test. (See Biochemical Lab for instructions.) If it is **positive**, your microbe is ***Proteus vulgaris***. If the methyl red test is negative, your organism is ***Pseudomonas aeruginosa***.

Step B: Antibiotic Sensitivity.

1. Using the Significance of Diameters of Zone of Inhibition table from the Effects of Chemical Agents exercise, determine the sensitivity of your bug to the various antimicrobial agents tested.

1. Examine and calculate colony forming units in original sample
2. Create Antibiotic sensitivity plates, Incubate
3. Identify bacteria and Bacterial effectiveness

Use the table below to determine the adverse side effects of antimicrobials.

CHARACTERISTICS OF SELECTED ANTIMICROBIALS

ANTIMICROBIAL AGENT	ACTION	MAJOR ADVERSE EFFECTS	OTHER PROBLEMS
PENICILLINS Penicillin-G Ampicillin Amoxicillin	Bactericidal. Inhibits proper formation of cell wall. G+ coverage with newer agents having both G+ and G- coverage.	Allergic reactions, rash, anaphylaxis. Seizures at very high doses in patients with renal failure.	Resistance (penicillinase).
AMINOGLYCOSIDES Gentamicin Streptomycin Neomycin	Bactericidal. Inhibits protein synthesis. Reserved for serious G- infections.	Ototoxicity, nephrotoxicity, irreversible 8th cranial nerve damage.	Must be given by injection.
TETRACYCLINES Tetracycline Doxycycline Minocycline	Bacteriostatic or bactericidal. Interferes with protein synthesis. Broad spectrum, some rickettsiae, mycoplasm, and *Chlamydia*.	Discoloration of tooth enamel if used in infants and children. Irritation at site of injection.	Superinfection. Oral agents are inactivated by polyvalent cations like Ca^{2+}, Mg^{2+}, etc.
CEPHALOSPORINS Cephalothin Cefazolin Ceftriaxone	Bactericidal. Inhibits proper formation of cell wall. Narrow to broad spectrum, depending on the agent being used.	Allergic reactions, rash, anaphylaxis. CNS effected at high doses.	Contraindicated for patients with true penicillin allergy.
MACROLIDES Erythromycin Azithromycin Clarithromycin	Bactericidal. Inhibits with protein synthesis. Primarily G+ coverage, with mycoplasma and *Chlamydia* coverage. Azithromycin covers some G- organisms.	Nausea, vomiting, diarrhea, severe abdominal cramping.	
QUINOLONES Ciprofloxacin Levofloxacin	Inhibition of nucleic acid replication and transcription. Broad spectrum, usually reserved for G- infections.	Photosensitivity, superinfection.	Indiscriminate and overuse is resulting in development of resistant organisms.
CHLOROMPHENICOL	Bactericidal. Inhibits cell wall synthesis or protein synthesis. Broad spectrum, also effective against some rickettsiae, mycoplasm, and *Chlamydia*.	Bone marrow suppression. Irreversible aplastic anemia. Indicated for severe intractable infections.	
SULFONAMIDES Sulfisoxazole Sulfamethoxazole Trimethoprim	Bacteriostatic. Broad spectrum. Inhibits folic acid synthesis in the microorganism.	Renal impairment due to crystal formation.	Vitamin K and folic acid deficiency with high dose and long duration of therapy.
IMIDAZOLES Ketoconazole	Static antifungal agent. Inhibits RNA and protein synthesis.	Anorexia, hepatotoxicity.	Requires gastric acid for absorption; do not use with agents that ↑ gastric pH.
TRIAZOLES Fluconazole Itraconazole	Static antifungal agent. Inhibits proper membrane synthesis.	GI and hepatic effects.	(Does not require gastric acid for absorption.)
POLYENES Amphotericin B Nystatin (oral/topical)	Fungicidal agents. Alters cell permeability.	Anorexia, hypokalemia, thrombophlebitits with Amphotericin B.	Shaking chills and malaise with IV infusion of Amphotericin B.
RIFAMPIN	Bactericidal. Inhibits RNA synthesis. Used to treat TB.	Hepatotoxicity.	
VANCOMYCIN	Bactericidal. Inhibits cell wall synthesis in G+ organisms.	Nephrotoxicity & ototoxic.	Systemic activity only when given by injection.
NOVOBIOCIN	Inhibits DNA synthesis	Hepatotoxicity	
KANAMYCIN	Inhibits protein synthesis	Hearing loss or ringing in the ears; kidney toxicity	

SAMPLE CLINICAL REPORT

Had you actually sent the patient's urine specimen to the clinical laboratory, you would likely receive a report that looks something like the one below:

URINALYSIS WITH MICROSCOPIC

Collected 12/04/2003 09:00

TEST	RESULT	NORMAL RANGE
COLOR	Yellow	
APPEARANCE	Clear	
SPECIFIC GRAVITY	1.012	1.005-1.030
pH	6.5	5.00-8.00
PROTEIN	Negative	Negative
GLUCOSE	Negative	Negative
KETONES	Negative	Negative
BILIRUBIN	Negative	Negative
OCCULT BLOOD	1+	Negative
NITRITE	Positive	Negative
LEUKO SCREEN	3+	Negative
RBC	6	None Seen
WBC	>100	None Seen
BACTERIA	OCC	None Seen
URINE CULTURE	IS INDICATED WATCH FOR SEPARATE REPORT	

URINE CULTURE

Collected 12/04/2003 09:00

SPECIMEN DESCRIPTION	URINE CATH	
PRELIMINARY RESULT	>100,000 CFU/ml	*Enterobacter sakazakii*
FINAL RESULT	>100,000 CFU/ml	*Enterobacter sakazakii*
	>100,000 CFU/ml	*Enterococcus* species

SENSITIVITY

ORGANISM	>100,000 CFU/ml	*Enterobacter sakazakii*
RESULTS	Ampicillin	< = 13 Resistant
	1st Gen. Cephalosporin	< = 14 Resistant
	Ciprofloxacin	> = 0.5 Susceptible
	Nitrofurantoin	> = 32 Susceptible
	Trimeth-Sulfamethoxazole	> = 12 Susceptible

Test performed at Fictitious Medical Center
5901 S. Calle Santa Cruz
Tucson, AZ 85709

Reported 12/06/2003 14:43

GOAL:

Bring it back to the clinical significance

Lab 9 Urinary Tract Pathogens Lab

Name_____Date _____

Patient name: _____

1. Describe your urine sample. (Discuss color, clarity and odor.)

_____

_____

_____

_____

2. What were the results of your Gram stain? (Discuss stain results, morphology, and arrangement.)

_____

_____

_____

_____

3. Which of the DLA and PEA plates had growth?

_____

4. Explain what each of these plates tells you about the **Gram reaction**, and therefore do the results of your Gram stain and the DLA/PEA plates correlate in terms of the **Gram reaction** of your organism?

_____

_____

5. How many bacteria (colony forming units, CFU) were in your original urine sample? (**Show your calculations and express your answer in scientific notation**.)

# of CFU/ml in original sample _____

6. List the tests you did to identify your organism along with the test results. **Describe the results of ONLY your group's tests for your organism:** whether or not bubbling occurred in the catalase; color of colonies and agar in DLA plates; color observed after performing the methyl red test, color of agar in citrate test. Do not just say "+" or "-".

_____

_____

_____

7. Identify your pathogen from the list of 6 possible organisms. (Full scientific name – genus capitalized and species lower case. Underline the name.)

⟹

8. To which antimicrobial agent(s) is it sensitive? List them all (abbreviate, if necessary).

_____

_____

9. List major side effects of the **4 most effective** of the antibiotic(s) to which your organism is sensitive. (If you organism was not sensitive to at least 4, then determine the side effects to the antibiotics to which it is sensitive.

_____

_____

_____

_____

10. Why is it important for the patient to take an antibiotic for the entire duration prescribed?

_____

_____

_____

_____

Antibiotic Resistance Lab

Lab 11 Evolution of Antibiotic Resistance in Bacteria

Objectives:

- Observe evolution in a population of bacteria.
- Understand the role of mutation and horizontal gene transfer in generating genetic variation.
- Explain the significance of genetic variation to natural selection.
- Learn basic sterile techniques in bacterial culture.

Introduction:

Antibiotic resistance is a major health care crisis. Deadly diseases like tuberculosis and pneumonia which used to be curable with antibiotics now have forms which are antibiotic-resistant. Even staph infections, a common malady of hospital patients caused by the bacterium *Staphylococcus aureus*, have turned deadly. MRSA, which stands for "Methicillin-resistant *Staphylococcus aureus* and is pronounced "mersa" has been in the news. Perhaps you've even seen information about it at your doctor's office or gym. Why is this happening?

The increased prevalence of antibiotic resistance is an outcome of evolution. Any population of organisms, bacteria included, naturally includes variants with unusual traits--in this case, the ability to withstand an antibiotic's attack on a microbe. When a person takes an antibiotic, the drug kills the defenseless (or susceptible) bacteria, leaving behind--or "selecting," in biological terms--those that can resist it (those that have higher relative fitness). These renegade bacteria then multiply, increasing their numbers a millionfold in a day, becoming the predominant microorganism.

Evolution is defined as the change in allele or gene frequency in a population over time. **Evolution is a fact** - it can be observed. The **theory of natural selection explains how evolution occurs** based upon **unequal reproductive success**. Many people refer to this as "survival of the fittest", but it would probably be more correct to say "reproduction of the fittest". Natural selection is the mechanism that describes HOW populations change over time.

The huge diversity of life that exists today is all influenced by this same process of natural selection. The canopy of a tall tree or a sea vent thousands of feet beneath the ocean surface represent two of the nearly infinite different environments present on Earth. These environments shape the 10-200 million species that dwell here.

How does natural selection shape populations?

Genetic variation exists within a population due to random **mutations**. If the population is sexually reproducing, then **genetic recombination** during meiosis also leads to variation. Natural selection favors a particular trait based upon the environment. Although the results of natural selection are staggering, the process itself is rather humble. **Natural selection is simply an editing process**. The process of natural selection cannot lead to the development of a new trait, it can only select from the existing variation. One common misconception about evolution is that organisms can evolve a particular trait because it is useful. This concept of "use and disuse" was proposed by Jean Baptiste de Lamarck as a mechanism of evolution 50 years before Darwin proposed natural selection as the mechanism of evolution.

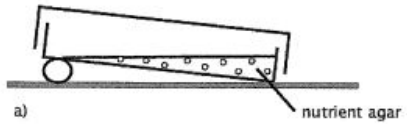
How do mutations and natural selection relate to antibiotic resistant bacteria? I'm glad you asked! **The antibiotic does not technically cause the resistance**, but allows it to happen by creating a situation where a mutant that is antibiotic-resistant can flourish. "Whenever antibiotics are used, there is **selective pressure for resistance** to occur. It builds upon itself. More and more organisms develop resistance to more and more drugs," says Joe Cranston, Ph.D., director of the department of drug policy and standards at the American Medical Association in Chicago (Lewis, 1995). This is the evolution of antibiotic-resistance in bacteria. For more information please read "Superbugs – superfast evolution". You can access it at http://evolution.berkeley.edu/evolibrary/news/080401_mrsa. This well-written article gives detailed information about horizontal gene transfer, a mechanism that bacteria can use to transfer genes for antibiotic resistance to unrelated bacterial species.

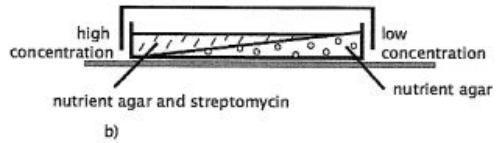
In this laboratory activity we will examine the evolution of streptomycin-resistance in *E. coli*, *Micrococcus luteus*, and *Bacillus thuringiensis*, a non-pathogenic bacterium commonly found in soil. *B. thuringiensis* causes disease in insects and is sometimes used as a biological control agent. Streptomycin is a widely used antibiotic and was the first antibiotic used to treat tuberculosis. Streptomycin prevents bacterial growth and division by binding to bacterial ribosomes and inhibiting protein synthesis.

B. thuringiensis reproduces rapidly, dividing every 45 minutes at body temperature (*E. coli* divides every 20 minutes and *M. luteus* each 30 min). Spontaneous mutations in a gene occur at an average frequency of one in every 10^9 generations (Krist and Showsh, 2007). Two mutations are possible that enable these bacteria to survive in the presence of streptomycin. One mutation changes the shape of the ribosomes. These altered ribosomes can still synthesize proteins but streptomycin can't bind to them, thus streptomycin cannot inhibit protein synthesis. The second possible mutation alters the transporter proteins that bind streptomycin and bring it into the cell so streptomycin cannot enter the bacterium.

We will use Petri plates with three types of media in our experiment. First, we will use a nutrient agar as a positive control – we expect resistant and susceptible strains of the bacteria to grow on this medium. Second, we will use nutrient agar + streptomycin as a negative control (we will refer to this as the streptomycin plate). We expect that no bacteria will grow on this plate but must verify this to ensure the results of our experiment. Third, we will use a streptomycin gradient plate to observe changes in streptomycin-resistance in a bacterial population. The concentration of streptomycin-containing agar increases and the amount of plain nutrient agar decreases over the surface of the gradient plate, as shown in the figure below.

Additional organisms to be used:
E. coli
M. luteus





1. Where on the streptomycin gradient plate would you be most-likely to see high concentrations of normal bacteria?

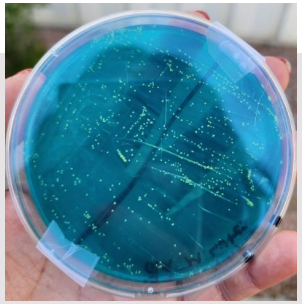
2. Where on this plate would you expect to see streptomycin-resistant bacteria?

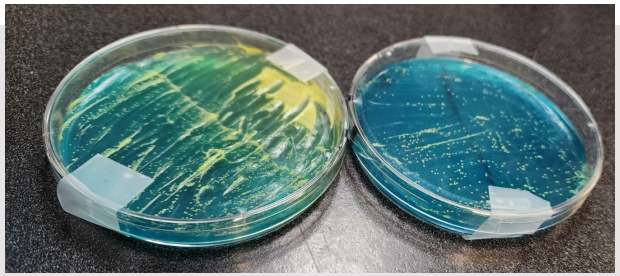
At one in every 10^9 generations, mutations may seem like rare events. However, in this experiment, each Petri plate will contain millions of bacteria, each of which will experience 16 million cell divisions over a period of one day!

Procedures:

- Demo plates of each organism growing on nutrient agar and streptomycin agar are provided.

Equipment for each person:
1 Petri plate of nutrient agar/streptomycin gradient (NA/SG)
1 Petri plate of Streptomycin agar.
1 slant culture of *B. thuringiensis or E. coli or M. luteus*





Day 1:

1. Each person will streak the assigned organism onto the nutrient agar/streptomycin gradient (NA/SG) plate.

2. Each student will streak the assigned organism onto the ½ of the Streptomycin agar.

- Be sure to label each plate with your name, name of organism, and date. Draw a line down the center of the Streptomycin plate to divide it in half.
- Obtain a test tube rack and an agar slant containing *B. thuringiensis or E. coli or M. luteus* and you will need your inoculating loop. Place your culture tube in the test tube rack at your work station.
- Sterilize the inoculating loop. **Keep it sterile!** Do not set it down on the countertop, wave in the air or blow on it. Hold the agar slant in your non-dominant hand. Curl the little finger of your dominant hand around the cap and pull it off the tube. Do not set down either the loop or the cap—hold them in the same hand.
- Lower the sterile loop into the culture tube and touch it to the bacterial growth (do not dig into the agar). Remove the loop from the tube. Recap the agar slant culture and set it back in the test tube rack.
- Open the lid of the streptomycin plate not all the way, but far enough so the loop can reach the media surface. Gently touch the loop to the media on one edge of the plate and gently move it side to side proceeding down the plate until **half of the surface area has been streaked**. Do not gouge the agar. Quickly close the Petri plate as soon as you finish streaking. Re-sterilize your loop.
- Look at the nutrient agar/streptomycin gradient plate (NA/SG) from the side. You should see the streptomycin gradient (blue agar = more streptomycin). Be sure to start your streak where the plate is straw colored (opposite from the blue side). This will ensure you start streaking in the area with little to no streptomycin and continue the streak in the areas of higher streptomycin concentration.
- Tape your plates together, invert them and place them on the prep table. The lab staff will incubate the plates at 37°C.
- Dispose of the slant agar in the labeled test tube rack in death row.

Day 2:

The Petri plates have incubated for approximately 24 hours.

- Observe your plates carefully but keep them closed.

If no bacteria were present in the streptomycin-containing portion of the gradient, you will need to re-streak your plate. You will take colonies from the nutrient agar region of the plate and streak them down into the streptomycin-containing portion of the plate. This physically separates bacterial colonies from one another. This way if any bacteria within a colony have resistance, they will be able to form entire new colonies on the streptomycin. Plus, breaking up the existing colonies allows colonies in the nutrient agar portion of the plate to divide and potentially mutate into resistant cells.

- Place a sterile loop over several colonies and streak them straight down into the streptomycin-containing portion of the gradient. Then glide your loop side-to-side across the gradient. This maximizes the area in which any new streptomycin-resistant bacteria can grow.

Day 3:

- Observe your plates carefully but keep them closed.

If you have streptomycin-resistant colonies present, you will inoculate them to the un-used ½ of the streptomycin-containing Petri plate.

- Use a sterile loop to touch any streptomycin-resistant colonies on the gradient plate and streak them on the un-used streptomycin plate. Be sure to label your plate!
- Re-inoculated streptomycin and gradient plates go in the box for incubation.

*Note that the mutation to become strep-resistant originated in the population growing in the nutrient agar region of the gradient plate. This mutation was already present when you streaked the plate, spreading bacteria into the streptomycin. Bacteria did NOT develop this mutation in response to streptomycin. Remember – natural selection only selects existing genetic variation – it cannot generate new genetic variation just because it would be useful (like Lamarck thought).

Day 4:

- Observe your plates carefully but keep them closed.
- After you are finished with all your plates, dispose of them in the biohazard bucket.

Complete the lab worksheet

References:

Krist, A. C. and S. A. Showsh. (2007). *Experimental Evolution of Antibiotic Resistance in Bacteria*. The American Biology Teacher, Vol. 69(2). Pages 94-97.

Lewis, R. (1995). "The Rise of Antibiotic-Resistant Infections" FDA Consumer Magazine. Vol. 29(7). Accessed online on 1/21/08 at http://www.fda.gov/Fdac/features/795_antibio.html.

Re-expose the bacteria until everyone gets resistant bacteria

Lab 11 Evolution of Antibiotic Resistance in Bacteria

Name _____ **Date:** _____

At one in every 10^9 generations, mutations may seem like rare events. However, in this experiment, each Petri plate will contain millions of bacteria, each of which will experience 16 million cell divisions over a period of one day!

1. What are some possible explanations for these observations? Be sure to use specific scientific terminology (i.e. resistant, susceptible, natural selection, evolution)

2. Did all groups have similar results as yours? What do you think best explains this?

*Note that the mutation to become strep-resistant originated in the population growing in the nutrient agar region of the gradient plate. This mutation was already present when you streaked the plate, spreading bacteria into the streptomycin. Bacteria did NOT develop this mutation in response to streptomycin. Remember – natural selection only selects existing genetic variation – it cannot generate new genetic variation just because it would be useful (like Lamarck thought).

3. Where did the new genetic variation for strep resistance come from?

4. Did the bacteria generate genes leading to streptomycin-resistance as a result of the presence of streptomycin? Why or why not?

5. In your own words, explain how mutation and horizontal transfer affect the genetic variation in a bacterial population and how natural selection leads to the evolution of streptomycin resistance in this population of bacteria.

Data Presentation

B. thuringiensis

Number of plates inoculated _____

Number of plates that produced strep resistance _____

Calculate the percentage of strep resistance _____

Describe colony characteristics of strep resistant organisms _____

_____

E. coli

Number of plates inoculated _____

Number of plates that produced strep resistance _____

Calculate the percentage of strep resistance_____

Describe colony characteristics of strep resistant organisms _____

_____

M. luteus

Number of plates inoculated _____

Number of plates that produced strep resistance _____

Calculate the percentage of strep resistance _____

Describe colony characteristics of strep resistant organisms _____

_____

6. What conclusions do you draw from your data?

Questions?