Project 2: Building Skills in the Microbiology Lab

Readings:

Links to the required readings can be found on VMC Main Page for this lab. See class schedule for related textbook reading.

### **Purpose**:

The purpose of this lab is to acquaint students with laboratory equipment and laboratory techniques in the microbiology lab. The students will employ the safety procedures introduced in the previous lab exercise. Students will practice microscopy techniques and digital photography also introduced in the previous laboratory session. Additionally, students will develop a level of proficiency when using the inoculating loop, Bunsen burner, and Bacti-Cinerator® to inoculate plates, pour media, and transfer bacteria to slides for staining.

### Outcomes:

After you complete this lab you will be able to:

* Identify all the equipment at the laboratory station and use it correctly
* Prepare and stain the slides correctly.
* Observe slides under the microscope.
* Aseptically transfer bacteria to slides and media plates
* Compare and contrast different laboratory techniques
* Properly use the Bunsen burner and the Bacti-Cinerator® and describe the benefits and limitations of both
* Transfer bacteria to microscopes slides and media plates
* Practice isolation streak plate inoculation techniques for media plates
* Employ laboratory safety techniques to perform the following tasks:
* Prepare and stain slides
* Inoculate media plates
* Observe slides and take digital photographs
* Identify the parts of the microscope.
* Given a picture of a microscope, write in the names of the parts.
* Point to an item on the microscope and identify it.
* Use the microscope properly.
* Consistently focus the microscope properly.
* Consistently use the mechanical control adjustment to move the specimen on the slide.
* Properly use the iris diaphragm and/or condenser to adjust the amount of light illuminating the specimen.
* Consistently find, observe and photograph specimens properly using low, high and oil immersion objectives.
* Consistently distinguish material for observation from any artifacts that may be present.
* Identify single bacterial cells on a simple stain
* distinguish between cocci and bacilli
* Consistently distinguish between the eukaryotic cells in blood and the prokaryotic bacteria identified in later laboratory exercises.
* Care for the microscope properly.
* Clean the microscope correctly after each use.
* Store the microscope properly after each use.
* Inspect stored microscopes to confirm proper storage.
* Apply terms used in the laboratory exercise.
* Write out the definition of all terms in the “Terms to Know” table below.
* Use all terms correctly and apply appropriate terms to specific techniques performed.

### Terms To Know

|  |  |
| --- | --- |
| Aseptic technique | light adjustment |
| Autoclave | microscope slide |
| Bacti-Cinerator®  | Petri plate |
| Bunsen burner | Pour plate |
| course adjustment knob | scanning, low and oil immersion powered objectives |
| culture media | sharpie marker |
| fine adjustment knob | vis-à-vis marker |
| inoculating loop | watch glass |
| iris diaphragm | wax pen |
| Isolation streak plate |  |

### Introduction

Laboratory equipment and many laboratory techniques were developed during the Golden Age of Microbiology beginning in the late 1800’s. The labs of Pasteur and Koch yielded great scientific discoveries in the field of microbiology. As Pasteur worked on fermentation theory, germ theory, and the practice referred to today as ‘pasteurization’, techniques were refined and equipment was developed as needed. Koch’s work in identifying the microbes causing tuberculosis, anthrax, and cholera gave rise to Koch’s Postulates, a systematic gold standard for linking a microbe to the disease it causes. Again, laboratory techniques and equipment were forged and refined, and many of these practices are still in use today.

The rich history of the discipline is connected to our work in the laboratory. Science is based on the examination of hypothesis and much of this exploration occurs in the laboratory, especially in the field of microbiology. The laboratory bench work we undertake as students prepares us for the skill sets needed for future careers in science and medicine. The manual dexterity and precise movements of the laboratory are ancillary skills that also benefit the future professional.

Some of the tools you will learn about in this lab are common to all microbiology labs, while some items are specific to the lab at KVCC. You will explore the equipment drawer and lab bench space to identify the following items: inoculating loop, Bacti-Cinerator® , Bunsen burner, watch glass, wax pen, sharpie marker, vis-à-vis marker, microscope slide, micropipette, Petri plates, test tubes, microbial culture media, warming oven, incubation bin, and incubator. In addition to the identity and function of each item, you will explore the practice of aseptic technique in the transfer of bacteria to a microscope slide and a media plate.

Today you will also begin to learn about **aseptic technique.** Aseptic technique means that in handling bacteria, you don’t introduce environmental or other contaminating microbes into your experiment. It also means that you do not become contaminated with the microbes you are working with. Microbiologists use aseptic technique when they pour TSY medium into Petri plates and when they inoculate specimens onto the media, as well. You also will aseptically make a streak plate. The practice of this technique is also an opportunity to develop skills to keep yourself and others safe in laboratory environments where the risk of contamination exists.

The medium that we use most often is designated "TSY" (**t**ryptic **s**o**y** agar). It isa ***complex*** ***nutrient medium that supports the growth of a wide variety of microbes***. When the lab personnel make a medium, they measure out a designated quantity of dry powdered medium, add a designated amount of water and check the pH. They dispense the medium into bottles, cap it and autoclave it. Autoclaving is a process similar to home canning techniques of food preservation. Once the medium is **autoclaved** (or pressure cooked), it is considered sterile. The autoclave exposes the medium to high temperature (121°C) and pressure (15 psi) for 20 minutes. This exposure has been demonstrated to result in **sterilization.** (Sterilization is the process of killing *all* life forms.)

Many normal-flora microorganisms and clinically important microbes can be grown either in liquid medium (sometimes called **broth**) or on Petri dishes (also called Petri plates, or just plates). When microbes are grown on plates, **agar** is added to the liquid medium so that, when cool, the medium has the consistency of very stiff gelatin. Agar is an inert (meaning the bacteria do not consume/eat it) seaweed extract that solidifies at room temperature. After the tubes are removed from the autoclave, the hot media is in a liquid form.

Sometimes the hot tubes are laid on their side, at about a 45o angle, and allowed to cool. As these tubes come to room temperature the agar solidifies the media on a slant. In today’s lab procedure, these ‘slant’ tubes have been inoculated with the bacteria you will need to perform the exercise. You will also aseptically pour media from the preparation bottles to Petri plates. In this case the agar will solidify in the plate allowing a large flat surface for the cultivation of microbes.

You will practice making **streak plates** in today’s lab. The streak-plate method is essentially a **dilution** technique that systematically spreads the bacterial cells over the surface of the medium to achieve growth of isolated colonies. Streaking a sample of bacteria on a plate as described in this lab results in dilution and separation of bacterial cells. When a plate is incubated after the sample has been streaked, each cell divides many, many times by binary fission and forms a **colony**. A colony that is not touching other colonies is said to be **isolated**, and all the cells in that colony are assumed to have arisen by division from a single cell. (FYI, a colony that is just barely visible to your eye contains at least a million cells!)

To identify the bacteria in a sample, you first make a streak plate — and then observe the plate after incubation. It is obvious from the appearance of the colonies (i.e., from the **colony morphology**) if the sample contained one or many kinds of bacteria. If only one colony morphology is present on the plate, then all the colonies will look the same (same color, shape, elevation, etc.). If a plate contains many colonies of only one colony morphology, then the plate is said to be a pure culture of bacteria. Colonies that appear different are, in fact, different genera and/or species of bacteria. If many colony morphologies are present, then the specimen contained a variety of microbes. If a sample contains multiple kinds of bacteria, making a streak plate is the first step in obtaining pure cultures of each so they can be identified.

### Procedures (Week 1):

1. Identify laboratory equipment:
2. Find and identify each of laboratory items listed below. After you have found all the items listed, take a picture of the item assigned to you.

|  |  |  |
| --- | --- | --- |
| * inoculating loop
 | * cover slips
 | * hot plate
 |
| * watch glass
 | * sharpie markers
 | * disinfectant bottle
 |
| * Bacti-Cinerator®
 | * vis-à-vis markers
 | * water bottle
 |
| * platform for air drying slides on Bacti-Cinerator®
 | * immersion oil
 | * hand wash station and soap
 |
| * microscopes
 | * staining rack
 |
| * Bunsen burner
 | * course adjustment
 | * stains
 |
| * gas nozzle
 | * fine adjustment
 | * forceps
 |
| * disinfecting tray
 | * iris diaphragm
 | * slide holders
 |
| * biohazard bags
 | * stage clip
 | * power cord and outlet for laptop
 |
| * glass/sharps disposal
 | * low power objective
 |
| * fume hood
 | * scanning power objective
 | * cord for camera in microscope
 |
| * warming oven
 |
| * incubators
 | * oil immersion objective
 | * eye wash station and emergency shower
 |
| * petri plates
 | * finger cot
 |
| * microscope slides
 | * high power objective
 | * incubation bin
 |

1. Post the picture of the item and a description of how or for what it is used in the appropriate upload in Moodle. Use APA formatting when inserting the picture of the item in the document.
2. The instructor will demonstrate the technique to aseptically pour media into the Petri plate. The technique is as follows:
3. Retrieve Petri plates from the storage bag careful to keep the plates closed at all times.
4. Set the plates, lid side up, in an area of the bench where they may sit for at least 15 minutes after the media is poured.
5. Hook the tubing from the Bunsen burner to the gas nozzle
6. Using the oven mitt if necessary, retrieve the TSY media from the warming oven.
7. Open the gas jet by moving the lever perpendicular to the gas nozzle. Light the Bunsen burner by striking a match and bring it up the side of the barrel of the Bunsen.
8. Using your non-dominant hand, twist open the cap of the media jar and remove (continue to hold the cap facing downward in your index finger); pass the lip of the media jar over the blue cone in the flame of the Bunsen.
9. Using your thumb and ring finger of your non-dominant hand, lift the lid of petri plate to a 90o angle. Pour about ¼ inch of media into the plate.
10. Replace the lid of the petri plate
11. Pass the lip of the media bottle through the flame and replace the cap.
12. Practice the technique. When you feel you have mastered the technique call the instructor to your bench and perform the technique. You will be evaluated on the following items: (Note: you will have two sterile TSY plates at the end of this procedure)

|  |  |  |
| --- | --- | --- |
| Item | Needs Improvement | Proficient |
| Handle petri plates |  |  |
| Manages media in jar |  |  |
| Pours appropriate amount of media |  |  |
| Aseptically pours media |  |  |
| Safe operation of Bunsen |  |  |
| Order in the work space |  |  |

1. The instructor will demonstrate the technique for aseptic transfer of bacteria to a microscope slide for simple staining.
2. Using a wax pen draw two circles about the size of a nickel on the microscope slide and turn the slide over. Place the slide, wax circle side down on a piece of white paper towel.
3. Light the Bunsen as previously directed.
4. Select a tube of sterile water from the test tube rack. Remove the cap using the little finger of your non-dominant hand. Pass the top of the tube through the blue cone of the Bunsen flame.
5. Sterilize the inoculating loop in the Bacti-Cinerator® for 5 seconds. Remove, cool, and insert into the tube of water, careful not to touch the sides of the tube.
6. Remove a loopful of water and transfer it to the circle.
7. Sterilize the loop, flame the top of the tube, and replace the cap.
8. Select the slant tube of *Staphylococcus epidermidis* (S. epi) from the test tube rack, remove the cap as previously directed, flame the top of the tube.
9. Sterilize the inoculating loop and insert it into the tube with the loop flat on the slant. Draw the loop along the slant for about ¼ of an inch.
10. Without touching the sides of the tube, withdraw the loop and transfer the bacteria from the slant to the drop of water by rubbing the loop in the drop of water on the slide. Use the loop to distribute the water and bacteria over the entire circle you have drawn.
11. Sterilize the inoculating loop.
12. Repeat items c-j, this time using *Escherichia coli* (E. coli) as the bacteria transferred to the second circle, as your instructor evaluates your performance.
13. Set the slide on the Bacti-Cinerator® platform to air dry and heat fix.
14. You will be evaluated on the following items:

|  |  |  |
| --- | --- | --- |
| Item | Needs Work | Proficient |
| Maintained safety |  |  |
| Maintained aseptic technique |  |  |
| Prepared slide correctly |  |  |
| Maintained order in the work space |  |  |
| Appropriate amount of bacteria transferred |  |  |

1. The instructor will demonstrate the isolation streak plate technique. Use the TSY media plate you poured in step 2, which should now be solidified at room temperature.
2. Place your sterile TSY media plate on the lab bench lid side down (media side up). Select the Sharpie marker and record the date, the specimen you transfer (S. epi), and your initials.
3. Sterilize the inoculating loop. While it cools, select the tube of S. epi from the test tube rack, remove the cap and flame the top of the tube.
4. Acquire a sample of S. epi from the test tube as previously directed.
5. Gently inoculate the first quadrant of the TSY media as illustrated in Figure 2.1.
6. Return the plate to the lid, and sterilize the loop.

**Figure 2.1**: First Quadrant Streak

Quadrant #1

1. Allow the inoculating loop to cool, remove the Petri plate and turn the plate 90°. Touch the loop to a corner of the culture in quadrant 1 and drag it several times across the agar in quadrant 2. Tease out of quadrant #1 only 2 or 3 times and then continue your streak, but do not dip back into quadrant #1 (Figure 2.3).

**Figure 2.2**: Second Quadrant Streak

Quadrant #1

Quadrant #2

1. Sterilize the inoculating loop in the Bacti-Cinerator®
2. Give the plate a 90° turn and begin the inoculation of quadrant #3 by placing your loop in quadrant #2. Again, tease out some of the bacteria into quadrant #3 in the manner indicated in Figure 2.3.

**Figure 2.3**: Third Quadrant Streak

Quadrant #2

Quadrant #3

Quadrant #1

1. Sterilize the inoculating loop in the Bacti-Cinerator®.

For your final quadrant streak, touch quadrant 3 only one time, and then streak quadrant 4 in the manner indicated in Figure 2.4

**Figure 2.4**: Fourth Quadrant Streak

Quadrant #1

Quadrant #2

Quadrant #3

Quadrant #4

1. Practice the technique by tracing the drawing on a piece of paper or the white board in the lab before your first attempt. Practice with the plate you poured in step 2 using *S. epi* cultures. When you are ready, call the instructor to observe your technique. Repeat the technique for your instructor using *Escherichia coli* (*E. coli*) as the bacteria you inoculate onto the TSY. Store both plates in the green ‘SAVE’ bin on the back bench in the lab. The plates will be incubated at 37o C for 24 hours and then stored until you return to lab next week.
2. You will be evaluated as follows:

|  |  |  |
| --- | --- | --- |
| Item | Needs Improvement | Proficient |
| Maintains safety |  |  |
| Maintains aseptic technique |  |  |
| Appropriate transfer of bacteria |  |  |
| Technique performed correctly |  |  |
| Plate labeled correctly |  |  |
| Obtains isolated colonies  |  |  |

1. Perform a simple stain of the bacteria you have transferred to the microscope slide.
2. After the slide has air-dried and heat fixed for at least 5 minutes, transfer the slide to the staining rack over the sink at your bench.
3. Select the crystal violet stain from the staining tray. Using the dropper tip, allow free-falling drops of stain to cover the circles containing your bacteria.
4. At the end of 1 minute, use a forceps or slide holder to grasp the end of the slide. With the slide at a 45o angle, rinse the slide gently with water from the faucet.
5. Place the slide on a piece of paper towel on the lab bench, fold the towel over the slide, and gently press the slide to dry.
6. Retrieve the microscope assigned to your station.

Place the slide in the stage clips of the microscope and observe each circle on your slide at oil immersion. Take digital photographs of cocci and bacilli, label, and save to your ‘O’ drive or email to yourself and your lab partner.

1. Call your instructor to your bench to observe your microscopy. You will be evaluated on the following:

|  |  |  |
| --- | --- | --- |
| Item | Needs Improvement | Proficient |
| Focuses microscope  |  |  |
| Identifies bacilli |  |  |
| Identifies cocci |  |  |
| Works in appropriate area of the slide |  |  |
| Navigates camera software |  |  |
| Saves work to O drive |  |  |

### Procedure (Week 2):

1. Retrieve the two isolation streak plates you set up last week from the ‘SAVE’ bin. Be careful not to remove the lid from the plate; hold the plate level to avoid contamination in case any condensation formed during incubation. Bring the plates back to your bench.
2. Record your level of proficiency in cultivating isolated colonies. Discuss the results with your instructor.
3. Use the *S. epi* and *E. coli* isolation streak plates as controls for the stains you perform in lab #3.

### Assessment:

You will receive points for this lab based on the following:

* Your wiki entry, which includes a picture, a description, and a citation when needed.
* The number of proficiencies you earn.
* Turning in the Lab Report (a partial lab report), as instructed, and which includes properly labeled photos that you took in lab.