5-2019

Developing a modern microbiology laboratory manual to enhance student learning

Alexander Yonchak
Ayonchak@uri.edu

Table of Contents

4. Cellular Morphologies
    a. Overview
    b. Learning outcomes
    c. Target Information and Skills
    d. Scenario Summary
    e. Materials and Methods
    f. General Scenario Procedure
    g. Student Assessment
5. Colony Morphologies
6. Physiologic/Metabolic Activity
7. Isolation of an Organism
8. Selective and Differential Testing
9. Serial Dilutions, Colony Forming Units, and Plaque Forming Units
10. Identification of an Unknown Organism

IV. Conclusion

Preamble

The following is a collection of experiment-oriented modules to be run ahead of a Microbiology laboratory-course. This collection of experiments will operate as a workshop for students, consisting of a three-part presentation which includes a lecture-based, online/pre-class, and practicum portion. The purpose of these is to promote students' smooth transition into a lab-course and provide students with an opportunity to learn by diverse means, including self-study, lecture-aided, and practical examination. To accomplish this, each experiment is designed to address a specific set of core learning-outcomes, based upon the laboratory competencies presented in the Continuum of the Competencies (Delany, 2011, Appendix B). A Risk Assessment (RA) associated with the module's practicum components, should be created to aid in the delivery of the lab-course. The completed RA will then provide a basis for the lab-course to design its own.

Risk Assessments (RA)

A complete risk assessment should contain the following:

1. Agent based hazards (organisms, etc.)
2. Procedural hazards
    (a) Chemicals & media by concentration
    (b) Equipment (e.g., burners and centrifuges)
    (c) SOP’s innate risk, (complexity, aerosols, contact with hazards)
3. Definitions for BSL; define your BSL
4. Evaluation for the existing staff, equipment, circumstance, and etc. in relation to the BSL
5. Have RA and any high-risk protocols reviewed by expert/IBC/IRB

Modules

1. Aseptic Technique

Glossary:

Hazards – Anything which can cause bodily harm, notably those which can cause acute or recurring illnesses.

Biological Hazards (biohazard, biohazardous materials) – Any biological material with hazardous qualities such as infectiousness, immunogenicity, or toxicity.

Biological Hazardous Waste (biohazardous waste) – Any waste materials contaminated by biological hazards.

a. Overview

i. the goal of the module - To isolate a living organism from a biohazardous material using aseptic technique

ii. the expectations of students - It is expected students understand the learning outcomes from the pre-lab materials and apply them while performing the Practicum Components

iii. the risks to be encountered - Aseptic isolation streaking has two key risks, use of a sterilization method (Bunsen burners or chemical disinfectant) which will be damaging to human tissue, and contact with a possibly pathogenic organism or biohazard.

iv. the relevance in future experiments - The students will be informed of how key the practice of aseptic isolation is, the importance of waste management by labeling human-samples, value of reducing wastefulness by effective isolation of an organism, and that identification of biohazards is a requirement for all biological laboratories.

b. Learning Outcomes

1. Students will be able to describe the concept of a biohazard and list those present in a laboratory.

2. Students will be able to identify the following potential traits associated with a biohazardous material: diseases associated with an infectious agent; the virulence and pathogenicity of a contaminant; and the principle exposure routes

3. Students will be able to demonstrate knowledge of Laboratory-Acquired Infections (LAIs) and the potential risks of working with a bacterial culture in which the identity of the organism(s) is unknown to them.

4. Students will be able to perform aseptic technique for the isolation of a microorganism via SOPs which minimizes the risk of biohazards

c. Target Information and Skills

Prerequisite information and skills for students :

• Able to follow a standardized lab manual

• Know basic information of diseases associated with common organisms

• Know the difference between opportunistic-pathogens & obligate pathogens

• Able to describe the typical progression of an infection (from exposure to host-cell death)

• Have a base-understanding for the identification a biohazard by labeling or warnings presented with them

Requisite information and skills for students to pass this module:

• Able to identify biohazards by labels presented with a material

• Able to describe the concept of a biohazard

• Able to identify typical diseases associated with an infectious agent; the virulence and pathogenicity of a contaminant; and describe the principle exposure routes

• Able to demonstrate knowledge of Laboratory-Acquired Infections (LAIs) and the potential risks of working with an unknown culture.

• Prepared to perform aseptic technique for the isolation of a microorganism

• Know the basis of pathogenesis and example LAIs

• Able to demonstrate knowledge of correct disposal methods for human-biohazardous materials

d. Scenario Summary

The practicum portion will be the performance of aseptic technique for the isolation and identification of an organism from a simulated blood culture. Further materials covered will be the examination of an organism's pathogenicity. SOPs will include isolation streaking, managing & labeling of a human-biohazardous materials with appropriate notation (tape/waste-disposal), and correct use of personal protective equipment (PPE) & chemical disinfectants for the lab. Use of a spread-bar will also be employed so students can practice use with spread plate technique. This scenario will allow students to perform SOPs for aseptic isolation of an organism while also evaluating what virulence factors should be considered to reduce risk posed by the organism. A simulated blood culture will provide an excellent example of how dangerous LAIs are, through referencing common blood-borne pathogens, and this will provide an opportunity for students to learn about typical clinical labs' SOPs; students will operate as if sample organism is an obligate-pathogen with low infectious doses.

A complete overview of the blood-borne pathogens will be presented along with this Practicum, focusing on the risks that exist with culturing an unknown sample. Moreover, because it is focused on aseptic technique, further explanation of the innate risks associated with aseptic technique will be given. The simulated aspects of the experiment will be also explained; a depiction of successful and failed results for isolation streaks & sample retrieval will be given as well.

e. Materials and Methods

Materials:

• Chemical – bench top disinfectant, ethanol

• Biological – one Gram-Positive organism, one Gram-Negative organism

• Equipment – micro-centrifuge tube, Bunsen burners, metal loops, spread-bars, nutrient agar (NA) plates (2x student pair)

Methods:

Isolation Streaking (4-quadrant method)

1. Flame a metal loop until it glows orange-red (3-5 seconds) to sterilize. Allow to cool in air
2. Pick up a small quantity of culture from micro-centrifuge tube with the metal loop
3. Place sample into center of first quadrant of a NA plate and streak in zig-zag till the quadrant is mostly filled
4. Sterilize metal loop as in Step 1
5. Drag a single line from the first quadrant into the second quadrant with the metal loop and, without entering into the first quadrant again, streak in a zig-zag motion throughout the second quadrant.
6. Sterilize metal loop as in Step 1
7. Repeat Steps 5 and 6 for the third and fourth quadrants as appropriate.

Spread Plate Technique

1. Pour or pipette sample from the micro-centrifuge tube onto the second NA plate
2. Dip a spread-bar into ethanol and allow excess to drip off
3. Quickly pass the spread-bar through the flame of a burner so the ethanol ignites
4. Allow spread-bar to cool briefly
5. Spread the sample around the NA plate with four-five single motions, always pull and do so in a straight line

f. General Scenario Procedure

Students will be given a sample of 0.5 mL simulated blood culture with a previously identified organism grown in it to a concentration of approximately 104 colony forming units per mL (CFU/mL) and two NA plates. Students will then perform an isolation streak on one plate and then a spread plate technique on the other, by procedure stated above. These plates will be incubated overnight in a 37C incubator. After completing the inoculations, students will dispose of the micro-centrifuge tubing by standard procedure for human biohazardous materials. The following class period, students will examine their plates and describe the different morphologies of organisms observed. Students will then compare their isolation plate to their spread plate to observe whether or not any contamination entered the plate during their inoculation.

g. Student Assessment

Students will be assessed on the amount of contamination observed compared to class average and the quality of their isolation streak; any significant errors or contamination will be discussed by their possible dangers and causes. A post-lab quiz will also be given where students must redraw out the streaking pattern for 4-quadrant streaking and answer questions derived from the biosafety / hazardous material management presentation.

Learning Outcomes will be confirmed via:

Post-lab Quiz Q/A- 1,2

Post-lab Quiz Written Response - 3

Post-lab Quiz Diagramming - 4

h. Worksheet & Summary

I. Organisms:

1. _____

Procedure:

4 Quadrant Isolation Streaking

1. Label 4Q plate and draw quadrant lines.
2. Sterilize loop 3-5 sec, let cool
3. Transfer small amount of culture to isolation plate and smear into small spot
4. Sterilize loop as in Step 1
5. Drag a line through culture smear and zig zag around quadrant 1; Sterilize loop
6. Drag a single line from the first quadrant into the second quadrant; Sterilize loop
7. Draw Zig-zags from the line throughout the second quadrant; Sterilize loop
8. Repeat Steps 6 and 7 for the third and fourth quadrants.

Spread Plate Technique

1. Label Spread Plate
2. Pour the remaining sample onto the second NA plate
3. Sterilize spread-bar with ethanol & fire – Do not leave in flame or will get too hot
4. Allow spread-bar to cool briefly (should not need long if flamed correctly)

5. Spread the sample around the NA plate by spreading the culture up and down then rotating the plate clockwise

Observations:

2. Microscopy Basics

Glossary:

Microscopy – the visual study of microbial samples

Microscope – a tool used to study specimens which are microscopic (smaller than the eye can see unaided)

a. Overview

i. the goal of the module – To develop the ability to use of microscopes for the identification and study of microbial life.

ii. the expectations of students – It is expected students understand the learning outcomes from the pre-lab materials and apply them while performing the Practicum Components. It is further expected that students preform the Practicum Components with an appreciation of the instrumentation being used.

iii. the risks to be encountered – Microscopy relies on aseptic technique and has three risks, contact an organism or biohazard on microscope slides, sharp glass of microscope slides, and ocular strain.

iv. the relevance in future experiments - The students will be informed of how microscopy is fundamental to Microbiology and its role in the identification and study of all microbial life. Further, students will be informed on their responsibility to use instruments correctly and efficiently.

b. Learning Outcomes

1. Students will be able to describe the concept of a microscopy and usage within a laboratory.

2. Students will be able to identify the components of a microscope and their function.

3. Students will be able to demonstrate knowledge of morphological diversity of prokaryotic organisms and identify them in situ.

4. Students will be able to perform an examination of microbial organisms using a microscope and record observations in a time-efficient manner.

c. Target Information and Skills

Prerequisite information and skills for students :

- Able to follow a standardized lab manual
- Is able to utilize aseptic technique
- Know basic information about microscopy and the principle of magnification.
- Know the difference between microbial organisms and macroorganisms.
- Able to describe the purpose and usage of a microscope.
- Have a base-understanding for the usage of microscopes.
- Have base-understanding of prokaryotic morphologies.

Requisite information and skills for students to pass this module:

- Able to identify the components of a microscope and their usage.
- Able to demonstrate correct usage of a microscope to preserve both sample and instrument condition.
- Know the importance and limitations of the available microscopes.
- Able to describe a microbial organism's morphology from visual observation.
- Prepared to perform microscopy of a given microorganism and record observations.

d. Scenario Summary

The practicum portion will consist of four parts: practicing microscopy, preparing a wet mount, preparing a dry mount, recording the morphologies of a bacterial sample, and cleaning microscopes. Each step must be completed adequately before students may progress to the next task. The purpose of this organization is to increase in complexity of the task and ensure that students are able to appropriately operate a microscope.

SOPs will include using a light field microscope, creating a wet mount slide, creating a dry mount slide, and cleaning a microscope after usage.

e. Materials and Methods

Materials:

- Chemical – None
- Biological – List provided ahead of lab
- Equipment – Bunsen burners, metal loops, light-phase microscopes

<u>Methods:</u>

Using a Light Field Microscope:

1. Turn on microscope and check if light source is functioning
2. Place slide onto the microscope stage and secure with angled clamps
3. Set microscope to lowest magnification and adjust ocular piece to your comfort
4. Place a single dot of either permanent marker or red wax onto the side of the slide
5. Use the state position knobs to move the slide so that the dot is directly centered in the view / above the light aperture
    - Be careful here as the slide will move in the opposite direction from what you observe.
6. Once the dot is in position, focus on it with the course knob until it is clear to see.
7. Now switch to a higher magnification and readjust focus with the fine adjustment knob.
8. Repeat steps f/g going up in magnification until enough detail can be seen

Dry Mounting a Slide

1. Use a clean slide and the NA plate culture

2. Place a single droplet of water onto the center of the slide

3. Sterilize a metal loop and take up a single colony from the plate (only a tiny amount is needed)

4. Place the end of the metal loop into the droplet of water and gently swirl until the droplet is evenly colored

5. Allow the droplet of water to dry, leaving a smear of bacteria behind

6. Quickly pass the slide through the flame of a Bunsen Burner, culture facing down to heat fix it

7. Slide is now prepared

Wet Mounting a Slide

1. Use a clean slide and the liquid media culture

2. Place a single droplet of water onto the center of the slide

3. Sterilize a metal loop and take up droplet of fluid from the culture (only a tiny amount is needed)

a. Alternatively, use a pipette and transfer 10 micro-liters to the slide

4. Place the end of the metal loop into the droplet of water and gently swirl until the droplet is evenly colored

5. Use a coverslip by placing a single edge of the slip on to the slide then gently lower it across the droplet, allowing it to evenly spread out under the glass

6. Slide is now prepared

Cleaning a Microscope

1. When moving a microscope, always carry by holding both the neck and base

2. Turn off microscope and unplug; identify which lens have oil on them

3. Switch to the lens with oil and lower stage to base

4. Using clean lens paper, wipe across the lens in a single motion

5. Repeat step c by using a new, clean portion of the lens paper for each wipe

6. Continue until no more oil is transferred to the paper in this process

7. For other lens and portions of the microscope, use either a lens paper or Kim wipe to remove dust or build up

8. For smears / smudges which will not be removed by wiping alone, put a few droplets of 195 proof ethanol onto the paper / wipe before using

9. No other maintenance should be done without permission of the TA / professor and the usage of the microscope manual

f. General Scenario Procedure

TAs will prepare a known organism on a Nutrient Agar (NA) plates and in liquid media cultures, along with cut pieces of paper with various contents printed on them. To begin, students will practice their light microscopy skills by navigating along a printed piece of paper (taped onto a slide) and then reporting their observations to the TA, if done correctly, students may progress. Students will then retrieve their cultures to create both a wet mount and dry mount of the organism by the above SOPs. From these cultures, students will record the morphologies by observation via wet mount. Lastly, students will clean their microscopes and return them to the shelves.

g. Student Assessment

Students will be assessed on the students' observations and the quality of their reporting. Focus will be placed on the precision in reporting & methodology over the accuracy of observations. A post-lab quiz will be given where students must summarize the procedures for each slide creation by diagram and respond to questions derived from presented materials.

Learning Outcomes will be confirmed via:

Post-lab Quiz Written Response - 1

Post-lab Quiz Q/A – 2,3

Practicum Results - 4

3. Chemical Hazards and Gram Staining

Glossary:

Hazards – Anything which can cause bodily harm, notably those which can cause acute or recurring illnesses.

Chemical Hazards – Any chemical material with hazardous qualities such as toxicity, carcinogenicity, or biological reactivity.

Chemical Hazardous Waste (BHW) – Any waste materials contaminated by chemical hazards

a. Overview

i. the goal of the module - To explain the selected learning outcomes and demonstrate them via Gram staining

ii. the expectations of students - It is expected students understand the learning outcomes from the pre-lab materials and apply them while performing the Practicum Components

iii. the risks to be encountered - Gram staining has two major risks, sharp glass from slides and contact with hazardous chemicals such as crystal violet and high-concentration Iodine.

iv. the relevance in future experiments - The students will be informed of the role of Gram staining in microbiology, the importance of proper waste management for risk mitigation, the value of reducing wastefulness, and how understanding chemical hazards via MSDS sheets will be expected in all future labs.

b. Learning Outcomes

1. Students will be able to identify chemical hazards and describe them by their chemical and biological activities.

2. Students will be able to demonstrate knowledge of control measures around hazardous materials of each major hazard type.

3. Students will be able to demonstrate knowledge of correct SOPs for hazardous waste management and disposal with a lab room.

4. Students will be able to perform a Gram stain correctly, and identify the morphologies present in a sample.

c. Target Information and Skills

Prerequisite information and skills for students:

- Able to follow a standardized lab manual
- Is able to utilize aseptic technique
- Is able to utilize microscopy in a time-efficient manner
- Is able to perform microscopy with proper technique and the correct SOPs

• Reading labels for common names

• Locating the MSDS sheets

• Reading the MSDS sheets (in English)

• Locating Satellite Waste Disposal locations (SWDs)

Requisites information and skills for students to pass this module:

• Able to identify chemicals used in a Gram stain by common name

• Able to read and understand the MSDS sheet for all chemicals used

• Prepared to apply the appropriate SOPs for chemicals used during an experiment post-use disposal

• Able to identify the reasons hazardous components must be disposed of in controlled systems by its hazard type

• Able to generate a Gram stained sample and describe its morphology (as observed with microscope)

d. Scenario Summary

The practicum scenario will be include preparing for and performing a Gram Stain of an isolated microbe followed by disposal of waste fluids. This scenario will have students to read and compare different MSDS sheets for chemicals ranging from environmental toxin to flammability, preform three Gram Stains, examine their products, and report their results. It will require the students to perform the SOPs efficiently so to minimize waste production and disposal of experiment waste correctly.

A complete overview of the chemicals in use, and procedures will be given ahead of student-driven investigation of the chemicals via MSDS sheets.

SOPs will include Gram staining, aseptic technique, and management of chemical waste.

e. Materials and Methods

Materials:

• Chemical - crystal violet, 95% ethanol, iodine, safranin

• Biological– one Gram-Positive organism, one Gram-Negative organism, list provided ahead of lab

• Equipment - glass slides, waste bins, microscopes, Bunsen burners, slide holders, wash bottles, metal loops

<u>Methods:</u>

Gram Staining Procedure

1. Place a small sample of the culture onto a glass slide with sterilized metal loop

(a) if culture is in liquid media, use loop to transfer small droplet of culture to slide

(b) if culture is on solid media, place a small droplet of water onto the slide and then transfer a single colony from the solid media into the droplet and mix till evenly distributed

2. Heat fix the culture

3. Apply crystal violet and wait 2 minutes, then wash off with water until no more dye leaches into the water.

4. Apply Iodine and wait 1 minute, then wash off with water.

5. Apply ethanol to the slide and wash off after 8 seconds, repeat this twice

6. Apply safranin dye and wait 45 seconds, then wash off slide and let dry.

7. Observe slide under a microscope.

Waste Disposal

**Detailed instructions given during lab

• Liquid waste must be disposed of in the Satellite Waste Disposal locations for liquid waste

• Used glass slides must either be washed for reuse or disposed of in the Biologically Hazardous Sharps disposal bin

f. General Scenario Procedure

TAs will prepare three samples of unknown organisms, one Gram Negative, one Gram Positive, and mixture. These cultures will be labeled as 1, 2, and 3, and being unknowns to the students. Students will then preform a Gram Stain of three given cultures as per the SOP stated above. After completing their Gram Stains but before observing, an explanation of the different possible results that the students may observe will be given along with the possible errors that may have happened, and students will then examine their Gram Stains & record their results. After reviewing their results with the class, students will be given a demonstration of waste disposal, discussing each of the different waste containers, and dispose of their produced waste accordingly.

g. Student Assessment

Students assessment will be based on their determination of which unknown culture is the Gram Positive, Negative, and a mixture. A post-lab quiz will also be given where students must summarize the procedure of a Gram Stain along with the purpose of each step and respond to questions derived from presented materials.

Learning Outcomes will be confirmed via:

Post-lab Quiz Q/A – 1,2

Post-lab Quiz Written Response - 3,4

Practicum Results - 4

h. Worksheet & Summary

• Organisms:

◦ Gram Negative -

◦ Gram Positive -

◦ Mixture -

• Gram Staining Procedure

1. Place a small sample of the culture onto a glass slide with sterilized metal loop

(a) if culture is in liquid media, use loop to transfer small droplet of culture to slide

(b) if culture is on solid media, place a small droplet of water onto the slide and then transfer a single colony from the solid media into the droplet and mix till evenly distributed

2. Heat fix the culture

3. Apply crystal violet and wait 2 minutes, then wash off with water until no more dye leaches into the water.

4. Apply Iodine and wait 1 minute, then wash off with water.

5. Apply ethanol to the slide and wash off after 8 seconds, repeat this twice

6. Apply safranin dye and wait 45 seconds, then wash off slide and let dry.

7. Observe slide under a microscope.

• Possible Gram Staining Results:

◦ Gram Negative (-) - Pink / Red from safranin dye

◦ Gram Positive (+) - Purple from crystal violet dye

◦ Unclear Results (na) - Mixture of purple, pink/red colonies or indistinct colonies from incomplete washing, contamination of culture, or unexpected organism

Observations:

Culture Number	Observations	Gram Stain Type (-),(+),(na
1		
2		
3		

4. Cellular Morphologies

Glossary:

Morphology – the shape, form, and physical structures of a cell.

a. Overview

i. the goal of the module – To learn the common visual characteristics of microbial life via microscopy.

ii. the expectations of students – It is expected students understand the learning outcomes from the previous modules and materials from the Pre-lab portion and apply this knowledge during the Practicum Components.

iii. the risks to be encountered – This unit will be examining multiple organisms by microscopy, which has three risks, contact an organism or biohazard on microscope slides, sharp glass of microscope slides, and ocular strain.

iv. the relevance in future experiments - The students will be informed of the limitations to information gathered by microscopy alone by examining organisms with multiple morphologies and organisms with similar morphologies.

b. Learning Outcomes

1. Students will be able to demonstrate knowledge of morphological diversity of prokaryotic organisms and identify them in situ.

2. Students will be able to utilize microscopy to identify the characteristics of a microbe.

3. Students will be able to distinguish different sects of organisms via their observable characteristics

4. Students will be able to perform an examination of microbial organisms and generate a report which notes all observed organisms, organized by their traits

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Is able to utilize microscopy in a time-efficient manner
- Is able to perform microscopy with proper technique and the correct SOPs

Requisite information and skills for students to pass this module:

- Able to identify the characteristics of an organism by visual observation
- Able to actively report these characteristics in a professional format
- Know the importance and limitations of the available microscopes.
- Prepared to perform microscopy of a given microorganism and record observations.

d. Scenario Summary

The practicum portion will consist of a series of demonstrations and student observations of multiple unknown organisms.

SOPs will include Gram staining, wet mounting slides, dry mounting slides, and light microscopy.

A complete overview of the organisms to be used will be provided ahead of the lab as this experiment will require a plethora of examples.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

• Chemical – crystal violet, 95% ethanol, iodine, safranin

• Biological – multiple organisms, list will be provided ahead of class

• Equipment – Bunsen burners, metal loops, light-phase microscopes, 3-5 micro-centrifuge tubes per student pair, NA plates / nutrient broth or other media variety as needed.

Methods:

• Gram staining (see module 2),

• Microscopy (see module 3), and

• Aseptic Technique (see module 1)

f. General Scenario Procedure

The TA will prepare a series of organisms which represent as large a range of morphologies as can be gathered. This series will consist of at least two types of coccus including a diplococcus, a bacillus, a vibrio, and a pleomorphic organism grown in multiple conditions. Each of these organisms will be shown to the students and their morphologies discussed alongside a general overview of the canonical morphologies.

Students will then be given between three and five different unknown samples, which they will have to organize and track. Each of the samples must be Gram stained, wet mounted, and dry mounted. After performing all of these observations, students must format a general report where they describe each of the tests and the implications on visual observations. Students will also have to generate a short-form report which organisms they think their samples is based off their testing.

g. Student Assessment

Assessment will be done on the students' ability to adequately report their observations, distinguish the effects of different sample preparations, and their justifications for choice of organisms (accuracy will not be counted, only precision will be judged).

Learning Outcomes will be confirmed via:

Post-lab Quiz Q/A – 1,3

Practicum Results – 2 4

5. Colony Morphologies

Glossary:

Colony – a collection of many microbial organisms which all came from a single initial cell

a. Overview

i. the goal of the module – To examine different colony morphologies by organism type and growth media.

ii. the expectations of students – It is expected students understand the learning outcomes from previous modules and pre-lab materials, and apply them while performing the Practicum Components.

iii. the risks to be encountered – This module will not require any instrumentation, so the only risks will consist of biohazards of the organisms being examined.

iv. the relevance in future experiments - The students will be informed of how the ability to distinguish different colony morphologies is key to clinical research.

b. Learning Outcomes

1. Students will be able to describe the concept of a colony morphology and its usage within a laboratory.

2. Students will be able to identify common colony morphological types by visual observation.

3. Students will be able to demonstrate knowledge of morphological diversity of prokaryotic colonies and identify them in situ.

4. Students will be able to perform an examination of a plate culture and report its characteristics.

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have general knowledge of colony morphologies

Requisite information and skills for students to pass this module:

- Able to identify typical colony types
- Able to demonstrate correct usage of terminology when reporting colony morphology
- Know the importance and limitations visual observations
- Know the impact of growth conditions on colony morphologies
- Prepared to perform macroscopic observation of a culture to examine colony growth

d. Scenario Summary

The practicum portion will consist of a series of demonstrations and student observations of multiple unknown organisms.

No SOPs will be required beyond aseptic technique.

A complete overview of colony morphological variation will be given along with the terminology to be used. Further overview will include the biological significant of different morphologies.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

- Chemical – None
- Biological – multiple organisms, list will be provided ahead of class
- Equipment – NA / other media plates as needed, dissecting scopes if requested by students

Methods:

Students will examine their plate cultures by the following morphology categories-

- Pigment: as compared to a sheet of white paper
- Form: circular, irregular, filamentous, rhizoid, etc.
- Size: in mm
- Opacity: hold up plate to a light
- Surface:smooth, glistening, rough, rugose, etc.
- Elevation: raised, flat , crateriform, umbonate, etc.
- Edge: complete, undulating, etc.
- Consistency:mucoid, fluffy, viscid, brittle, or butyrous
- Smell:as compared with growth conditions

f. General Scenario Procedure

The TA will prepare a series of organisms which represent as large a range of morphologies as can be gathered. This series will include a combination or rhizoid, mucoid, filamentous, and butyrous organisms. Of these organisms, some will be grown under multiple conditions so to examine the impact of conditions on morphologies. Each of these organisms will be shown to the students and their morphologies discussed alongside the biological significance of these traits.

Stations will be prepared around the lab, each with a different unknown sample. Each of the samples will be examined by macroscopic observation, which the students will have to record and organize. Students must format a general report where they describe each of the samples, by their observations, growth conditions, and media type. Students will also have to report which organisms they think each sample is, based off their testing and the opening lecture.

g. Student Assessment

Students will be assessed on their ability to adequately report observations, distinguish the effects of different media, and their justifications for choice of organisms (accuracy will not be counted, only precision will be judged). A post-lab quiz will be given where students must diagram four colony traits and respond to questions derived from presented materials.

Learning Outcomes will be confirmed via:

Post-lab Quiz Written Response - 1

Post-lab Quiz Q/A – 2,3

Practicum Results - 4

6. Physiologic and Metabolic Activity

a. Overview

i. the goal of the module – To examine the variations in media and growth conditions by their effects on organisms and usefulness within clinical research or diagnostics.

ii. the expectations of students – It is expected students understand the learning outcomes from previous modules and pre-lab materials, and apply them while performing the Practicum Components. It is further expected that students respect the importance in safety while preforming all protocols.

iii. the risks to be encountered – This module will include a large range of tests, each with the potential for chemical hazard, and a large range of organisms, increasing the possible number of biohazards.

iv. the relevance in future experiments - The students will be instructed the tests being performed will be used in future experiments, as well as how the interpretations of data can be used to infer important characteristics of organisms.

b. Learning Outcomes

1. Students will be able to describe the differential medias.
2. Students will be able to interpret the results of multiple new tests.
3. Students will be able to demonstrate knowledge of microbial metabolic and physiologic morphological diversity.
4. Students will be able to determine which tests can, and should, be used for different scenarios.

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have specific knowledge of colony morphologies

Requisite information and skills for students to pass this module:

- Able to describe colonies in relation to the media & growth conditions
- Able to demonstrate correct usage of a terminology
- Know the possible information which can be gathered from the tests performed
- Able to predict the results of a test for different organisms
- Prepared to utilize this information for differential testing

d. Scenario Summary

The practicum portion will consist of a series of demonstrations and student observations of multiple known organisms. This will be a largely hands-off experiment and it is the responsibility of the students to take adequate notes with descriptions of all observed tests, organisms, and medias.

No SOPs will be required beyond aseptic technique.

A complete overview of the media / growth conditions which were employed for each sample will be given alongside the biologic significance of the results. This module will be a strongly guided one, so each test and organism will be individually discussed, focusing on the metabolic or physiologic trait that is being tested.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

• Chemical – multiple medias and chemicals, list will be provided ahead of class

• Biological – multiple organisms, list will be provided ahead of class

• Equipment – NA / other media plates as needed, dissecting scopes if requested by students

Methods:

• A hand out will be generated with the possible results for each test and the strategies for observing them.

f. General Scenario Procedure

The TA will prepare a series of samples with different growth condition and organisms. The samples generated will be dependent on resources available and the tests which will be employed during the next units. Each of these samples will be shown to the students with the following information: type of growth conditions (e.g. media), organism, usage of test, and interpretations of results.

Students will move between stations, each of which consist of a single growth condition test for multiple organisms that show the variety of results that can be found. At each station, the student will observe an un-inoculated sample, a true positive, a true negative, and a false positive / negative, if possible. Students will then record this information and generate a table organized by organism and growth condition which will be used in later modules. Students will also prepare a short-form report that discusses what each growth condition tests for, and why it might be useful.

g. Student Assessment

Assessment will be based on the students' ability to adequately and accurately record their observations for each test. Key assessment topics will include the usage of specific growth conditions and medias, value of results for keystone growth conditions, and the scope of bacterial diversity observed. Further assessment will be done via the short-form report, giving focus to their ability to identify the key reasons when and why a test should be used (Accuracy will be judged over precision).

Learning Outcomes will be confirmed via:

Post-lab Quiz Written Response - 1

Post-lab Quiz Q/A – 2,3

Practicum Report - 4

7. Isolation of an Organism

a. Overview

i. the goal of the module – To learn the fundamental methods of isolating an organism by visual selection and re-streaking.

ii. the expectations of students – It is expected students understand the learning outcomes from previous modules and pre-lab materials, and apply them while performing the Practicum Components.

iii. the risks to be encountered – This module will work with multiple biological organisms, but no other hazards.

iv. the relevance in future experiments – This module and the methods reviewed in it will be featured as the primary step in all major modules to come as they are core practices for all of microbiology, regardless of setting.

b. Learning Outcomes

1. Students will understand the basis which isolation streaking works on.
2. Students will be able to demonstrate isolation streaking and rewrite the procedure on call.
3. Students will be able to perform isolation streaking to separate mixed cultures into individual organisms.
4. Students will be able to determine how many observable organisms are within a culture.
5. Students will have a baseline understanding of dilutions and Colony-Forming-Units (CFU/ml).

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have specific knowledge of colony morphologies
- Able to demonstrate correct usage of aseptic technique

Requisite information and skills for students to pass this module:

- Able to perform an isolation streak to locate a specified organism
- Able to use spread plate method to isolate individual colonies from a diluted culture
- Prepared to utilize this information alongside differential testing

d. Scenario Summary

The practicum portion will consist of two hands-on tasks for the students. The first will be isolation streaking from a dense, overgrown culture. Students must attempt to identify the different colony types in this plate, then selectively re-streak them for isolation. Afterwards, a brief overview of Colony

Forming Units (CFU/ml) and dilutions will be given. Once prepared, students will then perform a spread plate of a diluted liquid culture.

SOPs will include aseptic technique, isolation streaking (see module 1), and spread plating (see module 1).

This scenario will have limited guidance, as it is the students' responsibility to maintain their work area and perform the tasks requested, following all SOPs.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

- Chemical – None
- Biological – multiple organisms, list will be provided ahead of class
- Equipment – NA / other media plates as needed, dissecting scopes if requested by students

Methods:

See Module 1, Aseptic Technique

f. General Scenario Procedure

The TA will prepare samples of multiple organisms in diluted liquid cultures and overgrown NA plates. The samples generated will consist of organisms with significant difference in colony morphologies. Students will examine their NA plate culture and identify the different colony morphologies present to the best of their abilities. Students will then take two regions with different morphologies and perform two isolation streaks, attempting to isolate at least two organisms (greater than 2 will be present). Students will then transfer 100 ul of the diluted liquid culture onto the plate and spread it by spread-bar method. After incubating, students will examine their plates and compare the number of isolated, unique colonies present from their isolation streak to their spread-plate.

g. Student Assessment

Assessment will primarily be based on the students' ability to adequately isolate and report multiple organisms from their samples. Isolation of no organisms is a fail, one organism is a pass, two is an A, and three is an A+. Further assessment will be done via the short-form report, where students must write an annotated protocol for both aseptic isolation streaking and spread-plate method and provide summaries of the colony morphologies observed (where accuracy will be judged over precision). The report can be extended for students who were unable to isolate any bacteria where they must present:

- A diagram of their streaking patterns as observed on their plates
- A diagram of the organism distribution observed on the plate
- An explanation of their results by comparing their performed method to the original SOP

Learning Outcomes will be confirmed via:

Practicum Results – 3, 4

Post-lab Quiz Q/A – 1, 5

Practicum Report – 2

8. Selective and Differential Testing

a. Overview

i. the goal of the module – To examine the effects that growth media and conditions have on bacterial growth and how such conditions can be used to study or identify an organism.

ii. the expectations of students – It is expected students understand the learning outcomes from previous modules and pre-lab materials, and apply them while performing the Practicum Components. It is further expected that students respect the importance in safety while performing all protocols.

iii. the risks to be encountered – This module will include a range of chemical hazards depending on the tests performed, and a range of organisms, increasing the possible number of biohazards.

iv. the relevance in future experiments - The students will be informed the tests being performed will be used in future experiments, how the interpretations of data can be used to infer important characteristics of organisms, and the importance of these methods in clinical microbiology.

b. Learning Outcomes

1. Students will be able to describe the usage of different medias and interpretations of their results.

2. Students will be able to utilize the results of multiple new tests in order to identify an organism.

3. Students will be able to record the results of tests and explain their relevance to microbial metabolic and physiologic/morphological diversity.

4. Students will be able to assess whether the tests performed were effective at differentiating and identifying organisms.

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have specific knowledge of colony morphologies
- Have specific knowledge of the medias and growth conditions to be used

Requisite information and skills for students to pass this module:

- Able to describe the medias and their relevant usages
- Able to utilize the results of the tests performed
- Know the challenges and limitations of the tests performed
- Able to predict the results of a test by the known or predicted traits of an organism
- Prepared to utilize this information for differential testing of an unknown sample

d. Scenario Summary

The practicum portion will consist of hands on testing with a series of different medias and student observations for the identification of an unknown sample from a list of possible organisms. Several key traits which can be used to differentiate the organisms selected for this test will be highlighted, and each student will perform a test to determine said traits of their provided sample. After observing their results, students will compile them and deduce which organism they were growing from the list.

SOPs will include aseptic technique and specific protocols for each growth-condition being used.

A complete overview of the media / growth conditions which are employed will be given alongside the biologic significance of the results and the results students should expect. This module will be a loosely guided one, as only an overview of the tests and organisms will be given. It is up to the students to correctly follow the protocols and record / interpret their results.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

- Chemical – multiple chemical-growth conditions, list provided ahead of class
- Biological – multiple organisms, list will be provided ahead of class
- Equipment – NA / other media plates as needed, dissecting scopes if requested by students

Methods:

A hand out will be generated with the traits being tested, the protocols for the tests, and the possible results.

f. General Scenario Procedure

The TA will prepare 3-4 organisms as unknown samples and select 4-6 characteristics which can be used to separate and identify them. The TA will prepare a chart that explains each of the traits, what results are expected for a given organism, and how the traits will be examined. The organisms and characteristics selected will be dependent on resources available and the tests which will be employed during the next units.

Students will retrieve one of the unknown samples, on a plate or as a liquid culture, and record its number. Students will then move through the list of characteristics and inoculate all of the media being used. After incubation, these cultures will be examined, and any additional tests or processing will be completed. Students will then record this information and generate a recreation of the initial flow-chart, marking the results they observed and the significance of each. The flow chart should conclude with a single organism being identified. The flow chart will operate as a short-form report, which students will use as an aid for an in-class assessment quiz.

g. Student Assessment

Assessment will be done on the students' ability to adequately and accurately report their results, via flow-chart, and utilize this information within a quiz. The quiz will consist of a series of short-answer

questions where students must provide the rational for what each of the tests performed effectively determined and how a single organism was identified through the process. (Accuracy will be judged over precision).

Learning Outcomes will be confirmed via:

Post-lab Quiz – 1,2,4

Practicum Report – 2,3

9. Serial Dilutions, Colony Forming Units, and Plaque Forming Units

a. Overview

i. the goal of the module – To examine the mathematics and methods of serial dilutions used within Microbiology and apply it to observe Colony Forming Units (CFUs) and Plaque Forming Units (PFUs).

ii. the expectations of students – It is expected students refresh their knowledge of mathematical factors and fractions. It is further expected that students respect the importance in safety while performing all protocols.

iii. the risks to be encountered – This module will contain no major hazards, but will utilize both a bacterial organism and a bacteria phage.

iv. the relevance in future experiments - The students will be informed of the usages for serial dilution protocols within Microbiology and chemistry at large. Explanation of how both CFUs and PFUs are used for quantification of organisms will also be given.

b. Learning Outcomes

1. Students will be able to describe the usage of serial dilutions, CFUs, and PFUs.
2. Students will be able to utilize serial dilutions to determine CFUs/ml and PFUs/ml.
3. Students will be able to record the results of quantification testing as data tables.
4. Students will be able to assess whether the tests performed were effective, having accurate results and no contamination.

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have specific knowledge of colony morphologies
- Able to utilize a pipette efficiently and accurately

Requisite information and skills for students to pass this module:

- Able to describe the mathematics of serial dilutions
- Able to utilize the serial dilutions
- Know the definitions of CFUs and PFUs
- Able to predict the observed concentrations of a sample after dilution
- Prepared to utilize this information for quantification testing of an unknown sample

d. Scenario Summary

Serial dilutions is a practice of performing controlled and regular dilutions of a sample into a sterile media or solution. Serial dilutions can be applied to many different circumstances, most notably for the determination of CFUs/ml and PFUs/ml.

The practicum portion will consist of a guided practice of serial dilutions, then hands on testing for the determination of both CFUs/ml and PFUs/ml.

SOPs will include aseptic technique, serial dilution by pipette, and plate reading. Optionally, isolation of bacteria phages and creation of a bacterial lawn can be completed.

There will be simulated aspects of the practicum for the initial serial dilution practice only.

e. Materials and Methods

Materials:

• Chemical – none

• Biological – one organism, one bacteria-phage

• Equipment – 2ml micro centrifuge tubes with 900ul sterile water (6 per group), NA / other media plates as needed, dissecting scopes if requested by students, pipettes, pipette tips

Methods:

Serial Dilutions -

1. Label 6 tubes, 3 through 8; these will correspond to the virtual dilution factors
2. Remove 100 ul of the source sample and put into tube 2
3. Vortex / mix tube 3
4. Remove 100 ul of solution from tube 2 and put into tube 3
5. Vortex / mix tube 4
6. Repeat steps 4 and 5 for all remaining tubes

CFUs/ml determination -

1. Perform a serial dilution of an initial sample with virtual dilution factors ranging from 3 to 8
2. Divide a NA plate into 6 equal sections and label 3 through 8
3. Place 3 spots of 20 ul of tube 3 into region 3; keep the spots well separated, but within the lines of the region
4. Repeat step 3 for each of tubes and regions
5. Incubate plate
6. Using a dissecting scope, if needed, count the number of colonies present of each spot in every region of the plate

7. Record the number of colonies as Too Few to Count (TFC), Too Many to Count (TMC), or the number of observed colonies above each spot

8. Create a data table with all observed information, including the virtual dilution factor and counts from the three spots

9. Create a column within the data table that has the average of the three spots for each virtual dilution factor

PFUs/ml determination -

1. Perform a serial dilution of an initial sample with virtual dilution factors ranging from 3 to 8

2. Divide a NA plate into 6 equal sections and label 3 through 8

3. Place 3 spots of 20 ul of tube 3 into region 3; keep the spots well separated, but within the lines of the region

4. Repeat step 3 for each of tubes and regions

5. Incubate plate

6. Using a dissecting scope, if needed, count the number of colonies present of each spot in every region of the plate

7. Record the number of colonies as Too Few to Count (TFC), Too Many to Count (TMC), or the number of observed colonies above each spot

8. Create a data table with all observed information, including the virtual dilution factor and counts from the three spots

9. Create a column within the data table that has the average of the three spots for each virtual dilution factor

f. General Scenario Procedure

After an explanation of the math behind serial dilution, students will perform a practice with water and food coloring, treating the food coloring as a biohazardous agent. For this practice, once a dilution series is made, students will pipette a drop of each sample onto a piece of white paper, labeling each spot, creating a color gradient with the same scale as their bacterial dilution series.

The TA will prepare an overnight culture of a bacterial sample and a phage-culture. Students will retrieve 100 ul of both cultures in micro-centrifuges tubes. Students will then perform serial dilutions and plating of both samples as per the protocols above.

The students will gather their data and present it to the TA with a recreation of the serial dilution protocols as a diagram.

g. Student Assessment

Assessment will be done on the students' ability to adequately and accurately report their results and recreate the protocol. A quiz will also be given and consist of a series of multiple-choice questions of the formal definition of serial dilutions, CFUs, PFUs, and math-problems for example serial dilutions.

Learning Outcomes will be confirmed via:

Post-lab Quiz – 1

Practicum Results – 1,2,3,4

10. Identification of an Unknown Organism

a. Overview

i. the goal of the module – To utilize all materials and skills learned from previous modules to study and identify the unknown organisms present in a sample.

ii. the expectations of students – It is expected students understand the learning outcomes from all previous modules and apply them while performing the Practicum Components. It is further expected that students demonstrate proper techniques and SOPs to create a safe laboratory environment.

iii. the risks to be encountered – This module will include a range of chemical hazards depending on the tests selected, multiple possible biohazardous organisms, and physical risks by proximity to Bunsen Burners.

iv. the relevance in future experiments – Students will be informed that as the culminating module, this is a realistic scenario and experimental procedure for clinical microbiology. The skills in use are applied to many subjects, methodologies, and forms of research.

b. Learning Outcomes

1. Students will be able to demonstrate ability to perform all experimental protocols with correct SOP usage.

2. Students will be able to correctly report their results and present them all in a condensed, efficient format.

3. Students will be able to synthesis conclusions from the data they gather and present a clear defense of their conclusions.

4. Students will be able to demonstrate understanding of the tests they perform and correct interpretations of the results in order to discuss the physiologic and metabolic characteristics of the isolated organisms.

c. Target Information and Skills

Prerequisite information and skills for students :

- All information from previous modules
- Able to perform aseptic technique
- Have specific knowledge of colony morphologies
- Have specific knowledge of cellular morphologies
- Have specific knowledge of the medias and growth conditions to be used
- Able to apply all previous knowledge for the study of an unknown sample

Requisite information and skills for students to pass this module:

- Able to demonstrate ability to apply all previous learning outcomes

- Able to report data in a condensed format
- Able to generate a report which presents, explains, and utilizes all data collected

d. Scenario Summary

The practicum portion will consist of hands-on testing for the study of an unknown sample. Students will have to isolate one or more organisms from a sample via isolation streaking, study it by macroscopic observation, Gram stain it for microscopic observation, apply selective and differential testing for qualification, perform quantification testing of antibiotic resistance, and generate a complete report presenting all of this information. All major decisions of tests and organization within each section will be made by the students; it will be their responsibility to utilize the information given in previous modules and summary sheets provided for this experiment in order to select an optimal set of media and tests which will adequately study the organisms isolated. It will be the students' goal to isolate and study two organisms so that if issues arise with one, they will still have a complete flow-chart & report.

SOPs will include aseptic technique and specific protocols from each of previous modules.

Summary sheets which provide a topical list of all organisms that students may isolate and the results that each of the organisms would have for all tests available. This will include the following:

- Cellular morphologies
- Colony morphologies
- Physiologic activity (e.g., motility, temperature limits, pH limits, salinity limits)
- Metabolic activity (e.g., fermentation of sugars, nutrient requirements, Nitrate/Nitrite reduction, etc.)
- Antibiotic sensitivity (by zone of inhibition size)
- Phage type (if available)

This module will be further divided into specific sections of testing so that the TA may select which aspects to focus on. Each section will include a complete overview of the tests available, summary of the protocols to use, and overview of all hazards to be encountered. Beyond the initial instructions, students will operate independently for this module and the TA should only provide aid to ensure student safety and respond to direct questions about specific tests and results, if asked by a student.

There will be no simulated aspects of the practicum.

e. Materials and Methods

Materials:

1. Chemical – multiple medias, growth conditions, and antibiotics; list provided ahead of class
2. Biological – multiple organisms; list will be provided ahead of class
3. Equipment – Bunsen burners, temperature-controlled incubators or shaking baths, pipettes, dissecting scopes if requested by students, and an assortment of medias

Methods:

• Isolation Streaking (see modules 1,7)

• Gram Staining (see modules 2,3)

• Selective and Differential Testing (see modules 6,8)

Phage Typing

**Protocols of this will be provided with additional detail ahead of performing the test

1. Determine the PFU/ml of the phages available if not already completed

2. Overnight culture your organisms by spread plate on a NA

3. Divide the overnight culture plate into 4 or 5 regions (make one for each phage type being tested and one for control)

4. Label each region for a specific phage-type

5. Pipette a drop of each phage onto its corresponding zone

6. Incubate overnight

7. Record if plaques are formed for each phage-type

Antibiotic Resistivity Testing by Zones of Inhibition:

**Protocols of this will be provided with additional detail ahead of performing the test

1. From the data previously gathered, determine what to test with antibiotics

2. Divide an NA plate into 4 or 5 regions (make one for each antibiotic being tested and one for control), label each region with the antibiotic being applied

3. Spread plate out an overnight sample of the organism (see module 1) onto the labeled plate

4. Place antibiotic disks into the centers of each region, excluding the control

5. Incubate overnight

6. Measure the zones of inhibition in mm and record the diameter of the zone

For the determination of bactericidal effect:

1. Repeat procedure above except incubate the plate overnight before adding the antibiotic disks

2. Record zone of inhibitions

3. if zones appear clear through and large, then the antibiotic is bactericidal

4. if zones have less growth than control but still grew, then the antibiotic is bacteriostatic

f. General Scenario Procedure

This module will be done in 5 separate portions: isolation of organisms, qualification, quantification, phage-typing, and report writing.

1. Isolation of Organisms

The TA will prepare a series of unknown samples which can be separated and identified by a combination of morphological observations, physiologic / metabolic testing, antibiotic resistance testing, and phage-typing. Students will each be provided a sample and a summary sheet of the possible organisms and their testing results. Students will review the list of organisms then perform an isolation streak of their sample. The sample will be incubated overnight for the next class. Students will then perform a Gram stain of their initial unknown sample and record all morphologies and Gram types present.

2. Qualification

Students will retrieve their isolation streak plates and observe the results. Students should repeat their Gram Stain for each isolated colony type observed and create second isolation streaks for two observed colonies on a plate divided in half. From the observed colony morphologies and Gram staining results, students should decide which tests could be used to best identify the organisms isolated. Students will then retrieve the media they require and perform said tests. The following class period, students should observe their results and perform additional testing, if required. Ideally, each student will have 3-4 clear results of qualification.

*If students do not achieve isolated colonies to satisfaction, they may repeat their isolation streak on the original agar. In order to stay productive, they should only use plates for the testing and perform isolation streaks on them until isolated colonies are achieved.

Students should take the remaining of the following class period to determine what antibiotics to use in Quantification. If testing for bactericidal activity, spread NA plates with the organisms and incubate for next class period.

3. Quantification

Students will observe testing results from previous class if any additional ones were performed. Students should then perform an antibiotic resistivity test and incubate overnight. Before leaving, students should also prepare an overnight culture for phage-typing.

4.Phage Typing

Students will observe their antibiotic resistivity testing, repeating if plates were unreadable or disrupted in some way. Afterwards, students will perform phage-typing on their organisms and incubate for next class period.

5. Report Writing

After observing the results of the phage-typing, students should have all the data they require to properly identify the organisms isolated from their original sample.

As the final class period of this module, students will review all their data and create a drawn flow-chart showing their thought process for all tests performed. This flow chart should include all results and conclude with the organism they believe best fitting to their data. From this flowchart, students will

elaborate and create a formal lab-report which includes descriptions of all tests performed, the purpose of each test, their results, and the information which can be determined from the results. Students will be given a complete rubric of how their lab report will be graded, along with an example of how to format and organize their reports.

g. Student Assessment

Assessment will be done on the students' ability to adequately and accurately report their results, via flow-chart and report. Grading will not be done by the accuracy of their conclusion, but instead by the precision in their planning and execution. The ultimate purpose of the student reports will be to present their efforts in a concise, yet descriptive manner that justifies each choice made.

Learning Outcomes will be confirmed via:

In Practicum – 1

Flow Chart – 2

Practicum Report – 2,3,4

Example of Lab Report Content

Category: (quantification, qualification, isolation, phage-typing, etc.)

Description: (media type, growth conditions, tools used, etc.)

Protocol:

1. List steps performed

2. With all

3. Modifications done

Usage: (What is the purpose of this test?)

Results: (What did you observe? What does this tell you about the organism?)

--- repeat this format for all tests performed, each as individual sections---

Summary of Results:

(Copy formatting from summary sheets, present all results that will be included in the conclusion)

Conclusions:

From the tests performed, I have identified Name organisms as the organisms present within unknown sample (Your-Sample-Number). For organism 1, I observed (Insert-Results-Here). Of the results, each support the conclusion that organism 1 is Name organism. (Repeat for organism 2)

** if any results from tests are conflicting or failed, include a summary of the results and how they conflicted or failed with an explanation of what could have caused the problems **

Conclusion