OER - BIOL 2420 LABORATORY MANUAL

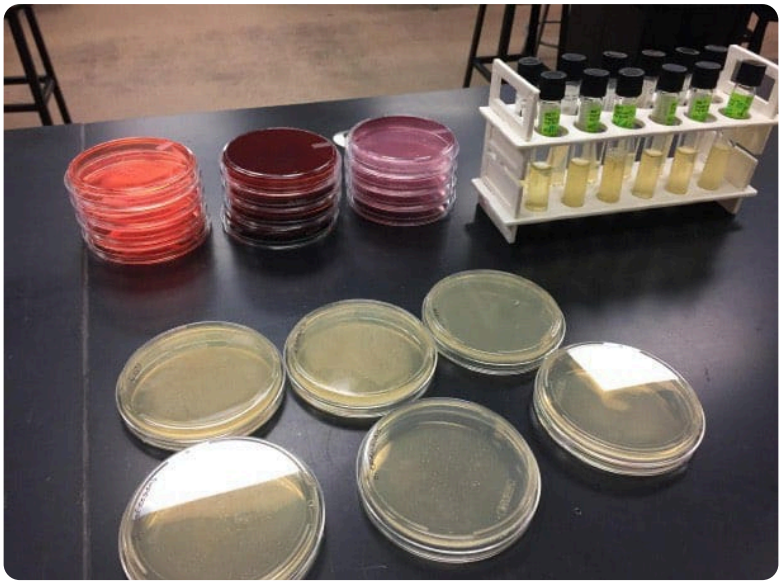


Sherry Smith
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A Laboratory Manual For Non-Science Majors Microbiology

Author: Sherry Smith



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Detailed Licensing

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Preface

This laboratory manual was written with the support of a Texas Higher Education Coordinating Board (THECB) Open Educational Resources Grant Program (OERGP). The purpose is to provide students in microbiology for non-science majors courses a no or low cost manual that would provide the content needed to successfully master the Academic Course Guide Manual (ACGM) course laboratory learning outcomes. The manual was written to provide laboratory exercises that would align with all required ACGM laboratory learning outcomes.

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THECB ACGM Course Laboratory Learning Outcome to Exercise Correlation Tables

ACGM Course Laboratory Learning Outcome to Exercise Correlation Tables

The following tables correlate the learning outcomes listed in the Texas Higher Education Coordinating Board (THECB) Academic Course Guide Manual (ACGM) for the microbiology for non-science majors laboratory courses with the exercises provided in this laboratory manual.

Course sections listed in the THECB ACGM that require these learning outcomes include the following:

- BIOL 2120 Microbiology for Non-Science Majors (Lab)
- BIOL 2420 Microbiology for Non-Science Majors (Lecture + Lab)

Lab course description:
This course covers basics of culture and identification of bacteria and microbial ecology. This course is primarily directed at pre-nursing and other pre-allied health majors and covers basics of microbiology. Emphasis is on medical microbiology, infectious diseases, and public health.

THECB ACGM Laboratory Learning Outcome #1

Use and comply with laboratory safety rules, procedures, and universal precautions.

Table 1. Correlation of Laboratory Learning Outcome #1 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #1	Content and terminology specific to laboratory safety is covered.
Exercise #2 - 33	Students are expected to utilize universal precautions, and comply with laboratory safety rules and procedures as the experiments are conducted.

THECB ACGM Laboratory Learning Outcome #2

Demonstrate proficient use of a compound light microscope.

Table 2. Correlation of Laboratory Learning Outcome #2 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #2	Content and terminology specific to the components and utilization of microscopes is covered.
Exercise #6 - 11, 25, 30 - 32	Students are expected to correctly utilize microscopes to examine slides for observation of samples.

THECB ACGM Laboratory Learning Outcome #3

Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

Table 3. Correlation of Laboratory Learning Outcome #3 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #7	Content and terminology related to specimen preparation and simple staining are covered. Students work to prepare and simple stain cheek smears, and bacterial smears.
Exercise #8	Content and terminology related to differential staining and the gram stain are covered. Students stain bacteria using the Gram stain procedure and make observations regarding their results.
Exercise #9	Content and terminology related to the gram acid-fast are covered. Students stain bacteria using the acid-fast stain procedure and make observations regarding their results.
Exercise #10	Content and terminology related to the endospore stain are covered. Students work bacteria using the Gram stain procedure and make observations regarding their results.
Exercise #11	Content and terminology related to the capsule stain are covered. Students stain bacteria using the capsule stain procedure and make observations regarding their results.

THECB ACGM Laboratory Learning Outcome #4

Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Table 4. Correlation of Laboratory Learning Outcome #4 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #4	Content and terminology related to aseptic protocols and the transfer of bacteria are covered. Students perform aseptic technique procedures transferring bacteria to demonstrate proficiency.
Exercise #5	Students utilize aseptic technique and transfer protocols to perform the streak plate isolation activity.
Exercise #7 - 11	Students utilize aseptic techniques to prepare bacterial smears for staining protocols.
Exercise #12 - 23	Students utilize aseptic techniques to inoculate media to perform biochemical tests for identification of clinically significant bacteria.
Exercise #24 - 25	Students utilize aseptic techniques while performing bacterial enumeration techniques.

THECB ACGM Laboratory Learning Outcome #5

Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Table 5. Correlation of Laboratory Learning Outcome #5 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #3	Content regarding types of media (chemical composition, physical composition, functional classification) are introduced.
Exercise #4, 5	Students utilize different physical forms of media (broth and solid) to practice aseptic transfers techniques and isolation techniques
Exercise #12 - 23	Students use various media types to complete biochemical tests.
Exercise #24	Students utilize agar to produce plates for viable plate counts to enumerate the number of bacteria in a provided sample.

THECB ACGM Laboratory Learning Outcome #6

Perform basic bacterial identification procedures using biochemical tests.

Table 6. Correlation of Laboratory Learning Outcome #6 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #12	Content and terminology related to the carbohydrate fermentation test are covered. Students perform the carbohydrate fermentation test.
Exercise #13	Content and terminology related to the triple sugar iron agar test are covered. Students perform the triple sugar iron agar test.
Exercise #14	Content and terminology related to the SIM medium test are covered. Students perform the SIM medium test.
Exercise #15	Content and terminology related to the IMViC tests (indole, methyl red, Voges-Proskauer, and citrate) are covered. Students perform the IMViC panel of tests.
Exercise #16	Content and terminology related to the respirations test are covered, including the oxidase test, catalase test, and nitrate reduction test. Students perform the oxidase test, catalase test, and nitrate reductase test.
Exercise #17	Content and terminology related to the urease test are covered. Students perform the urease test.
Exercise #18	Content and terminology related to the coagulase test are covered. Students perform the coagulase test.
Exercise #19	Content and terminology related to the casease test are covered. Students perform the casease test.
Exercise #20	Content and terminology related to the starch hydrolysis test are covered. Students perform the starch hydrolysis test.
Exercise #21	Content and terminology related to blood agar and hemolysis are covered. Students inoculate blood agar plates to observe hemolytic properties of bacteria.
Exercise #22	Content and terminology related to the use of MacConkey agar to determine lactose utilization abilities are covered. Students inoculate MacConkey agar plates to observe the growth of lactose fermenting and non-lactose fermenting gram negative bacteria on the agar.
Exercise #23	Content and terminology related to the use of mannitol salt agar to determine mannitol fermentation abilities are covered. Students inoculate mannitol agar plates to differentiate between various species of Staphylococcus.

THECB ACGM Laboratory Learning Outcome #7

Estimate the number of microorganisms in a sample using methods such as direct counts, viable plate counts, or spectrophotometric measurements.

Table 7. Correlation of Laboratory Learning Outcome #7 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #24	Content and terminology related to serial dilutions and viable plate counts are covered. Students perform serial dilutions and viable plate counts to determine the number of bacteria in a provided sample.
Exercise #25	Content and terminology related to direct microscopic counts are covered. Students enumerate bacteria in a provided sample using a Petroff-Hauser counting chamber and direct microscopic count procedures.

THECB ACGM Laboratory Learning Outcome #8

Demonstrate basic identification protocols based on microscopic morphology of some common fungi and parasites.

Table 8. Correlation of Laboratory Learning Outcome #8 to laboratory exercises

Exercise(s)	Correlation to Laboratory Manual
Exercise #30	Content and terminology related to fungal pathogens are covered. Students survey common fungal pathogens and visualize them utilizing prepared slides.
Exercise #31	Content and terminology related to protozoan parasites are covered. Students survey common protozoan parasites and visualize them utilizing prepared slides.
Exercise #32	Content and terminology related to helminth parasites are covered. Students survey common helminth parasites and visualize them utilizing prepared slides.

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Acknowledgements

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I'm grateful to the Texas Higher Education Coordinating Board (THECB) and the Open Educational Resources Grant Program (OERGP) for providing the funding that made this project possible. Their commitment to accessible, high-quality education benefits educators and students across Texas.

I'm thankful to Susan Decker in the Science Division at NCTC for sharing several illustrations that added to the manual. Likewise, I appreciate Yenny Mo and Josh Spradling from the NCTC eLearning team for creating videos that help connect the lab content to clinical practice in meaningful ways.

To the students who participated in the pilot during Fall 2024 and Spring 2025—thank you for your honest feedback and engagement. Your insights shaped this resource into a more effective and student-friendly tool. I also appreciate Dr. Diana Avila and Mattie Shah for piloting the manual in their classes and providing valuable instructional feedback.

Finally, I want to thank my husband for his unwavering encouragement and support at home—cheering me on through every late night and long revision.

This manual was written with students in mind, and I hope it serves as a helpful and meaningful resource for those studying microbiology and preparing for careers in the allied health sciences.

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1: SECTION ONE - GETTING STARTED IN THE MICROBIOLOGY LABORATORY

Section Introduction



Credit: Sherry Smith

In this section you will be introduced to foundational concepts needed to safely work in the microbiology laboratory. You will explore the concepts of laboratory safety, microscopy, and how to aseptically work with microbes and grow them. An understanding of these concepts is imperative for success in the laboratory activities of subsequent sections of this laboratory manual.

Why does this material matter? Watch the video to learn how this material applies to your future as a health-care professional.

YouTube Video

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Lab Foundations: Tools, Techniques, and Safety

Click on the video thumbnail or the linked title to access the video.

Credit: Yenny Mo, Josh Spradling, and Sherry Smith

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1.1: Exercise #1 - Laboratory Safety

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

Laboratory Exercise Learning Outcomes

1. Explain the purpose of following laboratory safety guidelines.
2. List and describe the expected laboratory rules and procedures
3. Locate and explain the use of laboratory safety equipment.
4. Demonstrate compliance with laboratory safety rules, procedures, and universal precautions.

Before we can begin working in the microbiology laboratory, we must address the topic of laboratory safety. Laboratory safety policies exist to protect you and those working with you. In the microbiology lab we must consider the potential of becoming contaminated with the microorganisms we are working with, carrying those microorganisms out of the laboratory setting, or contaminating the cultures we are working with.

Microorganisms are classified into four biosafety levels by the Centers for Disease Control and Prevention (CDC). These ***biosafety levels*** (BSL) require different measures to protect oneself when working with microorganisms. The higher the biosafety level the greater the number of procedures needed to protect oneself from the microorganism. We will be working with microorganisms that are BSL-1 and BSL-2 level microorganisms. Some of the microorganisms that will be used in the laboratory are known pathogens.

Pathogens are microorganisms capable of causing disease. Therefore, it will be imperative that you follow all safety protocols outlined for your laboratory.

Students will need to read lab exercises before attending laboratory sessions. This will allow the student to become familiar with what will be done during the laboratory session. This is extremely important in this course since students will be working with disease causing microorganisms, flammable materials, and caustic chemicals. By reading the exercises in advance of a lab session students will be less likely to experience a lab safety incidence and will also be more likely to successfully complete and understand the material covered.

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1.1.1: Microbiology Safety Guidelines

In this section of the laboratory exercise, you will read over the guidelines for the microbiology laboratory. It is important to know, understand, and adhere to these guidelines in order to keep yourself and others safe in the laboratory setting.

- Lab time is limited. Come prepared to lab having completed the pre-lab. A familiarity with what will be done in the lab session reduces the likelihood of confusion and accidents.
- Never work alone in the laboratory without permission and prior knowledge of the instructor.
- No children, visitors, or students not enrolled in this course are permitted in the lab.
- Do not engage in rowdy, playful, or unprofessional activities in the laboratory. This includes not being disrespectful of your instructor/lab facilitator and/or classmates.
- Know where the safety equipment is in the lab. (Eye Wash, Safety Shower, Fire Extinguisher, Safety Data Sheets, Gas Shut-Off, and the First Aid Kit).
- Students must wear closed-toe shoes that cover the top of the foot, and appropriate clothing at all times in the laboratory.
- Work surfaces must be disinfected at the beginning and at the end of every laboratory period.
- All students must wash their hands at the beginning of lab and at the end of every laboratory period.
- Keep hands away from your face, eyes, and mouth when working with chemicals or microorganisms. This includes not applying cosmetics, not adjusting contact lenses, and not biting your fingernails.
- No eating or drinking is permitted in the laboratory. No open food or beverage can be taken into the laboratory.
- Lab benches are to be kept free of extraneous items while conducting experiments. This includes personal items such as backpacks, cell phones, and unnecessary books.
- Never lay culture tubes on their sides on the table. They should remain upright in test tube racks to prevent the leakage of any bacteria onto the counter.
- If any chemicals or other agents splash into your eyes, immediately go to the eyewash and flush your eyes with water for 15 minutes.
- Cover any culture spills with paper towels. Soak the towels immediately with disinfectant and allow them to stand for 20 minutes. Report the spill to your instructor. When you are finished place the paper towels in the container marked for autoclaving.
- Students are to dispose of uncontaminated broken glassware in the marked cardboard box container. Broken glass containers are ***only*** to be used for broken glass. Always use a broom and dustpan if asked to clean up broken glassware.
- Biohazardous wastes must be disposed of in a biohazard waste container. Materials within this container will be autoclaved.
- Some experiments may require the use of examination gloves. It is important to wear gloves when instructed.
- While wearing examination gloves, students must not leave the laboratory and must not touch any equipment such as microscopes, any personal items such as cell phones, or any doorknobs.
- Take special precaution when working with open flames. Loose hair, clothing, dangling jewelry, and nearby paper must be secured. Do not leave a heat source (hot plate or Bunsen burner) unattended. Keep containers of alcohol, acetone, or other flammable liquids at a safe distance from flames.
- Report ***any*** and ***all*** accidents, spills, breakages, or injuries to the instructor, no matter how trivial they appear.
- Do not remove cultures, reagents, or other materials from the laboratory unless specific permission from the instructor has been granted.
- Do not use any lab equipment without instruction and authorization from the instructor. Report any damaged or broken equipment to your instructor immediately.
- Students must assume that all of the organisms that they work with in the laboratory are potential pathogens (disease-causing microbes). However, this laboratory does not require the use of any highly infectious human pathogens. All stock cultures are purchased commercially.

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1.1.2: Laboratory Safety Equipment

It is important to familiarize yourself with the purpose and location of laboratory safety equipment. You should know the importance of the following equipment, how it is utilized, and where it is located within your laboratory room. Look over the descriptions of the structures and their importance in the microbiology laboratory. As your instructor points these items out to you in your laboratory classroom, write down their location and specific instructions regarding their use as provided by your instructor.

Broken Glass Container -

Students in a microbiology lab will be working with glassware (i.e. test tubes, microscope slides, etc.) and thus must be prepared to handle broken glassware appropriately should an accident occur. Broken glass should ***never*** be placed in a trash can, because it could puncture the trash bag and cut the individual disposing of the bag. The broken glass container will generally be a cardboard container labeled "Broken Glass." This container serves to prevent injuries when the materials are being disposed of. ***Only uncontaminated broken glassware*** should be placed in this container. Broken contaminated glassware should be placed in an appropriate container to be autoclaved before it can be placed in this container.



Figure 1. Glass disposal box. Credit: Sherry Smith

Remember, broken glassware should never be handled with your bare hands. In the case of uncontaminated glassware, you should use a broom/ brush and dustpan to move it to the "Broken Glass" container.

Contaminated glassware on the other hand should be transported with autoclavable tongs to the appropriate autoclave/biohazard container.

Biohazard Container -

Biohazardous wastes are inherent in the microbiology laboratory. Therefore, you must be familiar with how to properly dispose of those wastes. Materials exposed to bodily fluids or microorganisms are considered biohazardous. Such materials should be placed in a biohazard container. Biohazard containers are typically red with a biohazard label, or are containers with the red biohazard emblem on them. Some examples of biohazardous materials you may work with this semester are contaminated disposable pipets, old petri dishes used to grow bacteria, etc. Materials placed in this biohazard container are autoclaved to sterilize the contents before it is disposed of.



Figure 2. Biohazard container. Credit: Sherry Smith

Safety Data Sheet Notebook –

Safety data sheets are documents that contain important information about chemicals, such as the chemical's properties, proper

precautions for handling, procedures for accidental exposure, etc. Your lab will have safety data sheets for all chemicals utilized in the lab. It is important that all individual's working with any chemicals in the lab know where these documents are in order to safely work with chemicals in the laboratory room.

Eyewash Station -

An emergency eye wash station will be present in the microbiology lab. Eye wash stations are important should an individual's eyes become exposed to chemicals, infectious agents, or other irritants. If your eyes are exposed to one of these items, you should go immediately to the eye wash station and flush your eyes for a minimum of 15 minutes.



Figure 3. Safety shower and eyewash station. Credit: Sherry Smith

Safety Shower -

Your lab will also have an emergency shower station. An emergency shower station is present in the lab should one's body be exposed to chemicals, infectious agents, or should clothing catch fire. Should you require use of the safety shower, you should remain under the shower for a minimum of 15 minutes.

Gas Shut-Off Valve -

Microbiology laboratory classrooms are equipped with gas jets to supply gas for the Bunsen burners used in some of your laboratory experiments. The emergency gas shut-off valve is important should there be a gas leak, or a fire break out in the lab. Activating the gas shut-off valve will cut off gas flow to all the gas jets in the classroom.



Figure 4. Gas shut-off valve. Credit: Sherry Smith

Fire Extinguisher -

Your lab should be equipped with a fire extinguisher. Some of your laboratory exercises will require the use of a Bunsen burner. Using flames does pose the potential of a fire, thus one needs to be familiar with the fire extinguisher's use and location.

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1.1.3: Exercise #1 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Describe how broken glassware should be disposed of. Consider the disposal of both contaminated and uncontaminated glassware.
2. Describe why open-toed shoes are not worn in the laboratory room.
3. Describe what should be done if you were to get a caustic chemical in your eyes.
4. When should lab bench tops be cleaned with disinfectant solution?
5. What protocol should be followed if you spill a bacterial culture on your lab bench? (List all of the steps).
6. How are microorganisms destroyed after they are placed into a biohazard container?
7. Is it permissible to have any food or drink in the laboratory? Explain your answer.

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1.2: Exercise #2 - Microscopy

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

Laboratory Exercise Learning Outcomes

1. Identify and label the components of the compound microscope and describe the function of each component.
2. Describe the proper care of the microscope and demonstrate the proper care of the microscope in the laboratory setting.
3. Describe the proper use of the microscope and demonstrate the proper use of the microscope in the laboratory setting.
4. Describe the purpose of the oil objective lens and describe how to properly utilize the oil objective lens.
5. Properly calculate total magnification.

Materials Needed:

- compound light microscope
- lens cleaner
- lens paper
- prepared microscope slides

Microscopes are one of the most important tools in microbiology. ***Microorganisms,*** the subject matter studied in microbiology cannot be observed without the aid of a microscope.

This laboratory exercise will introduce the student to the proper care and proper use of the compound microscope. Microbiology students must become very proficient with compound microscopes to be able to successfully complete other exercises in the laboratory.



Figure 1. Microscope storage cabinet – Microscopes should be properly stored to prevent damage. Credit: Sherry Smith

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1.2.1: Transporting the Microscope

The microscopes are stored in a microscope cabinet with their microscope covers on them to prevent dust from gathering and damaging the microscope components. Students will need to demonstrate how to properly obtain and transport the microscope to prevent damaging the microscopes. Follow the instructions listed below each time you are transporting a microscope in the laboratory.

1. Grasp the compound microscope by the arm with one hand and place your other hand under the base to support the microscope.
2. Place the microscope in front of your body as you transport the microscope. By transporting it in this way you will prevent bumping it against laboratory benches and reduce the likelihood that it would be knocked out of your hands should someone bump into you.
3. When you arrive at your table, gently set the microscope down on the table.





Transporting the microscope - Place one hand on the arm and one hand under the base to support the microscope. Credit: Sherry Smith

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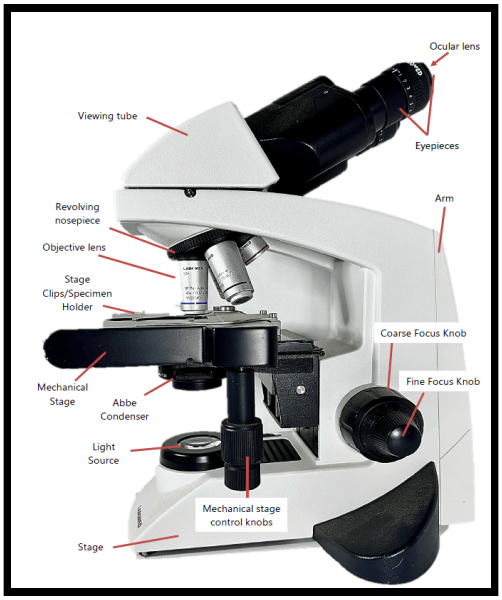
1.2.2: Identifying the Components of the Compound Microscope

The microscopes you will be using in lab are bright-field compound microscopes. ***Compound microscopes*** use two sets of lenses, ocular lens and objective lens, to magnify a specimen. ***Bright-field microscopy*** refers to the use of white light to visualize the specimen. The specimen absorbs the light causing the specimen to be darker than the surrounding background. Biological specimens usually lack the contrast (the ability to distinguish the object from the background) to observe details, and thus are generally stained before observation.

Microscopes allow the magnification of a specimen by refracting light rays as they pass through the magnifying lenses. Magnification alone will not produce a quality image. It is important that resolution and contrast be achieved in addition to magnification to generate a quality image. ***Resolution*** refers to the ability to distinguish two objects from one another. ***Contrast*** refers to the difference in light intensity of the specimen and the background.

You will need to have knowledge of the parts of the microscope to be able to follow instructions during future laboratory exercises. You will identify each of the following components on the microscope and describe the function of the component.

- Abbe Condenser – collects the light from the light source and focuses it onto the specimen
- Arm – connects the head of the microscope to the base; used to assist in carrying the microscope
- Base – bottom of the microscope that provides support for the microscope and houses the light source
- Coarse Focus (Adjustment) Knob – used to focus on the microscope
    - ***Note*** Should only be used with the 4x (scanning objective lens)
- Condenser Focus Knob – moves the condenser up and down to control the focus of the light on the specimen
- Eyepiece – houses the ocular lens; the component you look through at the top of the microscope
- Fine Focus (Adjustment) Knob – used to focus the microscope; it allows for fine tuning the focus; used with higher power objective lenses
    - ***Note*** Using ***only*** this focus knob with higher power objective lenses will help prevent driving the objective lens into the slide, possibly damaging the objective lens of breaking a slide
- Iris Diaphragm – controls the amount of light reaching the specimen
- Iris Diaphragm Aperture Lever – adjusts the aperture (opening) of the iris diaphragm to allow more or less light to reach the specimen
    - ***Note*** As you increase in objective magnification power you will need more light; you will need to adjust the amount of light reaching your specimen using this lever to ensure you have adequate contrast between your specimen and the background
- Mechanical Stage – platform where a microscope slide containing the specimen is placed; may be moved utilizing mechanical stage control knobs to move the slide around
- Objective Lenses – primary source of magnification; (scanning, low, high, and oil immersion). The oil immersion objective lens requires the use of immersion oil, which prevents the loss of light due to refraction, thereby preserving resolution (level of clarity/detail observed). A drop of immersion oil is added to the slide before rotating to the immersion oil lens. The scanning, low, and high objective lenses are dry objective lenses, meaning they do not require the addition of oil to maintain good resolution. ***Note*** when using the microscope to view a specimen you begin with the lowest power objective lens and then work to higher powers
- Ocular Lens – lens located within the eyepiece
- Revolving Nose-piece – houses the objective lenses; rotates to select for the different objective lenses
- Stage Control Knobs – moves the mechanical stage or stage clips to move the specimen; these knobs allow you to locate items not in the field of view or center a specimen within the field of view



Compound Microscope Components - LabOMed Binocular Compound Microscope Credit: Sherry Smith

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1.2.3: Calculating Total Magnification

Compound microscopes utilize two lenses to magnify the specimen, the ocular lens and the objective lens. The magnification that is achieved as a result of using these two lenses together to magnify the specimen is known as the ***total magnification (TM)***. You will need to know how to calculate total magnification. Total magnification is calculated by multiplying the magnification power of the ocular lens by the magnification power of the objective lens in use.

Total Magnification Example:

Ocular lens power of magnification X Objective lens power of magnification = TM

A student is observing a specimen using the scanning objective (power = 4x) on a compound microscope that has a 10x ocular lens.

Solution

The total magnification of the specimen being observed would be calculated as follows:

TM = 10x X 4x

TM = 40x

Total Magnification Practice:

Complete the following activities:

(A) A compound microscope has a 10x ocular lens. Calculate the total magnification for the following objective lenses:

Total magnification practice (10x ocular lens)

Objective Lens	Show Your Work	TM =
4x		
10x		
40x		
100x		

(B) A compound microscope has a 15x ocular lens. Calculate the total magnification for the following objective lenses:

Total magnification practice (15x ocular lens)

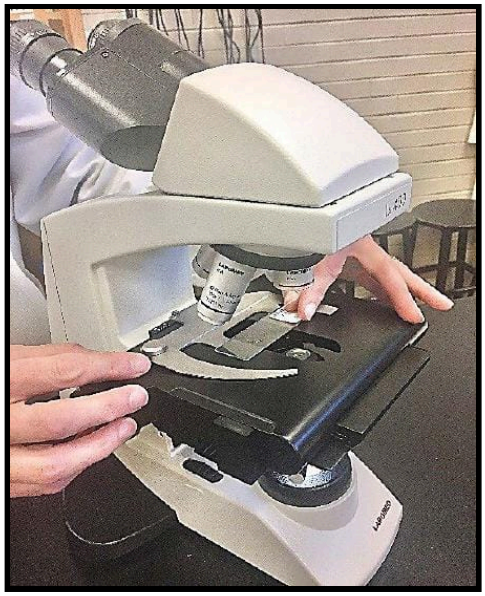
Objective Lens	Show Your Work	TM =
4x		
10x		
40x		
100x		

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1.2.4: Using the Compound Microscope

In this portion of your laboratory exercise, you will use the compound microscope to observe some prepared slides. It is important that you follow the instructions below to avoid damaging the microscope and to prevent the breakage of any microscope slides.

1. Secure your microscope from the microscope cabinet using the proper transport techniques described previously
2. Place your microscope on the laboratory bench gently and in a secure position
3. Remove the microscope cover, fold it neatly and place it out of the way
4. Plug the microscope in and position the cord so that it is not in the way and is less likely to be caught and drag the microscope off the laboratory bench
5. Turn the power on
6. Ensure the stage is in its lowest position and that the scanning (4x) objective lens is in position (if not use the revolving nose-piece to rotate the lens into position)
7. Place a prepared microscope slide on the stage of the microscope and secure it with the stage clips.
8. Rotate the coarse adjustment knob to move the stage closer to the objective lens
9. Looking through the ocular lens rotate the coarse adjustment knob away from the objective lens to bring the specimen into focus



Secure the microscope slide - use the stage clips, do not force the slide into place.

Credit: Sherry Smith



Coarse focus knob - should be used with lowest power objective lenses only to prevent damage to microscope slides and microscope objective lenses.

Credit: Sherry Smith

10. To view the specimen at a higher magnification, rotate the revolving nose-piece to bring the low power (10x) objective lens into position
11. Looking through the ocular lens use ***only the Fine Adjustment Knob*** to bring the specimen into focus; Your microscope objectives should be parfocal, meaning that when you are in focus using one objective lens, it should stay in focus when moving to another objective lens, thus a slight rotation of the fine adjustment knob should bring the sample into focus

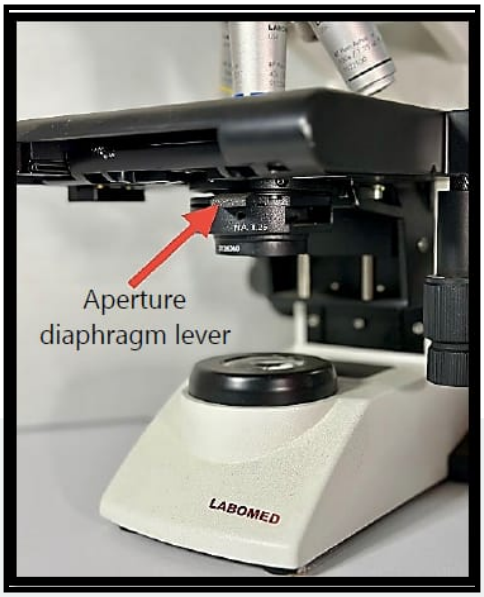


Fine focus knob - Used to sharpen the image and can safely be used with any objective lens.

Credit: Sherry Smith

12. Adjust your light intensity as needed to improve the quality of the image by using the iris diaphragm aperture lever
13. Repeat steps 10 and 11 to view the specimen with the high power (40x) objective lens...
14. When you are finished viewing the specimen on high power...
15. Rotate the revolving nose-piece so that the space between the high-power objective lens...
16. When you are finished viewing the specimen using the oil immersion lens...
17. Use the lens cleaner and a lens cleaning cloth/lens paper...
18. Remove the microscope slide from the stage.
19. If the slide is a prepared slide remove the oil if present...
20. Ensure that the stage and the lenses are clean.
21. Power the microscope off and position the cord...
22. Place the microscope cover back on the microscope and transport...

On the following pages, complete the activities to practice using the compound microscope. Record your observations as you progress from a large sample to a bacterial smear, the smallest specimen you will visualize this semester.



Aperture diaphragm lever is used to adjust the amount of light moving through the condenser to the specimen. Credit: Sherry Smith

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1.2.4.1: Observing the "Letter e"

Obtain a prepared slide with the letter “e.” Observe the specimen using the objective lenses assigned by your instructor. Draw your results in the spaces provided and record the total magnification for each objective lens observed.

> **Note:** Your instructor may have you observe and draw the specimen using only selected objective lenses. Follow your instructor’s directions for which objective lenses you are expected to view and record.

Microscope observation drawings and total magnification

Scanning Objective Lens TM =	**Low Power Objective Lens TM =**
High Power Objective Lens TM =	**Oil Immersion Objective Lens TM =**

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1.2.4.2: Observing a "whitefish blastodisc"

Obtain a prepared "whitefish blastodisc" slide. Observe the specimen using the objective lenses assigned by your instructor. Draw your results in the spaces provided and record the total magnification for each objective lens observed.

> **Note:** Your instructor may have you observe and draw the specimen using only selected objective lenses. Follow your instructor's directions for which objective lenses you are expected to view and record.

Microscope observation drawings and total magnification

Scanning Objective Lens TM =	**Low Power Objective Lens TM =**
High Power Objective Lens TM =	**Oil Immersion Objective Lens TM =**

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1.2.4.3: Observing a "human blood smear"

Obtain a prepared "human blood smear" slide. Observe the specimen using the objective lenses assigned by your instructor. Draw your results in the spaces provided and record the total magnification for each objective lens observed.

> **Note:** Your instructor may have you observe and draw the specimen using only selected objective lenses. Follow your instructor's directions for which objective lenses you are expected to view and record.

Microscope observation drawings and total magnification

Scanning Objective Lens TM =	**Low Power Objective Lens TM =**
High Power Objective Lens TM =	**Oil Immersion Objective Lens TM =**

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1.2.4.4: Observing "Balantidium coli"

Obtain a prepared "*Balantidium coli*" slide. Observe the specimen using the objective lenses assigned by your instructor. Draw your results in the spaces provided and record the total magnification for each objective lens observed.

> **Note:** Your instructor may have you observe and draw the specimen using only selected objective lenses. Follow your instructor's directions for which objective lenses you are expected to view and record.

Microscope observation drawings and total magnification

Scanning Objective Lens TM =	**Low Power Objective Lens TM =**
High Power Objective Lens TM =	**Oil Immersion Objective Lens TM =**

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1.2.4.5: Observing a "bacterial smear"

Obtain a prepared "bacterial smear" slide. Observe the specimen using the objective lenses assigned by your instructor. Draw your results in the spaces provided and record the total magnification for each objective lens observed.

> **Note:** Your instructor may have you observe and draw the specimen using only selected objective lenses. Follow your instructor's directions for which objective lenses you are expected to view and record.

Microscope observation drawings and total magnification

Scanning Objective Lens TM =	**Low Power Objective Lens TM =**
High Power Objective Lens TM =	**Oil Immersion Objective Lens TM =**

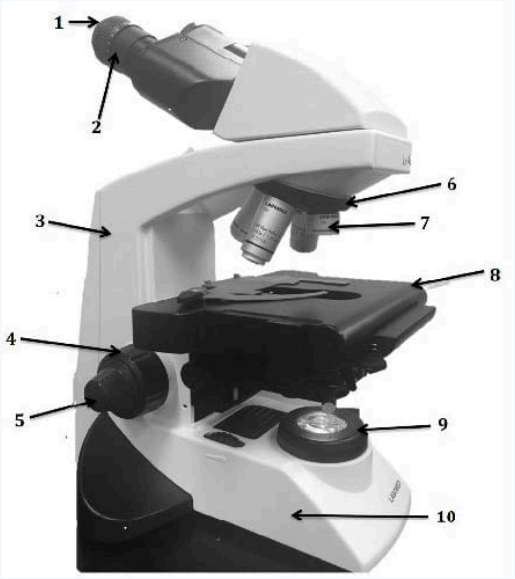
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1.2.5: Exercise #2 Lab Wrap-Up

Lab Wrap-Up Questions

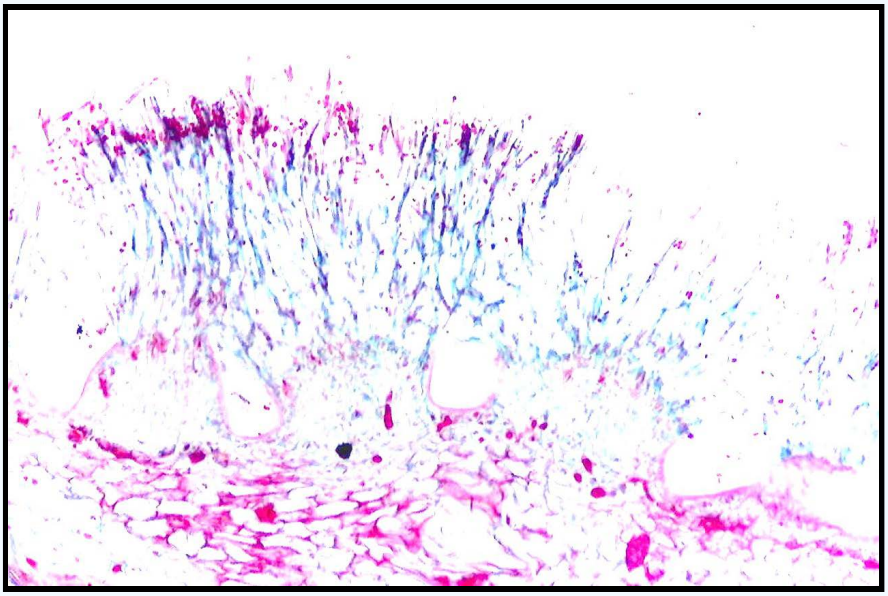
Answer the following questions:

1. Describe the proper way to transport a microscope.
2. Describe the proper way to store the microscope after you have finished looking at your last microscope slide.
3. Why is it important to use the fine adjustment knob only, when using the higher power objective lenses?
4. Describe how total magnification is determined?
5. Identify the indicated components of the microscope on the image below.



Credit: Sherry Smith

6. You finish adjusting the focus of the specimen you are observing. The object appears as seen in the image below. What adjustment should be made for an ideal image? Explain your answer.



Credit: Sherry Smith

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1.3: Exercise #3 - Growth Media and Inoculating Tools

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Define the difference between defined and complex media and list examples of each.
2. Describe the difference between liquid, semi-solid, and solid media.
3. Define and describe the purpose of selective media.
4. Define and describe the purpose of differential media.
5. Define and describe the purpose of transport media.
6. Define and describe the purpose of anaerobic media.
7. List and describe three tools used for inoculating media.

Microorganisms require a source of nutrients for growth. In a laboratory setting the nutrients are provided in a form known as media. Media can be classified based on its chemical composition, its physical characteristics, and/or its functional purpose. When introducing microorganisms to a growth medium an inoculating tool is used. Different types of media and methods require different types of inoculating tools. In this laboratory exercise, you will examine growth media based on their chemical classification, physical classification, and functional classification, and three tools used in the microbiology lab for the purpose of inoculation.

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1.3.1: Types of Media - Chemical Composition

Growth media supply nutrients for bacterial growth. Just as we need specific nutrients for healthy growth and development, bacteria need specific nutrients if they are expected to grow in the media. Different species of bacteria have different nutritional requirements for ideal growth. Therefore, microbiologists have different growth media recipes. There are many different recipes for growth media that meet the different nutritional requirements. Bacterial growth media can be classified based on its chemical composition, as either a defined media or a complex media. ***Defined (synthetic) media***, are growth media in which the exact chemical composition and quantities are known. ***Complex media*** are those in which the exact chemical composition is unknown due to the addition of yeast, plant, or animal products. Examples of complex media include tryptic soy media (contains plant products), brain heart media (contains animal products), and nutrient media (contains yeast or beef extract).

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1.3.2: Types of Media - Physical Characteristics

In this section, we will explore types of media based on their physical characteristics. When classifying media based on their physical characteristics, media may be classified as ***liquid media***, ***semi-solid media***, or ***solid media***. This difference in the physical nature of the media is due to the presence or absence of agar. Agar is a chemical substance derived from the cell walls of algal cells. Agar is a commonly used solidifying agent in microbiology laboratories due to the inability of microorganisms to digest it.

Liquid Media -

Liquid media are commonly referred to as broths. Broths contain nutrients that have been dissolved in liquid water and do not contain agar. Both plant-based and animal-based ingredients may be used to produce broths. Some common broths you may encounter this semester in laboratory session include nutrient broths, brain heart broths, and tryptic soy broths. Broths may be used in flasks, bottles, or test tubes.



Figure 1. Sterile broth media are shown on the left, and broth media with bacterial growth are shown on the right. Credit: Sherry Smith

There are some advantages as well as some disadvantages to utilizing broths for culturing microorganisms. A few of the advantages presented with the use of broths include:

1. Broths permit the growth of large numbers of bacteria within a single container. Bacterial growth in a broth can be detected due to cloudiness. Sterile broths remain clear, while broths containing bacteria are cloudy in appearance.

   The culture tube on the left in Figure 1 contains sterile broth solution, indicated by the clear color, while the broth on the right contains a sample of the bacterium *Staphylococcus aureus*. The bacteria growing in the broth tube on the right caused the solution to become cloudy.

2. Another advantage of utilizing broths is the ability to grow organisms at various concentrations of oxygen. Organisms vary in their ability to tolerate the presence of molecular oxygen, O_2. Oxygen levels are highest at the surface of the liquid and decrease as the depth of the broth increases.
3. Broths can be used to conduct a variety of tests and microbiological techniques such as carbohydrate fermentation tests and serial dilutions, both of which we will explore in more detail in later laboratory exercises.

Semi-Solid Media -

Semi-solid media are media that contain nutrients to meet the nutritional requirements of the microbe and a small percentage of agar, usually 0.5% or less. The semi-solid media allows for some movement of bacteria through the media if the bacteria are motile. Semi-solid media are commonly used for motility tests.

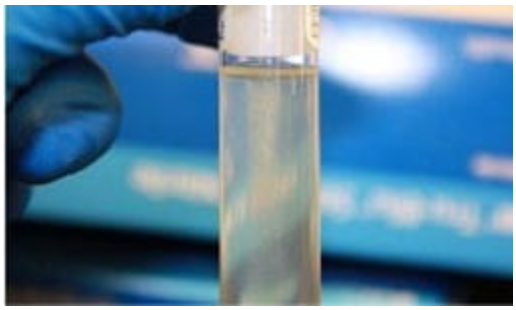


Figure 2. Motility test positive for motility. Source: VeeDunn, Flickr, CC BY 2.0.

Motility tests are performed by introducing the bacteria of interest to the media within a test tube using an inoculation needle. The needle with the sample is stabbed in the center of the tube. If growth remains near the site of inoculation, the bacteria are deemed non-motile. If growth appears to radiate outward from the site of inoculation, the bacteria are deemed motile.

Solid Media -

Solid media are prepared like semi-solid media, with the exception that it contains a higher concentration of agar. Solid media contain the required nutrients and 1.5%–2% agar. Solid media may be placed in test tubes or petri plates.

When solid media is introduced to test tubes, the tubes will be placed in a slanted position, which allows the media to solidify along the side of the tube, producing what is referred to as a slant. Slants provide a means of storing microbes for a longer period in cold storage.



Figure 3. Agar slant. Credit: Sherry Smith

When the media is poured into a petri plate and allowed to solidify, it produces a large surface area for the bacteria to be inoculated. Solid media in petri plates are used to perform several important isolation techniques, including the streak plate method. Solid media allow for the isolation of an individual species and allow the microbiologist to observe differences in the morphology, or form and appearance, of the colonies growing on the surface.

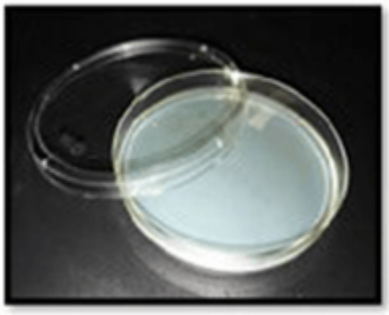


Figure 4. Sterile nutrient agar plate. Credit: Sherry Smith

Petri plates may also be used to observe differences in colony appearance, including color, size, shape, and other visible characteristics. These observations can help microbiologists compare bacterial growth and begin making decisions about how to further test or identify microorganisms.

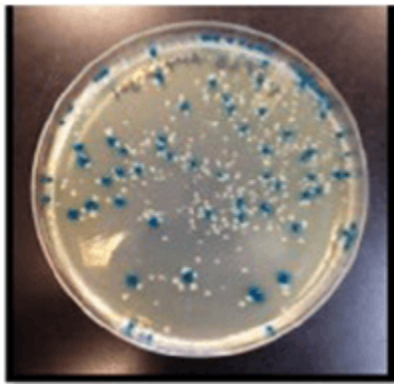


Figure 5. Petri plate with bacterial growth. Blue colonies are ampicillin-resistant Escherichia coli, while white colonies are ampicillin-susceptible. Credit: Sherry Smith

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1.3.3: Types of Media – Functional/Application Classifications

Media can also be classified based on their function in the laboratory setting. In this section, we will briefly examine five types of media classified based on their applications. We will explore selective and differential media in further detail in Section 3 of this laboratory and look at the specific role of each of the media covered.

Selective Media -

Selective media are media that contain ingredients that act to encourage the growth of some types of bacteria while inhibiting the growth of other types of bacteria. This characteristic of the media allows the microbiologist to isolate specific types of organisms on the media. Examples of selective media include mannitol salt agar media, MacConkey agar media, and eosin methylene blue agar.

Differential Media -

Differential media contain ingredients that cause distinct types of bacteria to grow differently on the same media. Those differences may include changes in the color of the media, changes in the color of the bacterial colonies, or other visible reactions. Blood agar is one type of differential media that allows bacteria to be differentiated based on if and how blood cells are processed by the bacteria growing on the media. Some media are both selective and differential, such as mannitol salt agar, MacConkey agar, and eosin methylene blue agar.

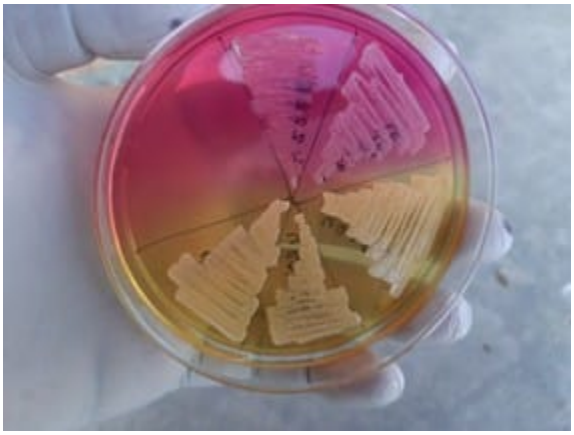


Figure 1. Mannitol salt agar. Source: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0.



Figure 2. MacConkey agar. Credit: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY-SA 4.0.

Selective and differential media are especially useful in clinical microbiology because they allow microbiologists to encourage the growth of certain organisms while also observing visible reactions that may help distinguish one group of bacteria from another.

Enriched Media -

Enriched media are used to grow fastidious microorganisms, which are microorganisms requiring additional nutrients and growth factors, such as amino acids, lipids, or other specialized nutrients. Enriched media contain special ingredients such as blood or serum. Examples of enriched media include blood agar and chocolate agar.

Anaerobic Media -

Anaerobic media are a specialized type of media used to grow bacteria that require low oxygen or the absence of oxygen. These bacteria are called anaerobes. Anaerobic media are made using an ingredient, such as sodium thioglycolate, that binds to and reduces oxygen in the media, thereby creating an environment favorable for anaerobic bacterial growth.

Transport Media -

Transport media are media specialized for the clinical setting. Transport media contain ingredients such as buffers and salts that serve to maintain the microorganism being transported without permitting its multiplication. There are a variety of transport media designed for the different types of microorganisms that must be identified in clinical laboratories.

Your instructor will have several examples of media of different physical characteristics available for your observation. You should observe each of the examples.

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1.3.4: Inoculating Tools

Microorganisms are introduced to media through a process known as inoculation. Inoculation is accomplished by transferring a sample, known as an inoculum, to a growth medium, allowing it to reproduce and grow there. In order to accomplish inoculation, an inoculating tool is employed. There are multiple types of inoculating tools that may be used for inoculation. Listed below are three types of inoculating tools used in microbiology laboratories.

Inoculating Loop -

An inoculating loop is the most common inoculating tool used in the microbiology laboratory. Inoculating loops exist in several types, including metal loops that may be reused and plastic sterile loops that are disposed of after use. Inoculating loops are frequently used in the transfer of bacteria from one type of media to another, such as broth to broth, broth to slant, or broth to agar.

Loops can also be used for the streak plate isolation method and for transferring samples to microscope slides to create a smear.

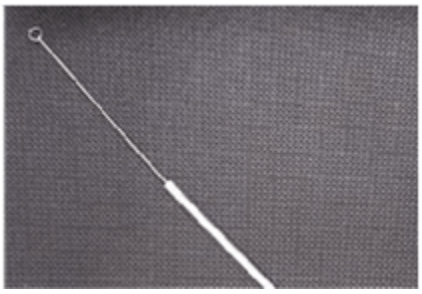


Figure 1. Inoculating loop. Credit: Sherry Smith

Inoculating Needle -

Inoculating needles are similar to loops in the way they are made, with the exception of a straight wire in place of the circular loop at the end. Like inoculating loops, they may be made of metal or plastic. Inoculating needles are used for stabbing media, as is needed in motility tests, or for obtaining very small colonies from solid media for transfer.

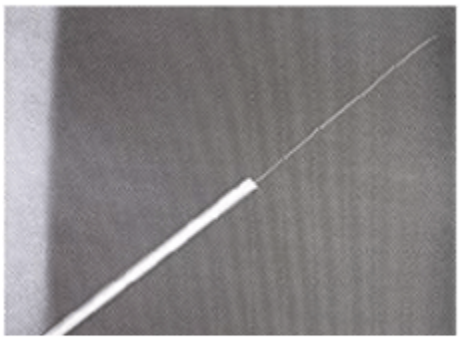


Figure 2. Inoculating needle. Credit: Sherry Smith

Glass Spreading Rod -

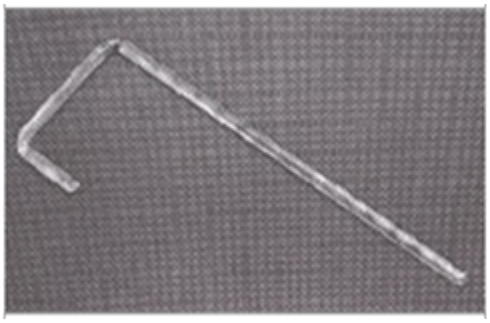


Figure 3. Glass spreading rod. Credit: Sherry Smith

Glass spreading rods, also called glass spreaders, are glass rods that have been bent into an L-shape. They appear much like a hockey stick and are used to spread an inoculum over the surface of a solid medium. The isolation technique known as the spread plate technique makes use of a glass spreading rod.

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1.3.5: Exercise #3 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. You are performing an experiment that requires the use of skim milk agar. What chemical classification would this agar belong to? Explain your selection.
2. List and describe three types of physical media.
3. What substance is used to make semi-solid media and solid media?
4. How do you know if bacteria are growing in a broth culture?
5. What is a slant and what can it be used for?
6. You have been asked to perform a motility test. What physical type of media will you use? What inoculation tool will you choose?
7. You are using a media that contains beef extract, pancreatic digest of gelatin, bile salts (inhibit gram-positive bacteria), methylene blue (inhibit gram-positive bacteria), sucrose, lactose. You inoculate your media which is contained in a petri plate with a sample. After incubation you are able to observe some colonies that have become a dark purple as a result of sugar fermentation, while others appear colorless from the lack of fermentation. Use the information provided, to pick all of the terms that would apply to this growth media (i.e. complex media, defined media, broth transport, etc.) and explain why the terms apply to this media.

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1.4: Exercise #4 - Aseptic Techniques and Aseptic Transfer of Bacteria

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Define aseptic techniques.
2. Describe the importance of aseptic techniques in the microbiology laboratory.
3. Demonstrate how to transfer microbes from various types of media using aseptic techniques.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Test tube rack
- China marker
- 1 sharpie marker
- Simulation broth culture
- Simulation sterile broth
- Stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)
- 1 sterile broth tube
- 1 sterile slant tube
- 1 Petri dish with nutrient agar

In this laboratory exercise students will become familiar with aseptic transfer techniques. Microorganisms are ubiquitous, meaning that they are everywhere. Microorganisms are present in the air around us, in and on our bodies, and on the laboratory benches at which you will be working. Therefore, we must use precautionary measures to ensure that our samples do not become contaminated, and we do not become contaminated with them and transport them out of the laboratory. Aseptic transfer techniques are techniques that are used by microbiologists to reduce the likelihood of such contamination.

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1.4.1: Aseptic Technique and Subculturing Bacteria

Aseptic techniques are procedures utilized to reduce microbial numbers in an attempt to prevent spreading the microbes. Aseptic techniques include things such as washing hands properly, disinfecting work surfaces, sterilizing inoculating tools, and other procedures used when transferring/subculturing bacteria microbes that are aimed at reducing the likelihood of contamination.

Subculturing is the process of moving bacteria from a media source in which they have been growing to a new sterile media source. This may be done simply to help keep some of the bacteria alive for study, but also done during isolation techniques and during the identification process as bacteria are introduced to different types of media to aid in the determination of the identity of the bacteria.

In this laboratory activity you will practice aseptic technique as you subculture bacteria to different types of media. Remember that the goals of aseptic techniques in the lab are to prevent the contamination of your sample, prevent contamination of your work space, and prevent contaminating yourself.

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1.4.2: Becoming Familiar with Aseptic Techniques

You will need to perform the following steps without error in front of the instructor/facilitator for a proficiency check before you will be given the materials to complete any additional exercises in this laboratory session.

1. Begin by disinfecting your work area.
2. Obtain the following items: Bunsen burner Inoculating loop Test tube rack Simulation broth culture Simulation sterile broth
3. Light your Bunsen burner. Be sure to adjust the Bunsen burner so that an inner blue cone is clearly visible. Remember, this is the hottest portion of the flame; you will sterilize your inoculation tool in this area.
4. Grasp your inoculating loop in your dominant hand holding it like a pencil. You will use the first three fingers of your dominant hand to manipulate your inoculating tool.

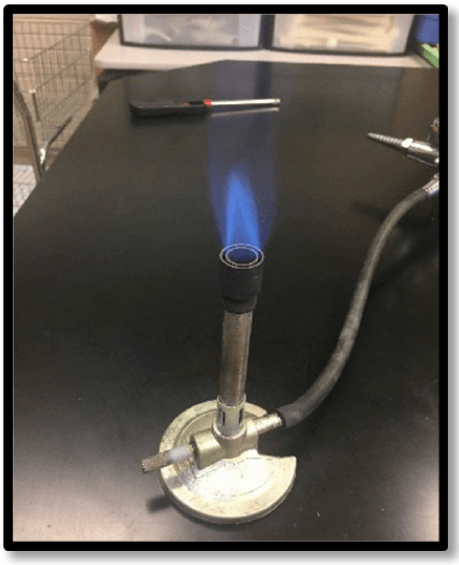


Figure 1. Bunsen burner with flame adjusted to have blue cone. Credit: Sherry Smith

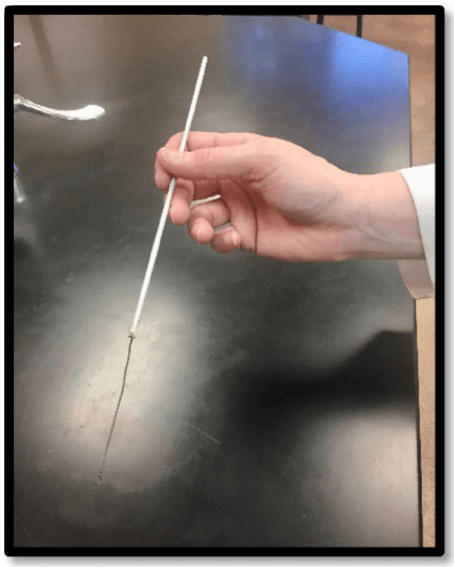


Figure 2. Proper technique for grasping the inoculating loop. Credit: Sherry Smith

5. Flame the inoculating loop ensuring that it turns red hot, and then let it cool for approximately 15-30 seconds. Do not allow the instrument to bump against anything and do not lay it down to allow it to cool.

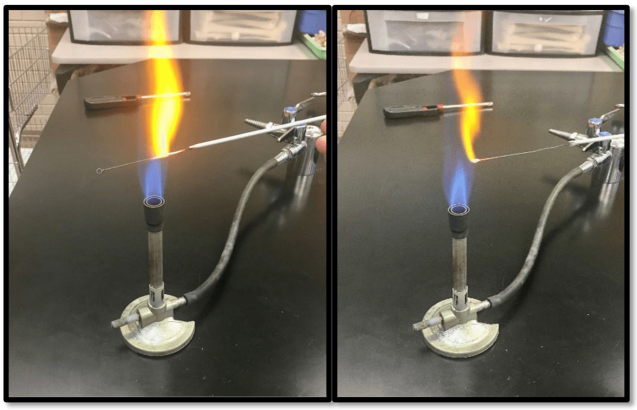


Figure 3. Proper flaming technique for sterilizing the inoculating loop. Ensure the entire wire becomes red hot. Credit: Sherry Smith

6. Use your non-dominant hand to pick up the test tube marked “stock culture.” This test tube is representative of a culture of bacteria from which you will collect a culture sample to transfer to another media source. Flick the test tube to practice suspending the bacteria in the culture. Generally, bacteria will settle in the tube and will need to be suspended for transfer.
7. Use the last two fingers of your dominant hand to remove the cap of the test tube. ***Do not set the cap down on the lab bench.***

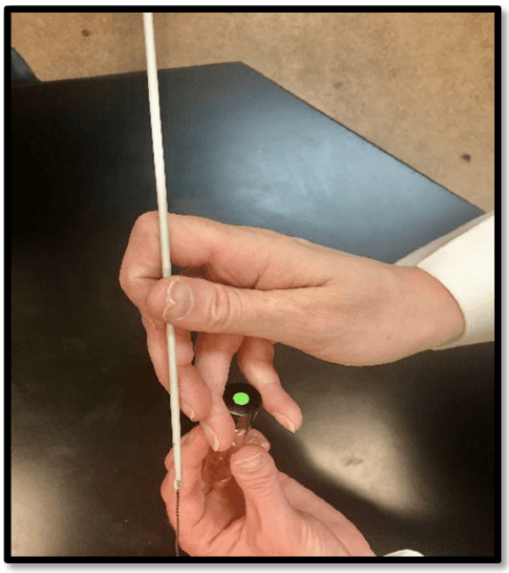


Figure 4. Proper technique for grasping the cap of a test tube. Credit: Sherry Smith

8. Pass the mouth of the test tube through the flame of the Bunsen burner.



Figure 5. Flaming the mouth of a test tube. Credit: Sherry Smith

9. Use the sterilized loop to obtain a loopful of sample, without touching the sides of the test tube.
10. Hold the loop steady and pass the mouth of the tube through the flame and then recap the test tube. Place the test tube back in the test tube rack.
11. Pick up the test tube marked “Sterile Broth.” This test tube of water is representative of the sterile media to which the sample of bacteria will be transferred. Remove the cap using the smallest two fingers of your dominant hand.
12. Flame the mouth of the test tube.
13. Insert the loop containing the inoculum and swirl the loop in the simulation “sterile broth”. Remove the loop without touching the sides of the test tube.
14. Flame the mouth of the tube. Recap the test tube and return it to the test tube rack.
15. Flame the loop until it is red hot. Flaming the loop kills any bacteria remaining on the instrument.

You should continue practicing the steps listed above until you feel competent to perform your proficiency check. Once you have successfully completed the proficiency check you will be granted access to the bacterial stock culture and media needed to complete the next activity.

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1.4.3: Aseptically Transferring Bacteria From Broth Culture to Sterile Broth

1. Begin by disinfecting your work area.
2. Obtain all the necessary items to complete the exercise:
   - Bunsen burner
   - Inoculating loop
   - Sharpie
   - Bacterial stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)
   - Sterile broth tube
3. Label the sterile broth tube with the following information using the sharpie:
   - Your initials
   - Your lab section
   - Date
   - Initials of the bacteria provided by your instructor
4. Light your Bunsen burner. Be sure to adjust the Bunsen burner so that an inner blue cone is clearly visible.
5. Grasp your inoculating loop in your dominant hand holding it like a pencil. You will use the first three fingers of your dominant hand to manipulate your inoculating tool.
6. Flame the inoculating loop ensuring that it turns red hot, and then let it cool for approximately 15-30 seconds. Do not allow the instrument to bump against anything and do not lay it down to allow it to cool.
7. Use your non-dominant hand to pick up the test tube containing the bacterial culture. Flick the test tube to suspend the bacteria in the culture.
8. Use the last two fingers of your dominant hand to remove the cap of the test tube. ***Do not set the cap down on the lab bench.***
9. Pass the mouth of the test tube through the flame of the Bunsen burner.
10. Use the sterilized loop to obtain a loopful of sample, without touching the sides of the test tube.
11. Hold the loop steady and pass the mouth of the tube through the flame and then recap the test tube. Place the test tube back in the test tube rack.
12. Pick up the sterile broth test tube. Remove the cap using the smallest two fingers of your dominant hand.
13. Flame the mouth of the test tube.
14. Insert the loop containing the inoculum and swirl the loop in the sterile broth. Remove the loop without touching the sides of the test tube.
15. Flame the mouth of the tube. Recap the test tube and return it to the test tube rack.
16. Flame the loop until it is red hot. Flaming the loop kills any bacteria remaining on the instrument.
17. Place your inoculated broth culture tube in the incubator in the designated location to incubate for 24 hours. You will examine your broth solution in the next laboratory session.

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1.4.4: Aseptically Transferring Bacteria From Broth Culture to Sterile Agar Slant

1. Begin by disinfecting your work area.
2. Obtain all the necessary items to complete the exercise:
   - Bunsen burner
   - Inoculating loop
   - Sharpie
   - Bacterial stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)
   - Sterile agar slant tube
3. Label the sterile agar slant with the following information using the sharpie:
   - Your initials
   - Your lab section
   - Date
   - Initials of the bacteria provided by your instructor
4. Light your Bunsen burner. Be sure to adjust the Bunsen burner so that an inner blue cone is clearly visible.
5. Grasp your inoculating loop in your dominant hand holding it like a pencil. You will use the first three fingers of your dominant hand to manipulate your inoculating tool.
6. Flame the inoculating loop ensuring that it turns red hot, and then let it cool for approximately 15-30 seconds. Do not allow the instrument to bump against anything and do not lay it down to allow it to cool.
7. Use your non-dominant hand to pick up the test tube containing the bacterial culture. Flick the test tube to suspend the bacteria in the culture.
8. Use the last two fingers of your dominant hand to remove the cap of the test tube. ***Do not set the cap down on the lab bench.***
9. Pass the mouth of the test tube through the flame of the Bunsen burner.
10. Use the sterilized loop to obtain a loopful of sample, without touching the sides of the test tube.
11. Hold the loop steady and pass the mouth of the tube through the flame and then recap the test tube. Place the test tube back in the test tube rack.
12. Pick up the sterile slant agar. Remove the cap using the smallest two fingers of your dominant hand.
13. Flame the mouth of the test tube.
14. Insert the loop containing the inoculum and streak the surface of the agar starting at the bottom of the tube moving upward.
15. Flame the mouth of the tube. Recap the test tube and return it to the test tube rack.
16. Flame the loop until it is red hot. Flaming the loop kills any bacteria remaining on the instrument.
17. Place your inoculated agar slant in the incubator in the designated location to incubate for 24 hours. You will examine your agar slant in the next laboratory session.

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1.4.5: Aseptically Transferring Bacteria From Broth Culture to Sterile Agar Plate

1. Begin by disinfecting your work area
2. Obtain all the necessary items to complete the exercise: Bunsen burner
    - Inoculating loop Sharpie
    - Bacterial stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)
    - Petri plate containing nutrient agar
3. Label the petri dish with the following information using the sharpie. Be sure to place the information on the bottom of the petri plate (that portion containing the agar)
    - Your initials Your lab section Date
    - Initials of the bacteria provided by your instructor
4. Light your Bunsen burner. Be sure to adjust the Bunsen burner so that an inner blue cone is clearly visible. Remember, this is the hottest portion of the flame; you will sterilize your inoculating tools in this area.
5. Grasp your inoculating loop in your dominant hand holding it like a pencil. You will use the first three fingers of your dominant hand to manipulate your inoculating tool.
6. Flame the inoculating loop ensuring that it turns red hot, and then let it cool for approximately 15- 30 seconds. Do not allow the instrument to bump against anything and do not lay it down to cool.
7. Use your non-dominant hand to pick up the bacterial Stock Culture. Flick the test tube to suspend the bacteria in the culture.
8. Use the last two fingers of your dominant hand to remove the cap of the test tube.
9. Pass the mouth of the test tube through the flame of the Bunsen burner.
10. Use the sterilized loop to obtain a loopful of sample, without touching the sides of the test tube.
11. Hold the loop steady and pass the mouth of the tube through the flame and then recap the test tube. Place the test tube back in the test tube rack.
12. Lift one side of the Petri plate lid. (***Do not*** completely remove the lid. Microbes present in the air will fall into the Petri plate and contaminate the sample. ***Always*** open the lid as seen in the image.) Introduce the inoculum to the surface of the media in streaking back and forth on the surface of the media.
13. Lift one side of the Petri plate lid. (***Do not*** completely remove the lid. Microbes present in the air will fall into the Petri plate and contaminate the sample. ***Always*** open the lid as seen in the image.) Introduce the inoculum to the surface of the media in streaking back and forth on the surface of the media.



Figure 1. Petri plate opened for inoculation. Credit: Sherry Smith

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1.4.6: Exercise #4 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What is subculturing and what is the purpose of subculturing?
2. Provide three reasons, covered in lab today, why the use of aseptic techniques is essential when handling microbial cultures in the laboratory.
3. Provide two different examples of how heat is used during the inoculation of a culture tube.
4. How is air contamination prevented when an inoculating loop is used to introduce or take a bacterial sample to/from an agar plate?
5. Why is it important to invert the petri plate when incubating it?

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1.5: Exercise #5 - Isolation Techniques

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Describe the streak plate method of isolation.
2. Perform a streak plate using a mixed culture.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Test tube rack
- China marker
- 1 sharpie marker
- Stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)
- 1 Petri dish with nutrient agar

One of the most important tasks microbiologists must achieve is the identification of microorganisms. Identification procedures/protocols are dependent upon the use of pure cultures. Pure cultures are cultures containing a single species. Most of the samples you will work with in lab will be pure cultures and will need to remain free of contamination for you to successfully complete the exercises for which they will be used. It is imperative that you become comfortable and proficient in working with bacterial samples aseptically.

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1.5.1: Streak Plate

Microbial samples analyzed in microbiology labs may come from several different sources. The samples introduced to the media are known as inoculum. Samples may be taken from a variety of sources including the environment (water and soil samples) and patients in the clinical setting (i.e., sputum, fecal, urine, blood, etc.). Samples sent to microbiology laboratories for identification and analysis are typically mixed samples, meaning that they have more than one microorganism present. The microbiologist must then employ techniques that permit them to separate/isolate the individual microorganism of interest and establish a pure culture.

There are several isolation techniques employed in the laboratory setting by microbiologists to accomplish this task. These techniques include a streak plate technique, the pour plate technique, and the spread plate technique. The most common of the isolation techniques utilized is the streak plate technique. The ***streak plate*** technique allows the microbiologist to isolate a species by spreading the microorganisms in the sample across the surface of a solid media in a Petri plate, in such a manner that the sample is diluted as it is streaked, allowing organisms to develop distinct isolated colonies following incubation.

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1.5.2: Performing the Streak Plate

Follow the steps listed below to complete your streak plate.

1. Begin by disinfecting your work area
2. Obtain all the necessary items to complete the exercise:

   Bunsen burner Inoculating loop Sharpie

   Bacterial stock culture (the identity of the bacteria present in the stock culture will be provided by the instructor during the laboratory session)

   Petri plate containing nutrient agar

3. Label the petri dish with the following information using the sharpie. Be sure to place the information on the bottom of the petri plate (that portion containing the agar)



Figure 1. Proper labeling of a Petri plate. Credit: Sherry Smith

   Your initials Your lab section Date

   MC

4. Light your Bunsen burner. Be sure to adjust the Bunsen burner so that an inner blue cone is clearly visible. Remember, this is the hottest portion of the flame; you will sterilize your inoculating tools in this area.
5. Grasp your inoculating loop in your dominant hand holding it like a pencil. You will use the first three fingers of your dominant hand to manipulate your inoculating tool.
6. Flame the inoculating loop ensuring that it turns red hot, and then let it cool for approximately 15- 30 seconds. Do not allow the instrument to bump against anything and do not lay it down to cool.
7. Use your non-dominant hand to pick up the bacterial Stock Culture. Flick the test tube to practice suspending the bacteria in the culture. Generally, bacteria will settle in the tube and will need to be suspended for transfer.
8. Use the last two fingers of your dominant hand to remove the cap of the test tube.
9. Pass the mouth of the test tube through the flame of the Bunsen burner.
10. Use the sterilized loop to obtain a loopful of sample, without touching the sides of the test tube.
11. Hold the loop steady and pass the mouth of the tube through the flame and then recap the test tube. Place the test tube back in the test tube rack.
12. Lift one side of the Petri plate lid. (***Do not*** completely remove the lid. Microbes present in the air will fall into the Petri plate and contaminate the sample. ***Always*** open the lid as seen in the image.) Introduce the inoculum to the surface of the media in the first quadrant by moving the loop across the surface area in as many tight streaks as



Figure 2. Proper technique for partially opening a Petri plate during inoculation. Credit: Sherry Smith

13. Replace the lid and rotate the plate to position the second quadrant in place for the next step. Flame the loop and let it cool.
14. Streak the second quadrant by lifting one side of the Petri plate lid and streaking a few times across the first quadrant with your sterile loop into the second quadrant. Streak the second quadrant several more times.
15. Replace the lid and rotate the plate to position the third quadrant in place for the next step. Flame the loop and let it cool.
16. Streak the third quadrant by lifting one side of the Petri plate lid and streaking a few times across the second quadrant with your sterile loop. Streak the third quadrant several more times.
17. Replace the lid and rotate the plate to position the fourth quadrant in place for the next step. Flame the loop and let it cool.
18. Streak the fourth quadrant by lifting one side of the Petri plate lid and streaking a few times across the third quadrant with your sterile loop. Streak the fourth quadrant several more times.
19. Replace the lid. Flame the loop until it is red hot to kill remaining bacteria.
20. Incubate the Petri plate in an inverted position in the incubator at 37°C for 24-48 hours. You will examine your plates in the next laboratory session.

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1.5.3: Exercise #5 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. You are performing an experiment that requires the use of skim milk agar. What chemical classification would this agar belong to? Explain your selection.
2. What is the purpose of a streak plate?
3. Examine the results of the streak plate in the image. Does this petri plate contain a pure culture or a mixed culture? Provide supporting evidence for your claim.



Figure 1. Streak plate used for evaluation of culture purity. Credit: Sherry Smith

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1.6: Section One Review

Review Purpose

This review helps you check what you learned in Section One before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you.....

- Explain why laboratory safety guidelines matter, and identify the safety equipment and universal precautions used in the lab?
- Describe the four biosafety levels (BSL) and the measure each requires?
- Identify and label the parts of the compound light microscope and state the function of each?
- Explain the purpose of the oil immersion objective lens and calculate total magnification?
- Explain the purpose of the oil immersion objective lens and calculate total magnification?
- Classify media by chemical composition (defined vs. complex), physical form (liquid, semi-solid, solid), and function (selective, differential, transport, anaerobic)?
- Name and describe three tools used to inoculate media?
- Define aseptic technique and explain why it is essential when transferring bacteria?
- Describe and perform the streak plate method to isolate bacteria from a mixed culture?

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1.6.1: Matching

Matching Questions

1. Match the terms regarding growth media on the left with their appropriate descriptions by placing the letter next to the correct term.

Question 1 Terms

Answer	Terms
_____	1. Selective media
_____	2. Solid media
_____	3. Complex media
_____	4. Liquid media
_____	5. Differential media
_____	6. Enriched media
_____	7. Anaerobic media
_____	8. Semi-solid media
_____	9. Defined media

Question 1 Descriptions

Letter	Description
a.	physical classification - commonly called broths, contain nutrients and lack agar
b.	functional classification - media containing special growth factors needed to grow fastidious microorganisms
c.	functional classification - contain ingredients that allow the differentiation of organisms based on production of difference in color, production of precipitate, or other physical changes
d.	physical classification - growth media containing nutrients and 0.5% or less agar
e.	functional classification - contain ingredients that act to encourage the growth of some organisms while inhibiting others

Letter	Description
f.	functional classification - media containing a reagent such as sodium thioglycolate which serves to remove oxygen from the media
g.	chemical classification - growth media in which the exact chemical contents are known
h.	physical classification - growth media in which 1.5% - 2% agar has been added along with the nutrients
i.	chemical classification - growth media in which the exact chemical composition is unknown due to the addition of yeast, plant, or animal products

Click to reveal answers

Answers:
1) e
2) h
3) i
4) a
5) c
6) b
7) f
8) d
9) g

2. Match the safety equipment term on the left with their appropriate descriptions by placing the letter next to the correct term.

Question 2 Terms

Answer	Terms
_____	1. Safety shower
_____	2. Eyewash station
_____	3. Gas shut off valve
_____	4. Biohazard container
_____	5. Safety data sheet notebook
_____	6. Broken glassware

Question 2 Descriptions

Letter	Description
a.	used to hold uncontaminated broken glassware to prevent injury during disposal
b.	container used to dispose of any materials that have been exposed to bodily fluids or infectious agents; including items such as contaminated glassware
c.	used to rinse chemicals, infectious agents, etc. from the body; or extinguish fire on the body
d.	used in times of fire or gas leak to stop flow of gas to gas jets
e.	used to remove chemical agents or other irritants from the eyes
f.	contains all necessary information regarding chemicals, including precautions for handling, and procedures for accidental exposure

Click to reveal answers

Answers:
1) c
2) e
3) d
4) b
5) f
6) a

3. Match the microscope part on the left with their appropriate descriptions by placing the letter next to the correct term.

Question 3 Terms

Answer	Terms
____	1. Abbe condenser
____	2. Course focus knob
____	3. Fine focus knob
____	4. Iris diaphragm
____	5. Iris diaphragm aperture lever
____	6. Objective lens
____	7. Ocular lens

Question 3 Descriptions

Letter	Description
a.	controls the amount of light reaching the specimen
b.	lens located within the eyepiece
c.	adjusts the aperture (opening) of the iris diaphragm to allow more or less light to reach the specimen
d.	collects the light from the light source and focuses it onto the specimen
e.	used to focus the microscope; it allows for fine tuning the focus and is used with higher power objective lenses
f.	lenses that provide the primary source of magnification; attached to the revolving nose-piece
g.	used to focus the microscope; used only with scanning objective lens

Click to reveal answers

Answers:
1) d
2) g
3) e
4) a
5) c
6) f
7) b

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1.6.2: Fill-In-The-Blank

Fill-in-the-Blank Questions

1. _____ are procedures utilized to reduce microbial numbers in an attempt to prevent spreading the microbes.

Click to reveal answer

Answer Aseptic techniques

2. The solidifying agent, _____ is used to create solid media for use in the microbiology lab.

Click to reveal answer

Answer agar

3. Microorganisms are classified into four _____, based on the level of measures needed to safely work with the organism.

Click to reveal answer

Answer biosafety levels

4. _____ microscopes use two sets of lenses, an ocular lens and objective lens to magnify the specimen.

Click to reveal answer

Answer Compound

5. The _____ method is used to isolate bacteria on the surface of solid media.

Click to reveal answer

Answer streak plate method

6. _____ is the process of moving bacteria from a media source in which they have been growing to a new sterile media source.

Click to reveal answer

Answer Subculturing

7. The _____ , is the magnification calculated by multiplying the magnifying power of the ocular lens with the magnify power of the objective lens.

Click to reveal answer

Answer total magnification

8. In the streak plate method, the _____ quadrant will show the greatest number of isolated colonies.

Click to reveal answer

Answer fourth

9. When using a compound microscope to view a specimen with the high or oil objective lens, you should use the _____ to bring the specimen into focus.

Click to reveal answer

Answer fine focus (adjustment) knob

10. A _____ is an instrument with a straight wire, which is used to stab media during the inoculating process.

Click to reveal answer

Answer inoculating needle

11. Always carry the microscope with one hand on the _____ and the other hand under the _____ .

Click to reveal answer

Answer arm; base

12. Unwanted microbes that find their way into our cultures are called _____ .

Click to reveal answer

Answer contaminants

13. Nutrient agar _____ are often used to store and transport microbes.

Click to reveal answer

Answer slants

14. The _____ concentrates light onto the slide and contains an iris diaphragm to control the angle and the size of the cone of light.

Click to reveal answer

Answer condenser

15. To increase the magnification, you must switch to a higher-power _____ .

Click to reveal answer

Answer objective lens

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1.6.3: True/False

True / False Questions

1. Used culture material (plates, glass slides, swabs, toothpicks, etc.) should be disposed of in the trash can.

_____ True _____ False

Click to reveal answer

Answer False

2. It is not necessary to wash hands before leaving the laboratory room.

_____ True _____ False

Click to reveal answer

Answer False

3. To keep the test tube cap from becoming contaminated you should keep it in the hand that holds the sterile inoculating loop or needle, with the inner aspects of the caps pointing away from the palm of the hand.

_____ True _____ False

Click to reveal answer

Answer True

4. As the magnification of the lens increases, the distance between the objective lens and slide, called working distance, increases.

_____ True _____ False

Click to reveal answer

Answer False

5. Once the inoculating loop has been heat sterilized, it should be allowed to cool 1 minute before inserting it into the bacterial culture to keep it from killing the bacteria in the culture.

_____ True _____ False

Click to reveal answer

Answer False

6. You knock over a glass culture tube containing bacteria, which shatters on the floor. You should then notify the instructor, soak the spill with disinfectant for at least 20 minutes, then dispose of the broken glass in a designated container for contaminated broken glass.

_____ True _____ False

Click to reveal answer

Answer True

7. You can use the course adjustment knob for focusing when viewing through the oil immersion lens.

_____ True _____ False

Click to reveal answer

Answer False

8. Students are permitted to wear open-toed shoes in the laboratory.

_____ True _____ False

Click to reveal answer

Answer False

9. Broth media allow bacteria to grow in such a way that different species can morphologically be distinguished from one another.

_____ True _____ False

Click to reveal answer

Answer False

10. To minimize contamination, the mouth of the culture tube is passed through the flame.

_____ True _____ False

Click to reveal answer

Answer True

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1.6.4: Multiple Choice / Multiple Select

Multiple Choice / Multiple-Select Questions

1. The Centers for Disease Control and Prevention (CDC) classifies microorganisms into how many biosafety levels (BSL)?

- a) Two
- b) Three
- c) Four
- d) Five
- e) Six

Click to reveal answer

> **Answer** c) Four
> The CDC classifies microorganisms into four biosafety levels (BSL).

2. The four biosafety levels classify microorganisms according to:

- a) The level of measures needed to safely work with the organism
- b) The temperature at which the organism grows best
- c) The type of growth media the organism requires
- d) The size of the colonies the organism produces

Click to reveal answer

> **Answer** a) The level of measures needed to safely work with the organism
> The CDC classifies microorganisms into four biosafety levels (BSL) based on the level of measures needed to safely work with the organism.

3. A microorganism that is capable of causing disease is best described as a:

- a) Fastidious organism
- b) Pathogen
- c) Inoculum
- d) Anaerobe
- e) Solidifying agent

Click to reveal answer

> **Answer** b) Pathogen
> Pathogens are microorganisms capable of causing disease.

4. If a chemical or other agent splashes into your eyes, you should flush your eyes at the eyewash station for a minimum of:

- a) 5 minutes

- b) 10 minutes
- c) 15 minutes
- d) 20 minutes
- e) 30 minutes

Click to reveal answer

> **Answer** c) 15 minutes
> Immediately go to the eyewash and flush your eyes with water for 15 minutes.

5. If your body or clothing is exposed to chemicals or infectious agents, you should remain under the emergency safety shower for a minimum of:

- a) 5 minutes
- b) 10 minutes
- c) 15 minutes
- d) 20 minutes

Click to reveal answer

> **Answer** c) 15 minutes
> Should you require use of the safety shower, you should remain under the shower for a minimum of 15 minutes.

6. While wearing examination gloves, a student must NOT do which of the following? **Select all that apply.**

- a) Leave the laboratory room
- b) Touch a microscope or other equipment
- c) Touch personal items such as a cell phone
- d) Touch doorknobs
- e) Handle the assigned culture at their own bench

Click to reveal answers

> **Answers:** a) Leave the laboratory room; b) Touch a microscope or other equipment; c) Touch personal items such as a cell phone; d) Touch doorknobs
> While gloved, students must not leave the lab or touch equipment, personal items, or doorknobs. Handling the assigned culture is the intended task.

7. How should UNCONTAMINATED broken glassware be handled?

- a) Picked up with bare hands and placed in the trash can
- b) Moved with a broom and dustpan into the container marked Broken Glass
- c) Picked up with bare hands and placed in the container marked Broken Glass
- d) Moved with autoclavable tongs into the biohazard container

Click to reveal answer

Answer b) Moved with a broom and dustpan into the container marked Broken Glass
Broken glassware should never be handled with your bare hands. Use a broom/brush and dustpan to move uncontaminated glass to the Broken Glass container.

8. How should CONTAMINATED broken glassware be handled?

- a) Placed directly into the container marked Broken Glass
- b) Transported with autoclavable tongs to the appropriate autoclave/biohazard container
- c) Rinsed with disinfectant and placed in the trash can
- d) Swept up with a broom and placed in the trash can

Click to reveal answer

Answer b) Transported with autoclavable tongs to the appropriate autoclave/biohazard container
Contaminated glassware should be transported with autoclavable tongs to the appropriate autoclave/biohazard container. It must be autoclaved before it can be placed in the broken glass container.

9. Which materials are considered biohazardous and must be placed in a biohazard container?

- a) Any glassware, whether or not it has contacted microorganisms
- b) Materials that have been exposed to bodily fluids or microorganisms
- c) Only materials that are visibly soiled
- d) Any item used during a laboratory session

Click to reveal answer

Answer b) Materials that have been exposed to bodily fluids or microorganisms
Materials exposed to bodily fluids or microorganisms are considered biohazardous and should be placed in a biohazard container.

10. How are the contents of a biohazard container treated before they are disposed of?

- a) They are soaked in disinfectant for 20 minutes
- b) They are autoclaved to sterilize the contents
- c) They are rinsed with deionized water
- d) They are placed in the broken glass container

Click to reveal answer

Answer b) They are autoclaved to sterilize the contents
Materials placed in the biohazard container are autoclaved to sterilize the contents before disposal.

11. You spill a bacterial culture on your lab bench. Which actions are part of the correct protocol? **Select all that apply.**

- a) Cover the spill with paper towels
- b) Soak the towels with disinfectant and allow them to stand for 20 minutes
- c) Report the spill to your instructor
- d) Place the used paper towels in the container marked for autoclaving
- e) Rinse the area with water and continue working

Click to reveal answers

Answers: a) Cover the spill with paper towels; b) Soak the towels with disinfectant and allow them to stand for 20 minutes; c) Report the spill to your instructor; d) Place the used paper towels in the container marked for autoclaving
Cover culture spills with paper towels, soak the towels immediately with disinfectant and allow them to stand for 20 minutes, report the spill to your instructor, and place the towels in the container marked for autoclaving.

12. Which precautions must be taken when working with open flames in the laboratory? **Select all that apply.**

- a) Secure loose hair and dangling jewelry
- b) Secure loose clothing and nearby paper
- c) Never leave a hot plate or Bunsen burner unattended
- d) Keep containers of alcohol or acetone at a safe distance from flames
- e) Lay the hot inoculating loop on the bench to cool

Click to reveal answers

Answers: a) Secure loose hair and dangling jewelry; b) Secure loose clothing and nearby paper; c) Never leave a hot plate or Bunsen burner unattended; d) Keep containers of alcohol or acetone at a safe distance from flames
Loose hair, clothing, dangling jewelry, and nearby paper must be secured; a heat source is never left unattended; and flammable liquids are kept at a safe distance from flames.

13. How should any microorganism handled in a microbiology laboratory be regarded?

- a) As harmless, provided it was purchased from a commercial supplier
- b) As a potential pathogen
- c) As a BSL-3 organism
- d) As harmless unless it is labeled with a biohazard symbol

Click to reveal answer

Answer b) As a potential pathogen
Any microorganism handled in the microbiology laboratory should be regarded as a potential pathogen, even though this laboratory does not require the use of any highly infectious human pathogens.

14. Bright-field microscopy uses white light to visualize the specimen. Because the specimen absorbs the light, it appears _____ than the surrounding background.

- a) Darker

- b) Brighter
- c) More magnified
- d) Colorless

Click to reveal answer

Answer a) Darker
In bright-field microscopy the specimen absorbs the light, causing the specimen to be darker than the surrounding background.

15. Which term refers to the ability to distinguish two objects from one another?

- a) Magnification
- b) Contrast
- c) Resolution
- d) Refraction

Click to reveal answer

Answer c) Resolution
Resolution refers to the ability to distinguish two objects from one another.

16. Which term refers to the difference in light intensity between the specimen and the background?

- a) Resolution
- b) Contrast
- c) Total magnification
- d) Working distance

Click to reveal answer

Answer b) Contrast
Contrast refers to the difference in light intensity of the specimen and the background.

17. Magnification alone will not produce a quality image. What else must be achieved? **Select all that apply.**

- a) Resolution
- b) Contrast
- c) A higher ocular lens power
- d) A thicker specimen

Click to reveal answers

Answers: a) Resolution; b) Contrast
It is important that resolution and contrast be achieved in addition to magnification to generate a quality image.

18. Why are biological specimens generally stained before they are observed?

- a) They usually lack the contrast needed to observe details
- b) Staining increases the total magnification
- c) Staining prevents the slide from breaking
- d) Staining is required before the coarse focus knob can be used

Click to reveal answer

Answer a) They usually lack the contrast needed to observe details
Biological specimens usually lack the contrast to observe details, and thus are generally stained before observation.

19. Immersion oil is used with the oil immersion objective lens because it:

- a) Increases the magnifying power of the lens
- b) Prevents the loss of light due to refraction, thereby preserving resolution
- c) Cleans the surface of the lens
- d) Kills bacteria present on the slide

Click to reveal answer

Answer b) Prevents the loss of light due to refraction, thereby preserving resolution
Immersion oil prevents the loss of light due to refraction, thereby preserving resolution.

20. Which objective lenses are dry objectives, meaning they do not require immersion oil to maintain good resolution? **Select all that apply.**

- a) Scanning
- b) Low power
- c) High power
- d) Oil immersion

Click to reveal answers

Answers: a) Scanning; b) Low power; c) High power
The scanning, low, and high objective lenses are dry objective lenses; only the oil immersion lens requires oil.

21. The coarse focus (adjustment) knob should be used only with which objective lens?

- a) The 4x scanning objective
- b) The 10x low-power objective
- c) The 40x high-power objective
- d) The 100x oil immersion objective

Click to reveal answer

Answer a) The 4x scanning objective
The coarse focus knob should only be used with the 4x scanning objective lens.

22. A compound microscope has a 10x ocular lens. What is the total magnification when the 40x high-power objective is in use?

- a) 40x
- b) 50x
- c) 400x
- d) 4000x

Click to reveal answer

Answer c) 400x
Total magnification = ocular lens power (10x) multiplied by objective lens power (40x) = 400x.

23. Your microscope objectives are parfocal. What does this mean?

- a) All objective lenses provide the same total magnification
- b) When the specimen is in focus with one objective, it should stay in focus when you move to another objective
- c) The objectives can be used without adding immersion oil
- d) The condenser automatically adjusts the light for each objective

Click to reveal answer

Answer b) When the specimen is in focus with one objective, it should stay in focus when you move to another objective
Parfocal means that when you are in focus using one objective lens, it should stay in focus when moving to another objective lens, so only a slight rotation of the fine adjustment knob is needed.

24. As you increase objective magnification power, what adjustment becomes necessary, and which component is used to make it?

- a) Less light is needed; the coarse focus knob is used
- b) More light is needed; the iris diaphragm aperture lever is used
- c) Less light is needed; the revolving nose-piece is used
- d) More light is needed; the stage control knobs are used

Click to reveal answer

Answer b) More light is needed; the iris diaphragm aperture lever is used
As you increase in objective magnification power you will need more light, adjusted using the iris diaphragm aperture lever to ensure adequate contrast between specimen and background.

25. What is the correct way to bring the oil immersion objective lens into use?

- a) Place a drop of oil on the slide while the high-power lens is in position, then rotate to the oil lens
- b) Rotate the nose-piece so the space between the high-power and oil immersion lenses is over the slide, place a small drop of oil on the slide, then slowly rotate the oil lens into place
- c) Rotate the oil immersion lens into place first, then add oil to the top of the lens
- d) Add oil to the condenser before rotating the oil immersion lens into place

Click to reveal answer

Answer b) Rotate the nose-piece so the space between the high-power and oil immersion lenses is over the slide, place a small drop of oil on the slide, then slowly rotate the oil lens into place
Rotate the revolving nose-piece so the space between the high-power objective and the oil immersion lens is over the slide, place a small drop of immersion oil on the slide, then slowly rotate the oil immersion lens into place.

26. After you finish viewing a specimen with the oil immersion lens, the oil is removed using:

- a) Deionized water and a paper towel
- b) Lens cleaner and a lens cleaning cloth or lens paper
- c) Disinfectant solution and a Kim wipe
- d) Immersion oil and a dustpan brush

Click to reveal answer

Answer b) Lens cleaner and a lens cleaning cloth or lens paper
Use the lens cleaner and a lens cleaning cloth/lens paper to remove the oil from the oil immersion lens.

27. Agar is derived from the cell walls of algal cells. Why is it useful as a solidifying agent in microbiology?

- a) It provides essential nutrients for all bacteria
- b) Microorganisms are unable to digest it
- c) It removes oxygen from the media
- d) It causes bacteria to produce a distinct color change

Click to reveal answer

Answer b) Microorganisms are unable to digest it
Agar is a commonly used solidifying agent due to the inability of microorganisms to digest it.

28. Which of the following are examples of complex media? **Select all that apply.**

- a) Tryptic soy media
- b) Brain heart media
- c) Nutrient media

- d) A medium in which the exact chemical composition and quantities are known

Click to reveal answers

Answers: a) Tryptic soy media; b) Brain heart media; c) Nutrient media
Tryptic soy, brain heart, and nutrient media are complex media. A medium of known exact chemical composition is a defined (synthetic) medium.

29. Semi-solid media contain what amount of agar, and for which test are they commonly used?

- a) 1.5%-2% agar; isolation on petri plates
- b) 0.5% or less agar; motility tests
- c) No agar; serial dilutions
- d) 2% or more agar; long-term cold storage

Click to reveal answer

Answer b) 0.5% or less agar; motility tests
Semi-solid media contain a small percentage of agar, 0.5% or less, and are commonly used for motility tests.

30. Which media are BOTH selective and differential? **Select all that apply.**

- a) Mannitol salt agar
- b) MacConkey agar
- c) Eosin methylene blue agar
- d) Sterile nutrient broth

Click to reveal answers

Answers: a) Mannitol salt agar; b) MacConkey agar; c) Eosin methylene blue agar
Mannitol salt agar, MacConkey agar, and eosin methylene blue agar are each both selective and differential.

31. Anaerobic media are made using an ingredient that binds to and reduces oxygen in the media. Which ingredient serves this purpose?

- a) Agar
- b) Sodium thioglycolate
- c) Immersion oil
- d) Methylene blue

Click to reveal answer

Answer b) Sodium thioglycolate
Anaerobic media are made using an ingredient such as sodium thioglycolate that binds to and reduces oxygen in the media, creating an environment favorable for anaerobe growth.

32. How can you tell that bacteria are growing in a broth culture?

- a) The broth becomes cloudy
- b) The broth remains clear
- c) The broth solidifies
- d) The broth separates into layers

Click to reveal answer

Answer a) The broth becomes cloudy
Sterile broths remain clear, while broths containing bacteria are cloudy in appearance.

33. One advantage of broth media is the ability to grow organisms at various concentrations of oxygen. Where are oxygen levels highest in a broth tube?

- a) At the surface of the liquid
- b) At the bottom of the tube
- c) Evenly distributed throughout the tube
- d) Oxygen is absent from all broth media

Click to reveal answer

Answer a) At the surface of the liquid
Oxygen levels are highest at the surface of the liquid and decrease as the depth of the broth increases.

34. What is the purpose of transport media?

- a) To encourage rapid multiplication of the sample during transport
- b) To maintain the microorganism being transported without permitting its multiplication
- c) To differentiate organisms based on color changes during transport
- d) To remove oxygen from the sample during transport

Click to reveal answer

Answer b) To maintain the microorganism being transported without permitting its multiplication
Transport media contain ingredients such as buffers and salts that maintain the microorganism being transported without permitting its multiplication.

35. Enriched media such as blood agar and chocolate agar are used to grow:

- a) Only anaerobic organisms
- b) Fastidious microorganisms requiring additional nutrients and growth factors
- c) Organisms that cannot digest agar
- d) Organisms being held for transport to a clinical laboratory

Click to reveal answer

Answer b) Fastidious microorganisms requiring additional nutrients and growth factors
Enriched media are used to grow fastidious microorganisms, those requiring additional nutrients and growth factors, and contain special ingredients like blood or serum.

36. A glass spreading rod is bent into an L-shape and is used to:

- a) Stab semi-solid media during a motility test
- b) Spread an inoculum over the surface of a solid medium
- c) Transfer a loopful of broth culture to a slant
- d) Remove broken glass from the laboratory bench

Click to reveal answer

Answer b) Spread an inoculum over the surface of a solid medium
Glass spreading rods are used to spread an inoculum over the surface of a solid medium, as in the spread plate technique.

37. For which tasks is an inoculating loop used? **Select all that apply.**

- a) Transferring bacteria from one type of media to another
- b) Performing the streak plate isolation method
- c) Transferring a sample to a microscope slide to create a smear
- d) Stabbing media during a motility test

Click to reveal answers

Answers: a) Transferring bacteria from one type of media to another; b) Performing the streak plate isolation method; c) Transferring a sample to a microscope slide to create a smear
Inoculating loops transfer bacteria between media, are used for the streak plate isolation method, and transfer samples to slides to create a smear. Stabbing media is done with an inoculating needle.

38. What advantage is gained when solid media is poured into a petri plate rather than a test tube?

- a) It produces a large surface area, allowing isolation and observation of colony morphology
- b) It allows bacteria to be stored longer in cold storage
- c) It permits the growth of motile bacteria only
- d) It removes the need to use aseptic technique

Click to reveal answer

Answer a) It produces a large surface area, allowing isolation and observation of colony morphology
Media poured into a petri plate produces a large surface area for inoculation, allowing isolation of individual species and observation of differences in colony morphology.

39. To say that microorganisms are ubiquitous means that they are:

- a) Present everywhere, including the air, on and in our bodies, and on laboratory benches
- b) Found only in clinical specimens
- c) Able to grow only on enriched media
- d) Harmless to healthy individuals

Click to reveal answer

Answer a) Present everywhere, including the air, on and in our bodies, and on laboratory benches
Microorganisms are ubiquitous, meaning they are everywhere: in the air around us, in and on our bodies, and on the laboratory benches.

40. Aseptic technique aims to prevent contamination of which of the following? **Select all that apply.**

- a) Your sample
- b) Your work space
- c) Yourself
- d) The microscopes stored in the cabinet

Click to reveal answers

Answers: a) Your sample; b) Your work space; c) Yourself
The goals of aseptic technique are to prevent contamination of your sample, prevent contamination of your work space, and prevent contaminating yourself.

41. Which of the following are examples of aseptic techniques? **Select all that apply.**

- a) Washing hands properly
- b) Disinfecting work surfaces
- c) Sterilizing inoculating tools
- d) Incubating plates in an inverted position

Click to reveal answers

Answers: a) Washing hands properly; b) Disinfecting work surfaces; c) Sterilizing inoculating tools
Aseptic techniques include washing hands properly, disinfecting work surfaces, sterilizing inoculating tools, and other procedures aimed at reducing the likelihood of contamination.

42. When adjusting the Bunsen burner, why is the inner blue cone important?

- a) It is the hottest portion of the flame, and is where the inoculating tool is sterilized
- b) It indicates the gas shut-off valve is closed
- c) It is the coolest portion of the flame, and is where the loop is allowed to cool

- d) It shows that the burner is safe to leave unattended

Click to reveal answer

Answer a) It is the hottest portion of the flame, and is where the inoculating tool is sterilized
The Bunsen burner is adjusted so an inner blue cone is clearly visible. This is the hottest portion of the flame, and is where you sterilize your inoculating tool.

43. After flaming an inoculating loop until it is red hot, you should:

- a) Use it immediately while it is still hot
- b) Let it cool approximately 15-30 seconds without laying it down or letting it bump anything
- c) Lay it on the lab bench for 1 minute to cool
- d) Rinse it with deionized water to cool it quickly

Click to reveal answer

Answer b) Let it cool approximately 15-30 seconds without laying it down or letting it bump anything
Flame the loop until red hot, then let it cool for approximately 15-30 seconds. Do not allow the instrument to bump against anything and do not lay it down to cool.

44. How is the cap of a culture tube handled during an aseptic transfer?

- a) It is set on the lab bench beside the tube
- b) It is removed using the last two fingers of the dominant hand and is never set down on the bench
- c) It is removed with the non-dominant hand and placed in a test tube rack
- d) It is left on the tube and the loop is inserted alongside it

Click to reveal answer

Answer b) It is removed using the last two fingers of the dominant hand and is never set down on the bench
Use the last two fingers of your dominant hand to remove the cap of the test tube. Do not set the cap down on the lab bench.

45. Why is a broth culture tube flicked before a sample is removed?

- a) To warm the culture to incubation temperature
- b) Because bacteria settle in the tube and need to be suspended for transfer
- c) To force air into the bottom of the tube for aerobic growth
- d) To check the cap for a tight seal

Click to reveal answer

Answer b) Because bacteria settle in the tube and need to be suspended for transfer
Generally, bacteria will settle in the tube and will need to be suspended for transfer, so the tube is flicked to suspend the bacteria in the culture.

46. What information must be written on media you have inoculated? **Select all that apply.**

- a) Your initials
- b) Your lab section
- c) The date
- d) The initials of the bacteria provided by your instructor
- e) The total magnification you will use to view it

Click to reveal answers

Answers: a) Your initials; b) Your lab section; c) The date; d) The initials of the bacteria provided by your instructor
Label inoculated media with your initials, your lab section, the date, and the initials of the bacteria provided by your instructor.

47. When labeling a petri dish, where should the information be placed?

- a) On the lid, so it can be read without disturbing the plate
- b) On the bottom, the portion containing the agar
- c) On a piece of tape placed across the seam of the plate
- d) On the side of the plate using a China marker

Click to reveal answer

Answer b) On the bottom, the portion containing the agar
Be sure to place the information on the bottom of the petri plate, the portion containing the agar.

48. When inoculating a petri plate, why is only one side of the lid lifted rather than removing it completely?

- a) Microbes present in the air would fall into the plate and contaminate the sample
- b) The agar would dry out too quickly
- c) The lid is difficult to reseat once removed
- d) Removing the lid would cool the media below growth temperature

Click to reveal answer

Answer a) Microbes present in the air would fall into the plate and contaminate the sample
Do not completely remove the lid. Microbes present in the air will fall into the petri plate and contaminate the sample.

49. Under what conditions are inoculated petri plates incubated?

- a) Inverted, at 37°C, for 24-48 hours
- b) Upright, at room temperature, for 24-48 hours
- c) Inverted, at 37°C, for 15-30 minutes

- d) Upright, at 37°C, for at least one week

Click to reveal answer

Answer a) Inverted, at 37°C, for 24-48 hours
Incubate the petri plate in an inverted position in the incubator at 37°C for 24-48 hours.

50. Inoculated petri plates are incubated in an inverted position primarily to:

- a) Prevent condensation from raining down onto the sample
- b) Increase the oxygen available to the culture
- c) Speed up bacterial growth
- d) Keep the labeling visible from above

Click to reveal answer

Answer a) Prevent condensation from raining down onto the sample
Plates are incubated inverted to prevent condensation from raining down on your sample.

51. A pure culture is a culture that contains:

- a) A single species
- b) Two or more species
- c) Only motile organisms
- d) Only organisms that can be grown on enriched media

Click to reveal answer

Answer a) A single species
Pure cultures are cultures containing a single species.

52. Identification procedures and protocols performed in the microbiology laboratory are dependent upon the use of:

- a) Mixed cultures
- b) Pure cultures
- c) Broth cultures only
- d) Transport media

Click to reveal answer

Answer b) Pure cultures
Identification procedures/protocols are dependent upon the use of pure cultures, which contain a single species.

53. The sample that is introduced to a growth medium is known as the:

- a) Inoculum
- b) Subculture
- c) Slant
- d) Pure culture

Click to reveal answer

Answer a) Inoculum
The samples introduced to the media are known as inoculum.

54. Samples sent to a microbiology laboratory for identification and analysis are typically:

- a) Pure samples containing a single organism
- b) Mixed samples, meaning more than one microorganism is present
- c) Sterile samples containing no organisms
- d) Samples that have already been streaked for isolation

Click to reveal answer

Answer b) Mixed samples, meaning more than one microorganism is present
Samples sent to microbiology laboratories for identification and analysis are typically mixed samples, meaning that they have more than one microorganism present.

55. From which of the following clinical sources might a microbial sample be taken? **Select all that apply.**

- a) Sputum
- b) Fecal material
- c) Urine
- d) Blood

Click to reveal answers

Answers: a) Sputum; b) Fecal material; c) Urine; d) Blood
Samples may be taken from the environment, such as water and soil, and from patients in the clinical setting, including sputum, fecal, urine, and blood samples.

56. The streak plate technique produces isolated colonies because the sample is:

- a) Progressively diluted as it is streaked across the surface of the solid media
- b) Stabbed into the depth of the agar
- c) Suspended evenly throughout a liquid broth
- d) Spread across the plate with a glass spreading rod

Click to reveal answer

Answer a) Progressively diluted as it is streaked across the surface of the solid media
The streak plate technique spreads the microorganisms across the surface of solid media in such a manner that the sample is diluted as it is streaked, allowing organisms to develop distinct isolated colonies.

57. During the streak plate procedure, why is the inoculating loop flamed and allowed to cool between quadrants?

- a) To continue diluting the sample so that isolated colonies can develop
- b) To keep the agar surface from melting
- c) To warm the loop before it touches the next quadrant
- d) To prevent the petri plate lid from becoming contaminated

Click to reveal answer

Answer a) To continue diluting the sample so that isolated colonies can develop
The loop is flamed and cooled between quadrants so the sample continues to be diluted as it is streaked, allowing organisms to develop distinct isolated colonies.

58. Besides the streak plate technique, which of the following are isolation techniques employed in the laboratory? **Select all that apply.**

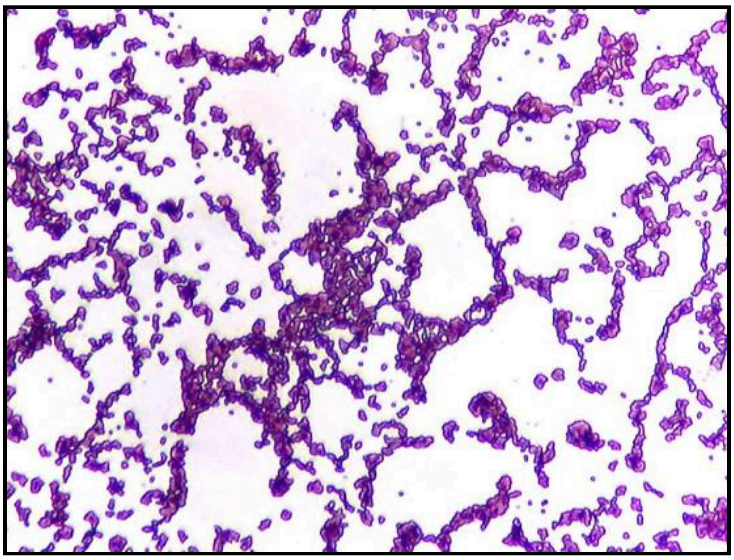
- a) The pour plate technique
- b) The spread plate technique
- c) The aseptic transfer technique
- d) The motility stab technique

Click to reveal answers

Answers: a) The pour plate technique; b) The spread plate technique
Isolation techniques include the streak plate technique, the pour plate technique, and the spread plate technique. The most common of these is the streak plate technique.

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2: SECTION TWO - VISUALIZING BACTERIA (STAINING BACTERIA)



Staphylococcus aureus. Credit: Sherry Smith

In this section you will be introduced to common staining techniques utilized in the microbiology laboratory. Cells must be stained to be visualized. The staining techniques that will be studied allow for visualization of the microbes as well as provide information regarding the structural make-up of the microbe. In addition to staining, you will examine the cellular structures of bacterial cells, cell shapes, and cell arrangements.

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Microscopic Clues: Bacterial Morphology and Staining Techniques - Click on the video thumbnail to be redirected to the video.

Credit: Yenny Mo, Josh Spradling, Sherry Smith

2.1: Exercise #6 - Bacterial Cellular Morphology and Structure

2.1.1: Cellular Morphology and Structure

2.1.2: Bacterial Cellular Arrangement

2.1.3: Exercise #6 Lab Wrap-Up

2.2: Exercise #7 - Specimen Preparation and Simple Staining

2.2.1: Specimen Preparation and Simple Staining

2.2.2: Cheek Smear Activity

2.2.3: Bacterial Smear Activity

2.2.4: Exercise #7 Lab Wrap-Up

2.3: Exercise #8 - Differential Staining- Gram Staining

2.3.1: Gram Stain

2.3.2: Gram Stain Activity

2.3.3: Smear Visualization

2.3.4: Exercise #8 Lab Wrap-Up

2.4: Exercise #9 - Differential Staining- Acid-Fast Stain

2.4.1: Acid-Fast Stain

2.4.2: Acid-Fast Stain Activity

2.4.3: Smear Visualization

2.4.4: Exercise #9 Lab Wrap-Up

2.5: Exercise #10 - Structural Stain- Endospore Stain

2.5.1: Endospore Stain

2.5.2: Endospore Stain Activity

2.5.3: Smear Visualization

2.5.4: Exercise #10 Lab Wrap-Up

2.6: Exercise #11 - Structural Stain- Capsule Stain

2.6.1: Capsule Stain

2.6.2: Capsule Stain Activity

2.6.3: Smear Visualization

2.6.4: Exercise #11 - Lab Wrap-Up

2.7: Section Two Review

2.7.1: Matching

2.7.2: Fill-In-The-Bank

2.7.3: True/False

2.7.4: Multiple Choice / Multiple Select

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2.1: Exercise #6 - Bacterial Cellular Morphology and Structure

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

Laboratory Exercise Learning Outcomes

1. Distinguish basic bacterial shapes.
2. Identify and describe the function of bacterial cellular structures.

Materials Needed:

- Compound light microscope
- Lens cleaner
- Lens paper
- Prepared microscope slides demonstrating various bacterial shapes

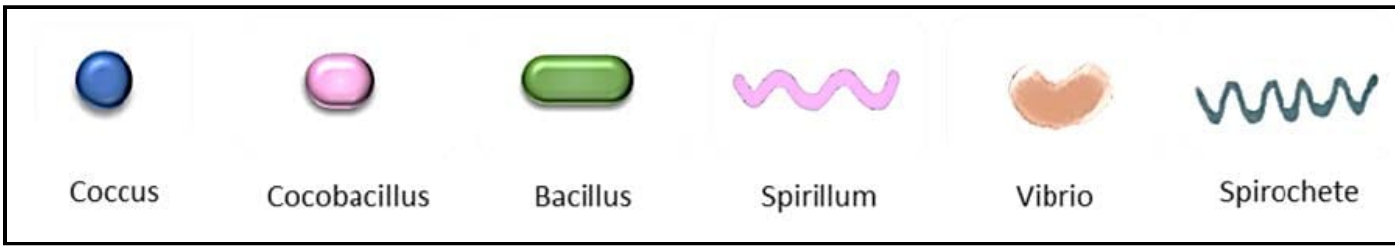
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2.1.1: Cellular Morphology and Structure

Morphology refers to the appearance or shape of a bacterium. All the cells of a given species will generally have the same shape and size. Three basic shapes are observed in bacteria: spheres, rods, and spirals.

Bacteria that are spherical in shape are referred to as ***cocci*** (singular, coccus). Those that are rod-shaped are referred to as ***bacilli*** (singular, bacillus). There are two categories of bacteria that appear spiral-shaped.

Those that have rigid cell walls are referred to as ***spirilla*** (singular, spirillum), while those that have flexible cell walls are known as ***spirochetes***. Spirilla can also be differentiated from spirochetes, due to the presence of external flagella. Spirochetes, on the other hand have structures known as axial filaments endoflagellum), that allow their cells to rotate in a "corkscrew" manner. ***Coccobacillus*** bacteria are cells that have a shape intermediate to that of a coccus and a bacillus; they appear as short plump rods. Cells that appear as curved rods are referred to as ***vibrio***. Some species will exhibit a variety of shapes and sizes, such bacteria are referred to as ***pleomorphic***.



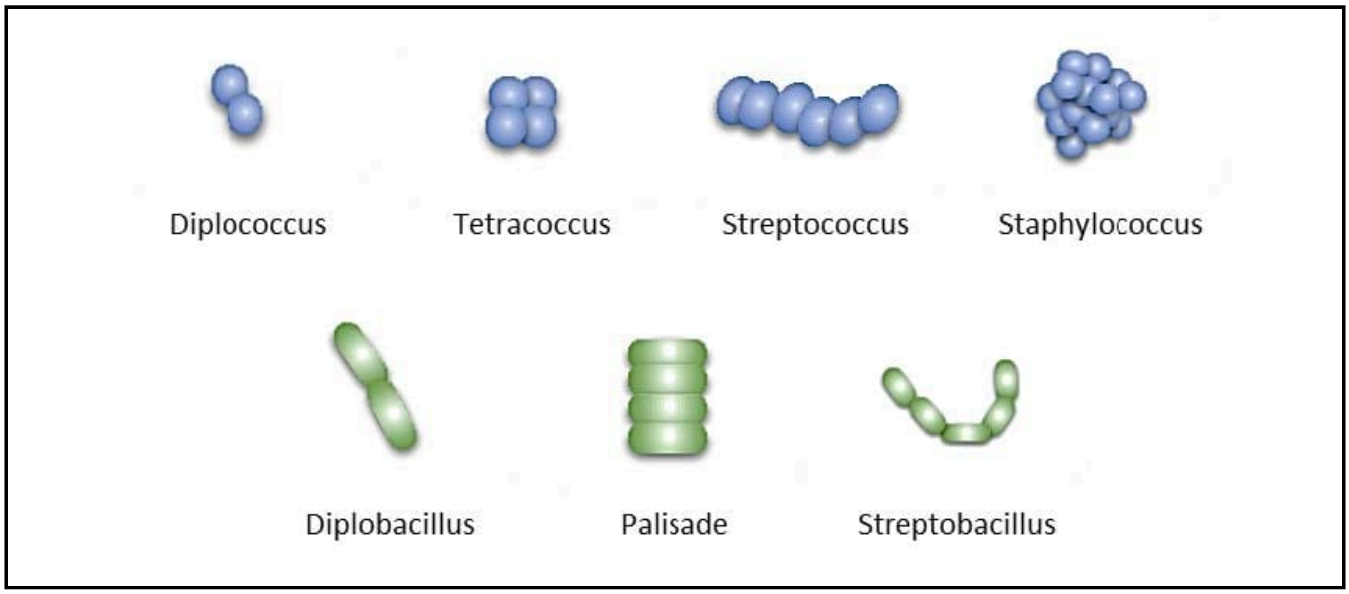
Common bacterial shapes. Credit: Sherry Smith

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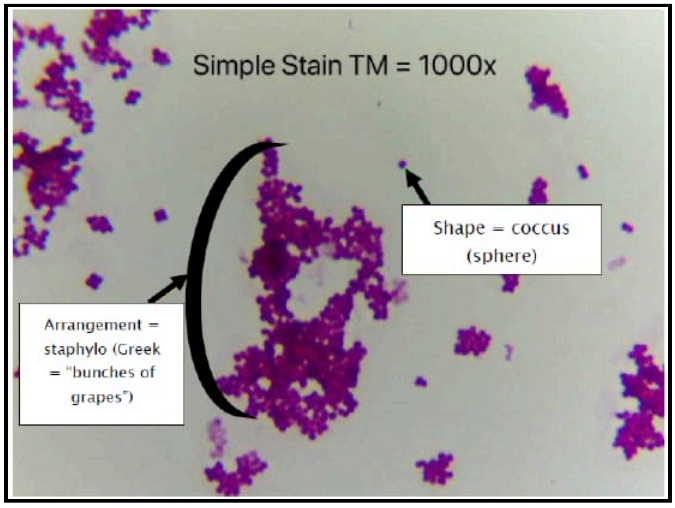
2.1.2: Bacterial Cellular Arrangement

Bacterial cellular arrangement refers to how bacterial cells of a species associate with one another. Cellular arrangement is dependent on the plane of cell division and whether cells remain attached to one another following cell division.

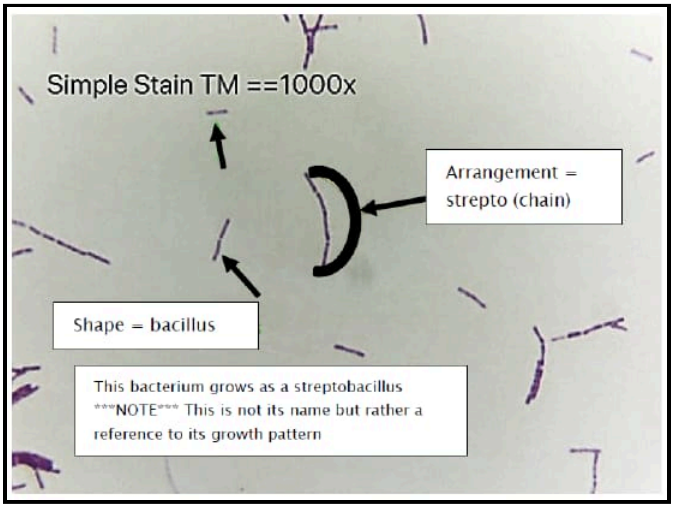
More arrangements are observed in cocci than are observed for bacilli. Some species of cocci will divide only in a single plane, while others divide in two perpendicular planes, and still others in many planes of division: resulting in a variety of cellular arrangements. Bacilli divide only in the transverse plane. Spirilla rarely remain attached, so they are generally observed as individual cells. Seen below are some examples of arrangements observed in some bacteria.



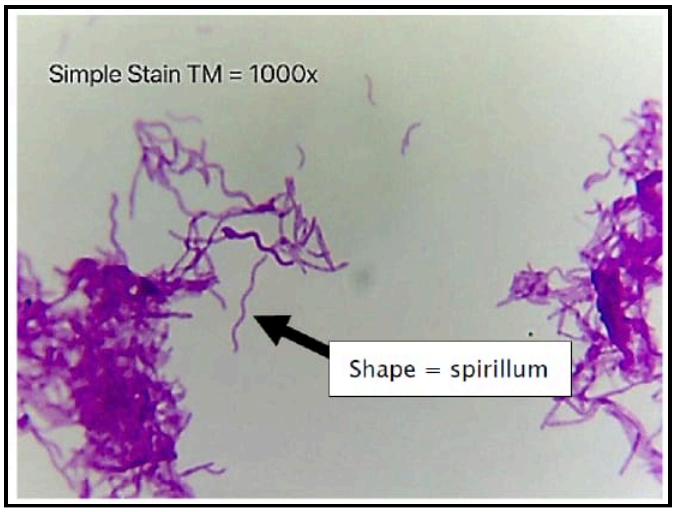
Common bacterial arrangements. Credit: Sherry Smith



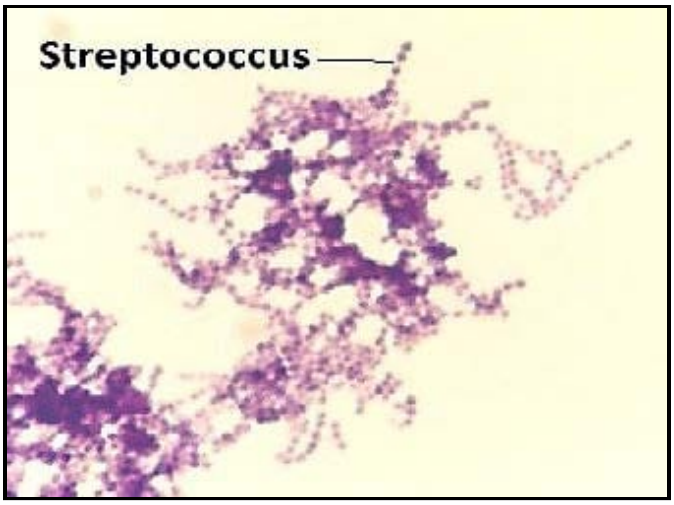
Staphylococcus Credit: Sherry Smith



Streptobacillus Credit: Sherry Smith



Spirillum Credit: Sherry Smith



Streptococcus Credit: Sherry Smith

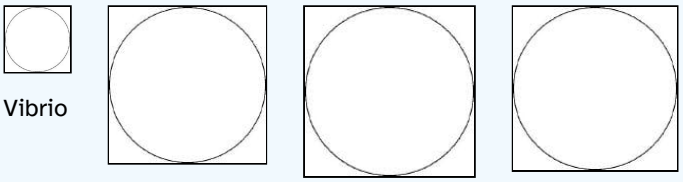
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2.1.3: Exercise #6 Lab Wrap-Up

Lab Wrap-Up Questions

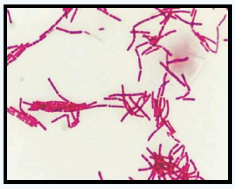
Answer the following questions:

1. You are performing an experiment that requires the use of skim milk agar. What chemical classification would this agar belong to? Explain your selection.
2. Draw a representation of each of the following shapes or arrangements:

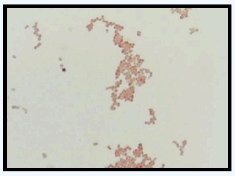


Vibrio | Streptococcus | Staphylococcus | Streptobacillus

3. What is the difference between spirillum and spirochete?
4. Identify the ***shape*** seen in each of the micrographs below:



Credit: Sherry Smith



Credit: Sherry Smith

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2.2: Exercise #7 - Specimen Preparation and Simple Staining

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #3 Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the following in the preparation of a specimen for microscopic viewing:
   a. smear
   b. heat fixation
   c. chemical fixation
2. Explain why specimens are stained.
3. Differentiate between acidic and basic dyes.
4. Describe simple staining and list the information that can be determined about a sample using simple staining.
5. Prepare and stain and oral smear.
6. Prepare and stain bacterial smears.
7. Explain why aseptic technique is important when preparing bacterial smears.

Materials Needed:

- Bunsen burner
- Staining tray (containing stains, stain rack, and DI water)
- Individually wrapped toothpick
- Saline solution
- Bacterial broth cultures
- Test tube rack
- Microscope slides
- Inoculating loop
- Microscope

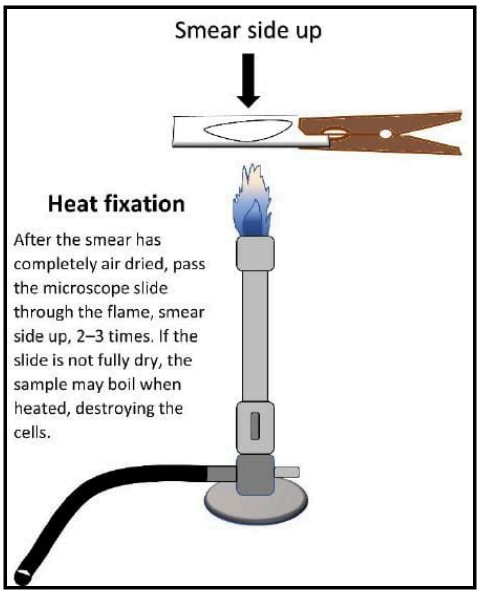
In this laboratory exercise we will be staining cells and viewing them. Students need to understand that there are different types of staining procedures utilized by microbiologist. In this lab we will employ what is known as a simple stain procedure to examine cells. We will also begin examining bacterial cellular morphology and structure.

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2.2.1: Specimen Preparation and Simple Staining

Bacterial samples must be placed on microscope slides for staining. Specimen preparation begins as a sample is taken from a culture and then introduced to the microscope slide and spread into a thin film. This thin film of bacterial sample is known as a ***smear***. As the specimen preparation is carried out it is important that those cells be attached or "fixed" to the slide, so they are not rinsed off the slide during the staining process. The process known as ***fixation,*** is done to attach the cells to the slide, but also to prevent distortion of cells that would alter their morphology. Fixation also results in the death of the cells, thereby making the sample safer to handle. There are two types of fixation, chemical fixation and heat fixation.

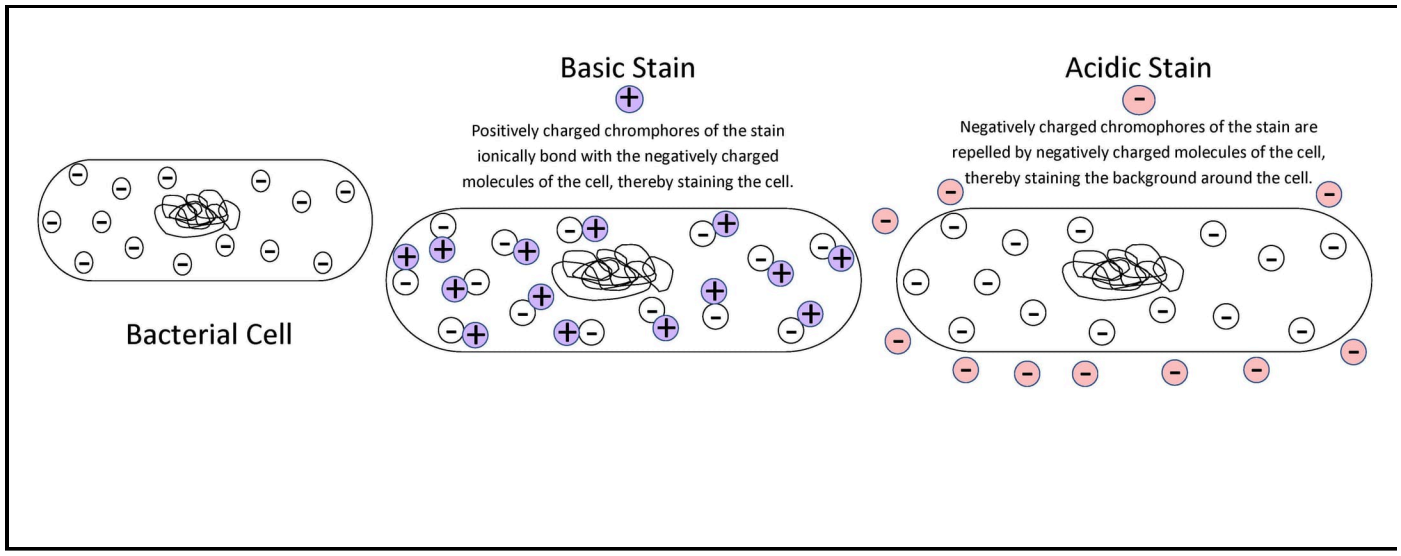
Chemical fixation is accomplished by applying a chemical reagent such as methyl alcohol or formaldehyde to the sample. ***Heat fixation*** is done by passing the air-dried smear back and forth over the flame of a Bunsen burner smear side up.



Heat fixation. Credit: Sherry Smith

Once the smear has been fixed the sample may then be stained. Samples are stained in order to add contrast to the cells, allowing them to be viewed using the microscope.

Staining techniques employ dyes in solution (stain) that will chemically interact with the cells in the smear. There are two types of dyes employed in the lab, acidic dyes and basic dyes. ***Acidic dyes***, also known as anionic dyes, are negatively charged and therefore are attracted to positively charged structures. ***Basic dyes***, also known as cationic dyes, are positively charged and thus will be attracted to negatively charged structures. It is important to understand that cells are filled with molecules, such as proteins and nucleic acids that are negatively charged, and thus the cell itself will carry an overall negative charge internally. When an anionic dye is applied to a cell it is repelled and thus would stain the surrounding background around the cell. A basic dye, however, would be attracted to the negatively charged molecules inside the cell and therefore ionically bond to those structures, staining the cell itself. Examples of acidic dyes include India ink, nigrosine, and eosin. Examples of basic dyes include methylene blue, crystal violet, and safranin.



Stain-cell interaction. Credit: Sherry Smith

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2.2.2: Cheek Smear Activity

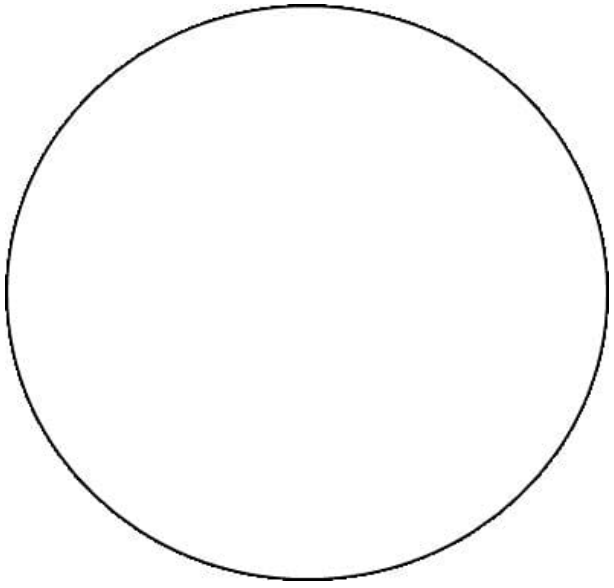
In this activity you will prepare a cheek smear sample for visualization. You will be obtaining your own cheek cells for this sample.

Follow the procedure below to prepare and stain a cheek smear.

1. Obtain one microscope slide, clean and dry the microscope slide using lens cleaner and Kim Wipes
2. Place a drop of isotonic saline solution on the clean, dry microscope slide
3. Using the end of a toothpick, scrape the inner surface of the cheek
4. Mount the scraping in the drop of saline solution by spreading the toothpick back and forth through the saline solution, spreading the saline solution out into a thin, even smear on the microscope slide
5. Allow the slide to air dry
6. Pass the air-dried smear, smear side up, through the flame of a Bunsen burner two or three times to heat fix the smear
7. Cover the smear with methylene blue stain for 1 minute
8. Rinse the stain from the slide using DI water
9. Blot the slide using blotting paper

Smear Visualization:

Obtain your assigned microscope. You will visualize your sample using a Total magnification of 400x. Use colored pencils to depict your observations in the space below. Label the components of your eu- karyotic cheek cell that can be distinguished. Label bacterial cells.

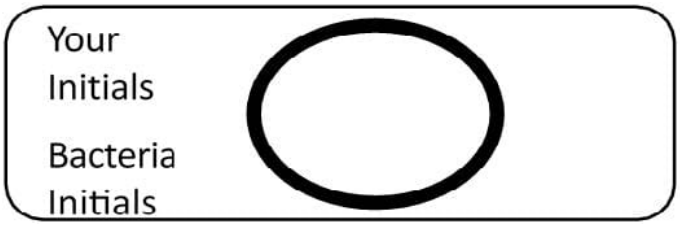


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2.2.3: Bacterial Smear Activity

In this activity you will prepare two bacterial samples for visualization. You will be staining and examining samples that exhibit the two most common bacterial shapes: coccus and bacillus.

1. Obtain two microscope slides, clean and dry the microscope slides using lens cleaners and Kim wipes
2. Label the slides using a China marker as seen below:



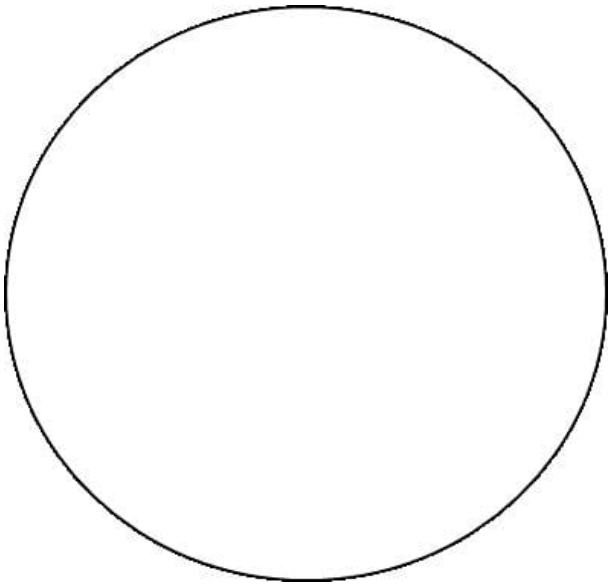
Credit: Sherry Smith

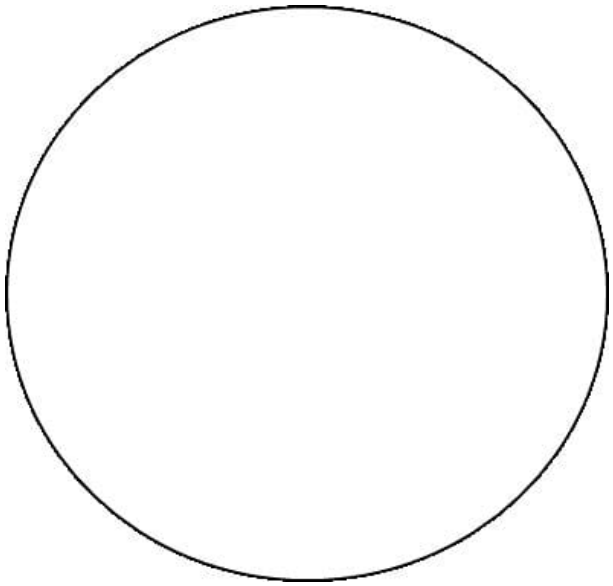
NOTE the circle must be placed at the bottom of the slide to prevent the marker from being mixed with the bacterial sample.

3. Obtain 1-2 loopfuls of bacteria from the appropriate broth culture and introduce the inoculum to the microscope slide, spreading it into a thin film
4. Allow the slide to air dry completely
5. Fix the smear passing the air-dried sample 2-3 times through the flame of the Bunsen burner smear side up
6. Cover the smear with crystal violet for 30-60 seconds
7. Rinse the slide with DI water
8. Gently blot the slide to dry it

Smear Visualization:

Obtain your assigned microscope. You will visualize your samples using a Total magnification of 400x. Use colored pencils to depict your observations in the spaces below.





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2.2.4: Exercise #7 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Why is it important to allow a slide to air dry completely before heat fixing a sample?
2. What will happen to your sample if you fail to heat fix it?
3. What is the difference between an acidic (anionic) stain and a basic (cationic) stain?
4. Explain at the chemical level how a basic stain like methylene blue interacts with a cell to stain it. (Include the charge of the cell, the charge of the dye, and the type of bond formed.)
5. Can you observe a nucleus in the bacterial cell? Why or why not?
6. List the names of the bacteria you stained and observed and describe their shapes and arrangements.

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2.3: Exercise #8 - Differential Staining- Gram Staining

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #3 Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Laboratory Exercise Learning Outcomes

1. Define differential staining.
2. Define primary stain, secondary stain (counter-stain), and mordant.
3. List and describe the differential stains that will be performed in the laboratory this semester
4. Describe the purpose of the Gram stain.
5. Describe the protocol for the Gram stain; properly identify all reagents.
6. Perform the Gram stain correctly in the laboratory setting.
7. Differentiate between Gram-positive and Gram-negative reactions.
8. Describe the importance of the Gram stain in disease detection and diagnosis.
9. Describe the Gram stain reaction for species studied in the laboratory.
10. Interpret possible causes for errors in the Gram stain procedure.

Materials Needed:

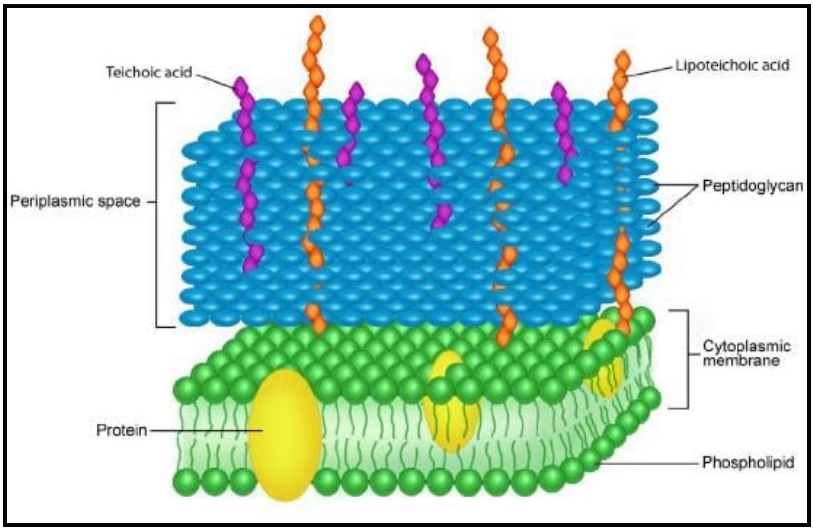
- Bunsen burner
- Microscope slides (one for each bacterial sample)
- Staining tray (Containing stains (crystal violet, Gram's iodine, safranin), decolorizer (acetone, alcohol or acetone/alcohol mixture), stain rack, and DI water)
- China marker
- Lens cleaner
- Lens paper
- Kim wipes
- Broth cultures of bacteria
- Test tube rack
- Inoculating loop
- Microscope

In this laboratory exercise, you will be performing the first of two types of differential stains. Differential staining procedures are staining procedures that allow an observer to differentiate or separate cells based on staining differences. These staining techniques allow the observer to examine cell shape, cell size, and cell arrangement like simple stains; but unlike simple stains allow the observer to obtain additional information about the sample. Differential stains allow for the detection of structural variations between two types of cells or the identification of parts of the same organism. Differential staining techniques also differ from simple stain, in that they make use of two stains rather than just one stain.

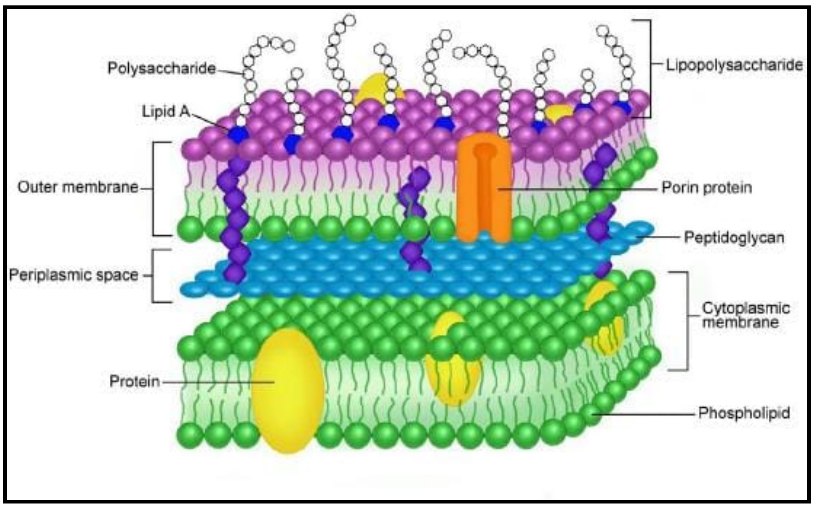
This page titled 2.3: Exercise #8 - Differential Staining- Gram Staining is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Sherry Smith.

2.3.1: Gram Stain

The ***Gram stain*** is one of the most widely employed staining techniques in microbiology. The Gram stain was developed by Han's Christian Gram in 1884. The technique separates bacterial cells into two types of cells based on structural differences in their cell walls. Those cells that are referred to as ***gram positive*** cells have thick peptidoglycan cell walls, while those that are known as ***gram negative*** cells have thin peptidoglycan cell walls with outer membranes. These structural differences in the cell walls of these two types of cells results in their having different susceptibilities to various antimicrobial drugs. Thus, knowing the Gram stain result aids in the selection appropriate treatments for bacterial infections.



A. Gram-positive cell wall structure



B. Gram-negative cell wall structure

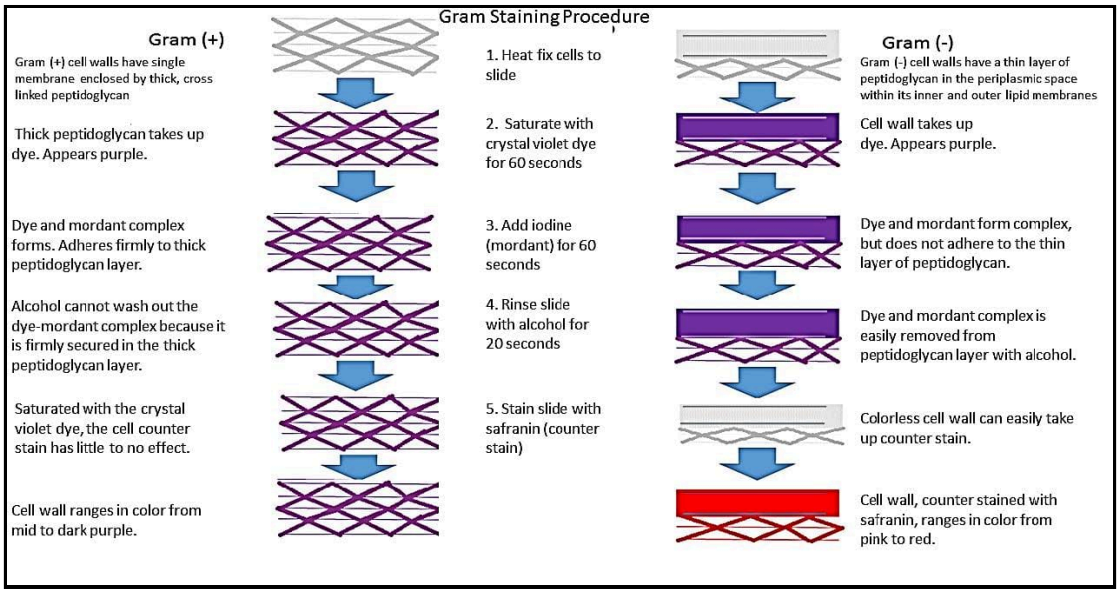
Bacterial Cell Walls - A. Gram-positive cell wall structure. B. Gram-negative cell wall structure

Credit: Susan Decker

The Gram stain is a differential stain and thus will employ more than one stain. The first stain (***primary stain***) employed in the

Gram stain procedure is crystal violet, a basic stain, that will stain both Gram-positive and Gram-negative cells. After the primary stain is rinsed from the smear the mordant, Gram's iodine, is added to the smear. ***Mordants*** help to fix the dyes into the samples. Thus, Gram's iodine binds to crystal violet forming an insoluble complex that is trapped within the cell. The next reagent used in the procedure is Gram's decolorizer. Decolorizers act as the differential step of a differential staining process. ***Decolorizers*** work based on differences in the cell to remove the primary stain from one cell type/structure and allowing it to remain inside another cell type/structure. The decolorizer used in the Gram stain procedure, Gram's decolorizer, may be alcohol and/or acetone. The decolorizer used in this lab is a 50/50 mixture of ethanol and acetone. This reagent reacts with the two cell walls very differently. In Gram-positive cell walls the peptidoglycan molecules become more tightly connected making it more difficult for the crystal-violet iodine complexes to leave the cell during the decolorization step. Gram-negative cells on the other hand have very little peptidoglycan. The decolorizer interacts with the outer membrane and cell membrane to cause them to become more porous because it dissolves some components of these two structures. As a result, the crystal violet iodine complex is washed out of the cell, leaving it colorless. You should be aware that it is important to use the decolorizer cautiously, as it is possible to remove the crystal violet from Gram-positive cells if too much decolorizer is utilized, thereby resulting in a false Gram-negative result. At this point in the procedure Gram-positive cells appear violet/purple, while Gram- negative cells are colorless.

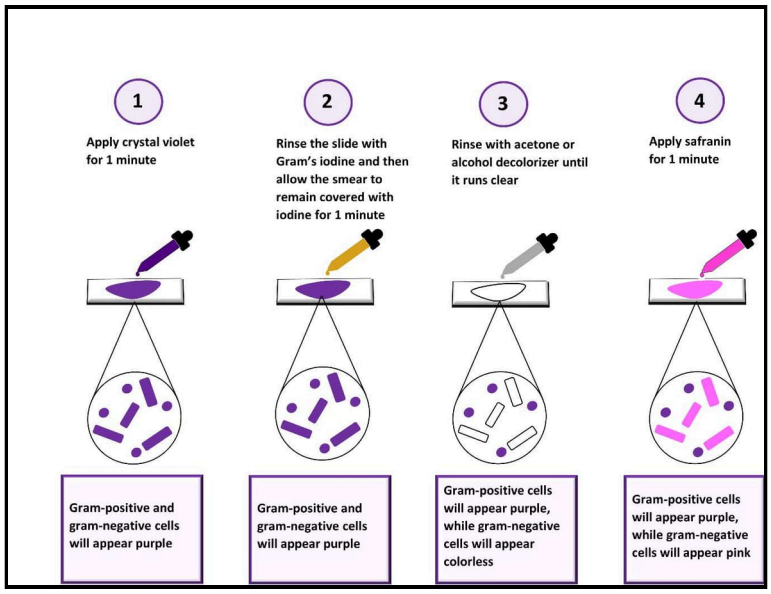
Differential staining procedures require a counter-stain be added after the differential step so that the cells that had the dye stripped from them can be observed. The counter-stain in this procedure is safranin, a red/pink dye. Safranin enters both cells. In Gram-positive cells the safranin is overshadowed by the darker crystal violet that is already present in the cell. Gram-negative cells however do not have any dye to compete with the red/pink safranin Thus at the end of the procedure Gram-positive cells will appear violet/ purple while Gram-negative cells appear light red/pink. The procedure for the Gram stain is provided in this exercise. It is vital that the stains/reagents be used in the correct order to obtain accurate results.



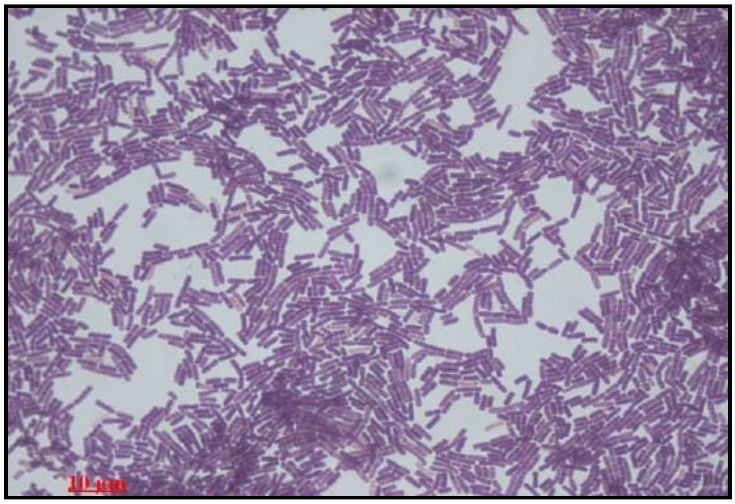
"The gram staining procedure and its effects on gram (-) and gram (+) cell wall types, respectively." Source: Paityn Donaldson, Wikimedia Commons, Creative Commons Attribution 4.0

There are several factors that you need to be aware of before beginning the procedure, that may affect the outcome of your Gram stain. The first of these is the age of the sample. As bacterial cells age their cell walls become weak. This is problematic more so with Gram-positive cells because as they age, they would fail to be capable of retaining the crystal violet-iodine complex and therefore may falsely appear Gram- negative. In order to avoid this problem, it is important to use cultures that are less than 24 hours old, preferably cultures that are 16-18 hours old.

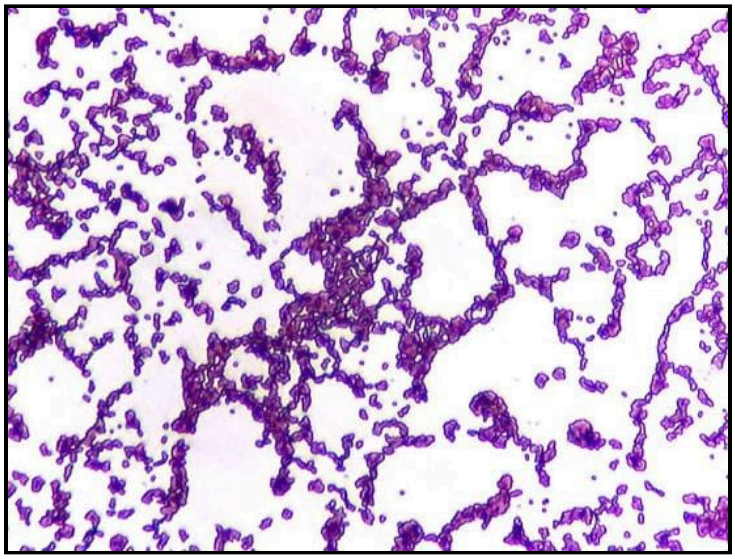
Another factor that may affect the results of the Gram stain is the thickness of the smear. If the smear is too thick the crystal violet may become trapped in the smear and not wash out during the decolorizing step. This would cause Gram-negative cells to falsely appear Gram-positive. Therefore, it is important that the smears to be stained are prepared correctly.



Gram stain procedure. Credit: Sherry Smith



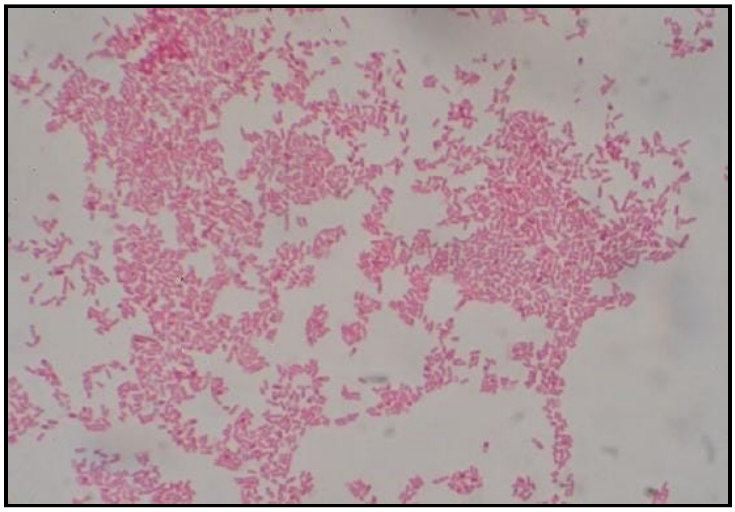
Bacillus subtilis
Credit: Dr. Sahay, Wikimedia Commons, CC BY-SA 3.0



Staphylococcus aureus
Credit: Sherry Smith



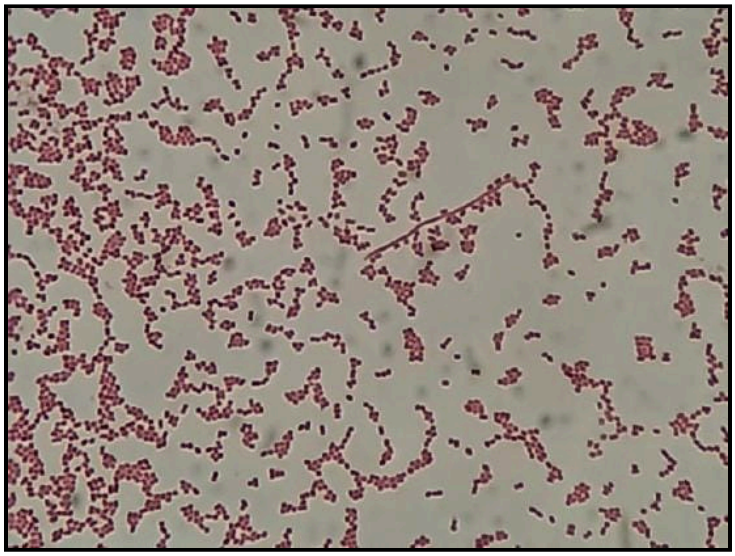
Klebsiella pneumoniae
Credit: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0



Escherichia coli
Credit: Dr Graham Beards, Wikimedia Commons, CC BY 4.0



Klebsiella pneumoniae
Credit: Y tambe, Wikimedia Commons, CC BY 4.0



Acinetobacter baumannii
Credit: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0

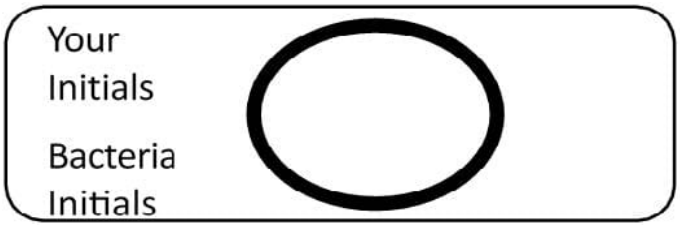
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2.3.2: Gram Stain Activity

In this activity you will prepare and stain bacterial samples using Grams stain procedure.

1. Obtain five microscope slides, clean and dry the microscope slides using lens cleaner and Kim wipes
2. Label the slides using a China marker as seen below

   NOTE the circle must be placed on the bottom of the slide to prevent the marker from being mixed with the bacterial sample



Credit: Sherry Smith

3. Obtain 1-2 loopfuls of bacteria from the appropriate broth culture and introduce the inoculum to the appropriate microscope slide, spreading it into a thin film
4. Allow the slides to air dry completely
5. Fix the smears by passing the air-dried samples 2-3 times through the flame of the Bunsen burner smear side up
6. Cover the smears with crystal violet for 1 minute
7. Rinse the slides slowly with DI water for 5 seconds or until the water runs clear
8. Rinse the smears with Gram's Iodine and then cover the smear with iodine and allow the slides to set for 1 minute
9. Rinse the slides slowly with DI water for 5 seconds or until the water runs clear
10. Apply Gram decolorizer slowly. Continue to add the Gram decolorizer until the dyes does not run off from the smears

    NOTE Gram decolorizer is a mixture of ethanol and acetone, which is ***highly flammable!!!***

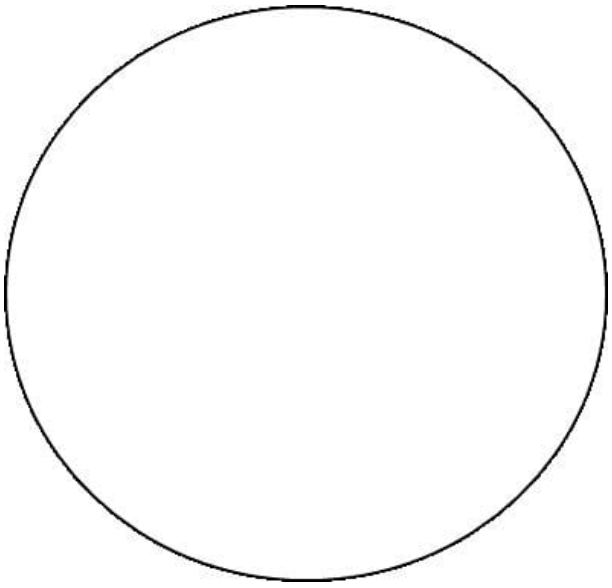
11. Rinse the slides slowly with DI water for 5 seconds or until the water runs clear
12. Cover the smear with safranin for 30 seconds
13. Rinse the slides slowly with DI water for 5 seconds or until the water runs clear
14. Use Kim wipes to blot the slides, smear side down. ***Blot, do not rub!!!***
15. Use your compound microscope to visualize your stained slides.

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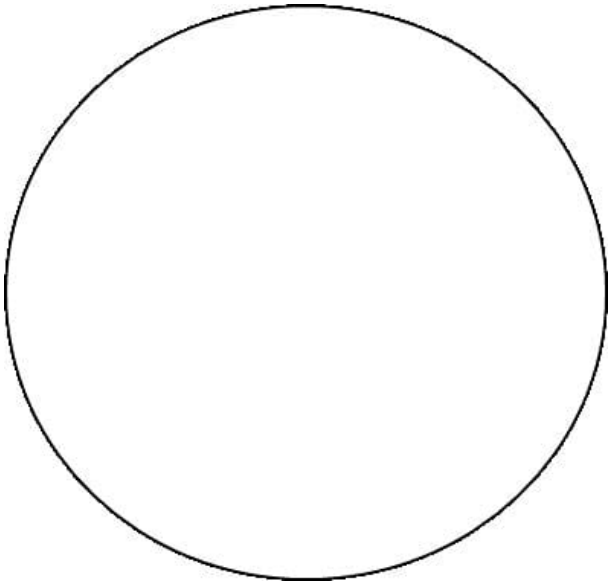
2.3.3: Smear Visualization

Obtain your assigned microscope. You will visualize your samples using a total magnification of 400x. Use colored pencils to depict your observations in the spaces below. Make note of shapes, sizes, arrangements, and stain results for each of your samples.

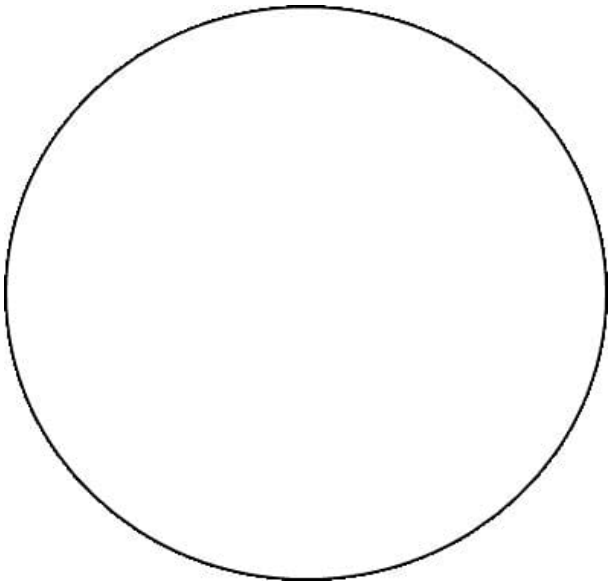
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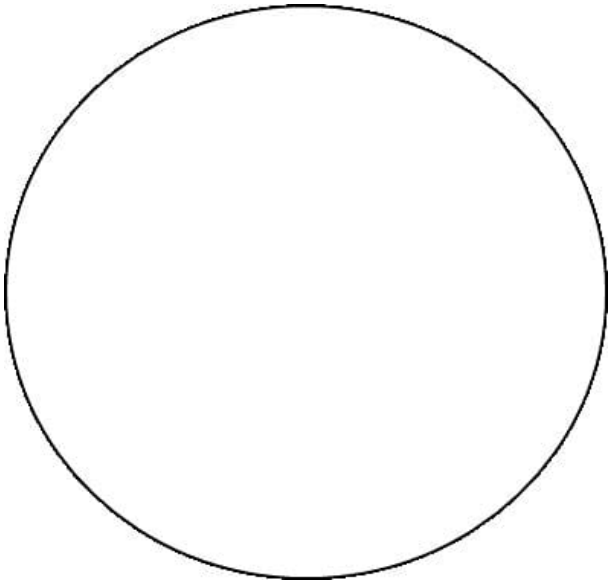
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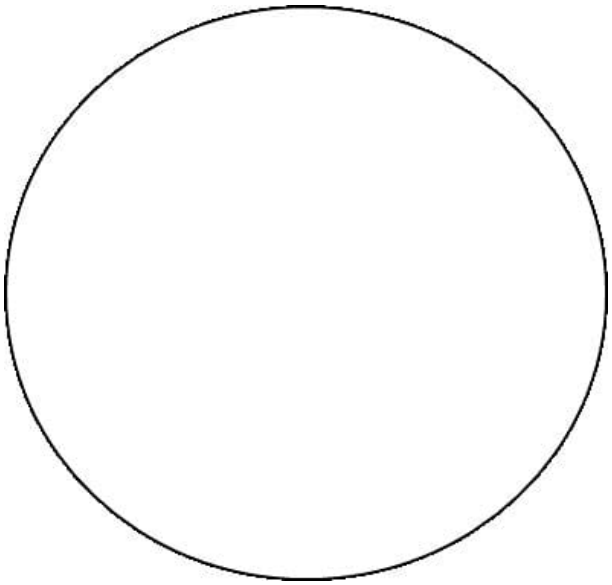
Specimen:



Specimen:



Specimen:



Complete the following chart for the results of each of the bacteria you stain using the Gram stain.

Gram stain observation results

Specimen (Species Name)	Shape	Color Following Gram Stain	Is the specimen Gram-positive (G+) or Gram-negative?

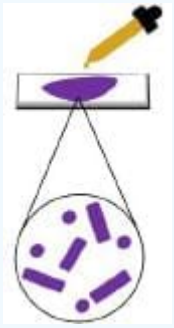
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2.3.4: Exercise #8 Lab Wrap-Up

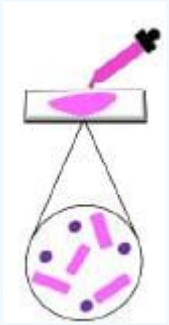
Lab Wrap-Up Questions

Answer the following questions:

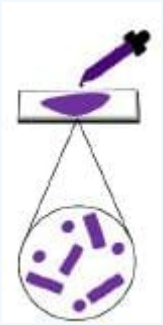
1. Why is the Gram stain considered a differential stain?
2. How does the age of a culture affect the Gram stain reaction? What is the optimum age for a valid Gram reaction?
3. Which step in the Gram stain procedure is most prone to error? If done incorrectly, how might that step affect the end result?
4. A wound culture is taken from a post-surgical patient showing signs of infection. The Gram stain shows purple clusters of round cells.
   a. what is the likely Gram reaction and morphology of this organism?
   b. How might this result guide the medical team's initial treatment decisions while waiting for full identification and sensitivity results?
5. Place steps of the Gram stain in the proper order by placing the appropriate number 1-4 under the images.



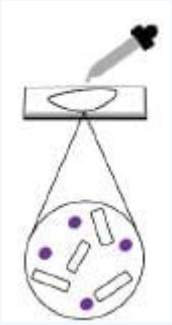
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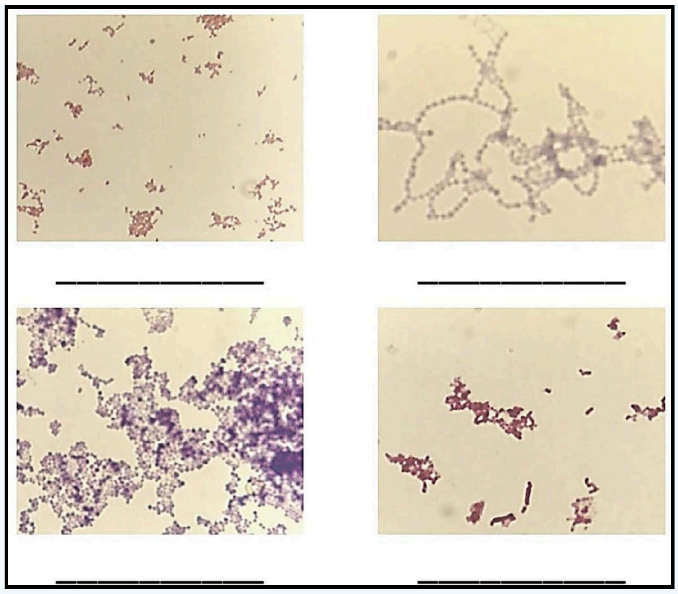
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_____

Credit: Sherry Smith

6. For each image indicate whether the result is gram-positive or gram-negative?



Credit: Sherry Smith

7. Describe how each type of cell will appear in the following scenarios in which the Gram stain was incorrectly performed.
    a. The decolorizer step was omitted.
        i. Gram positive
        ii. Gram negative
    b. The mordant was eliminated.
        i. Gram positive
        ii. Gram negative
    c. The counter-stain step was forgotten.
        i. Gram positive
        ii. Gram negative

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2.4: Exercise #9 - Differential Staining- Acid-Fast Stain

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #3 Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the acid-fast stain and what information can be obtained.
2. Explain what mycolic acids are, their relation to the acid-fast stain, and their role regarding bacterial structure and function.
3. Explain when and why the acid-fast stain is performed.
4. Describe the importance of the acid-fast stain procedure in disease detection and diagnosis.
5. List at least two bacterial species known to be acid-fast.
6. Describe the steps performed during an acid-fast stain.
7. Distinguish between acid-fast and non-acid-fast cells.
8. Perform the acid-fast stain and interpret the reactions for bacteria in the lab.

Materials Needed:

- Bunsen burner
- Microscope slides (one for each bacterial sample)
- Staining tray (Containing stains (carbolfuchsin, methylene blue), decolorizer (acid-alcohol), stain rack, and DI water)
- China marker
- Lens cleaner
- Lens paper
- Kim wipes
- Broth cultures of bacteria
- Test tube rack
- Inoculating loop
- Microscope

In this laboratory exercise, you will be performing the second of two types of differential stains, the acid-fast stain.

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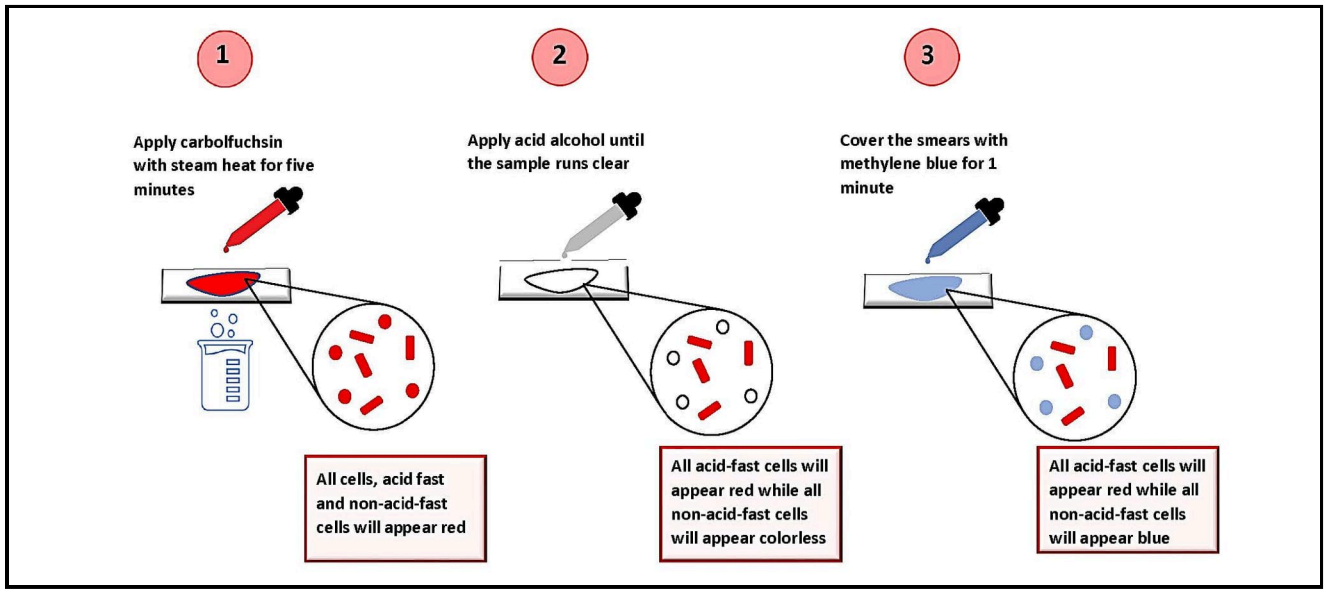
2.4.1: Acid-Fast Stain

The ***acid-fast stain***, while not as commonly used as the Gram stain, is an important diagnostic tool. The acid-fast stain is used to detect the presence of bacteria that contain waxy lipids known as mycolic acids. ***Mycolic acids*** are waxes found embedded in the thick cell walls of bacteria in the genera *Mycobacteria* and *Nocardia*. The waxes prevent the cells from desiccating and protect them from many chemical cleaners.

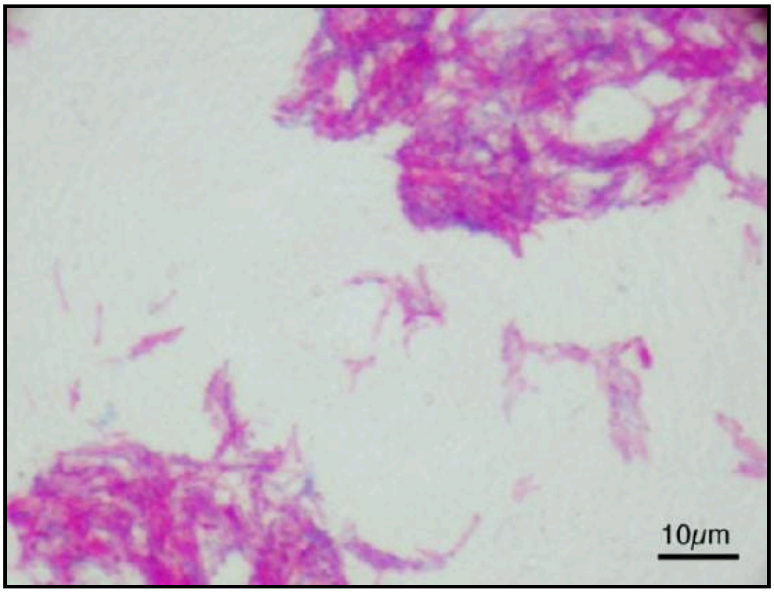
Some clinically noteworthy examples of species that contain mycolic acids are *Mycobacterium tuberculosis* and *Mycobacterium leprae,* which are known to cause tuberculosis and leprosy respectively.

The mycolic acids not only protect the bacteria from harsh environments but also make it more challenging to stain cells within these two genera. Many of the stains employed in microbiology are aqueous, meaning that they are mixed with water, thus causing the solution to be a polar solution. These do not interact well with the non-polar nature of the mycolic acids, and thus are not easily taken into the cells. The staining procedure you will employ to stain acid-fast cells is known as the ***Ziehl-Neelsen protocol***. The acid-fast staining procedure uses a primary stain known as carbolfuchsin. ***Carbolfuchsin*** is a dark red stain that is lipid soluble, thus allowing it to penetrate the waxy mycolic acid found in the cell walls. In addition to using a lipid soluble stain, heat is applied at the same time the primary dye is added to the smear. This is done by placing the slide over a steam bath and then applying the stain. The heat melts the waxy lipid, thereby facilitating the entry of the stain into the cell. The slide is then removed from the heat and allowed to cool, and the wax then solidifies trapping the primary stain in cells containing mycolic acid.

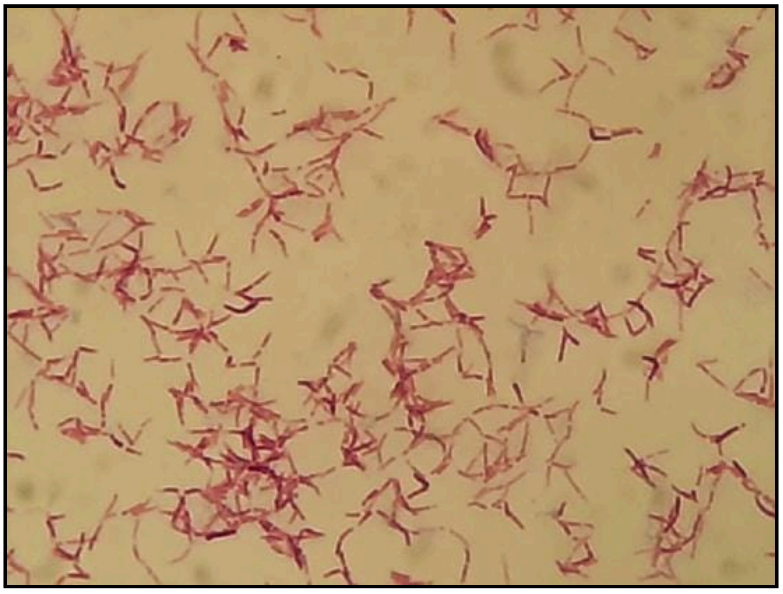
After rinsing with water, a decolorizer is applied to the smear. The decolorizer used in this protocol is acid-alcohol, a combination of ethyl alcohol and hydrochloric acid. The decolorizer is repelled by cells containing mycolic acid, because of its polar nature, thereby allowing the primary stain to remain in the cells. These cells are referred to as ***acid-fast cells*** because they have held fast or tightly to the primary stain. Cells that do not have mycolic acid are left colorless after the application of the decolorizer because the stain is removed from the cell, and thus are referred to as ***non-acid fast***. The counter-stain methylene blue is then applied. Methylene blue cannot enter the cells of acid-fast bacteria because it is repelled by the mycolic acid. Non-acid-fast cells, lacking mycolic acid, do not repel the stain, and thus are readily stained blue. Upon completion of the staining procedure, acid-fast bacteria will appear red, while non-acid-fast bacteria appear blue.



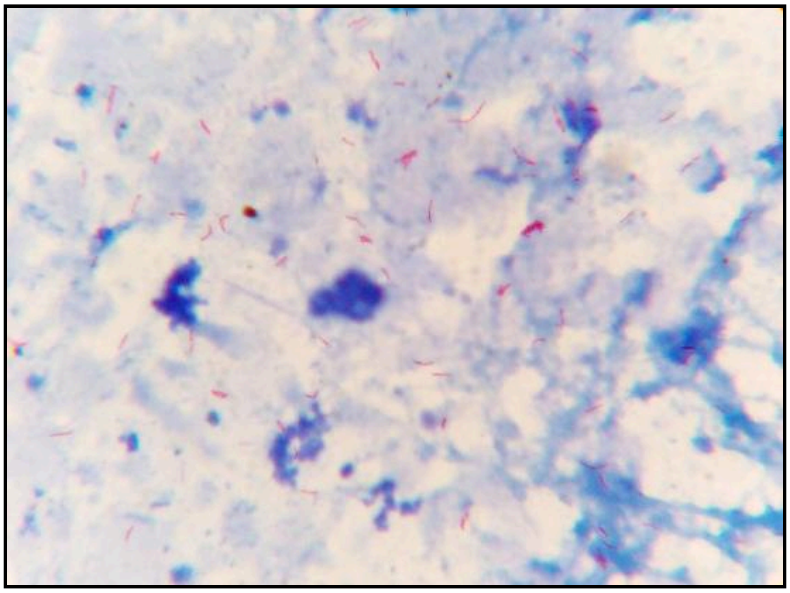
Acid-fast stain procedure. Credit: Sherry Smith



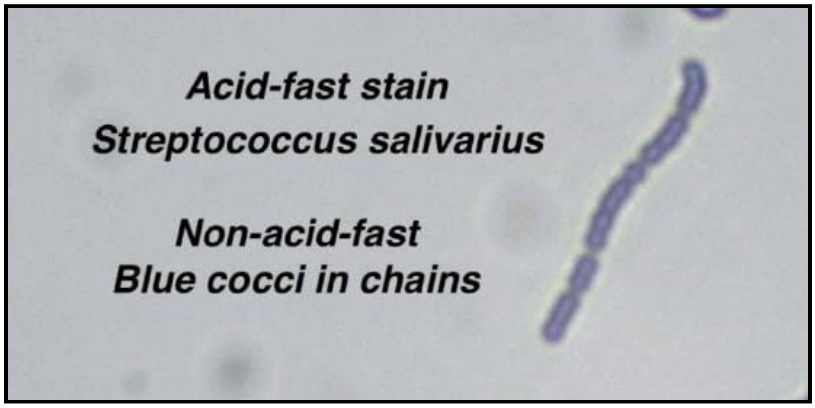
Ziehl-Neelsen staining. Magnification: 1,000.
Source: Y tambe, Wikimedia Commons, CC BY 3.0.



Modified acid-fast staining of Nocardia - typically weakly acid-fast.
Source: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0.



Sputum smear showing Acid Fast Bacilli (Mycobacterium tuberculosis)
Source: Microrao, Wikimedia Commons, CC BY 4.0.



Streptococcus salivarius acid-fast stain.
Credit: Sherry Smith

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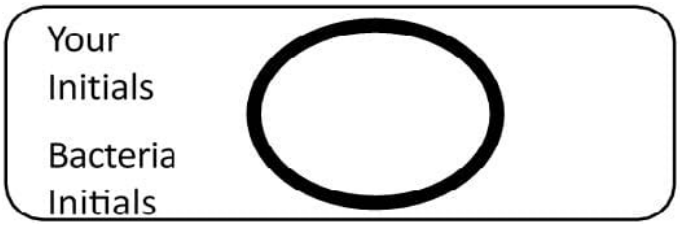
2.4.2: Acid-Fast Stain Activity

In this activity you will prepare five bacterial samples using the Acid-fast stain procedure. You will be using *Bacillus subtilis* (Bs), *Mycobacterium smegmatis* (Ms), *Escherichia coli* (Ec), *Streptococcus salivarius* (Ss) and *Staphylococcus aureus* (Sa) as your samples. Please utilize the proper precautions as you work with these samples.

*****NOTE***** *Staphylococcus aureus* and *Mycobacterium smegmatis* are BLS-2 organisms

1. Obtain five microscope slides, clean and dry the microscope slides using lens cleaner and Kim wipes
2. Label the slides using a China marker as seen below

   *****NOTE***** the circle must be placed on the bottom of the slide to prevent the marker from being mixed with the bacterial sample



Credit: Sherry Smith

3. Obtain 1-2 loopfuls of bacteria from the appropriate broth culture and introduce the inoculum to the appropriate microscope slide, spreading it into a thin film
4. Allow the slides to air dry completely
5. Fix the smears by passing the air-dried samples 2-3 times through the flame of the Bunsen burner smear side up
6. Use a clothes pin to place the slides smear side up on the rack above the steam pot
7. Place a steaming towel (small piece of paper towel) over the smear and cover with carbolfuchsin. Allow the slides to remain over the steam for 5 minutes. Add more dye as needed to prevent the steaming towel from drying.
8. Remove the steaming towel using the provided forceps and place it in the container provided for disposal. Remove the slides from the steaming rack and place them on your staining rack. Allow the slides to cool completely.
9. Rinse the slides slowly with DI water, aiming the water above the smears.
10. Decolorize the smears with acid-alcohol, applying slowly until dye does not run off the slides.

    *****NOTE***** Acid-alcohol is a mixture of ethyl alcohol and hydrochloric acid, which is ***highly flammable!!!***

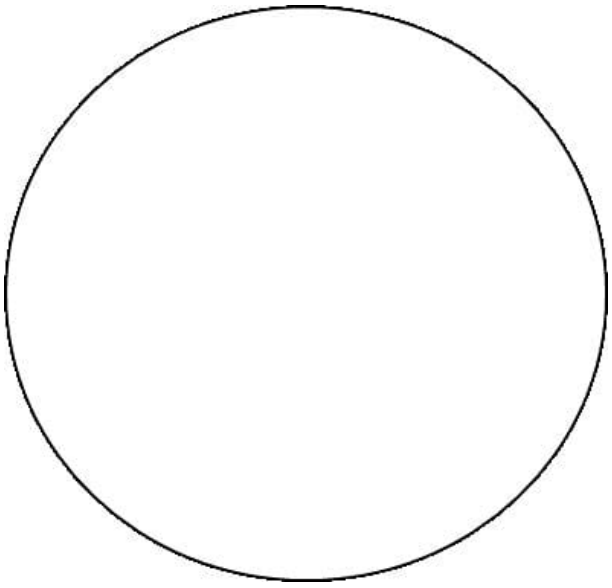
11. Rinse the slides slowly with DI water to remove the decolorizing agent.
12. Cover the smears with methylene blue for 1 minute
13. Rinse the slides slowly with DI water until the water runs clear
14. Use Kim wipes to blot the slides, smear side down. ***Blot, do not rub!!!***
15. Use your compound microscope to visualize your stained slides.

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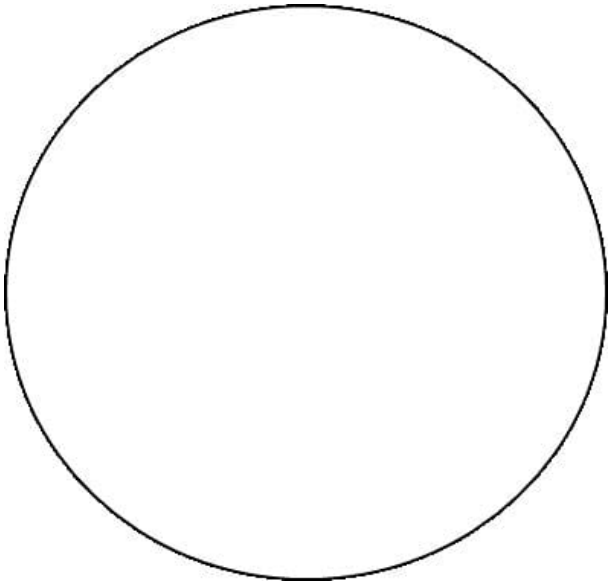
2.4.3: Smear Visualization

Obtain your assigned microscope. You will visualize your samples using a total magnification of 400x. Use colored pencils to depict your observations in the spaces below. Make note of shapes, sizes, arrangements, and stain results for each of your samples.

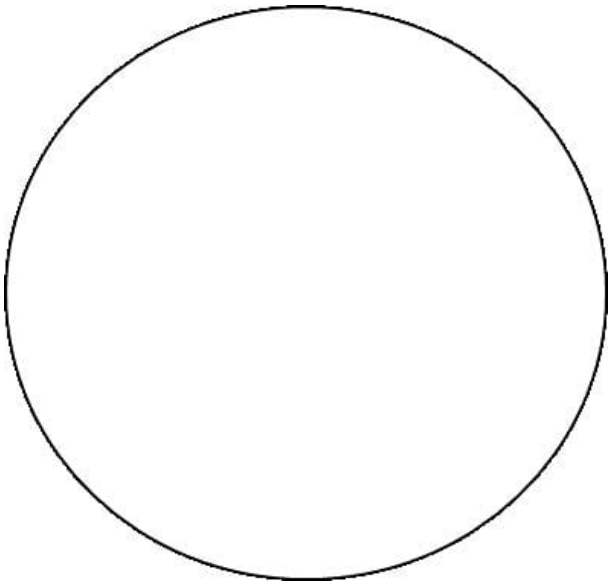
Specimen:



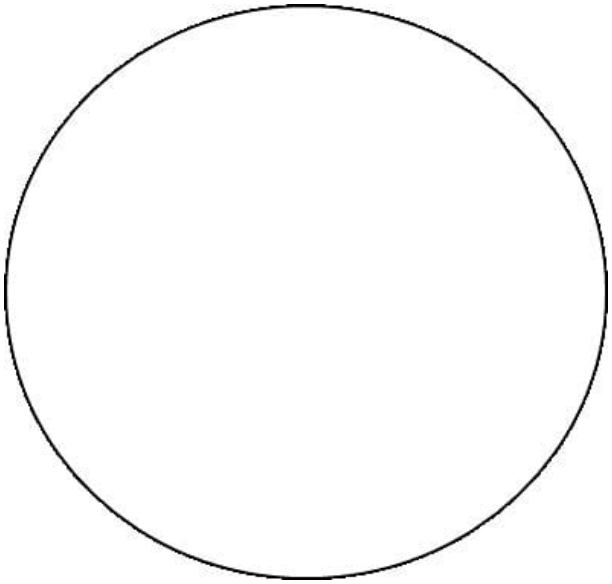
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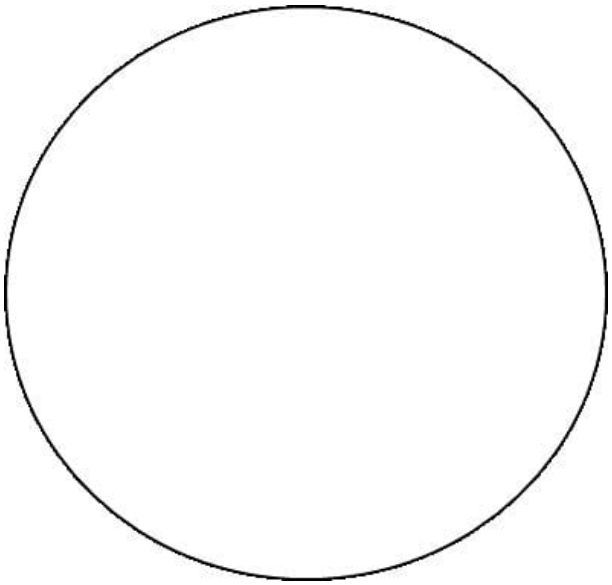
Specimen:



Specimen:



Specimen:



Complete the following chart for the results of each of the bacteria you stain using the acid-fast stain.

Acid-fast stain observation results

Specimen (Species Name)	Shape	Color Following acid-fast stain	Is the specimen acid-fast or non-acid-fast?

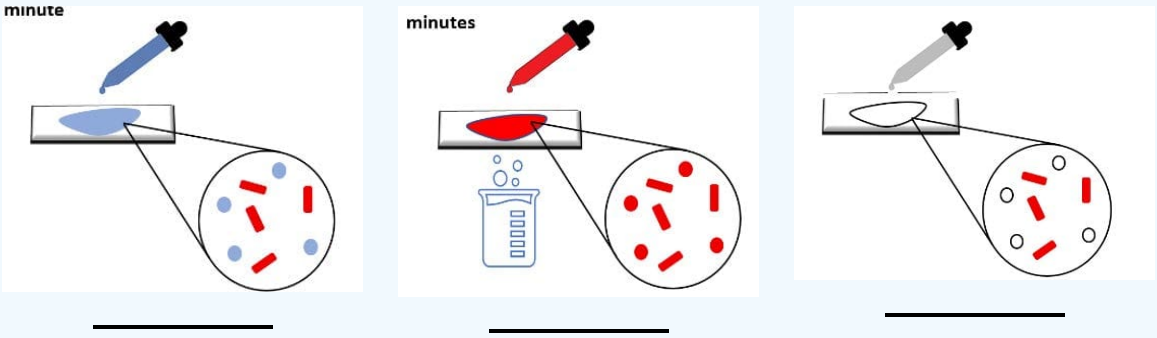
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2.4.4: Exercise #9 Lab Wrap-Up

Lab Wrap-Up Questions

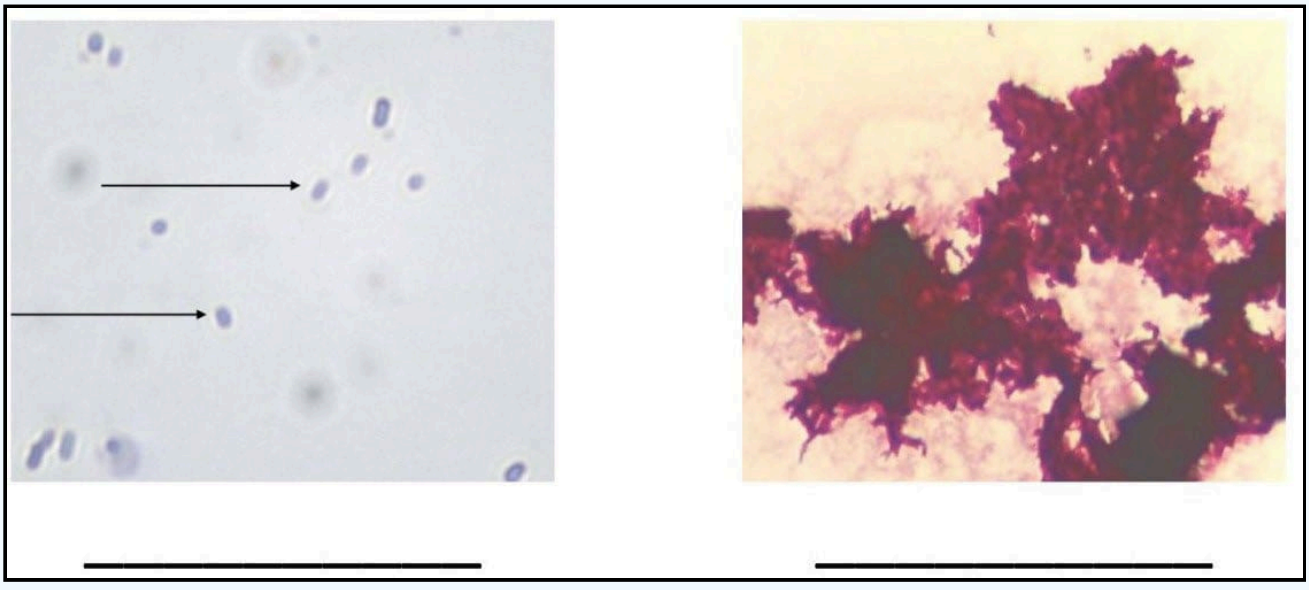
Answer the following questions:

1. Why is the acid-fast stain considered a differential stain?
2. What makes bacteria in the *Mycobacterium* genera resistant to staining?
3. If you conducted an acid-fast stain on a sputum sample from a person with tuberculosis, what would you expect to see on the slide? (Consider color, shape, etc.)
4. Why is it essential for healthcare workers to correctly identify acid-fast organisms like Mycobacterium tuberculosis in patient samples before initiating treatment? Consider the risks of misdiagnosis or delayed treatment.
5. Place the steps of the acid-fast stain in the proper order, by placing the appropriate number 1-4 under the images.



Credit: Sherry Smith

6. For each image indicate whether the result is acid-fast or non-acid fast.



Credit: Sherry Smith

7. Describe how each type of cell will appear in the following scenarios in which the Gram stain was incorrectly performed.
   a. The decolorizer step was omitted.
      i. Acid-fast cells
      ii. Non-acid-fast cells
   b. The counter-stain step was forgotten.

i. Acid-fast cells
ii. Non-acid-fast cells

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2.5: Exercise #10 - Structural Stain- Endospore Stain

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #3 Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Laboratory Exercise Learning Outcomes

1. Explain the difference between a vegetative cell and an endospore.
2. Describe the function of an endospore.
3. Name some endospore forming bacteria that are human pathogens and know the diseases they cause.
4. Explain the steps of the endospore stain.
5. Perform an endospore stain.
6. Detect the presence of bacterial endospores.
7. Distinguish between endospores and vegetative cells in a stained sample.
8. Explain what information can be gained by performing an endospore stain.
9. Explain the variations in size, shape, and location of endospores within the vegetative cell.

Materials Needed:

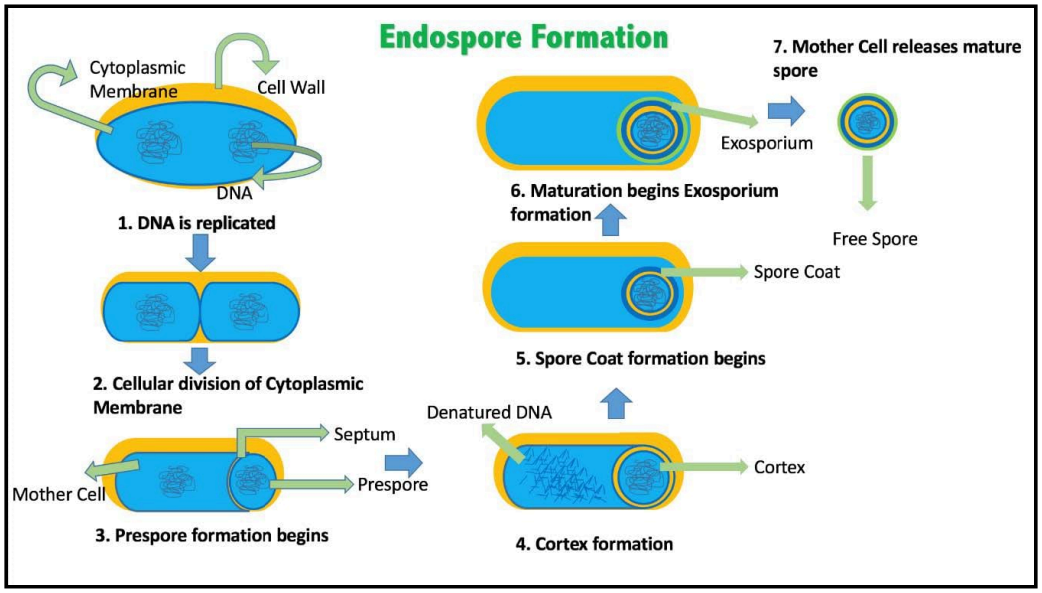
- Bunsen burner
- Microscope slide
- Staining tray (Containing stains (malachite green, safranin), stain rack, and DI water)
- China marker
- Lens cleaner
- Lens paper
- Kim wipes
- Broth culture of bacteria (*Bacillus subtilis* >72 hours old)
- Test tube rack
- Inoculating loop
- Microscope

In this laboratory exercise, we will explore the first of two staining techniques that are used to visualize cellular structures, the endospore stain.

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2.5.1: Endospore Stain

Endospores are metabolically inactive structures produced by members of the genera *Bacillus* and *Clostridium*. These structures serve to protect a copy of the genetic material in harsh environments and are resistant to extremes in temperature, radiation, and chemicals. Endospores are produced via a process called ***sporogenesis*** (sporulation), in which a ***vegetative cell*** (sporangium), a metabolic active cell, works to construct several protective layers around a copy of the cell's genetic material, producing what is known as the endospore.



Formation of an endospore through the process of sporulation. Source: Farah, Sophia, Alex, Wikimedia Commons, CC BY 4.0

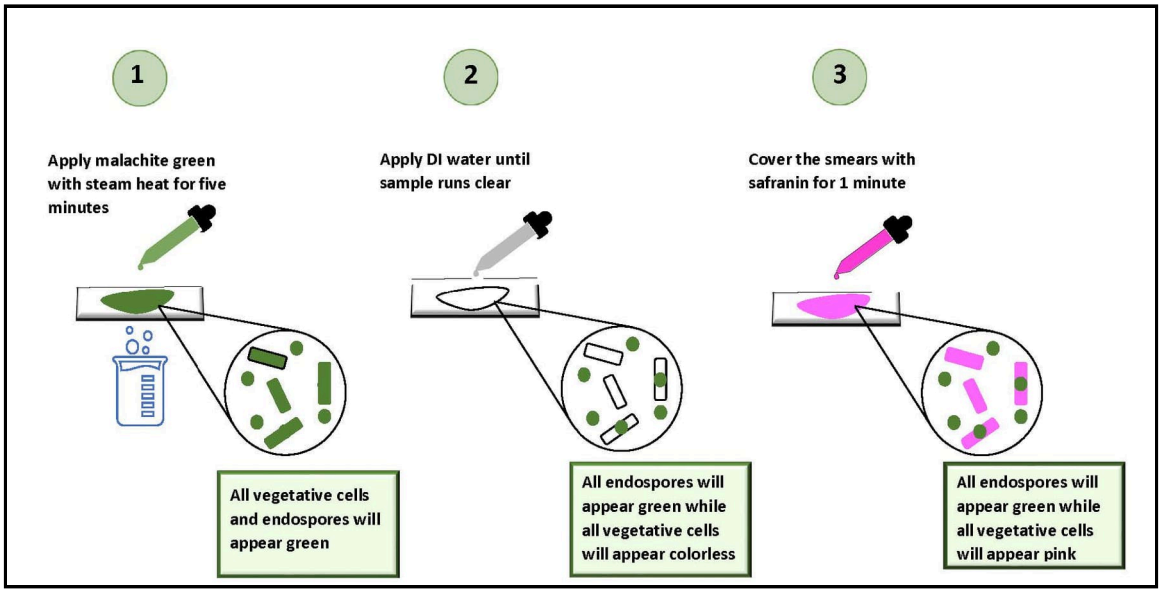
Endospores are clinically important because several disease-causing microbes can produce these protective structures. Some examples of endospore-forming pathogens include *Bacillus anthracis* (anthrax), *Clostridium tetani* (tetanus), *Clostridium botulinum* (botulism), *Clostridium perfringens* (gas gangrene), and *Clostridium difficile* (pseudomembranous colitis).

Endospores are resistant to staining and therefore if present appear as clear structures inside a cell after the Gram stain. In order to visualize endospores, we must use the endospore stain. The ***Shaeffer-Fulton*** method will be utilized in this exercise to stain and visualize endospores. This method begins with the addition of malachite green stain applied to the sample in the presence of steam heat. The heat serves to drive the stain through the thick protective layers of the endospore. The slide is removed from the heat and allowed to cool, thereby trapping the stain in the endospore. Malachite green does not have a great affinity for cellular structures, and therefore does not bind readily to the vegetative cell (sporangium) and can easily be washed out of the cell.

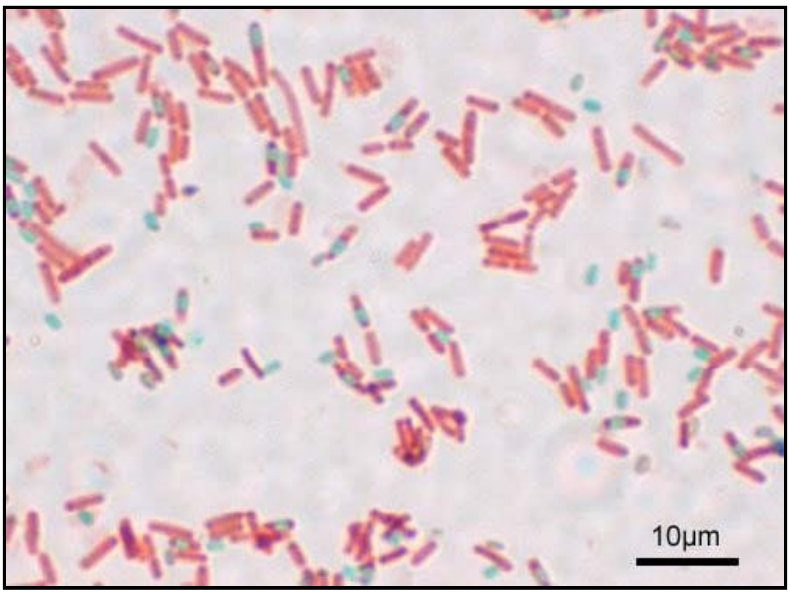
This decolorization is accomplished using water. The water is unable to penetrate the protective coat of the endospore, thus the endospore remains green, while the vegetative cell becomes colorless as the water washes the stain out. The counter-stain safranin is then applied to the sample. Safranin easily penetrates the cell wall of the vegetative cell staining it pink, while it is unable to penetrate the endospore coat.

Therefore, at the end of the staining procedure, vegetative cells will appear pink, while endospores appear green.

Endospores will have a spherical or elliptical appearance when observed. They may also be located at different areas of the vegetative cell depending on the species. There are three classifications based on the location of the endospore within the vegetative cell; central, terminal, and subterminal. Central endospores are located at the middle of the bacillus shaped cell, while terminal are located at the very end of the bacillus, and subterminal endospores is an intermediate to the central and terminal endospores. While still in the vegetative cell the endospore may or may not result in a bulging of the vegetative cell.



Endospore stain procedure. Credit: Sherry Smith



Bacillus subtilis stained by malachite green and safranin. Endospores in green and the bacteria in red. (x1000)
Source: Y tambe, Wikimedia Commons, CC BY 3.0

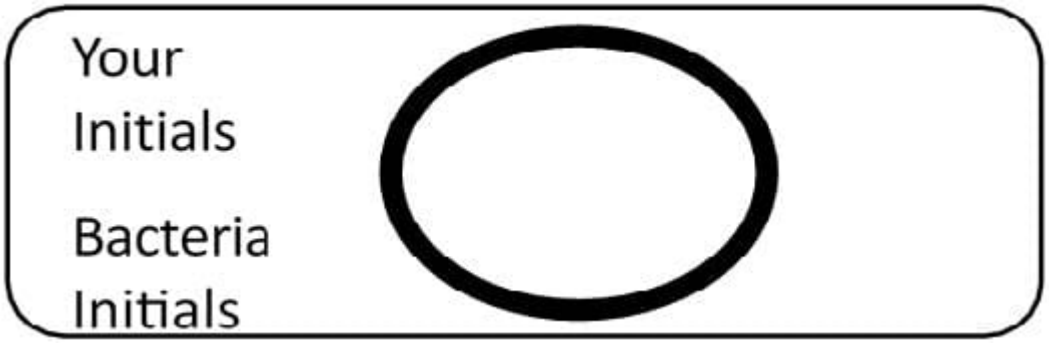
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2.5.2: Endospore Stain Activity

In this activity you will prepare one bacterial sample using the Schaeffer-Fulton endospore stain procedure. You will be using a culture of *Bacillus subtilis* (Bs) that is at least 72 hours old to ensure endospores have had an opportunity to form.

1. Obtain one microscope slide, clean and dry the microscope slide using lens cleaner and Kim wipes.
2. Label the slide using a China marker as seen below.

   NOTE The circle must be placed on the bottom of the slide to prevent the marker from being mixed with the bacterial sample.



Credit: Sherry Smith

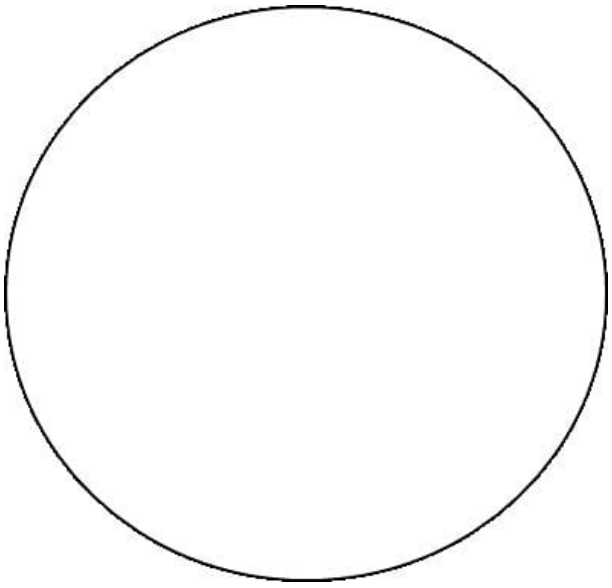
3. Obtain 1-2 loopfuls of bacteria from the appropriate broth culture and introduce the inoculum to the appropriate microscope slide, spreading it into a thin film.
4. Allow the slide to air dry completely.
5. Fix the smear by passing the air-dried sample 2-3 times through the flame of the Bunsen burner smear side up.
6. Use a clothes pin to place the slide smear side up on the rack above the steam pot.
7. Place a steaming towel (small piece of paper towel) over the smear and cover with malachite green. Allow the slide to remain over the steam for 5 minutes. Add more dye as needed to prevent the steaming towel from drying.
8. Remove the steaming towel using the provided forceps and place it in the container provided for disposal. Remove the slide from the steaming rack and place it on your staining rack. Allow the slide to cool completely.
9. Rinse the slide slowly with DI water, aiming the water above the smear, until the water runs clear.
10. Cover the smear with safranin for 1 minute.
11. Rinse the slide slowly with DI water until the water runs clear.
12. Use Kim wipes to blot the slide, smear side down. ***Blot, do not rub!!!***
13. Use your compound microscope to visualize your stained slide.

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2.5.3: Smear Visualization

Obtain your assigned microscope. You will visualize your samples using a total magnification of 400x. Use colored pencils to depict your observations in the spaces below. Label an endospore and a vegetative cell.

Specimen:



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2.5.4: Exercise #10 Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Explain the importance of applying steam heat while applying the primary stain in the Schaeffer-Fulton endospore staining technique.
2. How would endospores appear following a staining procedure such as a simple stain, or Gram stain procedure, which does not utilize steam heat during the staining process? Explain your answer.
3. Why is it important to use an older (at least 72-hour) culture of *Bacillus subtilis* when performing the endospore stain?
4. Imagine you performed the endospore stain on a sample known to form endospores and observed only pink vegetative cells and no green structures. List two possible reasons for this result and explain how each might have affected the outcome.
5. You are caring for a patient who has been diagnosed with *Clostridium difficile* colitis. Why might standard disinfectants used in hospitals be ineffective at eliminating this pathogen from surfaces? How does this relate to the endospore's structure?

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2.6: Exercise #11 - Structural Stain- Capsule Stain

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #3 Describe and prepare widely used stains and wet mounts and discuss their significance in identification of microorganisms.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

Laboratory Exercise Learning Outcomes

1. Describe what capsules are.
2. Explain how bacterial cells benefit from capsules.
3. Provide some examples of pathogens that have capsules.
4. Explain why the capsule stain is referred to as a negative staining technique.
5. Describe the protocol followed to stain for capsules.
6. Perform a capsule stain.

Materials Needed:

- Bunsen burner
- Microscope slides (one for each bacterial sample)
- Staining tray (Containing stains (congo red, Maneval's stain), stain rack, and DI water)
- China marker
- Lens cleaner
- Lens paper
- Kim wipes
- Broth culture of bacteria
- Test tube rack
- Inoculating loop
- Microscope

In this laboratory exercise, we will explore the second of two staining techniques that are used to visualize cellular structures, the capsule stain.

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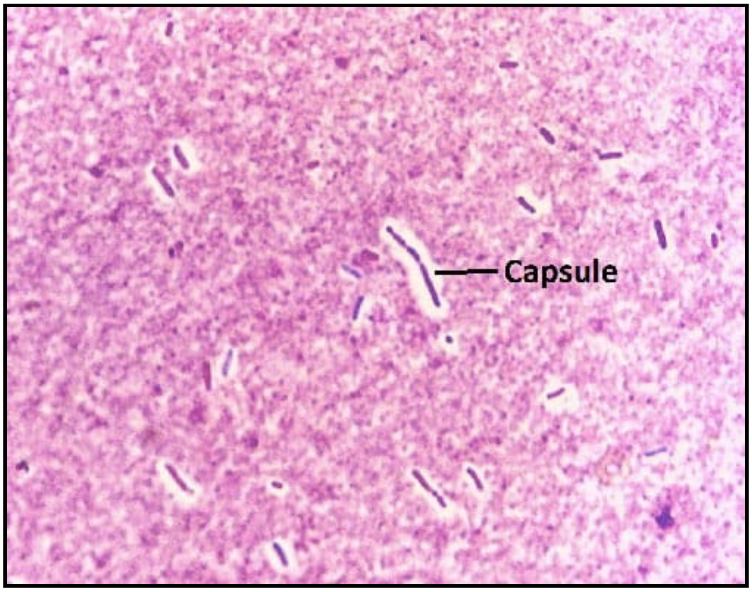
2.6.1: Capsule Stain

Capsules are one type of bacterial glycocalyx. Capsules are structures made of a combination of polysaccharides and proteins, arranged to form a protective structure around the cell. This structure serves to prevent the phagocytosis of bacterial cells by host's immune cells. They also help bacteria avoid desiccation and assist in the formation of biofilms by allowing cells to adhere to one another. Since capsules aid some bacteria in avoiding host's defenses, they are clinically important structures. Pathogens known to form capsules include bacteria such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Escherichia coli*.

Capsules repel most stains due to their chemical composition. Capsule staining is accomplished using a negative staining technique. In this staining procedure an acidic (anionic) stain is first applied to the sample. The stain that will be employed in your laboratory exercise is Congo Red. Congo Red acts to stain the background surrounding the cells, because the negatively charged cell repels the negatively charged dye.

The sample is then stained, with a basic (cationic) stain, which has a positive charge and is attracted to the negatively charged bacterial cell. In your laboratory procedure, you will use Maneval's stain. Maneval's stain is a mixture of acetic acid and acid fuchsin. The acidic pH of the Maneval's stain causes the Congo Red to appear gray blue. The acid fuchsin is responsible for causing the bacterial cells to appear pinkish red. The capsule is indirectly detected as a clear halo located between the gray-blue and the pinkish-red cell.

It is important to note that this procedure is carried without heat fixing the sample. Capsules may consist of up to 95% water. Heating the sample can cause the capsules to become dehydrated resulting in shrinkage and distortion of the sample.



Capsule stain - Klebsiella sp. Credit: Sherry Smith

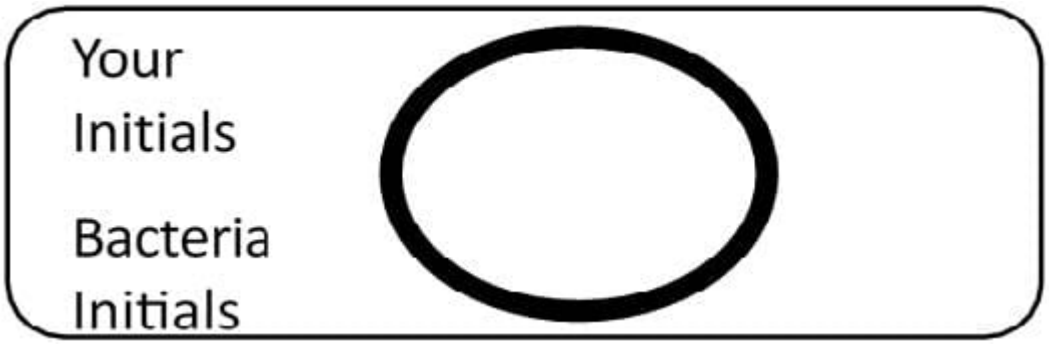
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2.6.2: Capsule Stain Activity

In this activity you will prepare one bacterial sample using the capsule stain procedure.

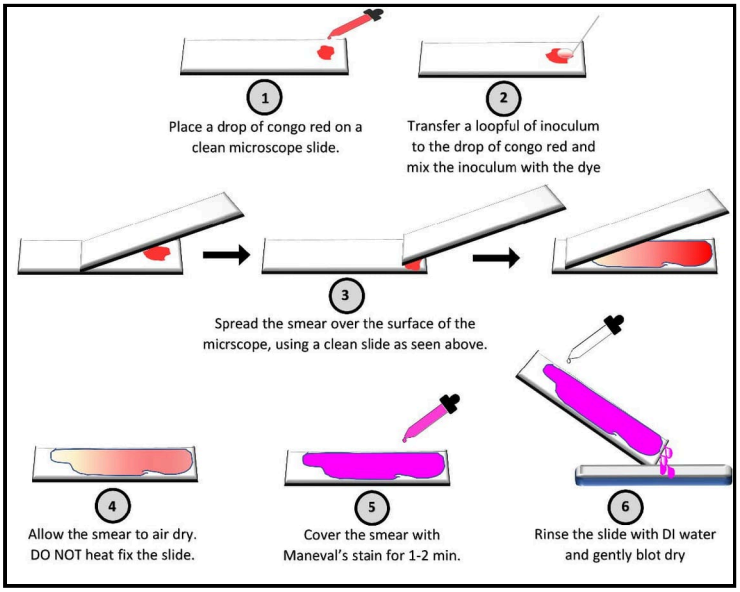
1. Obtain two microscope slides, clean and dry the microscope slide using lens cleaner and Kim wipes
2. Label the slides using a China marker as seen below

   NOTE the circle must be placed on the bottom of the slide to prevent the marker from being mixed with the bacterial sample



Credit: Sherry Smith

3. Place a drop of Congo Red on the slides
4. With an inoculating loop, transfer a loopful of inoculum to the Congo Red on each slide; Mix and spread out the dye-culture mixture into a thin even film using a clean microscope slide. Set the slides aside and let the slides air dry completely. ***Do not heat fix!!!***
5. When the slides are dry, apply Maneval's stain to the dried films on the slides so that they are completely covered and let stand for 1-2 minutes
6. Pour off the excess stain, wash the slides very gently with deionized water
7. Use Kim wipes to blot the slides, smear side down. ***Blot, do not rub!!!!***
8. Use your compound microscope to visualize your stained slides.



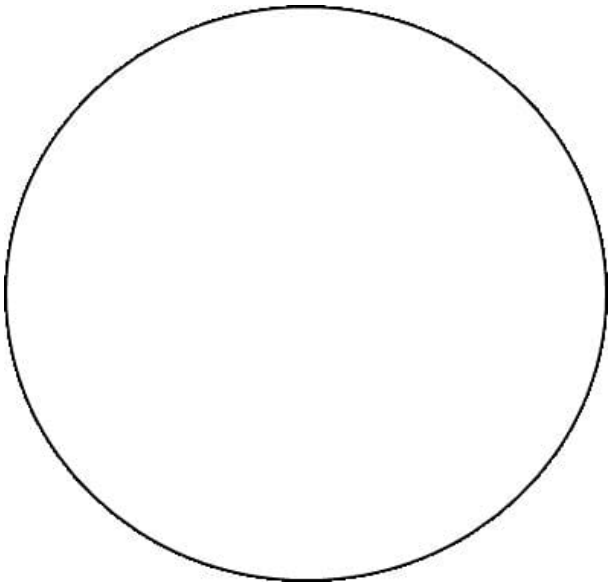
Capsule Stain Procedure. Credit: Sherry Smith

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2.6.3: Smear Visualization

Obtain your assigned microscope. You will visualize your samples using a total magnification of 400x. Use colored pencils to depict your observations in the spaces below. Label a cell and a capsule

Specimen:



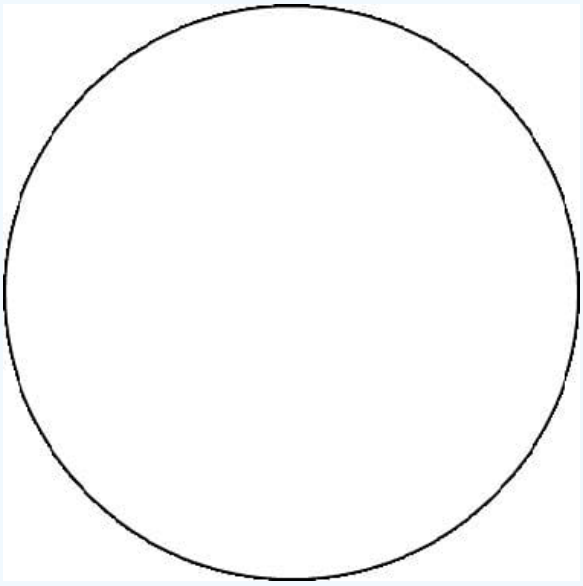
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2.6.4: Exercise #11 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Explain why it is important that the smear not be heat fixed for a capsule stain.
2. Draw and label a bacterial cell that has a capsule following the capsule stain.



3. Why are capsules clinically important? What does the capsule do for those bacteria that are pathogenic and possess a capsule?

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2.7: Section Two Review

Review Purpose

This review helps you check what you learned in Section Two before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you...

- Distinguish the basic bacterial shapes and identify the function of common bacterial cell structures?
- Explain the purpose of the smear and of heat versus chemical fixation, and why specimens are stained?
- Differentiate acidic and basic dyes, and describe what simple staining reveals about a sample?
- Define differential staining and the terms primary stain, secondary stain, and mordant?
- Describe the purpose of the Gram stain, its protocol, and its reagents (crystal violet, Gram's iodine, decolorizer, and safranin)?
- Differentiate Gram-positive from Gram-negative reactions, interpret common sources of error, and explain the Gram stain's importance in disease detection and diagnosis?
- Explain the purpose of the acid-fast stain, the role of mycolic acids, and name bacterial species that are acid-fast?
- Distinguish an endospore from a vegetative cell, describe the endospore stain, and name endospore-forming pathogens and the diseases they cause?
- Explain what a capsule is, why the capsule stain is a negative staining technique, and how capsules benefit the cell?

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2.7.1: Matching

Matching Questions

1. Match the cell type or structure to the appropriate description of its appearance following staining by placing the letter next to the correct term.

Question 1 Terms

Answer	Term
_____	1. Acid-fast cells
_____	2. Non-acid-fast cells
_____	3. Gram-positive cell
_____	4. Gram-negative cell
_____	5. Endospore
_____	6. Vegetative cell (mother cell)
_____	7. Capsule

Question 1 Descriptions

Letter	Description
a.	purple due to the presence of crystal violet and iodine
b.	pink due to the presence of safranin after decolorization with water
c.	pink due to the presence of safranin after decolorization with acetone and/or alcohol
d.	clear halo surrounding a cell
e.	blue due to presence of methylene blue
f.	red due to the presence of carbolfuchsin
g.	green due to the presence of malachite green

Click to reveal answers

Answers:
1-f
2-e
3-a
4-c
5-g
6-b
7-d

2. Match the staining procedure on the left with their appropriate descriptions by placing the letter next to the correct term.

Question 2 Staining Procedures

Answer	Staining Procedure
_____	1. Gram stain
_____	2. Acid-fast stain
_____	3. Endospore stain
_____	4. Capsule stain
_____	5. Simple stain
_____	6. Negative stain

Question 2 Descriptions

Letter	Description
a.	staining procedure that makes use of a single dye; allows one to determine a bacterium's shape, size, and arrangement
b.	staining procedure used to detect the presence of mycolic acid
c.	staining procedure in which only an acidic dye is applied to the sample
d.	staining procedure that separates bacteria into two groups based on difference in their cell wall structure/ thickness
e.	staining procedure that uses both an acidic and basic stain to detect a form of glycocalyx
f.	staining procedure used to detect the formation of metabolically inactive structures produced by a few genera of bacteria

Click to reveal answers

Answers:
1-d
2-b
3-f
4-e
5-a
6-c

3. Match the terms regarding bacterial shapes or arrangements on the left with their appropriate descriptions by placing the letter next to the correct term.

Question 3 Terms

Answer	Term
_____	1. Coccus
_____	2. Bacillus
_____	3. Palisade

Answer	Term
_____	4. Streptococcus
_____	5. Streptobacillus
_____	6. Staphylococcus
_____	7. Spirillum
_____	8. Spirochete
_____	9. Vibrio
_____	10. Pleomorphic

Question 3 Descriptions

Letter	Description
a.	spiral shaped bacterium with rigid cell wall
b.	chain of bacillus cells
c.	spherical shaped bacterial cells
d.	chain of spherical cells
e.	term used for bacteria that exhibit a variety of shapes and sizes
f.	clusters of spherical cells
g.	rod shaped bacterial cells
h.	spiral shaped bacterium with flexible cell walls
i.	rod shaped bacteria arranged in a pattern parallel to one another
j.	curved rods

Click to reveal answers

Answers:
1-c
2-g
3-i
4-d
5-b
6-f
7-a
8-h
9-j
10-e

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2.7.2: Fill-In-The-Bank

Fill-in-the-Blank Questions

1. _____ are procedures utilized to reduce microbial numbers in an attempt to prevent spreading the microbes.

Click to reveal answer

Answer Aseptic techniques

2. The solidifying agent, _____ is used to create solid media for use in the microbiology lab.

Click to reveal answer

Answer agar

3. Microorganisms are classified into four _____, based on the level of measures needed to safely work with the organism.

Click to reveal answer

Answer biosafety levels

4. _____ microscopes use two sets of lenses, an ocular lens and objective lens to magnify the specimen.

Click to reveal answer

Answer Compound

5. The _____ method is used to isolate bacteria on the surface of solid media.

Click to reveal answer

Answer streak plate method

6. _____ is the process of moving bacteria from a media source in which they have been growing to a new sterile media source.

Click to reveal answer

Answer Subculturing

7. The _____ , is the magnification calculated by multiplying the magnifying power of the ocular lens with the magnify power of the objective lens.

Click to reveal answer

Answer total magnification

8. In the streak plate method, the _____ quadrant will show the greatest number of isolated colonies.

Click to reveal answer

Answer fourth

9. When using a compound microscope to view a specimen with the high or oil objective lens, you should use the _____ to bring the specimen into focus.

Click to reveal answer

Answer fine focus (adjustment) knob

10. A _____ is an instrument with a straight wire, which is used to stab media during the inoculating process.

Click to reveal answer

Answer inoculating needle

11. Always carry the microscope with one hand on the _____ and the other hand under the _____ .

Click to reveal answer

Answer arm; base

12. Unwanted microbes that find their way into our cultures are called _____ .

Click to reveal answer

Answer contaminants

13. Nutrient agar _____ are often used to store and transport microbes.

Click to reveal answer

Answer slants

14. The _____ concentrates light onto the slide and contains an iris diaphragm to control the angle and the size of the cone of light.

Click to reveal answer

Answer condenser

15. To increase the magnification, you must switch to a higher-power _____ .

Click to reveal answer

Answer objective lens

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2.7.3: True/False

True / False Questions

1. During the heating step of the acid-fast stain, the carbolfuchsin is allowed to come to a boil and dry out. The bacteria are red after completion of the staining process when viewed under oil immersion, which means the bacteria are acid-fast positive.

_____ True _____ False

Click to reveal answer

Answer False

2. The endospore stain reveals internal structures within cells of the genera *Bacillus* and *Clostridium*.

_____ True _____ False

Click to reveal answer

Answer True

3. Heat fixing is not required when staining capsules because high temperatures may damage the capsule or lead to shrinkage of the cell that can be misread as a capsule.

_____ True _____ False

Click to reveal answer

Answer True

4. While acid-fast bacteria retain carbolfuchsin after acid-alcohol treatment, non-acid-fast bacteria are decolorized by acid-alcohol.

_____ True _____ False

Click to reveal answer

Answer True

5. The Gram stain allows for the determination of bacterial species based on their metabolic activities.

_____ True _____ False

Click to reveal answer

Answer False

6. A simple stain will allow you to determine the genus and species of an unknown bacterium.

_____ True _____ False

Click to reveal answer

Answer False

7. Because they have an extremely thick layer of peptidoglycan in their cell walls, *Mycobacterium* bacterial cells require the addition of heat in order for stains to penetrate the cells.

_____ True _____ False

Click to reveal answer

Answer False

8. Methylene blue can be used to stain DNA because it forms ionic bonds with DNA.

_____ True _____ False

Click to reveal answer

Answer True

9. Acidic stains are positively charged and are attracted to the positively charged bacterial cell wall proteins.

_____ True _____ False

Click to reveal answer

Answer False

10. Using a simple stain, you should be able to see multiple colors on a slide of mixed bacterial species.

_____ True _____ False

Click to reveal answer

Answer False

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2.7.4: Multiple Choice / Multiple Select

Multiple Choice / Multiple-Select Questions

1. Both spirilla and spirochetes appear spiral-shaped. Which structural feature distinguishes them?

- a) Spirilla have rigid cell walls; spirochetes have flexible cell walls
- b) Spirilla have flexible cell walls; spirochetes have rigid cell walls
- c) Spirilla are larger than spirochetes
- d) Spirilla are Gram-positive; spirochetes are Gram-negative

Click to reveal answer

Answer a) Spirilla have rigid cell walls; spirochetes have flexible cell walls
Spirilla have rigid cell walls, while spirochetes have flexible cell walls.

2. In addition to cell wall rigidity, spirilla can be differentiated from spirochetes by the presence of:

- a) Endospores
- b) External flagella
- c) A capsule
- d) Mycolic acids

Click to reveal answer

Answer b) External flagella
Spirilla can also be differentiated from spirochetes due to the presence of external flagella.

3. Spirochetes possess structures that allow their cells to rotate in a corkscrew manner. These structures are called:

- a) External flagella
- b) Axial filaments (endoflagella)
- c) Endospores
- d) Capsules

Click to reveal answer

Answer b) Axial filaments (endoflagella)
Spirochetes have structures known as axial filaments (endoflagella) that allow their cells to rotate in a corkscrew manner.

4. A bacterium that appears as a short, plump rod with a shape intermediate to that of a coccus and a bacillus is called a:

- a) Vibrio
- b) Coccobacillus
- c) Spirillum
- d) Palisade

Click to reveal answer

Answer b) Coccobacillus
Coccobacillus bacteria have a shape intermediate to that of a coccus and a bacillus; they appear as short plump rods.

5. Bacterial cellular arrangement is dependent on which of the following? **Select all that apply.**

- a) The plane of cell division
- b) Whether cells remain attached to one another following cell division
- c) The type of stain used to visualize the cells
- d) The magnification at which the cells are viewed

Click to reveal answers

Answers: a) The plane of cell division; b) Whether cells remain attached to one another following cell division
Cellular arrangement depends on the plane of cell division and whether cells remain attached to one another following cell division.

6. Why are more arrangements observed among cocci than among bacilli?

- a) Cocci reproduce more rapidly than bacilli
- b) Cocci may divide in a single plane, in two perpendicular planes, or in many planes, while bacilli divide only in the transverse plane
- c) Cocci are smaller and can pack together more tightly
- d) Bacilli never remain attached after division

Click to reveal answer

Answer b) Cocci may divide in a single plane, in two perpendicular planes, or in many planes, while bacilli divide only in the transverse plane
Some cocci divide in a single plane, others in two perpendicular planes, and still others in many planes, producing a variety of arrangements. Bacilli divide only in the transverse plane.

7. Spirilla are generally observed as individual cells because they:

- a) Rarely remain attached to one another after division
- b) Divide in many planes
- c) Are always motile
- d) Cannot be stained

Click to reveal answer

Answer a) Rarely remain attached to one another after division
Spirilla rarely remain attached, so they are generally observed as individual cells.

8. Fixation is performed on a smear for which of the following purposes? **Select all that apply.**

- a) To attach the cells to the slide so they are not rinsed off during staining
- b) To prevent distortion of the cells that would alter their morphology
- c) To kill the cells, making the sample safer to handle
- d) To increase the total magnification of the specimen

Click to reveal answers

Answers: a) To attach the cells to the slide so they are not rinsed off during staining; b) To prevent distortion of the cells that would alter their morphology; c) To kill the cells, making the sample safer to handle
Fixation attaches the cells to the slide, prevents distortion that would alter morphology, and kills the cells, making the sample safer to handle.

9. Chemical fixation is accomplished by applying which type of reagent to the sample?

- a) Methyl alcohol or formaldehyde
- b) Crystal violet or safranin
- c) Acid-alcohol
- d) Isotonic saline

Click to reveal answer

Answer a) Methyl alcohol or formaldehyde
Chemical fixation is accomplished by applying a chemical reagent such as methyl alcohol or formaldehyde.

10. Heat fixation is correctly performed by:

- a) Holding the wet smear directly in the flame until it steams
- b) Passing the air-dried smear back and forth over the flame of a Bunsen burner, smear side up
- c) Placing the slide on a steam bath for five minutes
- d) Submerging the slide in boiling water

Click to reveal answer

Answer b) Passing the air-dried smear back and forth over the flame of a Bunsen burner, smear side up
Heat fixation is done by passing the air-dried smear back and forth over the flame of a Bunsen burner, smear side up.

11. What is the primary reason specimens are stained before microscopic observation?

- a) To increase the resolution of the objective lens
- b) To add contrast to the cells, allowing them to be viewed with the microscope
- c) To kill any pathogens present on the slide

- d) To make the cells adhere to the slide

Click to reveal answer

Answer b) To add contrast to the cells, allowing them to be viewed with the microscope
Samples are stained in order to add contrast to the cells, allowing them to be viewed using the microscope.

12. Which of the following are examples of acidic (anionic) dyes? **Select all that apply.**

- a) India ink
- b) Nigrosine
- c) Eosin
- d) Methylene blue
- e) Crystal violet

Click to reveal answers

Answers: a) India ink; b) Nigrosine; c) Eosin
India ink, nigrosine, and eosin are acidic (anionic) dyes. Methylene blue and crystal violet are basic (cationic) dyes.

13. Why does the interior of a bacterial cell carry an overall negative charge?

- a) The cell wall is composed of peptidoglycan
- b) Cells are filled with negatively charged molecules such as proteins and nucleic acids
- c) The cell membrane is made of lipids
- d) Water molecules inside the cell are polar

Click to reveal answer

Answer b) Cells are filled with negatively charged molecules such as proteins and nucleic acids
Cells are filled with molecules such as proteins and nucleic acids that are negatively charged, giving the cell an overall negative charge internally.

14. When an anionic dye is applied to a bacterial smear, what is the expected result?

- a) The dye is repelled by the cell and stains the surrounding background
- b) The dye ionically bonds to structures inside the cell, staining the cell
- c) The dye stains only the cell wall
- d) The dye stains both the cell and the background equally

Click to reveal answer

Answer a) The dye is repelled by the cell and stains the surrounding background
An anionic dye is repelled by the negatively charged cell, so it stains the background surrounding the cell.

15. Differential staining procedures differ from simple staining procedures in that differential stains:

- a) Make use of two stains rather than just one
- b) Require the use of an oil immersion lens
- c) Do not require fixation of the smear
- d) Can determine the genus and species of a bacterium

Click to reveal answer

Answer a) Make use of two stains rather than just one
Differential staining techniques make use of two stains rather than just one, and allow the observer to obtain additional information about the sample.

16. The primary stain used in the Gram stain procedure is:

- a) Safranin, an acidic stain
- b) Crystal violet, a basic stain
- c) Carbolfuchsin, a lipid-soluble stain
- d) Malachite green, a basic stain

Click to reveal answer

Answer b) Crystal violet, a basic stain
The primary stain in the Gram stain is crystal violet, a basic stain that stains both Gram-positive and Gram-negative cells.

17. What is the function of the mordant, Gram's iodine, in the Gram stain procedure?

- a) It removes the primary stain from Gram-negative cells
- b) It binds to crystal violet, forming an insoluble complex that is trapped within the cell
- c) It stains cells that have lost the primary stain
- d) It dissolves the outer membrane of Gram-negative cells

Click to reveal answer

Answer b) It binds to crystal violet, forming an insoluble complex that is trapped within the cell
Mordants help fix dyes into the sample. Gram's iodine binds to crystal violet, forming an insoluble complex trapped within the cell.

18. Which reagent constitutes the differential step of the Gram stain procedure?

- a) Crystal violet
- b) Gram's iodine
- c) Gram's decolorizer

- d) Safranin

Click to reveal answer

Answer c) Gram's decolorizer
Decolorizers act as the differential step of a differential staining process, removing the primary stain from one cell type while allowing it to remain in another.

19. Why is the crystal violet-iodine complex washed out of Gram-negative cells during decolorization?

- a) Gram's iodine fails to form a complex inside Gram-negative cells
- b) The decolorizer dissolves components of the outer membrane and cell membrane, making them more porous
- c) Gram-negative peptidoglycan becomes more tightly connected
- d) The safranin counter-stain displaces the complex from Gram-negative cells

Click to reveal answer

Answer b) The decolorizer dissolves components of the outer membrane and cell membrane, making them more porous
The decolorizer interacts with the outer membrane and cell membrane, dissolving some of their components and making them more porous, so the complex washes out of the cell.

20. A student prepares a smear that is far too thick. What error is this most likely to produce?

- a) Gram-negative cells falsely appearing Gram-positive
- b) Gram-positive cells falsely appearing Gram-negative
- c) Complete loss of all cells from the slide
- d) The counter-stain failing to enter any cells

Click to reveal answer

Answer a) Gram-negative cells falsely appearing Gram-positive
If the smear is too thick, crystal violet may become trapped in the smear and not wash out during decolorization, causing Gram-negative cells to falsely appear Gram-positive.

21. What is the preferred age of a culture used for the Gram stain?

- a) 16-18 hours old
- b) 24-48 hours old
- c) At least 72 hours old
- d) One week old

Click to reveal answer

Answer a) 16-18 hours old
Cultures should be less than 24 hours old, preferably 16-18 hours old, because aging cell walls weaken and Gram-positive cells may falsely appear Gram-negative.

22. Safranin enters both Gram-positive and Gram-negative cells. Why do Gram-positive cells still appear violet/purple at the end of the procedure?

- a) Safranin cannot bind to peptidoglycan
- b) The safranin is overshadowed by the darker crystal violet already present in the cell
- c) Safranin is rinsed away from Gram-positive cells
- d) Gram-positive cells convert safranin to crystal violet

Click to reveal answer

> **Answer** b) The safranin is overshadowed by the darker crystal violet already present in the cell
> In Gram-positive cells the safranin is overshadowed by the darker crystal violet already present in the cell.

23. Knowing a bacterium's Gram stain result is clinically valuable primarily because Gram-positive and Gram-negative cells:

- a) Grow at different temperatures
- b) Have different susceptibilities to various antimicrobial drugs
- c) Produce different endospores
- d) Require different types of microscopes

Click to reveal answer

> **Answer** b) Have different susceptibilities to various antimicrobial drugs
> Structural differences in the cell walls result in different susceptibilities to antimicrobial drugs, so the Gram stain result aids in selecting appropriate treatment.

24. The acid-fast staining procedure is known as the:

- a) Shaeffer-Fulton protocol
- b) Ziehl-Neelsen protocol
- c) Maneval protocol
- d) Gram protocol

Click to reveal answer

> **Answer** b) Ziehl-Neelsen protocol
> The staining procedure used to stain acid-fast cells is known as the Ziehl-Neelsen protocol.

25. Carbolfuchsin, the primary stain of the acid-fast procedure, is best described as:

- a) A dark red, lipid-soluble stain
- b) A green, water-soluble stain
- c) A blue, acidic stain

- d) A colorless mordant

Click to reveal answer

Answer a) A dark red, lipid-soluble stain
Carbolfuchsin is a dark red stain that is lipid soluble, allowing it to penetrate the waxy mycolic acid in the cell wall.

26. Why do most aqueous stains fail to penetrate cells containing mycolic acids?

- a) Aqueous stains are too large to cross the cell wall
- b) Aqueous stains are polar and interact poorly with the non-polar mycolic acids
- c) Aqueous stains are positively charged and are repelled by the cell
- d) Mycolic acids chemically destroy aqueous stains

Click to reveal answer

Answer b) Aqueous stains are polar and interact poorly with the non-polar mycolic acids
Aqueous stains are mixed with water, making them polar solutions that do not interact well with the non-polar nature of mycolic acids.

27. What is the purpose of applying heat at the same time the primary stain is added in the acid-fast procedure?

- a) It sterilizes the sample before handling
- b) It melts the waxy lipid, facilitating entry of the stain into the cell
- c) It evaporates excess stain from the slide
- d) It fixes the cells to the slide

Click to reveal answer

Answer b) It melts the waxy lipid, facilitating entry of the stain into the cell
Heat melts the waxy lipid, thereby facilitating entry of the stain into the cell.

28. After the primary stain is applied with heat, the slide is removed from the heat and allowed to cool. Why is this step important?

- a) The wax solidifies, trapping the primary stain within cells containing mycolic acid
- b) Cooling allows the decolorizer to work more slowly
- c) Cooling causes the cells to adhere more firmly to the slide
- d) Cooling prevents the counter-stain from entering the cell

Click to reveal answer

Answer a) The wax solidifies, trapping the primary stain within cells containing mycolic acid
The slide is removed from the heat and allowed to cool so the wax solidifies, trapping the primary stain in cells containing mycolic acid.

29. Besides making cells difficult to stain, what do mycolic acids do for the bacteria that possess them? **Select all that apply.**

- a) Prevent the cells from desiccating
- b) Protect the cells from many chemical cleaners
- c) Allow the cells to form endospores
- d) Enable the cells to resist phagocytosis by forming a halo

Click to reveal answers

Answers: a) Prevent the cells from desiccating; b) Protect the cells from many chemical cleaners
The waxes prevent the cells from desiccating and protect them from many chemical cleaners.

30. Why is methylene blue, the counter-stain, unable to color acid-fast cells?

- a) It is repelled by the mycolic acid
- b) It is washed away during the decolorization step
- c) It is too dilute to be visible
- d) It only binds to endospores

Click to reveal answer

Answer a) It is repelled by the mycolic acid
Methylene blue cannot enter the cells of acid-fast bacteria because it is repelled by the mycolic acid.

31. The term acid-fast refers to the fact that these cells:

- a) Grow rapidly in acidic media
- b) Held fast to the primary stain despite treatment with the decolorizer
- c) Produce acid as a metabolic byproduct
- d) Are rapidly destroyed by acid-alcohol

Click to reveal answer

Answer b) Held fast to the primary stain despite treatment with the decolorizer
These cells are referred to as acid-fast because they have held fast, or tightly, to the primary stain.

32. *Mycobacterium leprae*, an organism detected using the acid-fast stain, is the causative agent of:

- a) Tuberculosis
- b) Leprosy
- c) Anthrax
- d) Tetanus

Click to reveal answer

Answer b) Leprosy
Mycobacterium tuberculosis and *Mycobacterium leprae* cause tuberculosis and leprosy respectively.

33. The endospore staining method is known as the:

- a) Ziehl-Neelsen method
- b) Shaeffer-Fulton method
- c) Gram method
- d) Maneval method

Click to reveal answer

Answer b) Shaeffer-Fulton method
The Shaeffer-Fulton method is used in this exercise to stain and visualize endospores.

34. During sporulation, the metabolically active cell that constructs the protective layers around a copy of the genetic material is called the:

- a) Endospore
- b) Vegetative cell (sporangium)
- c) Capsule
- d) Glycocalyx

Click to reveal answer

Answer b) Vegetative cell (sporangium)
A vegetative cell, also called the sporangium, is a metabolically active cell that constructs several protective layers around a copy of the cell's genetic material.

35. What is the function of an endospore?

- a) To increase the rate of bacterial reproduction
- b) To protect a copy of the genetic material in harsh environments
- c) To allow the cell to move through liquid media
- d) To prevent phagocytosis by host immune cells

Click to reveal answer

Answer b) To protect a copy of the genetic material in harsh environments
Endospores protect a copy of the genetic material in harsh environments.

36. Endospores are resistant to which of the following? **Select all that apply.**

- a) Extremes in temperature

- b) Radiation
- c) Chemicals
- d) Phagocytosis by host immune cells

Click to reveal answers

Answers: a) Extremes in temperature; b) Radiation; c) Chemicals
Endospores are resistant to extremes in temperature, radiation, and chemicals. Resisting phagocytosis is a function of the capsule, not the endospore.

37. How would an endospore appear on a slide that had been Gram stained rather than endospore stained?

- a) As a green structure inside the cell
- b) As a clear structure inside the cell
- c) As a dark purple structure inside the cell
- d) Endospores would not be visible at all

Click to reveal answer

Answer b) As a clear structure inside the cell
Endospores are resistant to staining, so if present they appear as clear structures inside a cell after the Gram stain.

38. Water serves as the decolorizing agent in the endospore stain because:

- a) Water is unable to penetrate the protective coat of the endospore, but washes the stain out of the vegetative cell
- b) Water chemically neutralizes malachite green
- c) Water dissolves the endospore coat
- d) Water is the only reagent safe to use with steam heat

Click to reveal answer

Answer a) Water is unable to penetrate the protective coat of the endospore, but washes the stain out of the vegetative cell
Malachite green does not bind readily to the vegetative cell and washes out with water, while the water is unable to penetrate the protective coat of the endospore, so the endospore remains green.

39. Endospores are classified based on their location within the vegetative cell. Which of the following are recognized locations? **Select all that apply.**

- a) Central
- b) Terminal
- c) Subterminal
- d) Elliptical

Click to reveal answers

Answers: a) Central; b) Terminal; c) Subterminal
There are three classifications based on location within the vegetative cell: central, terminal, and subterminal. Elliptical describes an endospore's shape, not its location.

40. Why must the *Bacillus subtilis* culture used for the endospore stain be at least 72 hours old?

- a) Older cells stain more readily with malachite green
- b) To ensure endospores have had an opportunity to form
- c) To allow the culture to become Gram-negative
- d) To reduce the number of vegetative cells to zero

Click to reveal answer

Answer b) To ensure endospores have had an opportunity to form
A culture of *Bacillus subtilis* that is at least 72 hours old is used to ensure endospores have had an opportunity to form.

41. Which of the following are endospore-forming pathogens? **Select all that apply.**

- a) *Bacillus anthracis*
- b) *Clostridium tetani*
- c) *Clostridium botulinum*
- d) *Streptococcus pneumoniae*
- e) *Klebsiella pneumoniae*

Click to reveal answers

Answers: a) *Bacillus anthracis*; b) *Clostridium tetani*; c) *Clostridium botulinum*
Endospore-forming pathogens include *Bacillus anthracis*, *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, and *Clostridium difficile*. *Streptococcus pneumoniae* and *Klebsiella pneumoniae* are capsule formers.

42. A capsule is one type of bacterial:

- a) Glycocalyx
- b) Endospore
- c) Flagellum
- d) Mycolic acid

Click to reveal answer

Answer a) Glycocalyx
Capsules are one type of bacterial glycocalyx.

43. Capsules are structures made of a combination of:

- a) Peptidoglycan and mycolic acids
- b) Polysaccharides and proteins
- c) Waxy lipids and agar
- d) Nucleic acids and phospholipids

Click to reveal answer

Answer b) Polysaccharides and proteins
Capsules are made of a combination of polysaccharides and proteins, arranged to form a protective structure around the cell.

44. In addition to preventing phagocytosis and avoiding desiccation, capsules assist bacteria by:

- a) Aiding in the formation of biofilms by allowing cells to adhere to one another
- b) Increasing the rate of cell division
- c) Allowing the cell to survive boiling
- d) Producing endospores under stress

Click to reveal answer

Answer a) Aiding in the formation of biofilms by allowing cells to adhere to one another
Capsules assist in the formation of biofilms by allowing cells to adhere to one another.

45. The capsule stain is described as a negative staining technique because:

- a) The capsule is stained a dark negative color
- b) An acidic dye is used that is repelled by the cell and stains the background instead
- c) The result is reported as either positive or negative
- d) No stain is applied to the slide at all

Click to reveal answer

Answer b) An acidic dye is used that is repelled by the cell and stains the background instead
Capsule staining uses a negative staining technique: an acidic (anionic) stain is applied that is repelled by the negatively charged cell and stains the background instead.

46. What is the role of Congo Red in the capsule stain procedure?

- a) It stains the bacterial cell pinkish red
- b) It stains the background surrounding the cells
- c) It stains the capsule directly
- d) It fixes the cells to the slide

Click to reveal answer

Answer b) It stains the background surrounding the cells
Congo Red stains the background surrounding the cells because the negatively charged cell repels the negatively charged dye.

47. Maneval's stain is a mixture of:

- a) Ethyl alcohol and hydrochloric acid
- b) Acetic acid and acid fuchsin
- c) Ethanol and acetone
- d) Malachite green and safranin

Click to reveal answer

Answer b) Acetic acid and acid fuchsin
Maneval's stain is a mixture of acetic acid and acid fuchsin.

48. Which component of Maneval's stain causes the bacterial cells to appear pinkish red?

- a) Acetic acid
- b) Acid fuchsin
- c) Congo Red
- d) Crystal violet

Click to reveal answer

Answer b) Acid fuchsin
The acid fuchsin is responsible for causing the bacterial cells to appear pinkish red.

49. Why does the Congo Red appear gray-blue at the end of the capsule stain procedure?

- a) The acidic pH of Maneval's stain causes the color change
- b) It is diluted by the deionized water rinse
- c) Heat fixation alters the pigment
- d) It reacts chemically with the capsule

Click to reveal answer

Answer a) The acidic pH of Maneval's stain causes the color change
The acidic pH of Maneval's stain causes the Congo Red to appear gray-blue.

50. Following a properly performed capsule stain, the capsule is detected as:

- a) A clear halo located between the gray-blue background and the pinkish-red cell

- b) A bright green structure within the cell
- c) A dark blue ring staining the outer edge of the cell
- d) A red structure at the end of the cell

Click to reveal answer

Answer a) A clear halo located between the gray-blue background and the pinkish-red cell
The capsule is indirectly detected as a clear halo located between the gray-blue background and the pinkish-red cell.

51. Heat fixation is omitted in the capsule stain procedure because capsules:

- a) May consist of up to 95% water, and heating causes dehydration, shrinkage, and distortion
- b) Are destroyed by contact with any glass surface
- c) Cannot adhere to a slide once heated
- d) Would be stained green rather than remaining clear

Click to reveal answer

Answer a) May consist of up to 95% water, and heating causes dehydration, shrinkage, and distortion
Capsules may consist of up to 95% water. Heating the sample can cause the capsules to become dehydrated, resulting in shrinkage and distortion of the sample.

52. Which of the following pathogens are known to form capsules? **Select all that apply.**

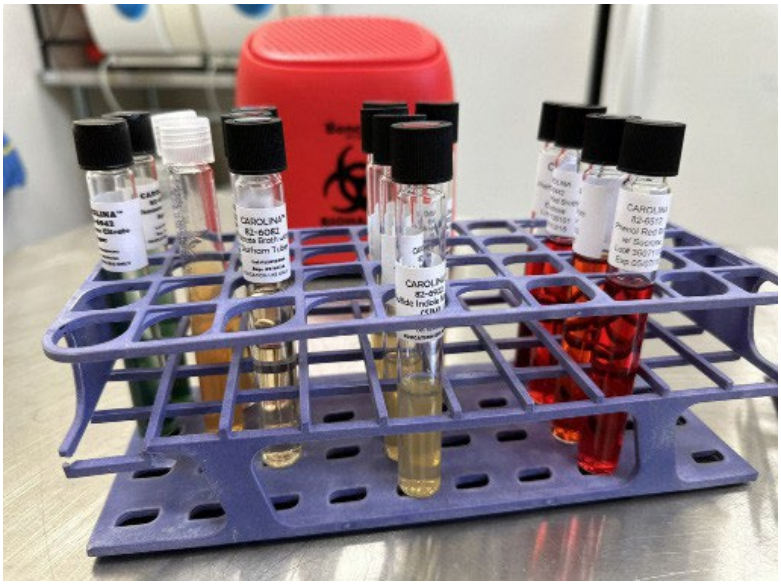
- a) *Streptococcus pneumoniae*
- b) *Klebsiella pneumoniae*
- c) *Escherichia coli*
- d) *Clostridium tetani*

Click to reveal answers

Answers: a) *Streptococcus pneumoniae*; b) *Klebsiella pneumoniae*; c) *Escherichia coli*
Pathogens known to form capsules include *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Escherichia coli*. *Clostridium tetani* is an endospore-forming pathogen.

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3: SECTION THREE ASSESSING METABOLIC CHARACTERISTICS OF BACTERIA (BIOCHEMICAL TESTS, SELECTIVE AND DIFFERENTIAL MEDIA)



Credit: Sherry Smith

Microscopy and staining techniques allow the observer to examine cell shape, cell size, and cellular arrangement of microbial specimens. Differential staining techniques allow a microbiologist to gather information about the presence or absence of specific structural features. These techniques, however, do not allow laboratory personnel to determine the bacterial sample's species.

Two different species within the same genus will look morphologically identical when stained and observed. For example, *Staphylococcus aureus* and *Staphylococcus epidermidis* would both present with the same Gram stain results and acid-fast stain results; both are cocci that grow in clusters and are approximately the same size. These two species cannot be differentiated using microscopy alone. In order to differentiate two species within the same genus, we must examine the physiological characteristics of the bacteria. Each species will be unique in its physiology, meaning that it will utilize a unique set of metabolic pathways. These metabolic pathways are catalyzed by protein molecules known as enzymes.

Since a bacterial species will have a unique set of enzymes, microbiologists can conduct what are known as biochemical tests to determine whether specific enzymes are present. After completing sets of biochemical tests, microbiologists interpret the data using a systematic key known as *Bergey's Manual*.

Selective and differential media are also used to aid in the isolation and identification of bacteria. ***Selective media*** contain ingredients that inhibit the growth of some organisms while encouraging the growth of others. ***Differential media*** allow the observer to differentiate between organisms on the media.

Differentiation may be the result of a color change in the media, a color change in the bacterial colony, the production of a product, or another observable change. Some media are classified only as selective media, others are classified only as differential media, and some combine both selective and differential properties.

In this section, you will examine a variety of biochemical tests, selective media, and differential media.

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Unmasking Microbes: Using Biochemical Tests and Media for Microbial Identification

Click on the video thumbnail or the linked title to access the video.

Credit: Yenny Mo, Josh Spradling, and Sherry Smith

3.1: Exercise #12 - Carbohydrate Fermentation Tests

3.1.1: Carbohydrate Fermentation Test

3.1.2: Carbohydrate Fermentation Test Activity

3.1.3: Exercise #12 - Lab Wrap-Up

3.2: Exercise #13 - Triple Sugar Iron Agar Test

3.2.1: Triple Sugar Iron Agar Test

3.2.2: Triple Sugar Iron Agar Test Activity

3.2.3: Exercise #13 - Lab Wrap-Up

3.3: Exercise #14 - SIM Medium Test

3.3.1: SIM Medium Test

3.3.2: SIM Medium Test Activity

3.3.3: Exercise #14 - Lab Wrap-Up

3.4: Exercise 15 - IMViC Tests

3.4.1: Indole Test

3.4.2: Indole Test Activity

3.4.3: MRVP

3.4.4: Methyl Red (MR) and Voges-Proskauer (VP) Test Activity

3.4.5: Citrate Test

3.4.6: Citrate Test Activity

3.4.7: Exercise #15 - Lab Wrap-Up

3.5: Exercise #16 - Respiration Tests

3.5.1: Oxidase Test

3.5.2: Oxidase Test Activity

3.5.3: Catalase Test

3.5.4: Catalase Test Activity

3.5.5: Nitrate Reduction Test

3.5.6: Nitrate Reduction Test Activity
3.5.7: Exercise #16 - Lab Wrap-Up

3.6: Exercise #17 - Urease Test
3.6.1: Urease Test
3.6.2: Urease Test Activity
3.6.3: Exercise #17 - Lab Wrap-Up

3.7: Exercise #18 - Coagulase Test
3.7.1: Coagulase Test
3.7.2: Coagulase Test Activity
3.7.3: Exercise #18 - Lab Wrap-Up

3.8: Exercise #19 - Casease Test
3.8.1: Casease Test
3.8.2: Casease Test Activity
3.8.3: Exercise #19 - Lab Wrap-Up

3.9: Exercise #20 - Starch Hydrolysis Test
3.9.1: Starch Hydrolysis Test
3.9.2: Starch Hydrolysis Test Activity
3.9.3: Exercise #20 - Lab Wrap-Up

3.10: Exercise #21 - Blood Agar
3.10.1: Blood Agar Test
3.10.2: Blood Agar Test Activity
3.10.3: Exercise #21 - Lab Wrap-Up

3.11: Exercise #22 MacConkey Agar
3.11.1: MacConkey Agar
3.11.2: MacConkey Agar Activity
3.11.3: Exercise #22 - Lab Wrap-Up

3.12: Exercise #23 - Mannitol Salt Agar
3.12.1: Mannitol Salt Agar Test
3.12.2: Mannitol Salt Agar Test Activity
3.12.3: Exercise #23 - Lab Wrap-Up

3.13: Section Three Review
3.13.1: Matching
3.13.2: Fill-In-The-Blank
3.13.3: True/False
3.13.4: Multiple Choice / Multiple Select

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3.1: Exercise #12 - Carbohydrate Fermentation Tests

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the carbohydrate fermentation test.
2. Explain key ingredients or reagents associated with the carbohydrate fermentation test.
3. Interpret the results of the carbohydrate fermentation test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Phenol red broth (1 or more different sugars may be tested; 2 of each sugar to be tested will be needed)
- Test tube rack

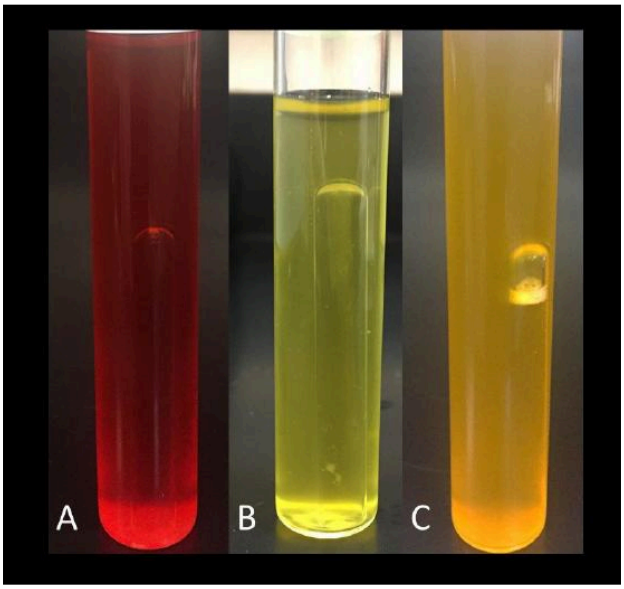
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3.1.1: Carbohydrate Fermentation Test

Carbohydrate fermentation tests are biochemical tests that determine the ability of an organism to ferment sugars. When organisms carry out fermentation of a carbohydrate, they may produce organic acids and/or gases as end-products. Organisms utilize carbohydrates differently depending on the enzymes present within a given organism.

Carbohydrate fermentation media contain a specific carbohydrate, such as glucose, lactose, or sucrose, and a pH indicator, phenol red, within a nutrient medium that provides other required nutrients that support bacterial growth. In addition to the media, a ***Durham tube***, which is a small test tube, is inverted within the media to serve as a trap for any gases that may be produced during fermentation.

Phenol red is a pH indicator that is red at alkaline pHs. Phenol red turns yellow at a slightly acidic pH, approximately pH 6.8. If an organism can metabolize the carbohydrate present, an acid is produced, and the indicator turns yellow. If a gas is produced as a result, a bubble will form in the Durham tube.



Carbohydrate fermentation. (A) Uninoculated phenol red broth media (B) Positive for fermentation (C) Positive for fermentation and gas produ
Credit: Sherry Smith

Carbohydrate tests are also considered a differential medium that allows differentiation of species within a genus. This permits more accurate identification of microorganisms. An understanding of the results of biochemical tests such as the carbohydrate fermentation test allows one to differentiate between two bacteria that morphologically appear similar under the microscope. For example, *Staphylococcus aureus* and *Staphylococcus epidermidis* both appear as Gram-positive cocci when stained with the Gram stain and visualized with the microscope. Carbohydrate fermentation tests would reveal the following:

Sample carbohydrate fermentation results for Staphylococcus aureus and Staphylococcus epidermidis

Organism	D-Glucose/Dextrose	Glucose	Lactose	Mannitol
Staphylococcus aureus	A	A	A	A
Staphylococcus epidermidis	A/G	A	A	-

A = acid production; **A/G** = acid and gas production; **-** = no change.

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3.1.2: Carbohydrate Fermentation Test Activity

In this activity you will test two bacterial samples. Label each phenol red broth tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner, recap the test tube, and return the tube to the test tube rack.
5. Pick up the phenol red broth tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the phenol red broth tube through the flame, recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner. Repeat steps 1 - 8 for each phenol red broth to be inoculated.
9. Incubate the inoculated phenol red broth tubes at 37°C for 24 hours.
10. At 24 hours, observe the broths for color changes and gas formation and record your results.

In the table below, write the name of the species tested, the name of the sugar(s) used, and the results for each of the tests performed.

Experimental Results

Bacterial Species	Carbohydrate Fermentation Media #1	Carbohydrate Fermentation Media #2	Carbohydrate Fermentation Media #3

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3.1.3: Exercise #12 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What information is determined regarding a bacterial cell after the carbohydrate fermentation test has been performed?
2. Why is it important to have only one sugar present in a single phenol red broth tube?
3. What ingredient allows for the detection of fermentation? What is the reagent detecting in the media?
4. How is the production of gas detected in a carbohydrate fermentation test?
5. Describe the appearance of a positive result in the carbohydrate fermentation test. What has occurred during the test to result in the positive result?
6. Why is it important to check the results of the test at 24 hours and not wait for a longer period of time?

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3.2: Exercise #13 - Triple Sugar Iron Agar Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the triple sugar iron agar test.
2. Explain key ingredients or reagents associated with the triple sugar iron agar test.
3. Interpret the results of the triple sugar iron agar test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Agar bacterial cultures (5 different species - instructor will provide name of species to be tested)
- Triple sugar iron agar tube (5 tubes)
- Test tube rack

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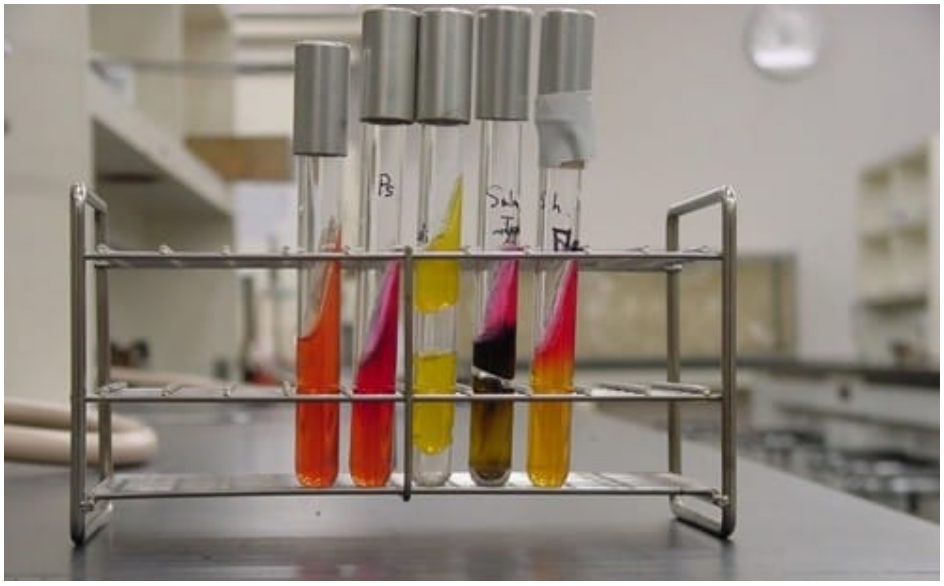
3.2.1: Triple Sugar Iron Agar Test

Triple sugar iron agar is an agar utilized to assess the ability of a bacterium to ferment sugars and produce hydrogen sulfide gas (H2S). Bacteria differ in their abilities to metabolize sugars. Some metabolize a greater variety of sugars than others. In metabolizing the sugars some species will produce gases. One such gas that may be produced during sugar metabolism is hydrogen sulfide gas.
Triple sugar iron agar contains three different sugars glucose (0.1%), sucrose (1%), and lactose (1%). In addition to the sugars the media contains the pH indicator phenol red, peptones, sodium thiosulfate, and ferrous ammonium sulfate.

After inoculation and incubation of the media, some bacteria may ferment glucose, sucrose, and/ or the lactose present in the media. In doing so they produce acidic products which lower the pH of the medium, causing the phenol red to turn yellow. Some bacteria however, can only metabolize the glucose. Glucose is present in a much lower concentration than the sucrose and lactose, therefore the bacteria would exhaust the sugars rather quickly as they ferment them. This would turn the media yellow initially. After exhausting the glucose, the bacteria can then metabolize the peptones present in the media producing ammonium as a result. Ammonium would increase the pH of the medium making it more alkaline, resulting in the media changing from yellow to red. This would occur in regions of the tube that are exposed to oxygen. The areas of the media that are anaerobic (lacking oxygen) would remain acidic, and thus continue to appear yellow.

In addition to assessing sugar metabolism the media can also reveal whether the bacterium produces hydrogen sulfide gas during the fermentation of the sugars. If the bacteria is capable of hydrogen sulfide gas production it will convert the sodium thiosulfate to hydrogen sulfide gas. Hydrogen sulfide gas is a colorless gas, so in order to detect its presence, the ingredient ferrous ammonium sulfate is included. The hydrogen sulfide gas interacts with the ferrous ammonium sulfate to produce a black precipitate. Other gases produced by fermentation, such as carbon dioxide or oxygen gas, will be detected due to bubbles or cracks in the media.

This test is an important test in helping differentiate members of the family Enterobacteriaceae.



TSI (Triple sugar iron) agar slant results. From left: (1) uninoculated (as control); (2) P. aeruginosa: Glc (-), Lac/Suc (-), H2S (-), Gas (-); (3) E. coli:Glc (+), Lac/Suc (+), H2S (-), Gas (+); (4) *Salmonella typhimurium*:Glc (+), Lac/Suc (-), H2S (+), Gas (+); (5) Shigella flexneri:Glc (+), Lac/Suc (-), H2S (-), Gas (-). Source: Y tambe, Wikimedia Commons, CC BY 3.0

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3.2.2: Triple Sugar Iron Agar Test Activity

In this activity you will test five bacterial samples.

1. Turn on the Bunsen burner and sterilize the inoculating needle by passing it through the flame of the burner.
2. Gently raise the lid to the agar plate containing the bacterial culture to be sampled and touch the inoculating needle to a bacterial colony to obtain a bacterial sample.
3. Pick up the triple sugar iron agar tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
4. Insert the inoculating needle by stabbing straight into the butt of the slant and then streak up the surface of the agar.
5. Pass the mouth of the triple sugar iron agar tube through the flame and recap it, and return it to the test tube rack.
6. Sterilize the inoculating needle by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 6 for the other four bacterial species and triple sugar iron agar tubes to be inoculated.

7. Incubate the inoculated nitrate broth tubes at 37°C for 24 hours.
8. Observe the triple sugar iron agar tubes for color changes, precipitation formation, and cracks in the media. Use the chart as a guide to interpret your results.

Triple Sugar Iron (TSI) Agar Reactions and Interpretations

Results			
Slant	**Butt**	**Abbreviations**	**Interpretation**
No change	No change	-/-	No fermentation
Red	Red	K/K	Protein catabolism yielded alkaline products
Red	Yellow	K/A	Glucose fermentation
Red	Yellow with bubbles	K/A, G	Glucose fermentation and gas production
Red	Yellow with black precipitate	K/A, H2S	Glucose fermentation and hydrogen sulfide gas production
Yellow	Yellow	A/A	Glucose and lactose or sucrose fermentation
Yellow	Yellow with bubbles	A/A, G	Glucose and lactose or sucrose fermentation; gas production
Yellow	Yellow with black precipitate	A/A, H2S	Glucose and lactose or sucrose fermentation; hydrogen sulfide gas production

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Results	Abbreviations	Interpretation

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3.2.3: Exercise #13 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What is the purpose of the ferrous ammonium sulfate in the triple sugar iron agar media?
2. How can you determine if the bacterium fermented only glucose or all three sugars present in the media?
3. What would the triple sugar iron agar medium look like if it were capable of fermenting glucose and sucrose and produced hydrogen sulfide gas? Explain your answer.
4. Following incubation, you observe a TSI agar, upon observation you note that the butt of the tube is yellow while the top is red and cracks are observed throughout the agar. Explain the results of TSI agar test.

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3.3: Exercise #14 - SIM Medium Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the SIM test.
2. Explain key ingredients or reagents associated with the SIM test.
3. Interpret the results of the SIM test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Agar bacterial cultures (5 different species - instructor will provide name of species to be tested)
- SIM tube (5 tubes)
- Kovac's reagent
- Test tube rack

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3.3.1: SIM Medium Test

SIM media is a biochemical media used to detect hydrogen sulfide gas production (S), the presence of tryptophanase, which converts tryptophan to indole (I), and detects the ability of a bacterium to move in its environment with the use of flagella (M). These tests are useful in differentiating members of the Enterobacteriaceae family.

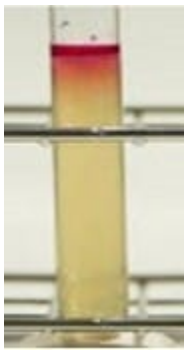
The media contains the ingredient sodium thiosulfate. If the bacteria is capable of hydrogen sulfide gas production it will convert the sodium thiosulfate to hydrogen sulfide gas. Hydrogen sulfide gas is a colorless gas, so in order to detect its presence, the ingredient ferrous ammonium sulfate is included. The hydrogen sulfide gas interacts with the ferrous ammonium sulfate to produce a black precipitate. Other gases produced by fermentation, such as carbon dioxide or oxygen gas, will be detected due to bubbles or cracks in the media.

The ***indole test*** is performed in order to assess a bacterium's ability to produce the enzyme tryptophanase. ***Tryptophanase*** converts the amino acid tryptophan to indole, pyruvic acid, and ammonia. The presence of indole can then be detected by adding Kovac's reagent. Kovac's reagent reacts with the indole to produce a red compound. Therefore, when Kovac's reagent is added to the medium and a red color change occurs it is indicative that the bacterium is capable of producing tryptophanase.

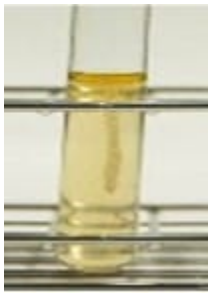
The final characteristic that can be assessed with this media is the ability of the bacterium to move in its environment using flagella. The media is known as a semi-solid deep. This is because it contains a small concentration of agar and is allowed to form the semi-solid consistency while the media cools in the tube in an upright position. The agar concentration in this media is approximately 3.5 g/L whereas to create solid agar media a concentration of 12-15 g/L is utilized. This creates an environment that favors movement if bacteria have flagella because a regular agar is too dense for the movement of the bacteria. In order to assess this characteristic, the bacterial culture is introduced to the media by stabbing it with an inoculating needle. If after incubation, bacteria are only found growing near the stab mark, they are non-motile. On the other hand, if the bacterial growth radiates out away from the stab mark the bacteria are motile. Some examples of SIM agar results are shown in the images below.



"Salmonella typhimurium in SIM agar, showing a positive result for H2S production, a negative result for the indole test (after Kovac's reagent has been added), and a positive result for motility." Source: A doubt, Wikimedia Commons, CC BY 4.0.



"Raoultella planticola in SIM agar, showing a negative result for H2S production, a negative result for the indole test (after Kovac's reagent has been added), and a negative result for motility." Source: A doubt, Wikimedia Commons, CC BY 4.0.



"*Escherichia coli* in SIM agar, showing a negative result for H2S production, a positive result for the indole test (after Kovac's reagent has been added), and a positive result for motility." Source: A doubt, Wikimedia Commons, CC BY 4.0.



"Pseudomonas fluorescens in SIM agar, showing a negative result for H2S production, a negative result for the indole test (after Kovac's reagent has been added), and a positive result for motility." Source: A doubt, Wikimedia Commons, CC BY 4.0.

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3.3.2: SIM Medium Test Activity

In this activity you will test five bacterial samples.

1. Turn on the Bunsen burner and sterilize the inoculating needle by passing it through the flame of the burner.
2. Gently raise the lid to the agar plate containing the bacterial culture to be sampled and touch the inoculating needle to a bacterial colony to obtain a bacterial sample.
3. Pick up the SIM tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
4. Insert the inoculating needle by stabbing straight down into the media all the way to the bottom of the media, and then gently remove the inoculating needle so as not to disturb the stab mark.
5. Pass the mouth of the SIM tube through the flame and recap it, and return it to the test tube rack.
6. Sterilize the inoculating needle by flaming the loop in the flame of the Bunsen burner. Repeat steps 1 - 8 for the other four bacterial species and SIM tubes to be inoculated.
7. Incubate the inoculated SIM tubes at 37°C for 24 hours.
8. At 24 hours add 5 – 6 drops of Kovac's reagent to each of the incubated SIM agar tubes.
9. After approximately 1 minute observe the SIM tubes for color changes, precipitation formation, and cracks in the media. Use the chart as a guide and record your results in the table below.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	H_2S Production (Color and Test Result)	Indole (Color and Test Result)	Motility Test (Growth pattern and Test Result)

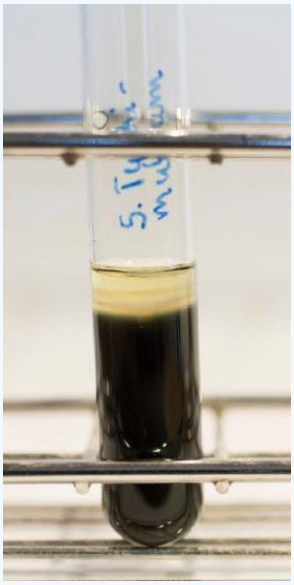
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3.3.3: Exercise #14 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What enzyme is produced by a bacterium that turns pink after the addition of Kovac's reagent? What does the enzyme do?
2. Why is it important that this agar have a lower concentration of agar than that used to make solid media agars?
3. How is the ability of a bacterium to produce hydrogen sulfide gas accomplished using SIM media?
4. How is a SIM test inoculated and why is it that this method of inoculation is needed?
5. Examine the image below of a SIM test. Explain the results of the test based on your observations.



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3.4: Exercise 15 - IMViC Tests

Exercise Outcomes

Course Laboratory Learning Outcomes

- THECB - ACGM Lab Learning Outcome #1: Use and comply with laboratory safety rules, procedures, and universal precautions.
- THECB - ACGM Lab Learning Outcome #4: Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.
- THECB - ACGM Lab Learning Outcome #5: Use different types of bacterial culture media to grow, isolate, and identify microorganisms.
- THECB - ACGM Lab Learning Outcome #6: Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. List the tests that are part of an IMViC panel of tests.
2. Explain what group of bacteria these tests aid in identifying.
3. Explain the purpose of the indole test.
4. Explain key ingredients or reagents associated with the indole test.
5. Interpret the results of the indole test.
6. Explain the purpose of the methyl red test.
7. Explain key ingredients or reagents associated with the methyl red test.
8. Interpret the results of the methyl red test.
9. Explain the purpose of the Voges-Proskauer test.
10. Explain key ingredients or reagents associated with the Voges-Proskauer test.
11. Interpret the results of the Voges-Proskauer test.
12. Explain the purpose of the citrate test.
13. Explain key ingredients or reagents associated with the citrate test.
14. Interpret the results of the citrate test.

IMViC Tests

The ***IMViC tests*** are a panel of tests that include the indole test (I), the methyl red test (M), the Voges-Proskauer test (Vi), and the citrate test (C). These tests are performed in order to differentiate members of the Enterobacteriaceae family.

Members of this family are commonly known as enterobacteria or enteric bacteria. This is because many members of this family reside within the intestines of humans or animals. The enterobacteria are gram-negative bacilli. While morphologically very similar, they can be distinguished by their biochemical properties. While most members of this family are not pathogenic, some do act as pathogens. Some medically important members of this family include bacteria such as *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., and *Escherichia coli*.

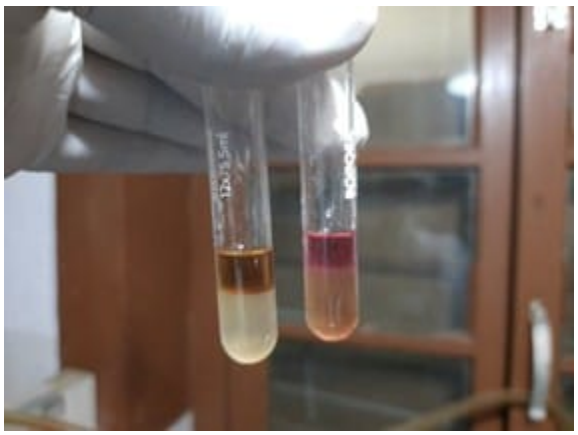
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3.4.1: Indole Test

The ***indole test*** is performed in order to assess a bacterium's ability to produce the enzyme tryptophanase. Tryptophanase converts the amino acid tryptophan to indole, pyruvic acid, and ammonia.

The presence of indole can be detected by adding Kovac's reagent. Kovac's reagent reacts with the indole to produce a red compound. Therefore, when Kovac's reagent is added to the medium and a red color change occurs it is indicative that the bacterium is capable of producing tryptophanase.

Indole Test Results	
Indole Positive (tryptophanase present)	**Indole Negative (tryptophanase not present)**
Examples: *Escherichia coli* *Proteus vulgaris*	Examples: *Enterobacter aerogenes* *Klebsiella pneumoniae* *Salmonella typhimurium*



"This indole test is useful for both aerobic and anaerobic gram-negative rods and really very useful in the routine identification of bacteria in Microbiology. Development of a brown-red to purple-red color - Test Positive (presence of indole) (right test tube), while colorless or slightly yellow- Negative (left test tube)." Source: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide names of species to be tested)
- Tryptone broth tube (2 tubes)
- Kovac's reagent
- Test tube rack

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3.4.2: Indole Test Activity

In this activity you will test two bacterial samples. Label each tryptone broth tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the tryptone broth tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the tryptone broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 8 for the second bacterial species and tryptone broth tube to be inoculated.

9. Incubate the inoculated tryptone broth tubes at 37°C for 24 hours.
10. At 24 hours add 5 – 6 drops of Kovac's reagent to each of the incubated tryptone broth tubes. Observe the top layer for color changes after approximately 1 minute.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color after addition of Kovac's reagent	Positive or Negative Result

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3.4.3: MRVP

MRVP broth is a broth utilized to perform two tests: the Methyl Red Test and the Voges-Proskauer test. These two tests are performed in order to differentiate members of the Enterobacteriaceae. Some members of the Enterobacteriaceae are capable of carrying out mixed-acid fermentation,

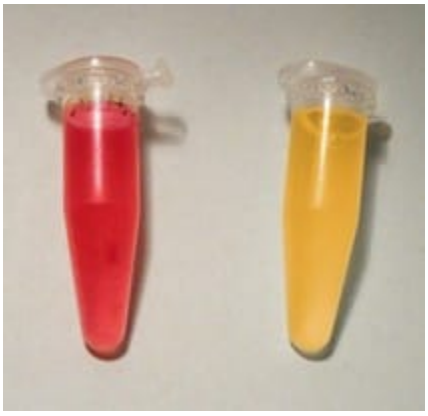
which results in the production of stable acid end products. Other members of this group do not carry out mixed-acid fermentation, but instead ferment glucose to produce a more neutral end product 2,3-butanediol. MRVP broth contains peptone, glucose, and a phosphate buffer. The bacterium is inoculated into the MRVP broth and then the broth is split to perform the two tests.

Methyl Red Test

The ***methyl red test*** is performed to determine if a bacterium is able to produce acid-end products as a result of the mixed-acid fermentation of glucose. The pH indicator methyl red is used to detect the presence of acids. Methyl red is red at pH 4.4 and yellow at pH 6.2. A positive methyl red test indicates that the organism is capable of mixed-acid fermentation.

In order to perform the Methyl Red test, a bacterium is introduced to a tube of MRVP broth using a sterile inoculating loop. The broth is then incubated for a period of 2-5 days. Following incubation, the pH indicator methyl red is introduced to the tube and the tube is observed for color changes. Examples of methyl red-positive bacteria include *Escherichia coli* and *Proteus vulgaris*.

Methyl Red Test Results	
Methyl Red Positive (positive for mixed-acid fermentation)	**Methyl Red Negative (negative for mixed acid fermentation)**
Examples: *Escherichia coli* *Proteus vulgaris* *Salmonella typhimurium*	Examples: *Enterobacter aerogenes* *Klebsiella pneumoniae*



Escherichia coli (left), showing a positive result, and Enterobacter cloacae (right) showing a negative result. Source: A doubt, Wikimedia Commons, CC BY 3.0.

Voges-Proskauer Test

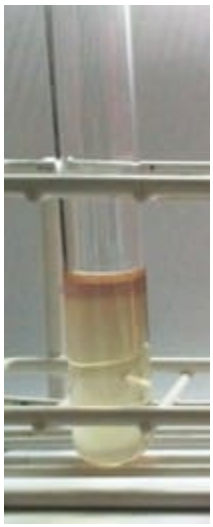
The ***Voges-Proskauer test*** is performed to identify the ability of a bacterium to produce acetoin from the catabolism of glucose. Some bacteria can produce neutral end-products such as

2,3-butanediol. These neutral end-products cannot readily be detected but can be converted to a product, acetoin via oxidation, which can then be observed with the addition of Barritt's reagent. Barritt's reagent (alpha-naphthol and KOH) reacts with acetoin to create a product that produces a red color. Thus, a color change from yellow to red indicates that neutral end products can be formed by the bacterium.

Voges-Proskauer (VP) Test Results	
VP Positive (positive for the production of neutral-end products)	**VP Negative (negative for the production of neutral end products)**
Examples: *Enterobacter aerogenes* *Klebsiella pneumoniae*	Examples: *Escherichia coli* *Proteus vulgaris* *Salmonella typhimurium*



Positive Voges-Proskauer result. Source: A doubt, Wikimedia Commons, CC BY 3.0.



Negative Voges-Proskauer result. Source: A doubt, Wikimedia Commons, CC BY 3.0.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide names of species to be tested)
- MRVP broth tubes (2 tubes)
- 2 sterile culture tubes
- Methyl red reagent
- Barritt's reagent A

- Barritt's reagent B
- Test tube rack

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3.4.4: Methyl Red (MR) and Voges-Proskauer (VP) Test Activity

In this activity you will test two bacterial samples. Label each MRVP broth tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the MRVP broth tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the MRVP broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 8 for the second bacterial species and MRVP broth tube to be inoculated.

9. Incubate the inoculated MRVP broth tubes at 37°C for 48 hours.
10. At 48 hours transfer 2.5 mL of each broth to new labeled sterile culture tubes. Methyl Red test:
11. To each of the new culture tubes add 5 drops of the methyl red reagent and then observe the color for the methyl red test and record your results.

Voges-Proskauer test:

12. In the remaining 2.5 ml of each culture add 0.6 mL of Barritt's reagent A and 0.2 ml of Barritt's reagent B. Shake the tube for 30 seconds to 1 minute. Wait 30 minutes and then observe the color of each tube and record your results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color after methyl red reagent	Methyl Red Test Results	Color after Barritt's Reagents A & B	Voges-Proskauer Test Results

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3.4.5: Citrate Test

The ***citrate utilization test*** is performed in order to determine if a bacterium is able to use citrate as a sole source of carbon. Citrate (citric acid) can be transported into some bacterial cells that have the enzyme citrate permease. Such bacteria are capable of importing citrate into their cells and then convert the citrate to pyruvate to be utilized as a carbon source. The citrate test is performed using Simmons Citrate agar, which contains sodium citrate as the only carbon source in the media, ammonium phosphate as a nitrogen source, and bromothymol blue as a pH indicator. Bromothymol blue is green at pH 6.9 and blue at 7.6. Bacteria capable of using citrate as a source of carbon produce sodium carbonate. These organisms are also able to metabolize the ammonium salts present in the media converting them to the alkaline product ammonia. Both the sodium carbonate and ammonia raise the pH of the media resulting in a color change from green to blue, indicating that the bacteria can use citrate as a carbon source.

Citrate Test Results	
Positive for Citrate Utilization	**Negative for Citrate Utilization**
Examples: *Enterobacter aerogenes* *Klebsiella pneumoniae* *Salmonella typhimurium*	Examples: *Escherichia coli* *Proteus vulgaris*



Citrate test - Negative citrate test result (left); positive citrate test result (right). Source: Jnjoffin, Wikimedia Commons, CC BY 4.0

Materials Needed:

- Bunsen burner
- Inoculating needle
- Bacterial agar cultures (2 different species - instructor will provide names of species to be tested)
- Simmons Citrate Agar Slants (2 tubes)
- Test tube rack

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3.4.6: Citrate Test Activity

In this activity you will test two bacterial samples. Label each Simmons citrate agar slant tube with the sample it is to be inoculated with.

1. Sterilize the inoculating needle by placing it in the Bunsen burner, then allow it to cool for 15 – 30 seconds.
2. Obtain a sample of bacteria #1 by touching a colony of the bacteria on the agar plate using a aseptic technique.
3. Pick up the Simmons Citrate Agar Slant labeled tube #1, remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
4. Insert the needle with the bacterial sample into the tube, stabbing into the bottom of the media and then gently streak up the surface of the media.
5. Pass the mouth of the tube through the flame, recap the tube and return it to the test tube rack.
6. Sterilize the inoculating loop.

Repeat steps 2 – 7 for bacterial sample #2.

7. Place the rack with both Simmons Citrate Agar Slant tubes in an incubator at 37°C for 24 hours.
8. After 24 hours observe your samples to determine your results and record your data in the table below.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color after Incubation	Citrate Test Results

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3.4.7: Exercise #15 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What test is performed to determine if members of the Enterobacteriaceae family can metabolize glucose producing neutral end products? How are those products detected in this test?
2. What is the function of the enzyme citrate permease?
3. Describe the appearance of a positive citrate test. Describe what has occurred in the media to cause the appearance?
4. You perform a methyl red test on an unknown bacterial sample. Following the addition of the methyl red reagent you observe your sample and note that it is yellow in color. What does this tell you about the unknown bacterium?
5. You perform a biochemical test in which you just added Kovac's reagent. You observe a red color at the top of the test tube. 1) What test has been performed? 2) Interpret the results of this test. 3) What does this tell you the bacterium that has been tested?

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3.5: Exercise #16 - Respiration Tests

Exercise Outcomes

Course Laboratory Learning Outcomes

- THECB - ACGM Lab Learning Outcome #1: Use and comply with laboratory safety rules, procedures, and universal precautions.
- THECB - ACGM Lab Learning Outcome #4: Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.
- THECB - ACGM Lab Learning Outcome #5: Use different types of bacterial culture media to grow, isolate, and identify microorganisms.
- THECB - ACGM Lab Learning Outcome #6: Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the oxidase test.
2. Explain key ingredients or reagents associated with the oxidase test.
3. Interpret the results of the oxidase test.
4. Explain the purpose of the catalase test.
5. Explain key ingredients or reagents associated with the catalase test.
6. Interpret the results of the catalase test.
7. Explain the purpose of the nitrate reductase test.
8. Explain key ingredients or reagents associated with the nitrate reductase test.
9. Interpret the results of the nitrate reductase test.

Respiration Tests

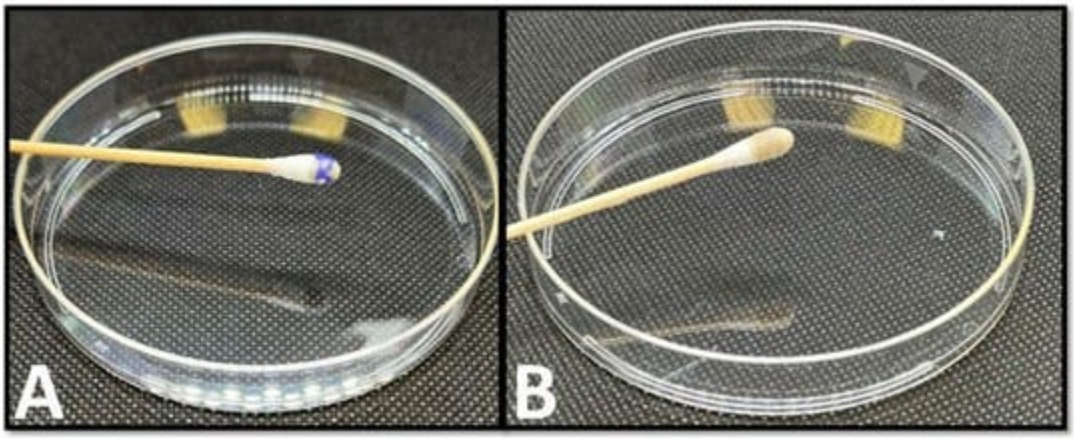
There are several tests utilized in the microbiology laboratory to assess the ability of microorganisms to carry out cellular respiration. Cellular respiration is the conversion of glucose to energy in the form of ATP, utilizing glycolysis, the citric acid cycle, and the electron transport chain. The electron transport chain requires the use of an inorganic molecule as a final electron acceptor. Some organisms use oxygen as their final electron acceptor while others utilize other inorganic molecules. Organisms that utilize oxygen as their final electron acceptor are known as aerobes and reduce the oxygen to produce water or other products. On the other hand, organisms that utilize other inorganic molecules such as nitrate, sulfate, or carbonate, are known as anaerobes, and produce a variety of products. Tests that are designed to assess an organism's ability to carry out cellular respiration assess the products that are formed as a result of the reduction of the final electron acceptor.

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3.5.1: Oxidase Test

The ***oxidase test*** is performed to determine if a bacterium contains the enzyme cytochrome c oxidase. Cytochrome c oxidase is an enzyme located at the end of the aerobic electron transport chain, which utilizes electrons from the electron transport chain to reduce molecular oxygen to water. The oxidase test makes use of a reduced reagent to detect the presence of cytochrome oxidase. The reagent used is tetramethyl-p-phenylenediamine (TMPD) which is colorless when it is reduced and changes to purple when oxidized. This reagent can be applied directly to a bacterial colony on solid medium or test strips containing the reagent may be utilized, to which a bacterial sample is introduced. If cytochrome c oxidase is present, it will oxidize the reagent causing it to turn a deep purple/blue color.

Oxidase Test Results	
Positive for Cytochrome c Oxidase	**Negative for Cytochrome c Oxidase**
Examples: *Pseudomonas aeruginosa* *Micrococcus luteus* *Alcaligenes faecalis*	Examples: *Escherichia coli* *Proteus vulgaris* *Enterococcus faecalis*



Oxidase Test - (A) Oxidase positive; (B) Oxidase negative. Credit: Sherry Smith

Materials Needed:

- Staining tray
- Sterile cotton swabs (2)
- Bacterial cultures (2 different species - instructor will provide names of species to be tested)
- Oxidase reagent

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3.5.2: Oxidase Test Activity

In this activity you will test two bacterial samples.

1. Obtain a sterile cotton swab and aseptically remove it from its packaging.
2. Gently raise the lid to the agar plate containing the bacterial culture to be sampled and touch the swab to several bacterial colonies to obtain a bacterial sample.
3. Hold the cotton swab with the bacterial culture over the staining tray and apply 2 – 3 drops of oxidase reagent to the cotton swab.
4. Wait for 15 – 30 seconds, observe the color of the swab, and record the results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color after oxidase reagent	Oxidase Test Results

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3.5.3: Catalase Test

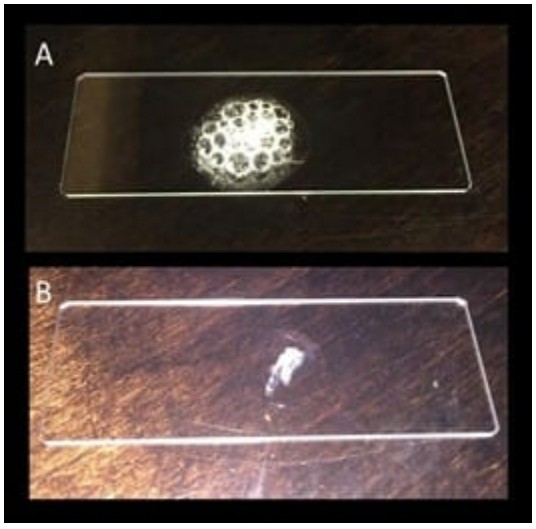
The electron transport chains of organisms capable of carrying out aerobic cellular respiration, produce two highly potent cellular toxins known as superoxide radical (O-) and hydrogen peroxide (H2O2). These products result from one of the electron carriers by-passing the last carrier and instead passing the electrons directly to oxygen. Organisms that perform aerobic cellular respiration produce enzymes (superoxide dismutase and catalase) that can break down the toxic by-products produced during cellular respiration. Superoxide dismutase converts the superoxide radical to hydrogen peroxide, while catalase converts hydrogen peroxide into water and gaseous oxygen.

Microorganisms that produce catalase are easily detected by introducing hydrogen peroxide to a culture of the organism. In the presence of hydrogen peroxide gas bubbles will immediately form if the organism is catalase positive. Organisms that lack catalase will not form any bubbles and are therefore catalase negative.

The ***catalase test*** is used to differentiate *Staphylococci* and *Micrococci* (catalase-positive) from *Streptococci* and *Enterococci* (catalase-negative). Catalase-positive bacteria include strict aerobes as well as facultative anaerobes, which all have the ability to respire using oxygen as a terminal electron acceptor. Catalase-negative bacteria may be anaerobes, or they may be aerotolerant anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (i.e., *Streptococci*).

Catalase Test Results

Catalase positive	Catalase negative
Examples:	Examples:
Staphylococcus aureus	*Streptococcus pyogenes*
Staphylococcus epidermidis	*Streptococcus salivarius*
Micrococcus luteus	*Streptococcus pneumoniae*
	Enterococcus faecalis



Catalase Test - (A) Catalase positive; (B) Catalase negative. Credit: Sherry Smith

Materials Needed:

- Bunsen burner
- Inoculating loop
- Staining tray with stain rack
- Microscope slides (2)
- Bacterial agar cultures (2 different species - instructor will provide names of species to be tested)
- Hydrogen peroxide (H_2O_2)

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3.5.4: Catalase Test Activity

In this activity you will test two bacterial samples.

1. Obtain a clean dry microscope slide and place it on a stain rack in a staining tray.
2. Place two drops of H2O2 onto the glass slide.
3. Sterilize the inoculating loop using the Bunsen burner and allow the loop to cool for 15-30 seconds.
4. Aseptically acquire a colony of bacteria from one of the provided bacterial samples.
5. Tap the sample into the drop of H2O2while the slide remains on the staining rack.
6. Sterilize the inoculating loop using the Bunsen burner.
7. Observe the H2O2 and bacterial sample for any signs of bubbling. Record your results in the table below.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Bubbles Present (Yes/No)	Catalase Test Results

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3.5.5: Nitrate Reduction Test

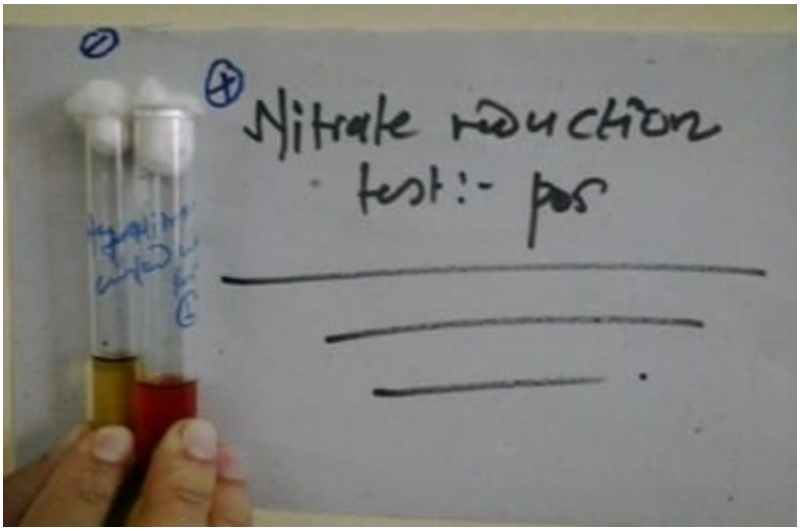
The ***nitrate reduction test*** is performed to determine if a bacterium can reduce nitrate, using the enzyme nitrate reductase. Nitrate reductase is an enzyme located within the electron transport chain of some bacteria that perform anaerobic cellular respiration. Bacteria containing nitrate reductase can utilize nitrate as a final electron acceptor in their electron transport chains. Nitrate can be reduced to produce nitrite ions, nitrous oxide, or nitrogen gas.

This test utilizes a nitrate broth, which is composed of beef extract, peptones, and potassium nitrate. The tube will have a Durham tube present to trap any gas produced during nitrate reduction. The broth is inoculated, incubated, and then observed for changes.

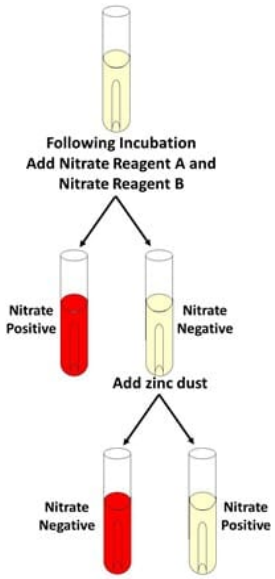
After 48 hours the tube is observed for gas production in the Durham tube. Reagents (sulfanilic acid and alpha-naphthylamine) are then added to the broth to detect the presence of nitrite. After 2 minutes if a red color appears the sample contains nitrite, indicating that nitrate has been reduced. If the tube is clear after two minutes, zinc dust is added. If nitrate is still present in the medium zinc will reduce it to nitrite causing the medium to change red/pink. This is a negative result for nitrate reduction. If there is no color change after the addition of zinc, then the nitrate has been reduced to some form other than nitrite.

Nitrate Reduction Test Results

Positive for Nitrate Reduction	Negative for Nitrate Reduction
Examples: *Enterobacter aerogenes* *Escherichia coli* *Staphylococcus aureus*	Examples: *Staphylococcus epidermidis* *Enterococcus faecalis* *Alcaligenes faecalis*



"A positive nitrate reduction test suggests that the bacterium possesses the enzyme nitrate reductase, which is responsible for the reduction of nitrate to nitrite. It's important to note that some bacteria, including *Mycobacterium tuberculosis*, are slow nitrate reducers, and the test may require extended incubation periods up to several weeks) to yield a positive result." Source: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0



Nitrate reductase test procedure. Credit: Sherry Smith

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (3 different species - instructor will provide names of species to be tested)
- Nitrate broth tubes (3 tubes)
- Nitrate reagent A
- Nitrate reagent B
- Zinc powder
- Test tube rack

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3.5.6: Nitrate Reduction Test Activity

In this activity you will test three bacterial samples. Label each nitrate broth tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the nitrate broth tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the nitrate broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 8 for the second and third bacterial species and nitrate broth tubes to be inoculated.

9. Incubate the inoculated nitrate broth tubes at 37°C for 24 hours.
10. For each nitrate broth tube, add 5 drops of nitrate reagent A, 5 drops of nitrate reagent B, and mix well. Observe the tubes for a color change.

If the tube remains clear

11. Using a spatula add a small quantity (just covering the tip) of zinc dust to the sample. Mix and observe for color change.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color after nitrate reagents A & B	Zinc dust added (Yes or Not required)	Color after addition of zinc	Nitrate Reduction Test Results

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3.5.7: Exercise #16 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What genera of bacteria can be distinguished based on their catalase results? List the expected results for each of the genera.
2. What does the enzyme catalase do for those bacteria capable of producing it?
3. You are performing a nitrate reduction test to determine if nitrite reductase is present. Two minutes after the addition of sulfanilic acid and alpha-naphthylamine, your tube remains clear. Can you safely conclude that nitrate reductase is not present at that point? Explain your answer.
4. You perform a biochemical test in which you have added the reagent, tetramethyl-p-phenylenediamine. After the addition of the reagent a purple color is observed. What test has been performed? What do your results indicate?

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3.6: Exercise #17 - Urease Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the urease test.
2. Explain key ingredients or reagents associated with the urease test.
3. Interpret the results of the urease test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Agar bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Urea agar tube (2 tubes)
- Test tube rack

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3.6.1: Urease Test

The ***urease test*** is performed to determine if a bacterium can produce urease. Urea is a metabolic waste product produced as a result of protein catabolism. Some bacteria can hydrolyze urea releasing ammonia and carbon dioxide. These bacteria use the enzyme urease to accomplish this hydrolysis, freeing ammonia to be used as a nitrogen source in the bacterium's metabolism.

The test is performed using urea broth, which contains urea, growth factors, and the pH indicator phenol red. Phenol red is pink (fuchsia) above pH 8.2 and yellow at pH 6.8. The media has a pH of 6.8 and therefore begins as a yellow color. The medium is inoculated, incubated, and then observed. If the bacterium has urease the production of ammonia will cause the media to become more alkaline therefore causing the media to change to a pink (fuchsia) color.

Urease Test Results	
Positive for Production of Urease	**Negative for Production of Urease**
Examples:	Examples:
Proteus vulgaris	*Escherichia coli*
Klebsiella pneumoniae	*Enterobacter aerogenes*
Staphylococcus aureus	*Enterococcus faecalis*
Staphylococcus epidermidis	*Alcaligenes faecalis*



Urease Test - (A) Urease positive; (B) Urease negative. Credit: Sherry Smith

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3.6.2: Urease Test Activity

In this activity you will test two bacterial samples. Label each urea agar tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating needle by passing it through the flame of the burner.
2. Gently raise the lid to the agar plate containing the bacterial culture to be sampled and touch the inoculating needle to a bacterial colony to obtain a bacterial sample.
3. Pick up the urea agar tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
4. Insert the inoculating needle by stabbing straight all the way down into agar. Pull the needle back out slowly without disturbing the stab
5. Pass the mouth of the urea agar tube through the flame and recap it, and return it to the test tube rack.
6. Sterilize the inoculating needle by flaming the loop in the flame of the Bunsen burner. Repeat steps 1 - 8 for the second bacterial species and urea agar tube to be inoculated.
7. Incubate the inoculated urea agar tubes at 37°C for 24 hours.
8. Observe the urea agar tube for color changes. Record your results in the table below.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Color of urea agar after incubation	Urease Test Results

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3.6.3: Exercise #17 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What product is produced when the enzyme urease acts on its substrate urea?
2. You inoculate a urea test medium with an unknown sample. After incubation you observe that the media has turned a pink (fuchsia) color. 1) What has happened to the pH of the media? 2) Why has the pH of the media changed? 3) What do these results mean?

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3.7: Exercise #18 - Coagulase Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the coagulase test.
2. Explain key ingredients or reagents associated with the coagulase test.
3. Interpret the results of the coagulase test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Agar bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Rabbit plasma culture tube (2 tubes)
- Test tube rack

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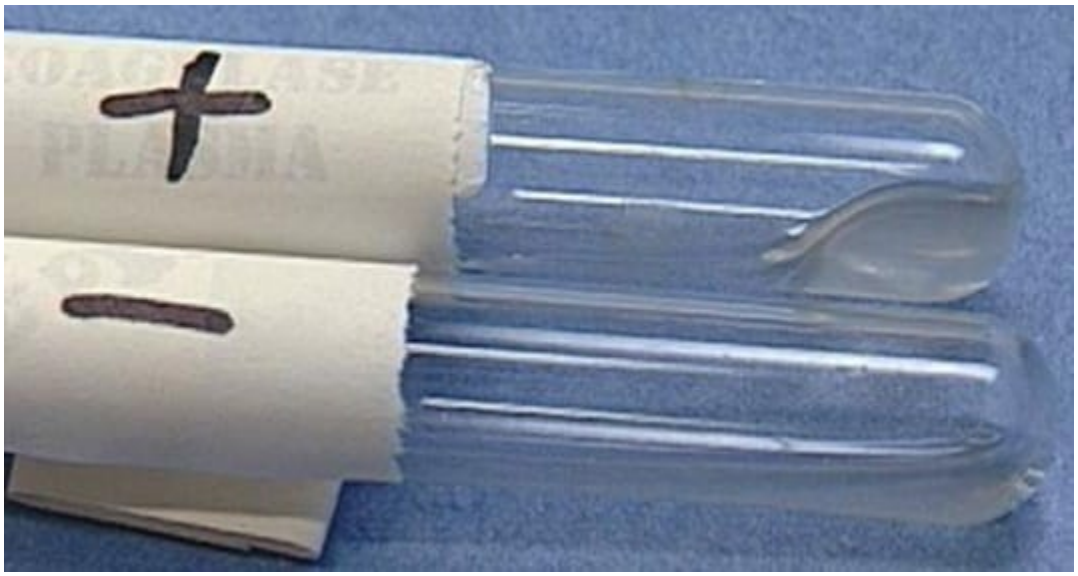
3.7.1: Coagulase Test

Coagulase is an exoenzyme produced by some species within the genera *Staphylococcus*, including *Staphylococcus aureus*. Coagulase contributes to the virulence of such species by permitting the bacteria to clot blood. The enzyme interacts with the enzyme prothrombin which then converts fibrinogen to fibrin in the plasma which in turn forms the clot. It has been hypothesized that the bacteria capable of doing this are protected by the network of fibrin proteins, preventing their phagocytosis by the immune cells of the host.

This test can assist in differentiating *Staphylococcus aureus* from other species of *Staphylococcus* such as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*.

The ***coagulase test*** is performed using rabbit plasma in a test tube. The bacteria is added to the media and then the media is observed to determine if the plasma coagulates.

Coagulase Test Results	
Coagulase positive	**Coagulase negative**
Examples: *Staphylococcus aureus* *Staphylococcus intermedius*	Examples: *Staphylococcus epidermidis* *Staphylococcus haemolyticus*



Coagulase test - Top culture tube shows a positive tube, bottom culture tube shows a negative result. Source: isis325, flickr, CC BY 2.0.

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3.7.2: Coagulase Test Activity

In this activity you will test two bacterial samples. Label each coagulase tube with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the rabbit plasma tube to which the sample is to be transferred. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the rabbit plasma tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 8 for the second bacterial species and rabbit plasma tubes to be inoculated.

9. Incubate the inoculated coagulase tubes at 37°C for 24 hours.
10. After 24 hours observe the tube for evidence of clot formation, any clumping.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	Coagulase Test Results

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3.7.3: Exercise #18 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Why is the coagulase test clinically important? What information is obtained?
2. A rabbit plasma tube was inoculated with an unknown bacterial sample isolated from a patient's infected wound. After incubation the tube is observed to have some clumping occurring within the plasma. What can be concluded from these results?

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3.8: Exercise #19 - Casease Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the casease test.
2. Explain key ingredients or reagents associated with the casease test.
3. Interpret the results of the casease test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Skim milk agar plate (2 plates)
- Test tube rack

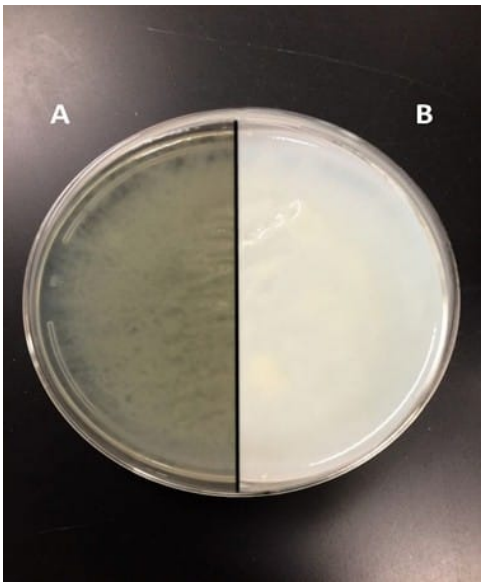
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3.8.1: Casease Test

The purpose of the ***casease test*** is to determine if a bacterium can hydrolyze the milk protein casein. Casein is a protein found in milk that is responsible for giving milk its white or cloudy appearance.

Some bacteria can produce the enzyme casease, which is used to catabolize the protein into smaller peptides that can then be taken up by the cell and used for the cell's metabolism. This test is performed by inoculating the bacteria onto a skim milk agar. If the bacteria have the enzyme casease, they will hydrolyze the casein causing the area around the bacteria to become clear.

Casein Hydrolysis Results	
Positive Casein Hydrolysis Results	**Negative Casein Hydrolysis Results**
Examples: *Bacillus subtilis* *Bacillus cereus*	Examples: *Escherichia coli* *Acinetobacter baumannii* *Salmonella enterica*



Casease Test - This digitally enhanced photo is the combined results of bacteria that are able to externally hydrolyze the milk protein, casein (side A), and bacteria that are unable to hydrolyze casein (side B). Source: Eunice Laurent, Wikimedia Commons, CC BY 4.0

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3.8.2: Casease Test Activity

In this activity you will test two bacterial samples. Label each plate with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Aseptically transfer the sample of bacteria to the surface of the skim milk agar plate, introduce the sample by streaking back and forth in the center of the plate to form a broad streak down the center of the surface of the plate.
6. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.
7. Invert your freshly streaked plate to prevent condensation from raining down on the surface of the agar. Repeat steps 1 - 8 for the second bacterial species and skim milk agar plate to be inoculated.
8. Incubate the inoculated skim milk agar plates in an inverted position at 37°C for 24 hours.
9. After 24 hours observe the plate and record your results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	Casease Test Results

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3.8.3: Exercise #19 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What is casein and what attribute does it impart to milk?
2. Describe the action of the enzyme casease.
3. Describe how a positive result is detected/determined when using a skim milk agar plate to assess the presence of casease.

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3.9: Exercise #20 - Starch Hydrolysis Test

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of the starch hydrolysis test.
2. Explain key ingredients or reagents associated with the starch hydrolysis test.
3. Interpret the results of the starch hydrolysis test.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Starch agar plates (2 plates)
- Test tube rack

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3.9.1: Starch Hydrolysis Test

Starch, also known as amylose, is a polysaccharide composed of many glucose monomers. The ***starch hydrolysis test*** is performed in order to determine if a bacterium can hydrolyze starch. Bacteria capable of hydrolyzing starch produce the enzyme amylase, which breaks the starch down releasing glucose.

A starch agar plate is inoculated with the bacterium. After incubation iodine is added to the plate. Iodine binds with starch producing a deep blue-black color. If there is a clear area around the bacterium then starch has been broken down to release glucose. A clear zone indicates positive starch hydrolysis.

Starch Hydrolysis Agar Results	
Positive Starch Hydrolysis Results	**Negative Starch Hydrolysis Results**
Examples: *Bacillus subtilis* *Bacillus stearothermophilus* *Streptococcus bovis* *Clostridium perfringens*	Examples: *Escherichia coli* *Clostridium difficile* *Clostridium botulinum*



Starch hydrolysis interpretation. Source: Rosanna Hartline (RosieScienceGal), Wikimedia Commons, CC BY 4.0

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3.9.2: Starch Hydrolysis Test Activity

In this activity you will test two bacterial samples. Label each plate with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Aseptically transfer the sample of bacteria to the surface of the starch agar plate, introduce the sample by streaking back and forth in the center of the plate to form a broad streak down the center of the surface of the plate.
6. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.
7. Invert your freshly streaked plate to prevent condensation from raining down on the surface of the agar. Repeat steps 1 - 7 for the second bacterial species and starch agar plate to be inoculated.
8. Incubate the plates in an inverted position at 37°C for 24 hours.
9. After 24 hours apply enough iodine to cover the surface of the plate. Observe the plate and record your results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	Starch Hydrolysis Test Results

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3.9.3: Exercise #20 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What reagent is used to determine if the bacteria have hydrolyzed starch?
2. What enzyme is responsible for hydrolyzing starch and what product results from the action of the enzyme?
3. Describe how a positive result is detected/determine when performing the starch hydrolysis test.

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3.10: Exercise #21 - Blood Agar

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the key ingredients or reagents associated with a blood agar plate.
2. Define fastidious bacteria and their relation to blood agar plates.
3. Describe what occurs during each of the following types of hemolysis and how they would appear when utilizing a blood agar plate.
    1. alpha hemolysis
    2. beta hemolysis
    3. gamma hemolysis
4. Interpret the type of hemolysis for bacterial samples when using a blood agar plate.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Bacterial cultures (3 different species - instructor will provide name of species to be tested)
- Blood agar plate (3 plates)
- Test tube rack

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3.10.1: Blood Agar Test

Blood agar is used to grow fastidious bacteria and to differentiate bacteria based on their blood lysis capabilities. ***Fastidious*** bacteria are bacteria that require a significant number of growth factors (vitamins, essential amino acids, etc.). Blood is an excellent source of nutrients to meet the needs of bacteria that have complex nutritional requirements; thus, this type of media can be utilized to grow fastidious bacteria.

Some bacteria are capable of producing enzymes known as hemolysins. ***Hemolysins*** cause the lysis of blood cells and may also cause the destruction of hemoglobin. There are three types of hemolysis that may be differentiated using blood agar: alpha (α) hemolysis, beta (β) hemolysis, and gamma (γ) hemolysis.

Alpha-hemolysis is a partial destruction of the red blood cell. Bacteria capable of alpha hemolysis, such as *Streptococcus pneumoniae*, do not lyse the red blood cells but rather produce hydrogen peroxide that interacts with heme producing a green discoloration of the media around the colonies. The cell membranes of the red blood cells remain intact, therefore alpha-hemolysis is not a "true" lysis of the blood cell.

Beta-hemolysis is the result of the action of hemolysins. *Streptococcus pyogenes* species are among the group of bacteria that can produce these hemolysins. There are two different hemolysins that can be produced; Streptolysin O, which is oxygen sensitive and Streptolysin S, which is stable in the presence of oxygen. These streptolysins function to completely lyse the red blood cell. This type of hemolysis leaves a clear area around the bacterial colonies and is a true lysis of the red blood cells.

Bacteria that are said to be ***gamma-hemolytic*** do not lyse red blood cells at all. Thus, the term gamma-hemolysis can be quite confusing for some. Gamma-hemolytic bacteria grow on the media but the media remains unchanged around the bacterial colonies. *Enterococcus faecalis* is an example of a bacterium that is gamma-hemolytic.

Blood Hemolysis Test Results

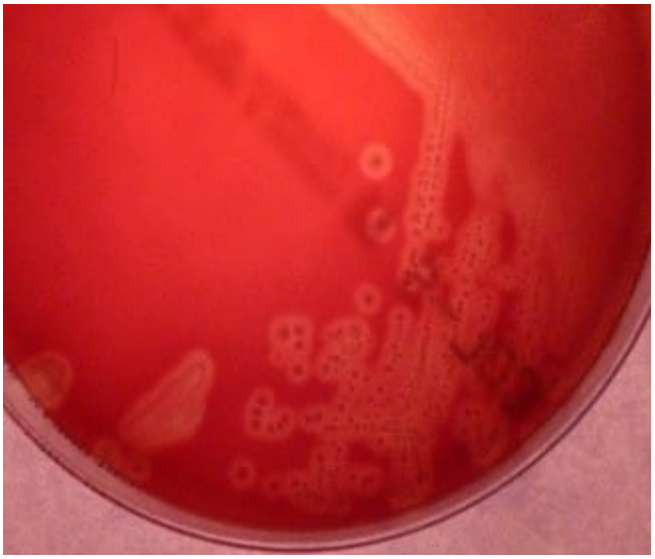
Alpha Hemolysis	Beta Hemolysis	Gamma Hemolysis
Examples: *Streptococcus pneumoniae* *Streptococcus mutans*	Examples: *Streptococcus pyogenes* *Streptococcus agalactiae*	Examples: *Enterococcus faecalis* *Enterococcus faecium*



Blood hemolysis types shown on a blood agar. Bacterial samples were inoculated in patterns of the Greek letters representing the type of hemolysis carried out by that bacterial species. Source: Y tambe, Wikimedia Commons, CC BY 2.0.



"Alpha hemolysis is observed where *Streptococcus pyogenes* is seen growing on the blood agar." Source: Nathan Reading, Wikimedia Commons, CC BY 2.0.



"*Streptococcus agalactiae* is observed to demonstrate beta hemolysis on the blood agar plate." Source: 43trevenque, Wikimedia Commons, CC BY 4.0.

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3.10.2: Blood Agar Test Activity

In this activity you will test three bacterial samples. Label each plate with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Aseptically transfer the sample of bacteria to the surface of the blood agar plate, introduce the sample by streaking back and forth in the center of the plate to form a broad down the center of the surface of the plate.
6. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.
7. Invert your freshly streaked plate to prevent condensation from raining down on the surface of the agar. Repeat steps 1 - 7 for the second and third bacterial species and blood agar plates to be inoculated.
8. Incubate the plates in an inverted position at 37°C for 24 hours.
9. After 24 hours observe the plate and record your results below.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	Blood Hemolysis Test Results

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3.10.3: Exercise #21 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Define fastidious bacteria and describe why blood agar may be utilized to grow them.
2. Describe the difference between alpha hemolysis and beta hemolysis. Describe what has occurred to cause the hemolysis observed.
3. You have been provided with a sample of bacteria to inoculate onto a blood agar sample. You are told that the organism you are working with is known to produce streptolysin O and streptolysin S. Describe what you should observe following incubation on a blood agar plate and why you expect those results.

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3.11: Exercise #22 MacConkey Agar

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of MacConkey agar.
2. Describe the ingredients that make MacConkey agar a selective media.
3. Describe why MacConkey agar is considered a selective media
4. Describe the ingredients that make MacConkey agar a differential media.
5. Describe why the MacConkey agar is considered a differential media.
6. Interpret the results when observing bacterial growth on MacConkey agar.

Materials Needed:

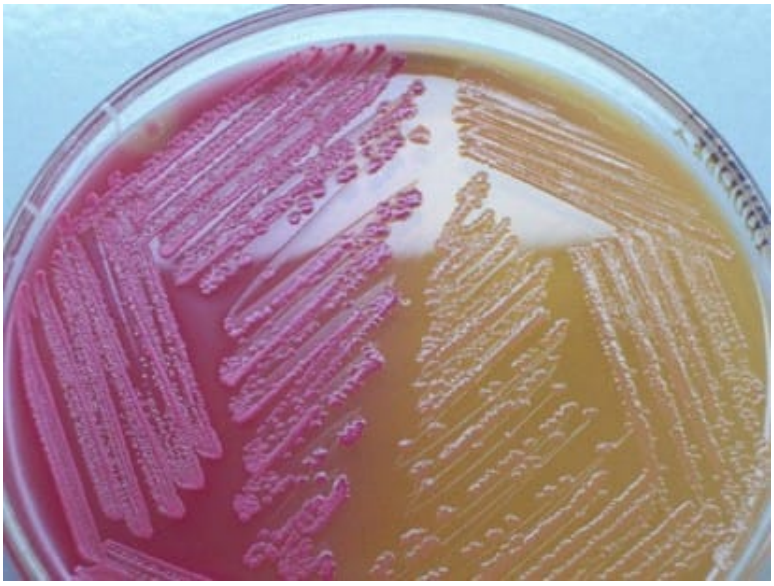
- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide name of species to be tested)
- MacConkey agar plate (2 plates)
- Test tube rack

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3.11.1: MacConkey Agar

MacConkey agar is both a selective and a differential media utilized in the differentiation of members of the Enterobacteriaceae family. The ingredients that cause this media to be selective are crystal violet and bile salts, which act to inhibit the growth of gram-positive bacteria. Therefore, only gram-negative bacteria will grow on MacConkey agar. The addition of lactose and the pH indicator neutral red allows the microbiologist to differentiate non-lactose fermenting Enterobacteriaceae from lactose fermenting Enterobacteriaceae. The pH indicator neutral red is clear above pH 6.8 and changes to red at a pH less than 6.8. The colonies of lactose fermenters appear red due to the accumulation of acids that result from the fermentation of the lactose. Non-lactose fermenting bacteria will appear cream, tan, or translucent.

MacConkey Agar Results	
Positive Lactose Fermentating *Enterobacteriaceae*	**Non-Lactose Fermentating *Enterobacteriaceae***
Examples: *Escherichia coli*. *Enterobacter spp.* *Klebsiella pneumoniae*	Examples: *Shigella spp.* *Salmonella spp.* *Serratia marcescens* *Yersinia pestis*



MacConkey agar - lactose fermenting bacteria seen on the left; non-lactose fermenting bacteria on the right. Source: Medimicro, Wikimedia Commons, Public Domain

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3.11.2: MacConkey Agar Activity

In this activity you will test two bacterial samples. Label each plate with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Aseptically transfer the sample of bacteria to the surface of the MacConkey agar plate, introduce the sample by streaking back and forth in the center of the plate to form a broad down the center of the surface of the plate.
6. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.
7. Invert your freshly streaked plate to prevent condensation from raining down on the surface of the agar. Repeat steps 1 - 7 for the second bacterial species and MacConkey agar plate to be inoculated.
8. Incubate the plates in an inverted position at 37°C for 24 hours.
9. After 24 hours observe the plate and record your results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	MacConkey Agar Test Results

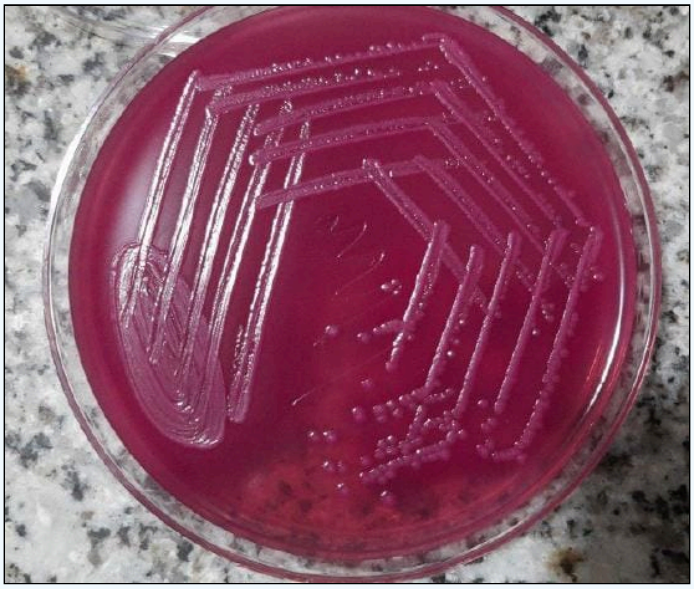
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3.11.3: Exercise #22 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What organisms does a MacConkey agar select for and why?
2. MacConkey agar is considered a differential medium. What can be differentiated using this media and why?
3. You inoculate a MacConkey agar plate and after incubation for 24 hours you observe the following plate. What can be concluded about the organism growing on the agar? Include all information that can be concluded.



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3.12: Exercise #23 - Mannitol Salt Agar

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #6 Perform basic bacterial identification procedures using biochemical tests.

Laboratory Exercise Learning Outcomes

1. Explain the purpose of mannitol salt agar.
2. Describe the ingredients that make mannitol salt agar a selective media.
3. Describe why mannitol salt agar is considered a selective media
4. Describe the ingredients that make mannitol salt agar a differential media.
5. Describe why the mannitol salt agar is considered a differential media.
6. Interpret the results when observing bacterial growth on mannitol salt agar.

Materials Needed:

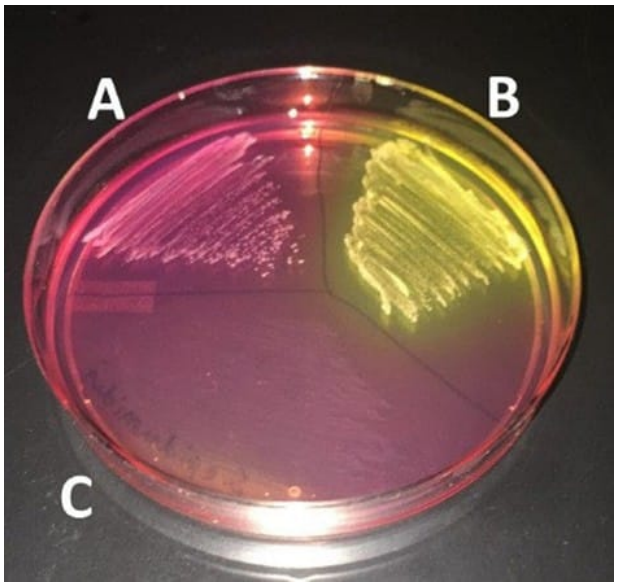
- Bunsen burner
- Inoculating loop
- Bacterial cultures (2 different species - instructor will provide name of species to be tested)
- Mannitol salt agar plate (2 plates)
- Test tube rack

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3.12.1: Mannitol Salt Agar Test

The ***mannitol salt agar*** is also a selective and differential media. This media contains 7.5% NaCl, which creates an environment on which only salt tolerant (***facultative halophiles***) can grow. This concentration of salt selects for organisms within the *Staphylococcus* genera, because most other bacteria cannot survive such high concentrations of salt. The media also contains the sugar mannitol and the pH indicator phenol red. These ingredients allow the microbiologists to differentiate between members of the *Staphylococcus* genus who are capable of fermenting mannitol and those who cannot. Pathogenic species of *Staphylococci* are capable of mannitol fermentation, whereas non-pathogenic species grow on the mannitol salt agar but do not ferment the mannitol.

Mannitol Salt Agar Results	
Positive Mannitol Fermenting Bacteria	**Non-Mannitol Fermenting Bacteria**
Examples: *Staphylococcus aureus* *Staphylococcus haemolyticus*	Examples: *Staphylococcus epidermidis*



Mannitol salt agar. A) *Staphylococcus epidermidis* - facultative halophile, negative for mannitol fermentation B) *Staphylococcus aureus* - facultative halophile, positive for mannitol fermentation C) No growth - *Escherichia coli* failed to grow - unable to tolerate salt concentration. Credit: Sherry Smith

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3.12.2: Mannitol Salt Agar Test Activity

In this activity you will test two bacterial samples. Label each plate with the sample it is to be inoculated with.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Aseptically transfer the sample of bacteria to the surface of the mannitol salt agar plate, introduce the sample by streaking back and forth in the center of the plate to form a broad down the center of the surface of the plate.
6. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.
7. Invert your freshly streaked plate to prevent condensation from raining down on the surface of the agar. Repeat steps 1 - 7 for the second bacterial species and mannitol salt agar plate to be inoculated.
8. Incubate the plates in an inverted position at 37°C for 24 hours.
9. After 24 hours observe the plate and record your results.

In the table below write the name of the species tested and the results for each of the tests performed.

Experimental Results

Bacterial Species	Appearance of media following incubation	Mannitol Salt Agar Test Results

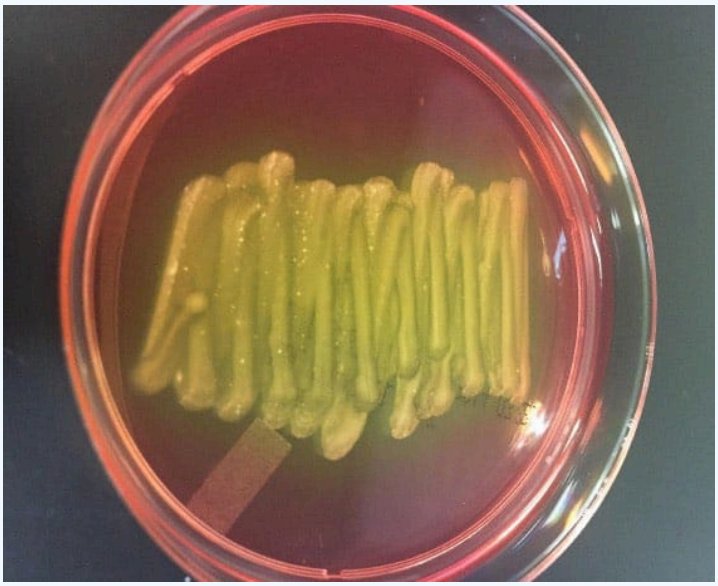
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3.12.3: Exercise #23 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What does a mannitol salt agar select for and why?
2. Mannitol salt agar is considered a differential medium, what cab be differentiated using this media and why?
3. You inoculate a Mannitol salt agar plate and after incubation for 24 hours you observe the following plate. What can be concluded about the organism growing on the agar? (Include all information that can be concluded.)



Credit: Sherry Smith

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3.13: Section Three Review

Review Purpose

This review helps you check what you learned in Section Three before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you...

Explain the purpose, identify the key reagents, and interpret the results for each biochemical test below?

- **Sugar utilization:** carbohydrate fermentation, triple sugar iron (TSI) agar
- **Multi-test media:** SIM, IMViC (indole, methyl red, Voges-Proskauer, citrate)
- **Respiration:** oxidase, catalase, nitrate reductase
- **Enzyme & hydrolysis:** urease, coagulase, casease, starch hydrolysis
- **Selective & differential agars:** blood, MacConkey, mannitol salt

And can you also...

- Name the group of bacteria the IMViC panel helps identify?
- Explain what coagulase is and how it relates to bacterial virulence?
- Define fastidious bacteria and distinguish alpha, beta, and gamma hemolysis on blood agar?
- Explain what makes MacConkey and mannitol salt agar each both selective and differential?

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3.13.1: Matching

Matching Questions

1. Match the enzyme on the left with the appropriate description of what it acts on and what it produces by placing the letter next to the correct term.

Question 1 Terms

Answer	Terms
_____	1. Tryptophanase
_____	2. Urease
_____	3. Casease
_____	4. Amylase
_____	5. Catalase
_____	6. Superoxide dismutase
_____	7. Nitrate reductase
_____	8. Cytochrome c oxidase
_____	9. Citrate permease
_____	10. Coagulase

Question 1 Descriptions

Letter	Description
a.	converts hydrogen peroxide into water and gaseous oxygen
b.	hydrolyzes urea, releasing ammonia and carbon dioxide
c.	transports citrate into the cell, where it is converted to pyruvate and used as a carbon source
d.	converts the amino acid tryptophan to indole, pyruvic acid, and ammonia
e.	reduces molecular oxygen to water at the end of the aerobic electron transport chain
f.	hydrolyzes the milk protein casein into smaller peptides the cell can take up

Letter	Description
g.	converts the superoxide radical to hydrogen peroxide
h.	interacts with prothrombin, which then converts fibrinogen to fibrin
i.	reduces nitrate, permitting it to serve as a final electron acceptor
j.	breaks starch down, releasing glucose

Click to reveal answers

Answers:
1) d
2) b
3) f
4) j
5) a
6) g
7) i
8) e
9) c
10) h

2. Match the media ingredient on the left with the appropriate description of its role by placing the letter next to the correct term.

Question 2 Terms

Answer	Terms
_____	1. Crystal violet and bile salts
_____	2. Neutral red
_____	3. 7.5% NaCl
_____	4. Mannitol
_____	5. Sodium thiosulfate
_____	6. Ferrous ammonium sulfate
_____	7. Durham tube
_____	8. Sodium citrate
_____	9. Ammonium phosphate
_____	10. Skim milk

Question 2 Descriptions

Letter	Description
a.	the sole carbon source present in Simmons citrate agar
b.	converted to hydrogen sulfide gas by bacteria capable of producing it
c.	inverted within the media to serve as a trap for gases produced during fermentation
d.	inhibit the growth of gram-positive bacteria, making MacConkey agar selective
e.	the sugar fermented by pathogenic Staphylococci on mannitol salt agar
f.	reacts with hydrogen sulfide gas to produce a black precipitate
g.	creates an environment on which only salt tolerant organisms can grow
h.	the nitrogen source present in Simmons citrate agar
i.	supplies the casein that is hydrolyzed in the casease test
j.	the pH indicator that changes to red at a pH less than 6.8 in MacConkey agar

Click to reveal answers

Answers:
1) d
2) j
3) g
4) e
5) b
6) f
7) c
8) a
9) h
10) i

3. Match the triple sugar iron agar result on the left with the appropriate abbreviation and interpretation by placing the letter next to the correct result.

Question 3 Terms

Answer	Terms
____	1. No change in the slant; no change in the butt
____	2. Red slant; red butt
____	3. Red slant; yellow butt

Answer	Terms
_____	4. Red slant; yellow butt with bubbles
_____	5. Red slant; yellow butt with black precipitate
_____	6. Yellow slant; yellow butt
_____	7. Yellow slant; yellow butt with bubbles
_____	8. Yellow slant; yellow butt with black precipitate

Question 3 Descriptions

Letter	Description
a.	A/A - glucose and lactose or sucrose fermentation
b.	K/A, H2S - glucose fermentation and hydrogen sulfide gas production
c.	-/- no fermentation
d.	A/A, G - glucose and lactose or sucrose fermentation; gas production
e.	K/K - protein catabolism yielded alkaline products
f.	A/A, H2S - glucose and lactose or sucrose fermentation; hydrogen sulfide gas production
g.	K/A - glucose fermentation
h.	K/A, G - glucose fermentation and gas production

Click to reveal answers

Answers:
1) c
2) e
3) g
4) h
5) b
6) a
7) d
8) f

4. Match the pH indicator on the left with the appropriate description of its color behavior by placing the letter next to the correct term.

Question 4 Terms

Answer	Terms
_____	1. Phenol red
_____	2. Methyl red
_____	3. Neutral red
_____	4. Bromothymol blue

Question 4 Descriptions

Letter	Description
a.	green at pH 6.9 and blue at pH 7.6
b.	red at alkaline pH and yellow at approximately pH 6.8
c.	clear above pH 6.8 and red at a pH less than 6.8
d.	red at pH 4.4 and yellow at pH 6.2

Click to reveal answers

Answers:
1) b
2) d
3) c
4) a

5. Match the biochemical test on the left with the appropriate description of its purpose by placing the letter next to the correct term.

Question 5 Terms

Answer	Terms
_____	1. Carbohydrate fermentation test
_____	2. Indole test
_____	3. Methyl red test
_____	4. Voges-Proskauer test

Answer	Terms
_____	5. Citrate test
_____	6. Oxidase test
_____	7. Catalase test
_____	8. Nitrate reduction test
_____	9. Urease test
_____	10. Coagulase test

Question 5 Descriptions

Letter	Description
a.	determines whether a bacterium can use citrate as its sole source of carbon
b.	differentiates *Staphylococcus aureus* from other species of *Staphylococcus*
c.	determines whether a bacterium can ferment a specific sugar, producing organic acids and/or gas
d.	determines whether a bacterium possesses the enzyme located at the end of the aerobic electron transport chain
e.	determines whether a bacterium can hydrolyze urea, releasing ammonia
f.	determines whether a bacterium ferments glucose to neutral end products, which are detected as acetoin
g.	differentiates Staphylococci and Micrococci from Streptococci and Enterococci
h.	determines whether a bacterium can convert tryptophan to indole, detected by adding Kovac's reagent
i.	determines whether a bacterium can use nitrate as a final electron acceptor
j.	determines whether a bacterium carries out mixed-acid fermentation, producing stable acid end products

Click to reveal answers

Answers:
1) c
2) h
3) j
4) f
5) a
6) d
7) g

8) i
9) e
10) b

6. Match the growth medium on the left with the appropriate description of its purpose by placing the letter next to the correct term.

Question 6 Terms

Answer	Terms
_____	1. Triple sugar iron agar
_____	2. SIM medium
_____	3. Skim milk agar
_____	4. Starch agar
_____	5. Blood agar
_____	6. MacConkey agar
_____	7. Mannitol salt agar

Question 6 Descriptions

Letter	Description
a.	selects for gram-negative bacteria and differentiates lactose fermenters from non-lactose fermenters
b.	determines whether a bacterium can hydrolyze the milk protein casein
c.	assesses the ability of a bacterium to ferment three different sugars and to produce hydrogen sulfide gas
d.	grows fastidious bacteria and differentiates organisms based on their blood lysis capabilities
e.	detects hydrogen sulfide production, indole production, and motility in a single tube
f.	selects for salt tolerant organisms and differentiates those able to ferment mannitol
g.	determines whether a bacterium can hydrolyze starch; read after iodine is added to the plate

Click to reveal answers

Answers:
1) c
2) e
3) b

4) g
5) d
6) a
7) f

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3.13.2: Fill-In-The-Blank

Fill-in-the-Blank Questions

1. An organism inoculated onto blood agar and observed to produce a clear zone surrounding the bacterial growth following incubation, this is known as _____ hemolysis.

Click to reveal answer

Answer beta

2. Catalase breaks down _____ into water and oxygen gas.

Click to reveal answer

Answer hydrogen peroxide

3. The indicator used in the carbohydrate fermentation test is _____ .

Click to reveal answer

Answer phenol red

4. An organism capable of producing citrate permease (citrase) will cause Simmons citrate media to turn _____ .

Click to reveal answer

Answer blue

5. The pH indicator used in the urease test is _____ .

Click to reveal answer

Answer phenol red

6. The _____ test determines a bacterium's ability to produce the enzyme amylase.

Click to reveal answer

Answer starch hydrolysis

7. The end product of the nitrate reductase reaction is _____ .

Click to reveal answer

Answer nitrite

8. The addition of hydrogen peroxide to bacterial sample to detect gas bubble formation is done in the _____ test.

Click to reveal answer

Answer catalase

9. The pH indicator neutral red used in MacConkey agar will cause a color change to _____ when acidic conditions from fermentation of lactose occur.

Click to reveal answer

Answer pink

10. The milk protein found in milk that makes it white and cloudy is _____.

Click to reveal answer

Answer casein

11. Mannitol salt agar is selective for salt tolerant bacteria, which are properly referred to as _____.

Click to reveal answer

Answer facultative halophile

12. The presence of starch is determined by a dark-blue/purple color when _____ is added.

Click to reveal answer

Answer iodine

13. _____ is the enzyme that reduces oxygen in the electron transport chain.

Click to reveal answer

Answer Cytochrome c oxidase

14. The _____ test determines a bacterium's ability to produce an enzyme that permits it to clot blood thereby enhancing its virulence.

Click to reveal answer

Answer coagulase test

15. The _____ test determines the ability of a bacterium to produce the enzyme tryptophanase.

Click to reveal answer

Answer indole

16. The _____ test is used to differentiate *Staphylococci* from *Streptococci* on the basis of their ability to produce the enzyme necessary to reduce hydrogen peroxide to water and oxygen gas.

Click to reveal answer

Answer catalase

17. The _____ test determines the ability of a bacterium to produce hydrogen sulfide gas its ability to produce the enzyme tryptophanase, and if it is capable of motility.

Click to reveal answer

Answer SIM agar

18. A bacterium capable of producing hydrogen sulfide gas will turn the color of the media _____ at the end of a triple sugar iron agar test.

Click to reveal answer

Answer black

19. A mannitol fermenting facultative halophile will cause the media of the mannitol salt agar to change in color to _____ .

Click to reveal answer

Answer yellow

20. _____ reagent is used to detect the presence of indole in both the SIM agar test and the indole test.

Click to reveal answer

Answer Kovac's

21. The pH indicator used in the MacConkey agar is _____ .

Click to reveal answer

Answer neutral red

22. The tests included in the IMViC panel of tests in order of their abbreviations are the

1 _____ 2 _____ 3 _____ 4 _____

Click to reveal answer

Answer Indole test, Methyl red test, Voges-Proskauer test, Citrate test

23. The _____ test determines a bacterium's ability to produce the enzyme needed at the end of the electron transport chain to reduce molecular oxygen to water.

Click to reveal answer

Answer Oxidase test

24. A positive catalase test is determined by the production of _____ after the addition of hydrogen peroxide to a bacterial sample.

Click to reveal answer

Answer bubbles

25. The enzymes that allow bacteria to lysis red blood cells on blood agar are called _____ .

Click to reveal answer

Answer hemolysins

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3.13.3: True/False

True / False Questions

1. The selective nature of the MacConkey agar is due to the presence of crystal violet and bile salts, which inhibits Gram-positive bacterial growth.

_____ True _____ False

Click to reveal answer

Answer True

2. The catalase test should have the same result whether the bacteria are on a slant, on a nutrient agar plate, or have been transferred to a glass slide.

_____ True _____ False

Click to reveal answer

Answer True

3. A phenol red result that gives an alkaline result but shows gas production in the Durham tube is most likely a result of reversion.

_____ True _____ False

Click to reveal answer

Answer True

4. All bacteria have the ability to ferment any sugar available.

_____ True _____ False

Click to reveal answer

Answer False

5. A phenol red dextrose tube can be incubated on Friday and then observed the following Monday.

_____ True _____ False

Click to reveal answer

Answer False

6. The methyl red test would be highly useful in differentiating *Staphylococcus* and *Streptococcus* species of bacteria.

_____ True _____ False

Click to reveal answer

Answer False

7. The oxidase test is used to distinguish enteric bacteria from non-enteric bacteria.

_____ True _____ False

Click to reveal answer

Answer True

8. In the catalase test, if possible, you should not test colonies from a blood agar plate because they may give a false positive due to the erythrocytes having catalase activity.

_____ True _____ False

Click to reveal answer

Answer True

9. Phenol red will turn yellow in the presence of acid.

_____ True _____ False

Click to reveal answer

Answer True

10. When looking at a blood agar plate with bacterial growth, beta hemolysis is complete lysis of the red blood cells, alpha hemolysis is partial lysis, and gamma hemolysis is no lysis.

_____ True _____ False

Click to reveal answer

Answer True

11. In order for an organism to give a positive result in a methyl red test, it must produce enough acid from fermentation to overcome a phosphate buffer.

_____ True _____ False

Click to reveal answer

Answer True

12. The urease test is used to test for gram-positive streptococci.

_____ True _____ False

Click to reveal answer

Answer False

13. A nitrate test that turns red after the addition of nitrate reagents A and B and the addition of zinc and HCl would be negative for nitrate reduction.

_____ True _____ False

Click to reveal answer

Answer True

14. Bubbles are expected to form if hydrogen peroxide is introduced to a sample of *Staphylococcus epidermidis* on a microscope slide.

_____ True _____ False

Click to reveal answer

Answer True

15. The ingredient added to the agar for the casein test that allows differentiation between positive and negative results is skim milk.

_____ True _____ False

Click to reveal answer

Answer True

16. A sample can safely be concluded to be negative if it remains clear after the addition of sulfanilic acid and alpha-naphthylamine in the nitrate reductase test.

_____ True _____ False

Click to reveal answer

Answer False

17. *Escherichia coli* bacteria would produce transparent colonies when growing on a MacConkey agar plate.

_____ True _____ False

Click to reveal answer

Answer False

18. A positive result for starch hydrolysis is observed when halos are observed around the bacterial cultures after the addition of iodine to the starch plate.

_____ True _____ False

Click to reveal answer

Answer True

19. Bacteria capable of lactose fermentation will change the media of a MacConkey agar to a pink color.

_____ True _____ False

Click to reveal answer

Answer True

20. The addition of Kovac's reagent onto a SIM agar that has been incubated with a bacterial species capable of producing the enzyme tryptophanase would result in a cherry red color change.

_____ True _____ False

Click to reveal answer

Answer True

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3.13.4: Multiple Choice / Multiple Select

Multiple Choice / Multiple-Select Questions

1. Why can microscopy and staining techniques alone fail to determine a bacterial sample's species?

- a) Stains cannot be applied to clinically significant organisms
- b) Two different species within the same genus will look morphologically identical when stained and observed
- c) Microscopes cannot achieve the magnification required to see bacteria
- d) Staining destroys the physiological characteristics of the sample

Click to reveal answer

Answer b) Two different species within the same genus will look morphologically identical when stained and observed
Two different species within the same genera will look morphologically identical when stained and observed, so they cannot be differentiated using microscopy alone.

2. The metabolic pathways that make each bacterial species physiologically unique are catalyzed by protein molecules known as:

- a) Enzymes
- b) Peptones
- c) Hemolysins
- d) Indicators

Click to reveal answer

Answer a) Enzymes
Each species will utilize a unique set of metabolic pathways, and these metabolic pathways are catalyzed by protein molecules known as enzymes.

3. After completing a set of biochemical tests, a microbiologist interprets the data using a systematic key known as:

- a) The ACGM manual
- b) Bergey's manual
- c) The IMViC panel
- d) Simmons citrate agar

Click to reveal answer

Answer b) Bergey's manual
After completing sets of biochemical tests, microbiologists interpret the data using a systematic key known as Bergey's manual.

4. When organisms carry out fermentation of a carbohydrate, what may be produced as end products? **Select all that apply.**

- a) Organic acids

- b) Gases
- c) Hydrogen sulfide only
- d) Immersion oil

Click to reveal answers

Answers: a) Organic acids; b) Gases
When organisms carry out fermentation of a carbohydrate, they may produce organic acids and/or gases as end-products.

5. Phenol red is red at alkaline pH. At approximately what pH does it turn yellow?

- a) pH 4.4
- b) pH 6.2
- c) pH 6.8
- d) pH 7.6
- e) pH 8.2

Click to reveal answer

Answer c) pH 6.8
Phenol red turns yellow at a slightly acidic pH of approximately pH 6.8.

6. What does carbohydrate fermentation media contain? **Select all that apply.**

- a) A specific carbohydrate such as glucose, lactose, or sucrose
- b) The pH indicator phenol red
- c) A nutrient medium supplying other required nutrients
- d) An inverted Durham tube
- e) Sodium thiosulfate

Click to reveal answers

Answers: a) A specific carbohydrate such as glucose, lactose, or sucrose; b) The pH indicator phenol red; c) A nutrient medium supplying other required nutrients; d) An inverted Durham tube
Carbohydrate fermentation media contain a specific carbohydrate, the pH indicator phenol red, and a nutrient medium, with an inverted Durham tube to trap any gases produced.

7. Carbohydrate fermentation tests are considered a differential medium because they allow:

- a) Differentiation of species within a genus
- b) Inhibition of gram-positive organisms
- c) Growth of fastidious organisms
- d) Isolation of a pure culture from a mixed sample

Click to reveal answer

Answer a) Differentiation of species within a genus
Carbohydrate tests are also considered a differential medium that allows differentiation of species within a genus, permitting more accurate identification of microorganisms.

8. A phenol red broth tube is yellow after incubation, but no bubble is present in the Durham tube. What does this indicate?

- a) The organism fermented the carbohydrate producing acid, but no gas
- b) The organism produced gas but no acid
- c) The organism did not ferment the carbohydrate
- d) The organism produced hydrogen sulfide gas

Click to reveal answer

Answer a) The organism fermented the carbohydrate producing acid, but no gas
If an organism can metabolize the carbohydrate present, an acid is produced and the indicator turns yellow. A bubble in the Durham tube would indicate gas was also produced.

9. Which three sugars are present in triple sugar iron agar? **Select all that apply.**

- a) Glucose
- b) Sucrose
- c) Lactose
- d) Mannitol

Click to reveal answers

Answers: a) Glucose; b) Sucrose; c) Lactose
Triple sugar iron agar contains three different sugars: glucose (0.1%), sucrose (1%), and lactose (1%).

10. How does the glucose concentration in triple sugar iron agar compare to the sucrose and lactose concentrations?

- a) Glucose is present at a much lower concentration than sucrose and lactose
- b) Glucose is present at a much higher concentration than sucrose and lactose
- c) All three sugars are present at equal concentrations
- d) Glucose is present only in the butt of the tube

Click to reveal answer

Answer a) Glucose is present at a much lower concentration than sucrose and lactose
Glucose is present at 0.1%, a much lower concentration than sucrose and lactose, which are each present at 1%.

11. In addition to the three sugars, which ingredients are found in triple sugar iron agar? **Select all that apply.**

- a) Phenol red
- b) Peptones
- c) Sodium thiosulfate
- d) Ferrous ammonium sulfate
- e) Bromothymol blue

Click to reveal answers

Answers: a) Phenol red; b) Peptones; c) Sodium thiosulfate; d) Ferrous ammonium sulfate
In addition to the sugars, the media contains the pH indicator phenol red, peptones, sodium thiosulfate, and ferrous ammonium sulfate.

12. An organism that can metabolize only glucose produces a red slant over a yellow butt. Why does the slant turn from yellow back to red?

- a) The glucose is exhausted, and the bacteria then metabolize the peptones producing ammonium, which raises the pH
- b) The sucrose and lactose are fermented later, producing alkaline products
- c) The phenol red is destroyed by prolonged incubation
- d) Hydrogen sulfide gas neutralizes the acid at the surface

Click to reveal answer

Answer a) The glucose is exhausted, and the bacteria then metabolize the peptones producing ammonium, which raises the pH
After exhausting the glucose, the bacteria metabolize the peptones present in the media, producing ammonium. Ammonium increases the pH, making it alkaline and changing the media from yellow to red.

13. When the slant of a triple sugar iron agar tube reverts to red, why does the butt remain yellow?

- a) The butt is anaerobic, so it remains acidic
- b) The butt contains a higher concentration of glucose
- c) Phenol red does not diffuse into the butt of the tube
- d) The ferrous ammonium sulfate settles into the butt

Click to reveal answer

Answer a) The butt is anaerobic, so it remains acidic
Reversion occurs in regions of the tube exposed to oxygen. The areas of the media that are anaerobic, lacking oxygen, remain acidic and continue to appear yellow.

14. Hydrogen sulfide gas is colorless. Which ingredient allows its presence to be detected?

- a) Sodium thiosulfate
- b) Ferrous ammonium sulfate

- c) Phenol red
- d) Peptones

Click to reveal answer

Answer b) Ferrous ammonium sulfate
Hydrogen sulfide gas is colorless, so the ingredient ferrous ammonium sulfate is included. The hydrogen sulfide gas interacts with it to produce a black precipitate.

15. How is a triple sugar iron agar tube inoculated?

- a) By streaking the surface of the slant only with an inoculating loop
- b) By stabbing straight into the butt of the slant with an inoculating needle, then streaking up the surface of the agar
- c) By swirling an inoculating loop through the media
- d) By adding a drop of broth culture to the surface of the agar

Click to reveal answer

Answer b) By stabbing straight into the butt of the slant with an inoculating needle, then streaking up the surface of the agar
Insert the inoculating needle by stabbing straight into the butt of the slant and then streak up the surface of the agar.

16. Which three characteristics does SIM media detect? **Select all that apply.**

- a) Hydrogen sulfide gas production
- b) The presence of tryptophanase, which converts tryptophan to indole
- c) The ability to move using flagella
- d) The ability to ferment lactose

Click to reveal answers

Answers: a) Hydrogen sulfide gas production; b) The presence of tryptophanase, which converts tryptophan to indole; c) The ability to move using flagella
SIM media detects hydrogen sulfide gas production (S), the presence of tryptophanase which converts tryptophan to indole (I), and the ability of a bacterium to move using flagella (M).

17. Tryptophanase converts the amino acid tryptophan into which products? **Select all that apply.**

- a) Indole
- b) Pyruvic acid
- c) Ammonia
- d) Acetoin

Click to reveal answers

Answers: a) Indole; b) Pyruvic acid; c) Ammonia
Tryptophanase converts the amino acid tryptophan to indole, pyruvic acid, and ammonia.

18. SIM media is a semi-solid deep containing approximately 3.5 g/L agar, whereas solid agar media uses 12-15 g/L. Why is the lower concentration used?

- a) It creates an environment that favors movement if the bacteria have flagella, because regular agar is too dense
- b) It allows the media to be poured into petri plates
- c) It prevents hydrogen sulfide gas from escaping the tube
- d) It keeps the media from solidifying before it is inoculated

Click to reveal answer

Answer a) It creates an environment that favors movement if the bacteria have flagella, because regular agar is too dense
The lower agar concentration creates an environment that favors movement if bacteria have flagella, because a regular agar is too dense for the movement of the bacteria.

19. How is a SIM tube inoculated?

- a) By streaking back and forth across the surface of the media
- b) By stabbing straight down into the media all the way to the bottom, then gently removing the needle without disturbing the stab mark
- c) By swirling an inoculating loop through the media
- d) By pouring a broth culture over the surface of the media

Click to reveal answer

Answer b) By stabbing straight down into the media all the way to the bottom, then gently removing the needle without disturbing the stab mark
Insert the inoculating needle by stabbing straight down into the media all the way to the bottom, and then gently remove the needle so as not to disturb the stab mark.

20. After incubation, bacteria are found growing only near the stab mark of a SIM tube. What does this indicate?

- a) The bacteria are non-motile
- b) The bacteria are motile
- c) The bacteria produced hydrogen sulfide gas
- d) The bacteria produced tryptophanase

Click to reveal answer

Answer a) The bacteria are non-motile
If bacteria are only found growing near the stab mark they are non-motile. If growth radiates out away from the stab mark the bacteria are motile.

21. The IMViC panel of tests is performed in order to differentiate members of which family?

- a) The Enterobacteriaceae family
- b) The *Staphylococcus* genus
- c) The *Mycobacterium* genus
- d) The *Streptococcus* genus

Click to reveal answer

Answer a) The Enterobacteriaceae family
The IMViC tests are performed in order to differentiate members of the Enterobacteriaceae family.

22. Members of the Enterobacteriaceae are commonly called enteric bacteria because:

- a) Many members reside within the intestines of humans or animals
- b) They are all enteric pathogens
- c) They can only be grown on enriched media
- d) They all produce endospores

Click to reveal answer

Answer a) Many members reside within the intestines of humans or animals
Members of this family are commonly known as enterobacteria or enteric bacteria because many members of this family reside within the intestines of humans or animals.

23. Morphologically, the enterobacteria are described as:

- a) Gram-negative bacilli
- b) Gram-positive cocci
- c) Acid-fast bacilli
- d) Gram-positive bacilli

Click to reveal answer

Answer a) Gram-negative bacilli
The enterobacteria are gram-negative bacilli that, while morphologically very similar, can be distinguished by their biochemical properties.

24. Which of the following are medically important members of the Enterobacteriaceae family? **Select all that apply.**

- a) *Klebsiella*
- b) *Shigella*
- c) *Salmonella*
- d) *Escherichia coli*

- e) *Staphylococcus aureus*

Click to reveal answers

Answers: a) *Klebsiella*; b) *Shigella*; c) *Salmonella*; d) *Escherichia coli*
Some medically important members of this family include *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., and *Escherichia coli*.

25. MRVP broth, used for both the methyl red and Voges-Proskauer tests, contains which ingredients? **Select all that apply.**

- a) Peptone
- b) Glucose
- c) A phosphate buffer
- d) Sodium citrate

Click to reveal answers

Answers: a) Peptone; b) Glucose; c) A phosphate buffer
MRVP broth contains peptone, glucose, and a phosphate buffer. The bacterium is inoculated into the broth, which is then split to perform the two tests.

26. The methyl red test determines whether a bacterium is able to produce acid end products as a result of:

- a) The mixed-acid fermentation of glucose
- b) The hydrolysis of urea
- c) The reduction of nitrate
- d) The catabolism of citrate

Click to reveal answer

Answer a) The mixed-acid fermentation of glucose
The methyl red test is performed to determine if a bacterium is able to produce acid end products as a result of the mixed-acid fermentation of glucose.

27. Methyl red is red at which pH, and yellow at which pH?

- a) Red at pH 4.4; yellow at pH 6.2
- b) Red at pH 6.2; yellow at pH 4.4
- c) Red at pH 6.8; yellow at pH 8.2
- d) Red at pH 7.6; yellow at pH 6.9

Click to reveal answer

Answer a) Red at pH 4.4; yellow at pH 6.2
Methyl red is red at pH 4.4 and yellow at pH 6.2.

28. Members of the Enterobacteriaceae that do NOT carry out mixed-acid fermentation instead ferment glucose to produce which more neutral end product?

- a) 2,3-butanediol
- b) Pyruvic acid
- c) Indole
- d) Hydrogen sulfide

Click to reveal answer

Answer a) 2,3-butanediol
Other members of this group do not carry out mixed-acid fermentation, but instead ferment glucose to produce a more neutral end product, 2,3-butanediol.

29. Neutral end products such as 2,3-butanediol cannot readily be detected. What are they converted to so they can be observed?

- a) Acetoin, via oxidation
- b) Indole, via tryptophanase
- c) Nitrite, via nitrate reductase
- d) Ammonia, via urease

Click to reveal answer

Answer a) Acetoin, via oxidation
These neutral end-products cannot readily be detected but can be converted to acetoin via oxidation, which can then be observed with the addition of Barritt's reagent.

30. Barritt's reagent, used in the Voges-Proskauer test, is composed of:

- a) Alpha-naphthol and KOH
- b) Sulfanilic acid and alpha-naphthylamine
- c) Alpha-naphthol and hydrochloric acid
- d) Sodium citrate and ammonium phosphate

Click to reveal answer

Answer a) Alpha-naphthol and KOH
Barritt's reagent, alpha-naphthol and KOH, reacts with acetoin to create a product that produces a red color.

31. Which enzyme allows citrate to be transported into a bacterial cell?

- a) Citrate permease
- b) Tryptophanase
- c) Nitrate reductase

- d) Amylase

Click to reveal answer

Answer a) Citrate permease
Citrate can be transported into some bacterial cells that have the enzyme citrate permease. Such bacteria import citrate and convert it to pyruvate to be used as a carbon source.

32. Simmons citrate agar contains which of the following? **Select all that apply.**

- a) Sodium citrate as the only carbon source
- b) Ammonium phosphate as a nitrogen source
- c) Bromothymol blue as a pH indicator
- d) Phenol red as a pH indicator

Click to reveal answers

Answers: a) Sodium citrate as the only carbon source; b) Ammonium phosphate as a nitrogen source; c) Bromothymol blue as a pH indicator
Simmons citrate agar contains sodium citrate as the only carbon source, ammonium phosphate as a nitrogen source, and bromothymol blue as a pH indicator.

33. In a positive citrate test, which products raise the pH of the media and cause the color change?

- a) Sodium carbonate and ammonia
- b) Pyruvic acid and indole
- c) Hydrogen sulfide and ammonium
- d) Acetoin and 2,3-butanediol

Click to reveal answer

Answer a) Sodium carbonate and ammonia
Bacteria capable of using citrate produce sodium carbonate, and also metabolize the ammonium salts converting them to ammonia. Both raise the pH, changing the media from green to blue.

34. Cellular respiration is the conversion of glucose to energy in the form of ATP. Which pathways does it utilize? **Select all that apply.**

- a) Glycolysis
- b) The citric acid cycle
- c) The electron transport chain
- d) Mixed-acid fermentation

Click to reveal answers

Answers: a) Glycolysis; b) The citric acid cycle; c) The electron transport chain
Cellular respiration is the conversion of glucose to energy in the form of ATP, utilizing glycolysis, the citric acid cycle, and the electron transport chain.

35. Organisms that utilize oxygen as their final electron acceptor are known as:

- a) Aerobes
- b) Anaerobes
- c) Facultative halophiles
- d) Fastidious organisms

Click to reveal answer

Answer a) Aerobes
Organisms that utilize oxygen as their final electron acceptor are known as aerobes, and reduce the oxygen to produce water or other products.

36. Anaerobes use inorganic molecules other than oxygen as a final electron acceptor. Which molecules may they use? **Select all that apply.**

- a) Nitrate
- b) Sulfate
- c) Carbonate
- d) Glucose

Click to reveal answers

Answers: a) Nitrate; b) Sulfate; c) Carbonate
Organisms that utilize other inorganic molecules such as nitrate, sulfate, or carbonate are known as anaerobes.

37. Where is cytochrome c oxidase located, and what does it do?

- a) At the end of the aerobic electron transport chain; it reduces molecular oxygen to water
- b) At the beginning of glycolysis; it converts glucose to pyruvate
- c) In the cell wall; it hydrolyzes urea to ammonia
- d) In the cytoplasm; it converts hydrogen peroxide to water and oxygen

Click to reveal answer

Answer a) At the end of the aerobic electron transport chain; it reduces molecular oxygen to water
Cytochrome c oxidase is an enzyme located at the end of the aerobic electron transport chain, which utilizes electrons from the chain to reduce molecular oxygen to water.

38. The oxidase reagent tetramethyl-p-phenylenediamine (TMPD) is _____ when reduced and _____ when oxidized.

- a) Colorless; purple
- b) Purple; colorless
- c) Yellow; red
- d) Green; blue

Click to reveal answer

Answer a) Colorless; purple
TMPD is colorless when it is reduced and changes to purple when oxidized. If cytochrome c oxidase is present, it will oxidize the reagent causing a deep purple/blue color.

39. The electron transport chains of organisms carrying out aerobic cellular respiration produce which two highly potent cellular toxins? **Select all that apply.**

- a) Superoxide radical
- b) Hydrogen peroxide
- c) Nitrous oxide
- d) Acetoin

Click to reveal answers

Answers: a) Superoxide radical; b) Hydrogen peroxide
Aerobic electron transport chains produce two highly potent cellular toxins: superoxide radical and hydrogen peroxide.

40. What does the enzyme superoxide dismutase do?

- a) Converts the superoxide radical to hydrogen peroxide
- b) Converts hydrogen peroxide into water and gaseous oxygen
- c) Reduces molecular oxygen to water
- d) Reduces nitrate to nitrite

Click to reveal answer

Answer a) Converts the superoxide radical to hydrogen peroxide
Superoxide dismutase converts the superoxide radical to hydrogen peroxide, while catalase converts hydrogen peroxide into water and gaseous oxygen.

41. The catalase test is used to differentiate which groups of bacteria?

- a) Staphylococci and Micrococci, which are catalase-positive, from Streptococci and Enterococci, which are catalase-negative
- b) Streptococci and Enterococci, which are catalase-positive, from Staphylococci and Micrococci, which are catalase-negative
- c) Lactose fermenters from non-lactose fermenters

- d) Motile organisms from non-motile organisms

Click to reveal answer

Answer a) Staphylococci and Micrococci, which are catalase-positive, from Streptococci and Enterococci, which are catalase-negative
The catalase test is used to differentiate Staphylococci and Micrococci, which are catalase-positive, from Streptococci and Enterococci, which are catalase-negative.

42. Nitrate reductase is located within the electron transport chain of bacteria that perform:

- a) Anaerobic cellular respiration
- b) Aerobic cellular respiration
- c) Mixed-acid fermentation
- d) Starch hydrolysis

Click to reveal answer

Answer a) Anaerobic cellular respiration
Nitrate reductase is an enzyme located within the electron transport chain of some bacteria that perform anaerobic cellular respiration, allowing them to use nitrate as a final electron acceptor.

43. Nitrate may be reduced to which of the following? **Select all that apply.**

- a) Nitrite ions
- b) Nitrous oxide
- c) Nitrogen gas
- d) Ammonia

Click to reveal answers

Answers: a) Nitrite ions; b) Nitrous oxide; c) Nitrogen gas
Nitrate can be reduced to produce nitrite ions, nitrous oxide, or nitrogen gas.

44. Nitrate broth is composed of which ingredients? **Select all that apply.**

- a) Beef extract
- b) Peptones
- c) Potassium nitrate
- d) Sodium thiosulfate

Click to reveal answers

Answers: a) Beef extract; b) Peptones; c) Potassium nitrate
The nitrate broth is composed of beef extract, peptones, and potassium nitrate, with a Durham tube present to trap any gas produced.

45. A nitrate broth remains clear after the addition of the nitrate reagents. Zinc dust is added and the medium turns red. How is this interpreted?

- a) Nitrate is still present and was not reduced, which is a negative result
- b) Nitrate was reduced to nitrite, which is a positive result
- c) Nitrate was reduced to a form other than nitrite, which is a positive result
- d) The test is invalid and must be repeated

Click to reveal answer

Answer a) Nitrate is still present and was not reduced, which is a negative result
If nitrate is still present in the medium, zinc will reduce it to nitrite causing the medium to change red/pink. This is a negative result for nitrate reduction.

46. A nitrate broth remains clear after the nitrate reagents AND remains clear after zinc dust is added. How is this interpreted?

- a) The nitrate has been reduced to some form other than nitrite
- b) No nitrate was present in the medium to begin with
- c) The organism is a strict aerobe
- d) The reagents were added in the wrong order

Click to reveal answer

Answer a) The nitrate has been reduced to some form other than nitrite
If there is no color change after the addition of zinc, then the nitrate has been reduced to some form other than nitrite.

47. Urea is a metabolic waste product produced as a result of:

- a) Protein catabolism
- b) Carbohydrate fermentation
- c) Nitrate reduction
- d) Starch hydrolysis

Click to reveal answer

Answer a) Protein catabolism
Urea is a metabolic waste product produced as a result of protein catabolism.

48. When a bacterium hydrolyzes urea using urease, which products are released? **Select all that apply.**

- a) Ammonia
- b) Carbon dioxide
- c) Hydrogen sulfide

- d) Pyruvic acid

Click to reveal answers

Answers: a) Ammonia; b) Carbon dioxide
Some bacteria can hydrolyze urea using the enzyme urease, releasing ammonia and carbon dioxide.

49. How does a bacterium benefit from hydrolyzing urea?

- a) The freed ammonia is used as a nitrogen source in the bacterium's metabolism
- b) The carbon dioxide is used as a final electron acceptor
- c) The reaction produces ATP directly
- d) The reaction protects the cell from phagocytosis

Click to reveal answer

Answer a) The freed ammonia is used as a nitrogen source in the bacterium's metabolism
These bacteria use urease to hydrolyze urea, freeing ammonia to be used as a nitrogen source in the bacterium's metabolism.

50. Urea broth has a pH of 6.8. What color is the media before it is inoculated?

- a) Yellow
- b) Pink (fuchsia)
- c) Green
- d) Colorless

Click to reveal answer

Answer a) Yellow
Phenol red is yellow at pH 6.8. The media has a pH of 6.8 and therefore begins as a yellow color.

51. Coagulase is an exoenzyme produced by some species within which genus?

- a) *Staphylococcus*
- b) *Streptococcus*
- c) *Escherichia*
- d) *Klebsiella*

Click to reveal answer

Answer a) *Staphylococcus*
Coagulase is an exoenzyme produced by some species within the genera *Staphylococcus*, including *Staphylococcus aureus*.

52. How does coagulase contribute to the virulence of the bacteria that produce it?

- a) It permits the bacteria to clot blood
- b) It allows the bacteria to lyse red blood cells
- c) It allows the bacteria to tolerate high salt concentrations
- d) It allows the bacteria to survive without oxygen

Click to reveal answer

Answer a) It permits the bacteria to clot blood
Coagulase contributes to the virulence of such species by permitting the bacteria to clot blood.

53. What is the sequence of events by which coagulase produces a clot?

- a) Coagulase interacts with prothrombin, which then converts fibrinogen to fibrin, forming the clot
- b) Coagulase directly converts fibrin to fibrinogen, forming the clot
- c) Coagulase lyses red blood cells, releasing clotting factors
- d) Coagulase converts plasma to serum, which then solidifies

Click to reveal answer

Answer a) Coagulase interacts with prothrombin, which then converts fibrinogen to fibrin, forming the clot
The enzyme interacts with the enzyme prothrombin, which then converts fibrinogen to fibrin in the plasma, which in turn forms the clot.

54. Why is the resulting network of fibrin proteins thought to benefit the bacterium?

- a) It is hypothesized to prevent phagocytosis by the immune cells of the host
- b) It provides an additional carbon source for the bacterium
- c) It protects the bacterium from desiccation
- d) It allows the bacterium to move through host tissue

Click to reveal answer

Answer a) It is hypothesized to prevent phagocytosis by the immune cells of the host
It has been hypothesized that bacteria capable of doing this are protected by the network of fibrin proteins, preventing their phagocytosis by the immune cells of the host.

55. The coagulase test is performed using:

- a) Rabbit plasma in a test tube
- b) Skim milk agar plates
- c) Simmons citrate agar slants
- d) Blood agar plates

Click to reveal answer

Answer a) Rabbit plasma in a test tube
The coagulase test is performed using rabbit plasma in a test tube. The bacteria are added and the media is observed to determine if the plasma coagulates.

56. What does the enzyme casease do to casein, and why is this useful to the bacterium?

- a) It catabolizes casein into smaller peptides that can be taken up by the cell and used for metabolism
- b) It converts casein into glucose that can be fermented
- c) It clots the casein, forming a protective network around the cell
- d) It oxidizes casein, releasing hydrogen peroxide

Click to reveal answer

Answer a) It catabolizes casein into smaller peptides that can be taken up by the cell and used for metabolism
Some bacteria produce the enzyme casease, which catabolizes the protein into smaller peptides that can then be taken up by the cell and used for the cell's metabolism.

57. Starch is also known by which name, and what is it composed of?

- a) Amylose; many glucose monomers
- b) Amylase; many peptide monomers
- c) Casein; many lactose monomers
- d) Mannitol; many sucrose monomers

Click to reveal answer

Answer a) Amylose; many glucose monomers
Starch, also known as amylose, is a polysaccharide composed of many glucose monomers.

58. Bacteria capable of hydrolyzing starch produce amylase. What does this enzyme release?

- a) Glucose
- b) Ammonia
- c) Pyruvic acid
- d) Acetoin

Click to reveal answer

Answer a) Glucose
Bacteria capable of hydrolyzing starch produce the enzyme amylase, which breaks the starch down releasing glucose.

59. Fastidious bacteria are best described as bacteria that:

- a) Require a significant number of growth factors such as vitamins and essential amino acids
- b) Grow only in the absence of oxygen
- c) Tolerate high concentrations of salt
- d) Grow only at temperatures above 37 degrees

Click to reveal answer

Answer a) Require a significant number of growth factors such as vitamins and essential amino acids
Fastidious bacteria are bacteria that require a significant number of growth factors such as vitamins and essential amino acids. Blood is an excellent source of nutrients to meet these complex requirements.

60. What occurs during alpha hemolysis?

- a) The bacteria do not lyse the red blood cells, but produce hydrogen peroxide that interacts with heme, producing a green discoloration
- b) The bacteria completely lyse the red blood cells, leaving a clear zone
- c) The bacteria do not affect the red blood cells at all
- d) The bacteria clot the blood, forming a fibrin network

Click to reveal answer

Answer a) The bacteria do not lyse the red blood cells, but produce hydrogen peroxide that interacts with heme, producing a green discoloration
Alpha hemolysis is a partial destruction of the red blood cell. The bacteria do not lyse the red blood cells but produce hydrogen peroxide that interacts with heme, producing a green discoloration of the media around the colonies.

61. Why is alpha hemolysis not considered a true lysis of the blood cell?

- a) The cell membranes of the red blood cells remain intact
- b) No hemolysins are produced by any bacteria
- c) The red blood cells are removed from the media before observation
- d) The green color is caused by the media, not the cells

Click to reveal answer

Answer a) The cell membranes of the red blood cells remain intact
The cell membranes of the red blood cells remain intact, therefore alpha hemolysis is not a true lysis of the blood cell.

62. Which two hemolysins may be produced by bacteria capable of beta hemolysis? **Select all that apply.**

- a) Streptolysin O, which is oxygen sensitive

- b) Streptolysin S, which is stable in the presence of oxygen
- c) Catalase, which is oxygen dependent
- d) Coagulase, which is oxygen sensitive

Click to reveal answers

Answers: a) Streptolysin O, which is oxygen sensitive; b) Streptolysin S, which is stable in the presence of oxygen There are two different hemolysins that can be produced: Streptolysin O, which is oxygen sensitive, and Streptolysin S, which is stable in the presence of oxygen. Both completely lyse the red blood cell.

63. Which organism is given as an example of a bacterium capable of alpha hemolysis?

- a) *Streptococcus pneumoniae*
- b) *Streptococcus pyogenes*
- c) *Enterococcus faecalis*
- d) *Staphylococcus aureus*

Click to reveal answer

Answer a) *Streptococcus pneumoniae*
Bacteria capable of alpha hemolysis, such as *Streptococcus pneumoniae*, produce hydrogen peroxide that interacts with heme, producing a green discoloration.

64. Which organism is given as an example of a gamma-hemolytic bacterium?

- a) *Enterococcus faecalis*
- b) *Streptococcus pyogenes*
- c) *Streptococcus pneumoniae*
- d) *Escherichia coli*

Click to reveal answer

Answer a) *Enterococcus faecalis*
Enterococcus faecalis is an example of a bacterium that is gamma-hemolytic. Gamma-hemolytic bacteria grow on the media but the media remains unchanged around the colonies.

65. Because of its selective ingredients, which bacteria will grow on MacConkey agar?

- a) Only gram-negative bacteria
- b) Only gram-positive bacteria
- c) Only salt-tolerant bacteria
- d) Only fastidious bacteria

Click to reveal answer

Answer a) Only gram-negative bacteria
Crystal violet and bile salts inhibit the growth of gram-positive bacteria. Therefore, only gram-negative bacteria will grow on MacConkey agar.

66. The pH indicator neutral red is clear above which pH, and changes to red below it?

- a) pH 6.8
- b) pH 4.4
- c) pH 7.6
- d) pH 8.2

Click to reveal answer

Answer a) pH 6.8
The pH indicator neutral red is clear above pH 6.8 and changes to red at a pH less than 6.8.

67. How do non-lactose fermenting bacteria appear when grown on MacConkey agar?

- a) Cream, tan, or translucent
- b) Bright red
- c) Deep blue-black
- d) Green with a metallic sheen

Click to reveal answer

Answer a) Cream, tan, or translucent
The colonies of lactose fermenters appear red due to the accumulation of acids. Non-lactose fermenting bacteria will appear cream, tan, or translucent.

68. What concentration of NaCl does mannitol salt agar contain?

- a) 7.5%
- b) 0.1%
- c) 1.5%
- d) 15%

Click to reveal answer

Answer a) 7.5%
Mannitol salt agar contains 7.5% NaCl, which creates an environment on which only salt tolerant organisms, facultative halophiles, can grow.

69. The salt concentration of mannitol salt agar selects for organisms within which genus?

- a) *Staphylococcus*
- b) *Streptococcus*
- c) *Escherichia*
- d) *Klebsiella*

Click to reveal answer

Answer a) *Staphylococcus*
This concentration of salt selects for organisms within the *Staphylococcus* genera, because most other bacteria cannot survive such high concentrations of salt.

70. Which ingredients make mannitol salt agar a differential medium? **Select all that apply.**

- a) The sugar mannitol
- b) The pH indicator phenol red
- c) 7.5% NaCl
- d) Bile salts

Click to reveal answers

Answers: a) The sugar mannitol; b) The pH indicator phenol red
The media contains the sugar mannitol and the pH indicator phenol red. These ingredients allow differentiation between members of the *Staphylococcus* genus who can ferment mannitol and those who cannot.

71. On mannitol salt agar, how do pathogenic species of Staphylococci differ from non-pathogenic species?

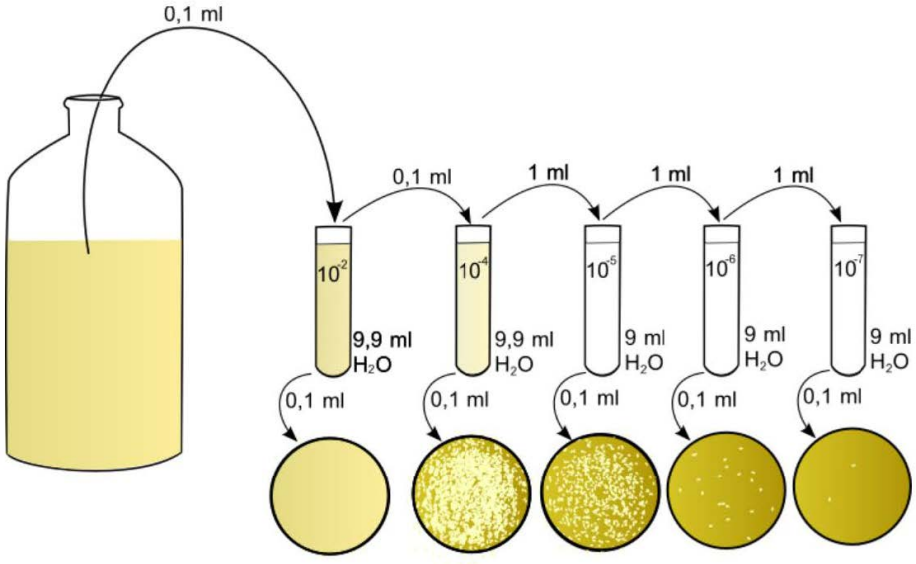
- a) Pathogenic species are capable of mannitol fermentation, while non-pathogenic species grow but do not ferment the mannitol
- b) Pathogenic species fail to grow, while non-pathogenic species grow and ferment mannitol
- c) Pathogenic species produce a black precipitate, while non-pathogenic species do not
- d) Pathogenic species are inhibited by the salt, while non-pathogenic species tolerate it

Click to reveal answer

Answer a) Pathogenic species are capable of mannitol fermentation, while non-pathogenic species grow but do not ferment the mannitol
Pathogenic species of Staphylococci are capable of mannitol fermentation, whereas non-pathogenic species grow on the mannitol salt agar but do not ferment the mannitol.

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4: SECTION FOUR - ENUMERATING BACTERIA



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In all fields of microbiology, the ability to measure microbial growth is extremely important. Microbial cells are extremely small and grow rapidly. It would be impossible to count every microbial cell in a population. Microbiologists utilize a number of enumerating methods to estimate the number of cells in a microbial sample. In this laboratory section, you will examine two of the methods employed by microbiologists to enumerate bacteria: viable plate counts and direct microscopic counts. As you move through the section, compare the accuracy, estimate of living cells, use of materials, and time required for the techniques surveyed.

YouTube Video

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Counts Shape Clinical Care: Bacterial Enumeration

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Credit: Yenny Mo, Josh Spradling, Sherry Smith

4.1: Exercise #24 - Viable Plate Counts

4.1.1: Serial Dilutions and Viable Plate Counts

4.1.2: Serial Dilutions and Viable Plate Counts Activity

4.1.3: Exercise #24 - Lab Wrap-Up

4.2: Exercise #25- Direct Microscopic Count

4.2.1: Direct Microscopic Count

4.2.2: Direct Microscopic Count Activity

4.2.3: Exercise #25 - Lab Wrap-Up

4.3: Section Four Review

4.3.1: Matching

4.3.2: Fill-In-The-Blank

4.3.3: True/False

4.3.4: Multiple Choice / Multiple Select

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4.1: Exercise #24 - Viable Plate Counts

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

THECB - ACGM Lab Learning Outcome #7 Estimate the number of microorganisms in a sample using methods such as direct counts, viable plate counts, or spectrophotometric measurements.

Laboratory Exercise Learning Outcomes

1. Define serial dilution.
2. Define colony and colony forming unit.
3. Describe the steps involved in performing a serial dilution and viable plate count.
4. Demonstrate the ability to calculate serial dilutions.
5. Demonstrate the ability to determine the number of bacteria in a sample using serial dilutions and viable plate counts.

Materials Needed:

- Test tube rack
- E. coli culture (24-48 hours old)
- 7 sterile water blank tubes
- 6 nutrient agar deep tubes
- Water bath (45°C)
- Sterile mL serological pipettes (11)
- Pipette pump
- Biohazard container for sharps

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4.1.1: Serial Dilutions and Viable Plate Counts

Viable plate counts, also known as standard plate counts, are a method of counting bacteria in which samples are diluted, plated, and then colonies counted following incubation. This technique requires that a sample be diluted before being introduced to the petri plates, because undiluted samples would not yield isolated colonies that could be easily counted. Prior to introducing an inoculum to petri plates, ***serial dilutions*** are carried out by transferring a sample to a sterile water blank, and then in a in a series of transfers of known volume transferred to additional water blanks. So, in essence each container will be a dilution of the previous dilution.

After the serial dilutions have been achieved a known volume from each of several dilutions will be transferred to separate petri plates. Agar at 45°C is then introduced to the sample within the petri dish. The temperature of the agar is important. If the agar is too cool it will solidify making it impossible to mix with the diluted bacterial samples. If the agar is too hot, it will kill the bacteria in

the sample, and thus nothing would grow after the plates are incubated. After the addition of agar, plates are allowed to solidify. They are then inverted and incubated for 24 hours.

At 24 hours the plates can be evaluated for growth. The plates will have colonies present on the surface of the agar. An isolated ***colony*** will contain genetically identical bacterial cells, due to the cell(s) originally deposited in that location of the agar undergoing many rounds of binary fission to produce the observable growth. Plates for counting should have between 30 and 300 colonies. This is because plates with less than 30 colonies will not provide a statistically reliable determination of bacteria in the original sample. Plates with greater than 300 colonies, will have some colonies too closely arranged together, and may also be miscounted, yielding results that are not statistically reliable. Once the colonies have been counted the number of bacteria in the original sample is calculated by multiply the number of colonies by the dilution factor of the plate counted. Results are recorded as colony forming units/ml (CFU/ml). This is because some bacteria naturally form arrangements (i.e. *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, etc.) and thus may have a few genetically identical cells attached to one another, that are referred to as a ***colony-forming unit***, that act to give rise to a colony.

The viable plate is a very accurate method of enumerating bacteria. This is because only viable (living) cells will give rise to colonies. This technique does pose some disadvantages. One of the disadvantages is that the technique takes a minimum of 24 - 48 hours to complete. Another disadvantage is that more laboratory materials are required than some of the other enumerating techniques utilized by microbiologist. A third disadvantage is that it requires more steps than other techniques, and thus creates more opportunities for the introduction of errors and the process is carried out.

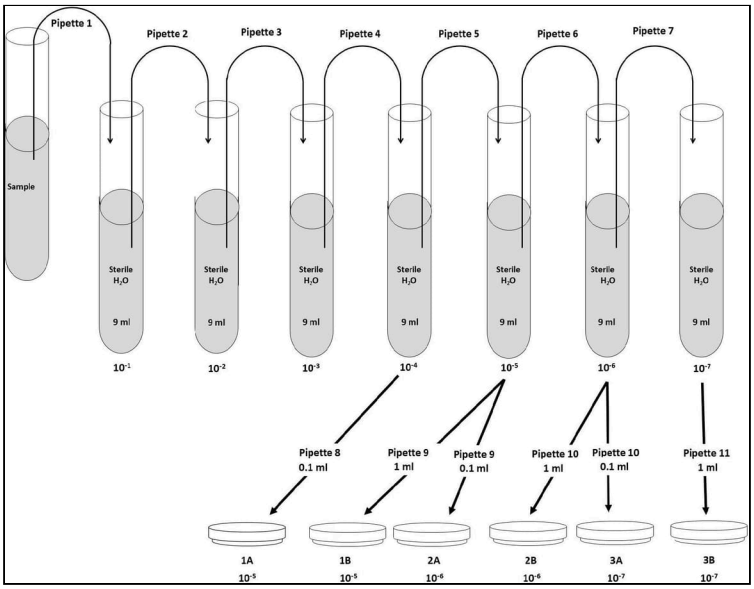
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4.1.2: Serial Dilutions and Viable Plate Counts Activity

Activity Preparation

1. Melt the agar deep tubes by placing them in boiling water or by autoclaving. After they are melted place them in the hot water bath at 45C to prevent them from solidifying.
2. Label your sterile water blank tubes # 1 - 7.
3. Label your petri dishes as follows: 1A, 1B, 2A, 2B, 3A, 3B

The following diagram shows the steps you will carry out to complete the serial dilutions of your stock sample and the transfer of samples to petri dishes.



Credit: Sherry Smith

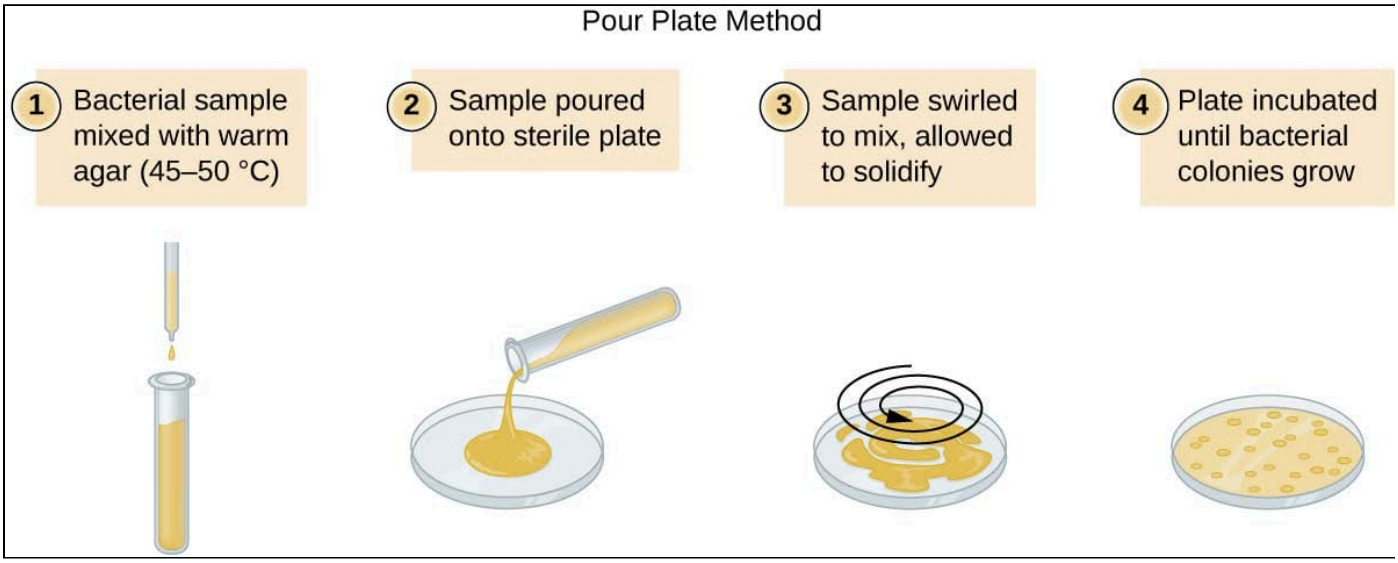
Serial Dilutions

1. Obtain the *E. coli* stock culture and flick the tube until the bacteria are evenly suspended throughout the media.
2. Use a sterile pipette and pipette pump to aseptically obtain 1 ml of the sample and transfer it to the sterile blank tube #1.
3. Mix tube #1 to evenly distribute the sample throughout the solution.
4. Aseptically obtain 1 ml of sample from tube #1 using a fresh sterile pipette and transfer it to sterile blank tube #2.
5. Mix tube #2 to evenly distribute the sample throughout the solution.
6. Aseptically obtain 1 ml of sample from tube #2 using a fresh sterile pipette and transfer it to sterile blank tube #3.
7. Mix tube #3 to evenly distribute the sample throughout the solution.
8. Aseptically obtain 1 ml of sample from tube #3 using a fresh sterile pipette and transfer it to sterile blank tube #4.
9. Mix tube #4 to evenly distribute the sample throughout the solution.
10. Aseptically obtain 1 ml of sample from tube #4 using a fresh sterile pipette and transfer it to sterile blank tube #5.
11. Mix tube #5 to evenly distribute the sample throughout the solution.
12. Aseptically obtain 1 ml of sample from tube #5 using a fresh sterile pipette and transfer it to sterile blank tube #6.
13. Mix tube #6 to evenly distribute the sample throughout the solution.
14. Aseptically obtain 1 ml of sample from tube #6 using a fresh sterile pipette and transfer it to sterile blank tube #7.
15. Mix tube #7 to evenly distribute the sample throughout the solution.

Inoculation of Petri Dishes

You will use the serial dilution tubes #4, #5, #6, and #7 to inoculate the plates that were labeled to begin this activity.

1. Mix tube #4 to ensure the bacteria are evenly distributed throughout the solution. Use a fresh pipette to aseptically transfer 0.1 ml of the sample to petri dish 1A.
2. Mix tube #5 to ensure the bacteria are evenly distributed throughout the sample. Then using a fresh pipette transfer 1 ml of sample to petri dish 1B.
3. Using the same pipette obtain 0.1 ml of sample from tube #5 and transfer to petri dish 2A.
4. Mix tube #6 to ensure the bacteria are evenly distributed throughout the sample. Then using a fresh pipette transfer 1 ml of sample to petri dish 2B.
5. Using the same pipette obtain 0.1 ml of sample from tube #6 and transfer to petri dish 3A.
6. Mix tube #7 to ensure the bacteria are evenly distributed throughout the solution. Use a fresh pipette to aseptically transfer 1 ml of the sample to petri dish 1A.
7. Obtain one 45°C molten agar tube, wipe moisture from the outside of the tube.
8. Using sterile technique pour the molten agar into petri dish 1A. Close the lid to the petri dish and gently rotate the plate to move the agar through the sample until it has been evenly distributed throughout the dish.
9. Repeat steps 7 & 8 for petri dishes 1B, 2A, 2B, 3A, and 3B and then allow the agar to solidify within the dishes.
10. After the agar has solidified, invert the petri dishes and place them in an incubator at 37°C for 24 hours.



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Counting Colonies

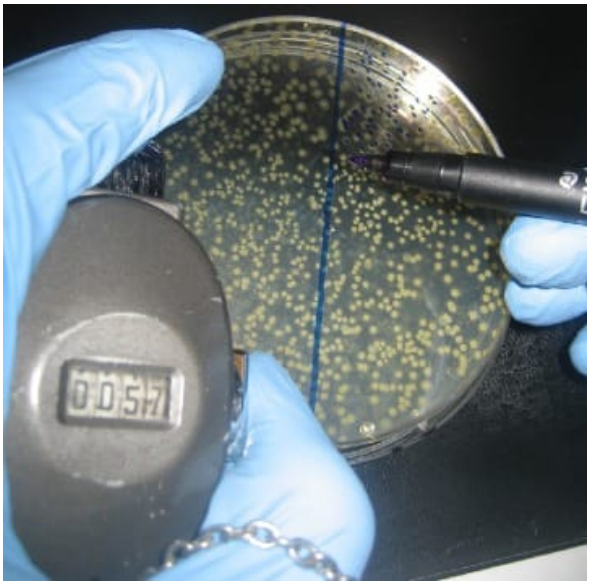
After incubation obtain all of your petri plates Examine them and select those plates with 30 - 300 colonies for counting. If a plate has less than 30 colonies use a marker to write TFTC on the plate. TFTC stands for “too few to count.” If a plate has greater than 300 colonies use a marker to write TNTC on the plate. TNTC stands for “too numerous to count.”

If the plate has TNTC or TFTC you will also record that as the “# bacterial colonies in your results table.

1. Count the number of colonies on each of the plates yielding 30 - 300 colonies. If one is available you can use a Quebec colony counter (digital colony counter). Your instructor will show you how to utilize the device. If not, a handheld counter can be utilized.



Digital colony counter.
Source: Srini297, Wikimedia Commons, CC BY 4.0



"The traditional way of enumerating CFUs with a "click counter" and a pen."
Source: Quentin Geissmann, Wikimedia Commons, CC BY 3.0

2. Calculate the number of bacteria in the original sample. Do this by multiplying the #of colonies by the dilution factor. Record your data in the provided table.

Example: You count 190 colonies on a plate that was diluted 1:1,000,000 (10-7). The dilution factor is the reciprocal of the dilution. So, the dilution factor would be 107. Answers will be denoted using scientific notation

190 x 107 = 1.9 x 109

Results:

Serial Dilution Viable Plate Count Results

Plate	# bacterial colonies	dilution factor	Total # CFU per ml original sample
1A			
1B			
2A			
2B			
3A			
3B			

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4.1.3: Exercise #24 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. State the formula for determining the number of CFUs per mL of sample when using the viable plate count technique.
2. What is the acceptable range of colonies on a plate to be counted?
3. A student performs a series of dilutions, using a stock solution of *M. luteus*. The stock solution he started with was turbid. He carried out his serial dilutions to produce 5 dilutions (10^{-1},10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). He labeled 5 petri dishes, one for each dilution. He then transferred 1 ml of the dilutions to the appropriate dish. He then poured melted agar into each of the Petri dishes and mixed the sample by gently swirling the dish. The petri dishes were incubated for 48 hours at 37°C. Upon observation he found no growth in any of the dishes. What could explain these results?
4. You are asked to prepare a serial dilution in which the final dilution is 10^{-8}. Describe and diagram how this can be achieved.

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4.2: Exercise #25- Direct Microscopic Count

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions.

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #7 Estimate the number of microorganisms in a sample using methods such as direct counts, viable plate counts, or spectrophotometric measurements.

Laboratory Exercise Learning Outcomes

1. Describe the steps for a direct microscopic count of bacteria.
2. Demonstrate the ability to determine the number of bacteria in a sample using a direct microscopic count.

Materials Needed:

- Test tube rack
- E. coli culture (16-24 hours old)
- 1 mL pipette & Pipette pump or micropipettor with tips
- Methylene blue dye
- Test tube
- Microscope
- Immersion oil

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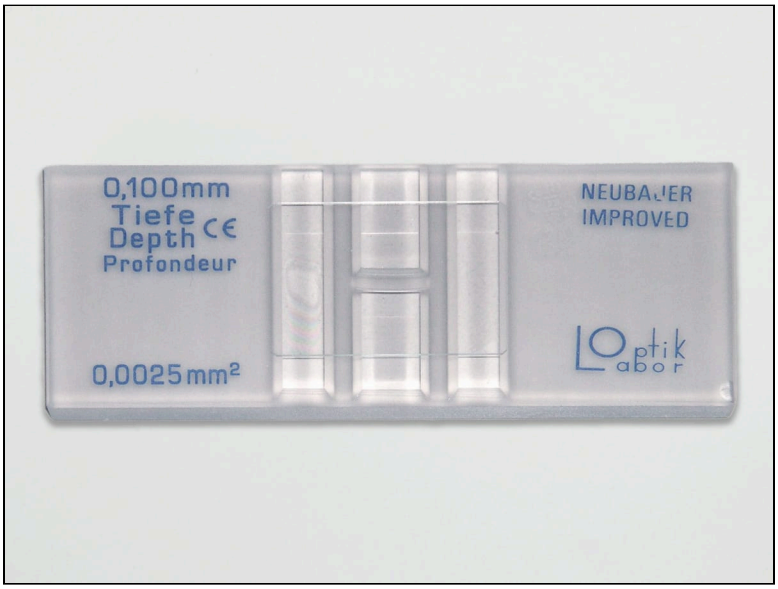
4.2.1: Direct Microscopic Count

The ***direct microscopic count*** is a method in which microbial cells are directly counted using a special counting chamber, known as a Petroff-Hausser counting chamber and a microscope. A stain is added to a sample and then applied to the chamber and visualized using oil immersion to count the number of cells present in the sample. This technique does not differentiate living cells from dead cells.

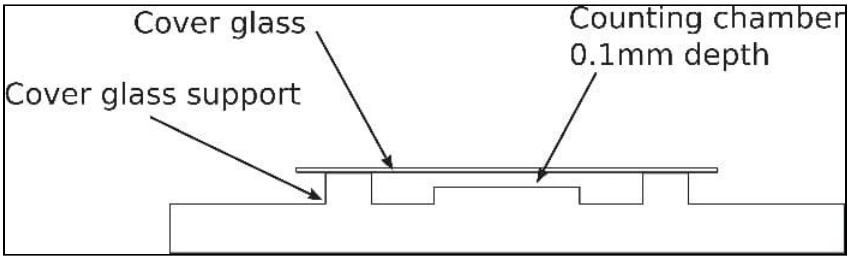
The ability to enumerate bacteria with this method, lies in the construction of the Petroff-Hauser counting chamber. The counting chamber is built in a manner that it has etched grids in a depress region of the slide,

over which cover slip is placed. A small volume of sample to be counted is added at the edge of the cover slip using a pipette. The sample moves into the chamber area by capillary action. The counting chamber is built in a manner such that when a sample is introduce a 0.02 mm3 volume of the sample will be present in each 1 mm2 of the chamber. Each 1 mm2 region has 25 large squares etched into the chamber. In total, when a sample is applied to this reservoir its dilution is 1:1,250,000. To complete a count the number of bacteria in several of the large squares are counted, and then the average taken. To determine the total number of cells in the original sample the number of cells must be multiplied by the dilution factor of the chamber and the dilution factor produced as a result of dying the sample.

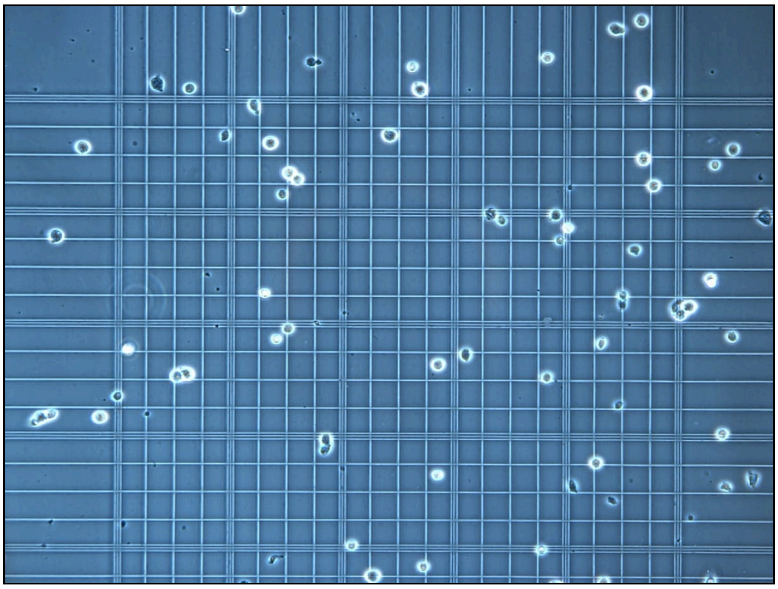
Formula for Direct Microscopic Count: Avg, #cells x 1,250,000 x dye dilution factor = #cells/cm3(ml)



Counting chamber.
Source: Alcibiades, Wikimedia Commons, CC0



Counting chamber components.
Source: Ewen, Wikimedia Commons, Copyrighted free use



Phase contrast micrograph of cells in a counting chamber.
Source: Alcibiades, Wikimedia Commons, Public Domain

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4.2.2: Direct Microscopic Count Activity

1. Obtain the *E. coli* stock culture and flick the tube until the bacteria are evenly suspended throughout the media.
2. Use a sterile pipette to aseptically obtain 1 ml of the sample and transfer it to the clean test tube.
3. Use a fresh pipette to transfer 1 ml of methylene blue dye to the sample in the test to, creating a 1/2 dilution.
4. Ensure the counting chamber is clean, then place the cover slip over the chamber.
5. Use a pipette to place one drop of the dyed sample at the edge of the cover slip. Allow time for the sample to move completely into the chamber as a result of capillary action.
6. Utilize a compound light microscope on oil immersion to count the number of bacterial cells present. You will count five of the large squares and then determine the average number of cells present.
7. Calculate the total number of cells present in the original sample using the formula below and then record your answer in the provided space.

Avg, #cells x 1,250,000 x dye dilution factor = #cells/cm3(ml)

_____ cells/ml

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4.2.3: Exercise #25 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. State the formula for determining the number of CFUs per mL of sample when using the direct microscopic method of enumeration.
2. How does the accuracy of the direct microscopic count compare to that of the viable plate count?
3. A mL sample of a bacterial is mixed with a 1 ml sample of crystal violet. After counting bacterial cells in 5 large squares, it is determined that the average number of cells is 35 cells. Calculate the number of cells present in the original sample. Show your work.

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4.3: Section Four Review

Review Purpose

This review helps you check what you learned in Section Four before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you...

- Define serial dilution, colony, and colony-forming unit (CFU)?
- Describe the steps of a serial dilution and viable plate count, and calculate a serial dilution?
- Determine the number of bacteria in a sample using a viable plate count?
- Describe the steps of a direct microscopic count using a counting chamber?
- Determine the number of bacteria in a sample using a direct microscopic count?

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4.3.1: Matching

Matching Questions

1. Match the dilution on the left with its correct dilution factor by placing the letter next to the correct dilution.

Question 1 Terms

Answer	Terms
_____	1. 1:100
_____	2. 1:1,000
_____	3. 1:10,000
_____	4. 1:100,000
_____	5. 1:1,000,000
_____	6. 1:10,000,000

Question 1 Descriptions

Letter	Description
a.	a dilution factor of 10^5
b.	a dilution factor of 10^2
c.	a dilution factor of 10^7
d.	a dilution factor of 10^3
e.	a dilution factor of 10^6
f.	a dilution factor of 10^4

Click to reveal answers

Answers:
1) b
2) d
3) f
4) a
5) e
6) c

2. Match the value on the left with the appropriate description of what it refers to by placing the letter next to the correct value.

Question 2 Terms

Answer	Terms
_____	1. 30 - 300
_____	2. 45 degrees C
_____	3. 37 degrees C
_____	4. 24 hours

Answer	Terms
_____	5. 25
_____	6. 0.02 mm cubed
_____	7. 1,250,000
_____	8. 2

Question 2 Descriptions

Letter	Description
a.	the temperature at which molten agar is held before it is poured into the petri dish
b.	the number of large squares etched into each 1 mm squared region of the counting chamber
c.	the acceptable range of colonies on a plate selected for counting
d.	the dye dilution factor created when 1 ml of sample is mixed with 1 ml of methylene blue
e.	the volume of sample present in each 1 mm squared of the counting chamber
f.	the incubation temperature used for the poured plates
g.	the dilution factor of the Petroff-Hausser counting chamber
h.	the length of time the poured plates are incubated before colonies are counted

Click to reveal answers

Answers:
1) c
2) a
3) f
4) h
5) b
6) e
7) g
8) d

3. Match the procedure step on the left with the reason it is performed by placing the letter next to the correct step.

Question 3 Terms

Answer	Terms
____	1. Flick the stock culture tube before removing a sample
____	2. Hold the melted agar tubes in a 45 degrees C water bath
____	3. Gently rotate the plate after the molten agar is poured in
____	4. Invert the plates before placing them in the incubator
____	5. Add the sample at the edge of the cover slip
____	6. Combine 1 ml of sample with 1 ml of methylene blue

Question 3 Descriptions

Letter	Description
a.	moves the agar through the sample until it has been evenly distributed throughout the dish
b.	suspends the bacteria evenly throughout the media so a representative sample can be taken
c.	allows the sample to move into the chamber by capillary action
d.	keeps the agar molten without making it hot enough to kill the bacteria in the sample
e.	prevents condensation from raining down onto the sample
f.	stains the cells and creates a 1/2 dilution that must be included in the calculation

Click to reveal answers

Answers:
1) b
2) d
3) a
4) e
5) c
6) f

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4.3.2: Fill-In-The-Blank

Fill-in-the-Blank Questions

1. A group of genetically identical cells attached to one another that give rise to a single colony on the surface of solid media is referred to as a _____.

Click to reveal answer

Answer colony-forming unit

2. The device used to hold the sample for a direct microscopic count is called a _____.

Click to reveal answer

Answer Petroff-Hausser counting chamber

3. When using the viable plate count method to count bacteria in a sample, it is important to perform a serial _____ of the original sample so there is a statistically valid number of colonies to count.

Click to reveal answer

Answer dilution

4. A _____ is a growth of bacteria on the surface of solid media that contain genetically identical cells.

Click to reveal answer

Answer colony

5. When performing a standard plate count (viable plate count), plates that contain _____ to _____ colonies are selected for counting.

Click to reveal answer

Answer 30, 300

6. Viable plate counts are also known as _____.

Click to reveal answer

Answer standard plate counts

7. The dilution factor is the _____ of the dilution.

Click to reveal answer

Answer reciprocal

8. Cells originally deposited on the agar undergo many rounds of _____ to produce the observable growth of a colony.

Click to reveal answer

Answer binary fission

9. In a direct microscopic count, the sample moves from the edge of the cover slip into the chamber by _____.

Click to reveal answer

Answer capillary action

10. The direct microscopic count does not differentiate _____ cells from _____ cells.

Click to reveal answer

Answer living, dead

11. Bacterial cells in the counting chamber are visualized and counted using the _____ objective lens.

Click to reveal answer

Answer oil immersion

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4.3.3: True/False

True / False Questions

1. It's feasible to count the number of colonies on a plate if they are going to exceed 500.

_____ True _____ False

Click to reveal answer

Answer False

2. The dilution factor of a particular bacterial culture made when 30 microliters (µL) is added to 270 µL of sterile broth is the same dilution factor as when 10 milliliters (mL) is added to 90 mL of sterile broth.

_____ True _____ False

Click to reveal answer

Answer True

3. A petri dish with the abbreviation "TNTC" marked on it indicates that there are fewer than 30 colonies present.

_____ True _____ False

Click to reveal answer

Answer False

4. Plates should not be counted if they have more than 100 colonies present on them.

_____ True _____ False

Click to reveal answer

Answer False

5. Viable plate counts are the most accurate method of counting bacteria, because only viable (living) cells are counted.

_____ True _____ False

Click to reveal answer

Answer True

6. The direct microscopic count allows the observer to distinguish living cells from dead cells.

_____ True _____ False

Click to reveal answer

Answer False

7. A sample is drawn into the Petroff-Hausser counting chamber by capillary action.

_____ True _____ False

Click to reveal answer

Answer True

8. Every cell in the counting chamber must be counted in order to determine the number of cells present.

_____ True _____ False

Click to reveal answer

Answer False

9. To determine the number of cells in the original sample, the average cell count is multiplied only by the dilution factor of the chamber.

_____ True _____ False

Click to reveal answer

Answer False

10. A single colony forming unit may consist of more than one bacterial cell.

_____ True _____ False

Click to reveal answer

Answer True

11. An undiluted sample may be plated directly, since the colonies would still be isolated enough to count.

_____ True _____ False

Click to reveal answer

Answer False

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4.3.4: Multiple Choice / Multiple Select

Multiple Choice / Multiple-Select Questions

1. Why do microbiologists use methods that estimate the number of cells in a population rather than counting them all?

- a) Microbial cells are extremely small and grow rapidly, making it impossible to count every cell in a population
- b) Counting every cell would require equipment unavailable in a clinical laboratory
- c) Bacterial populations change too slowly for a direct count to be meaningful
- d) Estimation is required by the standard plate count protocol

Click to reveal answer

> **Answer** a) Microbial cells are extremely small and grow rapidly, making it impossible to count every cell in a population
> Microbial cells are extremely small and grow rather rapidly. It would be impossible to count every microbial cell in a population, so microbiologists use enumerating methods to estimate the number of cells.

2. In a serial dilution, what is the relationship between each container and the one before it?

- a) Each container is a dilution of the previous dilution
- b) Each container is diluted directly from the original stock culture
- c) Each container is more concentrated than the one before it
- d) Each container holds the same concentration in a larger volume

Click to reveal answer

> **Answer** a) Each container is a dilution of the previous dilution
> Serial dilutions are carried out by transferring a sample to a sterile water blank, and then in a series of transfers of known volume to additional water blanks. In essence, each container will be a dilution of the previous dilution.

3. Why are plates holding fewer than 30 colonies not used for counting?

- a) They will not provide a statistically reliable determination of the bacteria in the original sample
- b) The colonies are too small to be seen without a colony counter
- c) A dilution factor cannot be calculated for such a plate
- d) The bacteria on those plates are not viable

Click to reveal answer

> **Answer** a) They will not provide a statistically reliable determination of the bacteria in the original sample
> Plates with less than 30 colonies will not provide a statistically reliable determination of bacteria in the original sample.

4. Why are plates holding more than 300 colonies not used for counting?

- a) Some colonies will be too closely arranged together and may be miscounted, so the result is not statistically reliable

- b) The agar cannot supply nutrients to that many colonies
- c) Colonies at that density stop undergoing binary fission
- d) The excess colonies alter the dilution factor of the plate

Click to reveal answer

Answer a) Some colonies will be too closely arranged together and may be miscounted, so the result is not statistically reliable
Plates with greater than 300 colonies will have some colonies too closely arranged together, and may also be miscounted, yielding results that are not statistically reliable.

5. A plate is marked TFTC. What does this abbreviation stand for?

- a) Too few to count
- b) Too numerous to count
- c) Total fraction to count
- d) Transferred for total count

Click to reveal answer

Answer a) Too few to count
If a plate has less than 30 colonies use a marker to write TFTC on the plate. TFTC stands for too few to count.

6. A plate has been marked TNTC. What should be entered in the results table for that plate?

- a) TNTC is recorded as the number of bacterial colonies
- b) The entry is left blank
- c) An estimated count of 300 is recorded
- d) The plate is recounted with a colony counter and that number recorded

Click to reveal answer

Answer a) TNTC is recorded as the number of bacterial colonies
If the plate has TNTC or TFTC you will also record that as the number of bacterial colonies in your results table.

7. Three plates from the same serial dilution are examined. The 1:1,000 plate holds more than 400 colonies, the 1:10,000 plate holds 145 colonies, and the 1:100,000 plate holds 8 colonies. Which plate should be used to calculate the original sample?

- a) The 1:1,000 plate
- b) The 1:10,000 plate
- c) The 1:100,000 plate
- d) All three plates should be averaged together

Click to reveal answer

Answer b) The 1:10,000 plate
Only plates holding 30 to 300 colonies are selected for counting. The 1:1,000 plate would be marked TNTC and the 1:100,000 plate TFTC, leaving the 1:10,000 plate with 145 colonies as the countable plate.

8. A plate that received 1 ml of the 1:10,000 dilution yields a final result of 2.4 x 10 to the sixth power CFU/ml for the original specimen. How many colonies were counted on that plate?

- a) 24
- b) 240
- c) 2,400
- d) 24,000

Click to reveal answer

Answer b) 240
The number of colonies multiplied by the dilution factor gives the original count. The dilution factor for 1:10,000 is 10 to the fourth power, so the colony count is 2.4 x 10 to the sixth power divided by 10 to the fourth power, which is 240 colonies.

9. Why are results reported as colony forming units per ml rather than as cells per ml?

- a) Some bacteria naturally form arrangements, so several genetically identical cells attached to one another may give rise to a single colony
- b) Each colony may contain more than one species of bacterium
- c) Dead cells contribute to the size of each colony
- d) The dilution factor cannot account for individual cells

Click to reveal answer

Answer a) Some bacteria naturally form arrangements, so several genetically identical cells attached to one another may give rise to a single colony
Results are recorded as colony forming units/ml because some bacteria naturally form arrangements and thus may have a few genetically identical cells attached to one another, referred to as a colony-forming unit, that act to give rise to a colony.

10. Which organisms are given as examples of bacteria that naturally form arrangements? **Select all that apply.**

- a) *Bacillus subtilis*
- b) *Staphylococcus aureus*
- c) *Streptococcus pneumoniae*
- d) *Escherichia coli*

Click to reveal answers

Answers: a) *Bacillus subtilis*; b) *Staphylococcus aureus*; c) *Streptococcus pneumoniae*
Some bacteria naturally form arrangements, for example *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*.

11. What disadvantages does the viable plate count technique present? **Select all that apply.**

- a) It takes a minimum of 24 to 48 hours to complete
- b) It requires more laboratory materials than some other enumerating techniques
- c) It requires more steps, creating more opportunities for the introduction of errors
- d) It cannot distinguish one colony from another

Click to reveal answers

Answers: a) It takes a minimum of 24 to 48 hours to complete; b) It requires more laboratory materials than some other enumerating techniques; c) It requires more steps, creating more opportunities for the introduction of errors
The disadvantages are that the technique takes a minimum of 24 to 48 hours, requires more laboratory materials than some other techniques, and requires more steps, creating more opportunities for the introduction of errors.

12. Which device may be used to assist in counting the colonies on a plate?

- a) A Quebec colony counter
- b) A Petroff-Hausser counting chamber
- c) A pipette pump
- d) A water bath

Click to reveal answer

Answer a) A Quebec colony counter
If one is available you can use a Quebec colony counter, a digital colony counter. If not, a handheld counter can be utilized.

13. A student completes the pour plate procedure correctly but finds no growth at all on any of the plates after incubation. What is the most likely explanation?

- a) The agar was too hot when it was poured, killing the bacteria in the sample
- b) The agar was too cool and solidified before it could be mixed with the sample
- c) The plates were incubated upright rather than inverted
- d) The sample was diluted only to 1:100

Click to reveal answer

Answer a) The agar was too hot when it was poured, killing the bacteria in the sample
If the agar is too hot, it will kill the bacteria in the sample, and thus nothing would grow after the plates are incubated.

14. How is the Petroff-Hausser counting chamber constructed?

- a) It has etched grids in a depressed region of the slide, over which a cover slip is placed
- b) It has a raised platform with a sample well at each corner
- c) It is a sealed chamber filled with solidified agar
- d) It uses a rotating disk that separates cells by size

Click to reveal answer

Answer a) It has etched grids in a depressed region of the slide, over which a cover slip is placed
The counting chamber is built in a manner that it has etched grids in a depressed region of the slide, over which a cover slip is placed.

15. Which statements are true of the direct microscopic count but NOT of the viable plate count? **Select all that apply.**

- a) The result is obtained without waiting for an incubation period
- b) Both living and dead cells are included in the count
- c) Only viable cells contribute to the result
- d) The sample must be serially diluted before it can be used

Click to reveal answers

Answers: a) The result is obtained without waiting for an incubation period; b) Both living and dead cells are included in the count
The direct microscopic count does not differentiate living cells from dead cells, and requires no incubation. The viable plate count takes a minimum of 24 to 48 hours and counts only viable cells, because only living cells give rise to colonies.

16. A direct microscopic count and a viable plate count are performed on the same culture, and the direct count gives a much higher number. What is the most likely reason?

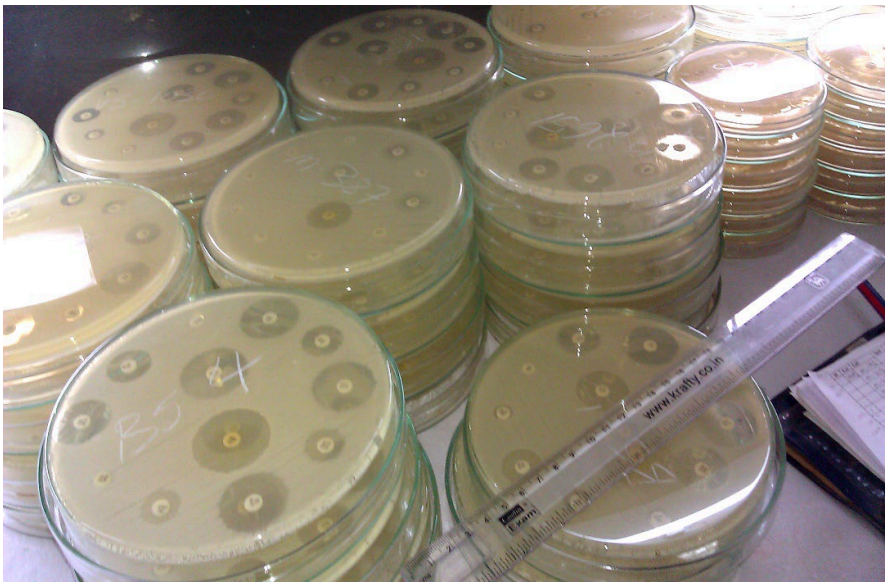
- a) The direct microscopic count includes dead cells, while only living cells give rise to colonies
- b) The counting chamber magnifies the sample more than the microscope used to count colonies
- c) The stain used in the direct count causes the cells to divide
- d) The viable plate count uses a larger dilution factor

Click to reveal answer

Answer a) The direct microscopic count includes dead cells, while only living cells give rise to colonies
The direct microscopic count does not differentiate living cells from dead cells, so dead cells are included in the total. The viable plate count is accurate for living cells because only viable cells will give rise to colonies.

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5: SECTION FIVE - FACTORS AFFECTING MICROBIAL GROWTH



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Microbial growth can be impacted by a variety of factors including temperature, pH, and the presence of antimicrobial agents. Structural differences in microorganisms cause them to respond to these factors differently. These differences can be used to classify and characterize the microorganisms we work with in the lab. Knowing how such factors affect bacteria also helps microbiologists understand what conditions are required to encourage growth for study, as well as how to alter those conditions and make environments less conducive to microbial growth. In this section, you will explore how temperature, pH, and antiseptics/disinfectants affect microbial growth. You will also examine a clinically important procedure, known as the antimicrobial sensitivity test (Kirby-Bauer method), that aids in the selection of antimicrobials for treatment of infections in patients.

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Power to Control: Factors Affecting Microbial Growth

Click on the video thumbnail or the linked title to access the video.

Credit: Yenny Mo, Josh Spradling, Sherry Smith

5.1: Exercise #26 - Effect of Temperature on Microbial Growth

5.1.1: Temperature and Microbial Growth

5.1.2: Assessing Effects of Temperature on Microbial Growth Activity
5.1.3: Assessing Effects of Temperature on Phenotypic Expression Activity
5.1.4: Exercise #26 - Lab Wrap-Up

5.2: Exercise #27 - Effect of pH on Microbial Growth

5.2.1: pH and Microbial Growth
5.2.2: Assessing Effects of pH on Microbial Growth Activity
5.2.3: Exercise #27 - Lab Wrap-Up

5.3: Exercise #28 - Effect of Antiseptics and Disinfectants on Microbial Growth

5.3.1: Antiseptics and Disinfectants
5.3.2: Assessing the Effects of Antiseptics and Disinfectants on Microbial Growth Activity
5.3.3: Exercise #28 - Lab Wrap-Up

5.4: Exercise #29 - Antimicrobial Sensitivity Test (Kirby-Bauer Method)

5.4.1: Antimicrobial Susceptibility Test
5.4.2: Antimicrobial Sensitivity Test (Kirby-Bauer Method) Activity
5.4.3: Exercise #29 - Lab Wrap-Up

5.5: Section Five Review

5.5.1: Matching
5.5.2: Fill-In-The-Blank
5.5.3: True/False
5.5.4: Multiple Choice / Multiple Select

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5.1: Exercise #26 - Effect of Temperature on Microbial Growth

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Define serial dilution.
2. Define the following temperature groups used to characterize bacteria, psychrophile, psychrotroph, mesophile, thermophile.
3. Describe how microorganisms are affected by temperature.
4. Perform an experiment to determine temperature preferences for a given set of bacterial species, and appropriately classify the bacteria on those preferences.

Materials Needed:

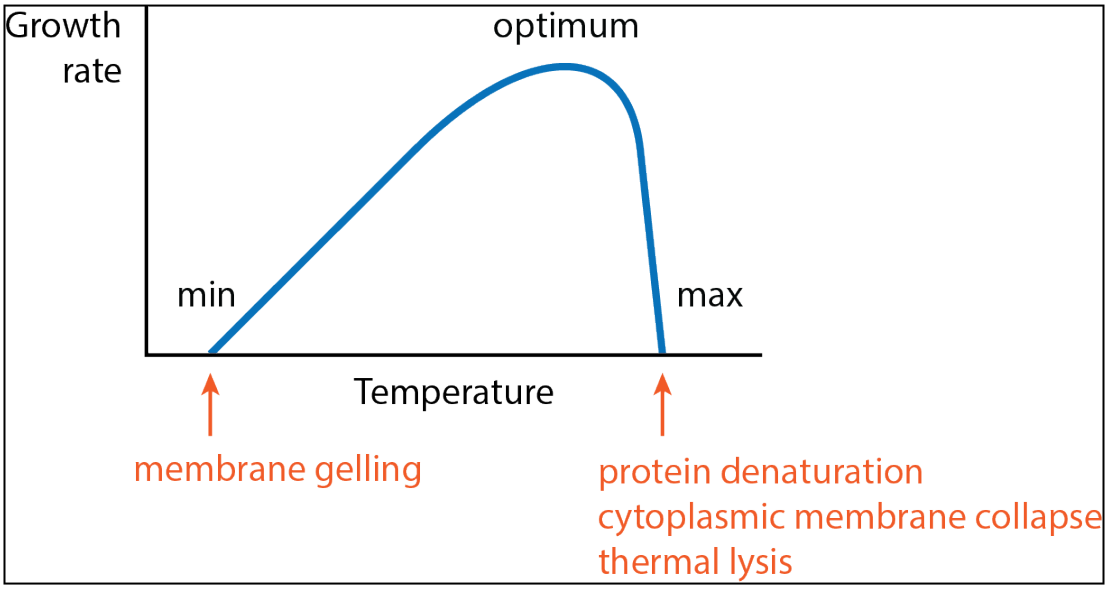
- Bunsen burner
- Inoculating loop
- Broth bacterial cultures (*Pseudomonas fluorescens, Serratia marcescens, Escherichia coli, Bacillus stearothermophilus*)
- 20 sterile broth cultures

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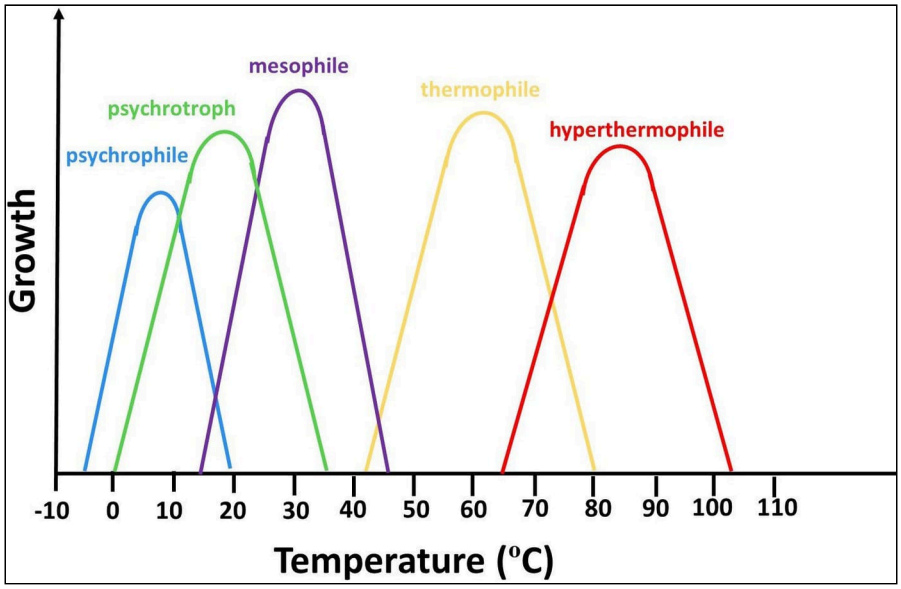
5.1.1: Temperature and Microbial Growth

Microorganisms require specific temperature ranges in order to grow. These growth requirements exist because these are the temperatures at which their enzymes and molecules that make up their cellular structures work best. Deviations from these temperatures can be detrimental to the cell. Temperatures above a cells optimum temperature may result in the denaturation of enzymes needed for metabolism and the destruction of lipids needed for membranous structures, such as the cell membrane, causing them to become too fluid. Temperatures that are lower than that of the optimum for a microorganism may impede the activity of enzymes in their ability to function, and alter lipids in such a manner that membranes become rigid, thereby affecting membrane transport.

Microorganisms can be categorized into groups based on their optimal temperatures. The groups include the psychrophiles, psychrotroph, mesophiles, thermophiles, and hyperthermophiles. Each of these groups demonstrate a range that has a minimum temperature, a maximum temperature, and optimal temperature for the organism's growth. ***Psychrophiles***, are cold-loving bacteria that have a minimum growth temperature of 0°C or lower, and a maximum temperature around 15C. They are not capable of growth above 20°C**.** ***Psychrotrophs*** are able to tolerate growth temperatures around 0°C - 5°C up to around 35°C. ***Mesophiles*** are adapted to moderate temperatures and have optimal growth temperatures between approximately 20°C - 45 °C. Pathogenic (disease-causing) microorganisms generally fall within this group, because the human body temperature is 37°C. Organisms that are adapted to high temperatures are referred to as ***thermophiles*** if they tolerate up to 80°C and ***hyperthermophiles*** if they have optimal temperatures greater then 80°C.



Temperature and growth rate Credit: Microbialmatt, Wikimedia Commons, CC BY 4.0



Microbial growth at various temperatures. Credit: Sherry Smith

In this exercise you will inoculate broths with bacterial samples and incubate them at five different temperatures. After allowing them to incubate you will assess the test tube for bacterial growth. You will do this for four different species, so you can determine the temperature ranges that each of the four organisms prefer.

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5.1.2: Assessing Effects of Temperature on Microbial Growth Activity

Inoculation and Incubation of Broth Tubes

Label five sterile broths tubes for each of the bacteria to be assessed.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the broth tube to be incubated at 5°C. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner.

Repeat steps 1 - 8 for the tubes that are to be incubated at 25°C, 37°C, 42°C, and 55°C for this same bacterium.

9. Incubate the inoculated broth tubes at the temperature labeled on the tube for 24 hours.

Repeat the above steps for each of the bacteria to be assessed.

Following incubation, you will measure microbial growth and report the data for the growth observed. You will then determine what type of organism each bacterium is based on its temperature preferences, i.e., psychrophile, psychrotroph, mesophile, thermophile.

Two methods have been provided for assessing the microbial growth. The choice of method will depend on what tools are available in your laboratory.

Measuring Microbial Growth:

You will choose your method of analyzing bacterial growth within the broth cultures and then record your data in the provided table.

Spectrophotometric Analysis

If a spectrophotometer is available for analysis, you may use the following steps to obtain your data.

Your instructor will provide instructions for calibrating your spectrophotometer. Calibrate the spectrophotometer using an uninoculated medium. Set the mode to absorbance and the wavelength to 600 nm.

After calibrating the spectrophotometer, you will pipette some of the sample to be measured into a clean cuvette. Make sure to wipe the sides of the cuvette with a Kim wipe to ensure no skin oils or fingerprints interfere with the measurement. Place the cuvette into the spectrophotometer and close the door. Record the absorbance for your sample. Repeat this process until you have measured all of your samples.

Visual Analysis

If a spectrophotometer is available for analysis, you may use the following steps to obtain your data. You will visually examine the sample for growth. You will record your observations based on the following scale.

- - = no growth
- + = minimal growth (minimal turbidity)
- ++ = moderate growth (moderate turbidity)
- +++ = maximal growth (maximum turbidity)

Microbial Growth at Different Incubation Temperatures

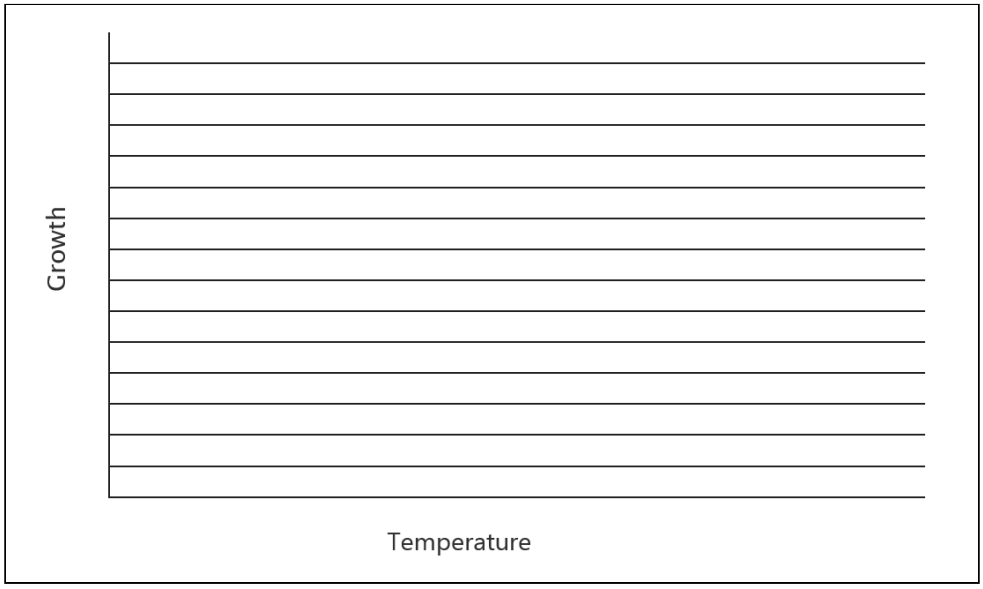
Bacterial Species	5°C	25°C	37°C	42°C	55°C

Measuring Microbial Growth: Spectrophotometric Analysis

You will now create a scatter plot from your data using colored pencils. Select a color and place the name of the bacteria in the legend. Then using the same color chart, the data obtained from your experiment. Repeat this for all bacteria assessed.

Graph Legend

Color	Bacterial Species



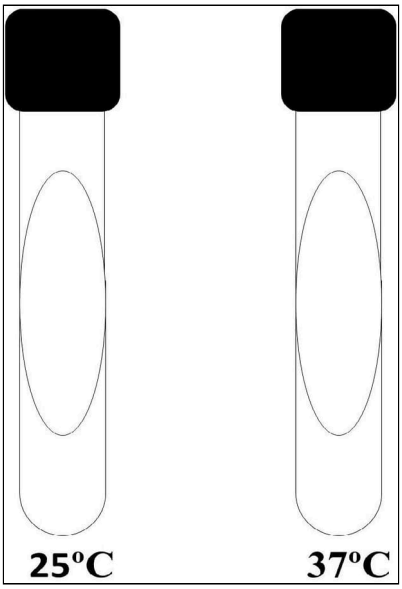
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5.1.3: Assessing Effects of Temperature on Phenotypic Expression Activity

Label two sterile agar slant tubes for each of the temperatures to be assessed

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a broth culture of *Serratia marcescens*. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the nutrient agar slant to be incubated at 25°C. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner. Repeat steps 1 - 8 for the tube that is to be incubated at 37°C.
9. Incubate the inoculated broth tubes at the temperature labeled on the tube for 24 hours.

Following incubation, you will examine the growth on the surface of the agar. Record your observations using the images below.



Credit: Sherry Smith

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5.1.4: Exercise #26 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. You sampled four different species in your experiment. List the organism tested and identify the temperature group to which each belongs based on your observations.
2. *Serratia marcescens* was grown on agar slants and incubated at two different temperatures. Describe your observations and explain what you observed.
3. Describe the difference between a psychrophile and a psychrotroph.
4. Explain why psychrotrophic organisms would be a concern for food-born illness.
5. There are number of methods in which temperature is used to control microbial growth. Describe three different ways in the home, clinical environment, or laboratory environment in which temperature is utilized for microbial control. Describe how that mechanism works and why it is important.

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5.2: Exercise #27 - Effect of pH on Microbial Growth

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Define the following groups used to characterize bacteria based on their pH preferences; acidophile, neutrophile, alkaliphile.
2. Describe how microorganisms are affected by pH.
3. Perform an experiment to determine the pH preferences for a given set of bacterial species and appropriately classify the bacteria based on those preferences.

Materials Needed:

- Bunsen burner
- Inoculating loop
- Broth bacterial cultures (*Lactobacillus acidophilis, Staphylococcus aureus, Alcaligenes faecalis*)
- 3 sterile nutrient broth cultures pH 3
- 3 sterile nutrient broth cultures pH 5
- 3 sterile nutrient broth cultures pH 7
- 3 sterile nutrient broth cultures pH 9
- 3 sterile nutrient broth cultures pH 11

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5.2.1: pH and Microbial Growth

Microorganisms can be found growing at all pH ranges. However, each specific microorganism will have range at which it can grow. The range consists of a minimum that allows growth, a maximum pH for growth, and an optimal pH between these two extremes where growth is most prolific.

pH is a measurement of the concentration of hydrogen ions in a sample or environment. pH is measured on a scale of 0 to 14. As hydrogen ion concentrations increase, the measurement moves toward 0 on the pH scale and is said to be acidic. On the other hand, when hydrogen ions decrease, the measurement moves toward 14 and is alkaline. pH 7 is considered neutral. This information is important to note when attempting to understand the classification of microorganism based on their pH preferences. Microorganisms that prefer a neutral pH range near pH 7 are called ***neutrophiles***. Most microorganisms belong to the classification.

Microorganisms whose optimal pH range is less than pH 5.5 are referred to as ***acidophile***. A clinically important example of acidophiles are the *Lactobacillus* species that are part of the normal flora of the vagina. *Lactobacillus* species require a pH less than 5.5 and produce products that help maintain that pH within the vagina. In doing so the *Lactobacillus* bacteria inhibit the growth of microorganisms that require a higher pH, i.e. yeast. There also microorganisms that prefer alkaline conditions, with a pH of 8 or greater.

Such microorganisms are referred to as ***alkaliphiles***. *Vibrio cholerae* is an example of clinically significant alkaliphile. *Vibrio cholerae* grows best at a pH slightly above pH 8.

The pH of the microorganism's environment is important because changes in pH can detrimentally affect cellular function. When the hydrogen concentration is too high (acidic) or too low (alkaline) hydrogen bonding in the cell may be impacted. The is problematic for both DNA and proteins. The double helix of DNA is held together by hydrogen bonds. Proteins, many of which act as enzymes within the cell to facilitate cellular metabolism are also dependent on hydrogen bonding for their secondary and tertiary structure.

Interrupting the hydrogen bonds due changes in pH can cause proteins to denature permanently. This can halt metabolism and thus kill the cell. In addition to affecting protein and DNA structure, alterations in pH can affect the process of cellular respiration. Some bacteria depend on cellular respiration to produce their needed ATP. The process requires that a gradient of hydrogen ions be present across the cell membrane so that they can enable ATP synthase to function in producing ATP.

In this exercise you will examine three different species of bacteria in different pH environments to determine each microorganism's optimal pH for growth.

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5.2.2: Assessing Effects of pH on Microbial Growth Activity

Inoculation and Incubation of Broth Tubes

Label five sterile broths tubes for each of the bacteria to be assessed.

1. Turn on the Bunsen burner and sterilize the inoculating loop by passing it through the flame of the burner.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the loop into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Pick up the pH3 broth tube. Remove the cap and pass the mouth of the tube through the flame of the Bunsen burner.
6. Insert the loop into the tube to introduce the bacterial sample to the media.
7. Pass the mouth of the broth tube through the flame and recap it, and return it to the test tube rack.
8. Sterilize the inoculating loop by flaming the loop in the flame of the Bunsen burner. Repeat steps 1 - 8 for the pH 5, pH 7, pH 9, and pH 11 broth tubes for this same bacterium.
9. Incubate the inoculated broth tubes at 37°C for 24 hours.

Repeat the above steps for each of the bacteria to be assessed.

Following incubation, you will measure microbial growth and report the data for the growth observed. You will then determine what type of organism each bacterium is based on its pH preferences, i.e. acidophile, neutrophile, alkalinophile.

Two methods have been provided for assessing the microbial growth. The choice of method will depend on what tools are available in your laboratory.

Measuring Microbial Growth:

You will choose your method of analyzing bacterial growth within the broth cultures and then record your data in the provided table.

Spectrophotometric Analysis

If a spectrophotometer is available for analysis you may use the following steps to obtain your data.

Your instructor will provide instructions for calibrating your spectrophotometer. Calibrate the spectrophotometer using an uninoculated medium. Set the mode to absorbance and the wavelength to 600 nm.

After calibrating the spectrophotometer, you will pipette some of the sample to be measured into a clean cuvette. Make sure to wipe the sides of the cuvette with a kim wipe to ensure no skin oils or fingerprints interfere with the measurement. Place the cuvette into the spectrophotometer and close the door. Record the absorbance for your sample. Repeat this process until you have measured all of your samples.

Visual Analysis

If a spectrophotometer is available for analysis you may use the following steps to obtain your data. You will visually examine the sample for growth. You will record your observations based on the following scale.

- - = no growth
- + = minimal growth (minimal turbidity)
- ++ = moderate growth (moderate turbidity)
- +++ = maximal growth (maximum turbidity)

Microbial Growth at Different pH Levels

Bacterial Species	pH 3	pH 5	pH 7	pH 9	pH 11

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5.2.3: Exercise #27 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Describe the consequences of a neutrophile being grown in a medium with pH 5.5. Include what occurs at the molecular level within the cell.
2. The organism *Vibrio cholerae* thrives at a pH above 8. Would a patient taking a proton-pump inhibiting drug be more or less likely to acquire an infection with this bacterium? Explain your answer.
3. When bacteria carry out fermentation, they produce organic acids. Explain how this could impact the growth of bacteria in a culture medium and why?
4. Some bacteria produce the enzyme urease, which acts to convert urea to ammonium. Describe how this process could impact bacterial growth in the medium.

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5.3: Exercise #28 - Effect of Antiseptics and Disinfectants on Microbial Growth

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Define the following terms related to control of microbial growth: antiseptic, disinfectant, sterilant, sanitizer, microbicidal, microbiostatic, bactericidal, bacteriostatic.
2. Define zone of inhibition.
3. Complete an experiment in which a variety of chemical agents (i.e. alcohol, bleach, lysol, etc.) are tested and compared for their relative effectiveness.

Materials Needed:

- Bunsen burner
- Sterile swabs
- Broth bacterial cultures (*Staphylococcus aureus, Pseudomonas aeruginosa*)
- 2 nutrient agar plates
- Sterile disks of filter paper (1/2" diameter)
- Microcentrifuge tubes with the antiseptic or disinfectant samples for analysis (sterile water, lysol, alcohol, bleach, hydrogen peroxide, mouthwash, hand sanitizer, pine sol, iodine, etc.)
- Forceps
- Beaker 70% alcohol
- Biohazard container

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5.3.1: Antiseptics and Disinfectants

Controlling microbial growth in the environment around us is important in our homes, food industry, clinical and hospital setting among other areas. Antimicrobial chemicals are one tool that can be utilized to control microbial growth. Antimicrobial agents work to either kill or inhibit the growth of microorganisms. Agents that act to kill microbes are known as ***microbicidal*** (***-cidal = kill***) agents. Chemicals that work only to inhibit the growth of microorganisms are known as ***microbiostatic*** (***-stasis*** =). The suffixes -cidal and -stasis, can be combined with other terms to provide more detailed information about a specific type of microorganism being target. For example, chemicals that are referred to as ***bactericidal***, specifically work to kill bacteria. ***Bacteriostatic*** chemicals would work to inhibit the growth of bacteria. ***Virucidal*** agents would specifically target and destroy viruses. *Sporicidal* chemicals, also known as ***sterilants*** would have the ability to destroy endospores.

Chemical agents do not work equally against all forms of microorganisms. This is because some organisms are more resistant due to their structural make up. For example, the two types of animal viruses, naked and enveloped viruses are not equally resistant to chemicals. Naked viruses are considered more resistant.

Enveloped viruses have a lipid membrane derived from their hosts that could be disrupted by any chemical that is damaging to lipids. Naked viruses, lacking the lipid envelope, would be unaffected by such agents. The most resistant form of microorganism is the endospore. Endospores, due to the protective layers around the genetic material, are resistant to heat, radiation, and many chemicals. In the microbiology lab, it is assumed that if the method is capable of killing/destroying endospores, then all other less resistant forms will have also been destroyed. Such methods are known as sterilizing methods because all forms of microorganisms have been killed.

Antiseptics are chemical agents that are used to reduce microbial numbers on living tissues. Antiseptics include substances such as isopropyl alcohol, iodine, and hydrogen peroxide. ***Disinfectant***, on the other hand are reduce microbial numbers on inanimate objects, i.e. lab benches, kitchen counters, stethoscopes, etc. Bleach, Lysol, iodine, and alcohol are some examples of chemicals that can be utilized as disinfectants. Notice that some of the examples were listed as both an antiseptic and disinfectant. Not all chemical agents can classify as both, due to the fact that some of the agents used solely as disinfectants are too harsh to be applied to living tissues. Another group of chemical agents, known as sanitizers, are used primarily in the food industry, to reduce microbes to a safe level.

In this exercise, you test a variety of chemical agents that can be found in the household or in an introductory lab. You will soak filter disks in the chemical agents and then place the disks on agar plates that have been inoculated with a bacterium. After incubation, if the chemical agent has been successful in inhibiting or killing the microorganism, a clear zone will appear around the filter disk, known as a zone of inhibition. To assess the effectiveness of the chemical agents, you will measure the zones of inhibition.

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5.3.2: Assessing the Effects of Antiseptics and Disinfectants on Microbial Growth Activity

Inoculation of Nutrient Agar Plates

Label three nutrient agar plates for each of the bacteria to be assessed.

1. Turn on the Bunsen burner and aseptically obtain a cotton swab.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the swab into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Use the cotton swab applicator to introduce the bacterial sample to the surface of the nutrient agar. Swab the surface multiple times rotating in the plate in between swabs. The surface of the agar should be completely covered in bacteria in order to create a lawn of growth.
6. Place the used cotton swab into a biohazard bin or temporarily into a beaker of disinfectant until they can be sterilized.
7. Turn off the burner and let the plates dry for 3-5 minutes.

Application of Antiseptics and Disinfectant Disks

Use the following steps to prepare antiseptic or disinfectant disks and apply them to the surface of the agar. Obtain a microcentrifuge tube with sterile water and then select four different antiseptics or disinfectants for evaluation. You will use the same antiseptics/disinfectants for both bacteria for purposes of comparison.

1. Sterilize the forceps by dipping them in a beaker of 70% of ethanol and then passing them through the flame of a Bunsen burner.
2. Pick up a filter disk with the sterile forceps and dip it into the microcentrifuge tube containing sterile water. (Sterile water will serve as your control)
3. Remove the disk from the sterile water and place the disk onto the surface of your nutrient agar plate. Use the forceps to gently press on the disk to ensure that it adheres to the surface of the agar.
4. Dip the forceps into the alcohol and then pass them through the flame to sterilize them.
5. Repeat steps 1 -4 for each antiseptic/disinfectant to be assessed.

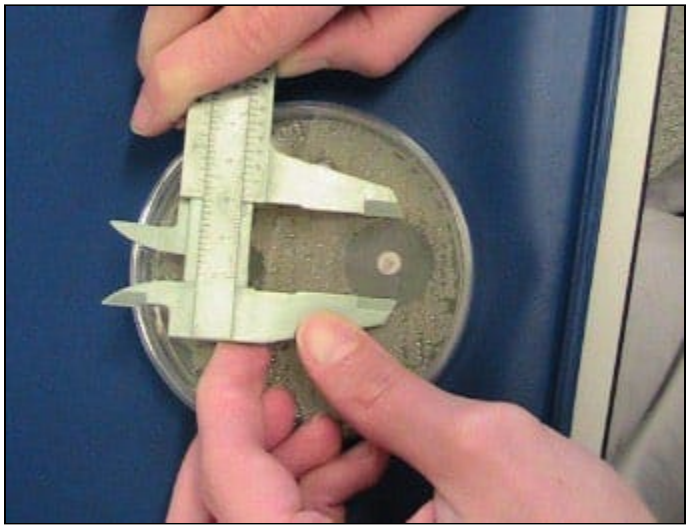
******Note****** Ensure that the antimicrobial disks are at least 15 mm from the edge of the plate and that the disks are spaced at equal distances from one another.

6. Invert the plate and incubate it at 37°C for 24-48 hours. Repeat this process for each plate inoculated.

Measuring Zones of Inhibition

Obtain the agar plates that were incubated.

1. Use the provided metric ruler to measure the zone of inhibition for each filter disk on each plate. Measure the diameter of the zone of inhibition across the center of the filter disk. Write your answer in the provided table.



Using calipers to measure bacterial zone of inhibition. Source: Viv Rolfe, Wikimedia Commons, CC BY-SA 4.0

Repeat this for each plate.

Zone of Inhibition Measurements

Bacterial Species	Antiseptic/Disinfectant	Zone Diameter	Notes/Additional Observations
Staphylococcus aureus			
Pseudomonas aeruginosa			

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5.3.3: Exercise #28 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Describe the difference between antiseptics and disinfectants.
2. You tested two different organisms with the same antiseptics/disinfectants. Describe your observations. Explain possible causes for any differences in microbial growth.
3. You are working in a clinical environment. You are asked to assist in cleaning a room that had been occupied by a *Mycobacterium tuberculosis* patient. Can you choose any cleaner (antiseptic/disinfectant) available in the cleaning closet? Explain your answer.

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5.4: Exercise #29 - Antimicrobial Sensitivity Test (Kirby-Bauer Method)

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions

THECB - ACGM Lab Learning Outcome #4 Perform basic microbiology procedures using aseptic techniques for transfer, isolation, and observation of commonly encountered, clinically significant bacteria.

THECB - ACGM Lab Learning Outcome #5 Use different types of bacterial culture media to grow, isolate, and identify microorganisms.

Laboratory Exercise Learning Outcomes

1. Describe the importance of Mueller-Hinton agar to the antimicrobial sensitivity test (Kirby-Bauer method).
2. Define zone of inhibition.
3. Define antimicrobial resistance.
4. Describe how an antimicrobial sensitivity test is performed.
5. Describe how antimicrobials are classified as susceptible (S), intermediate (I), or resistant (R), following and antimicrobial sensitivity test.
6. Correctly interpret a Kirby-Bauer plate to determine if an organism is susceptible, intermediate, or resistant to an antimicrobial agent.
7. Describe the clinical importance of an antimicrobial sensitivity test (Kirby-Bauer method).

Materials Needed:

- Bunsen burner
- 3 Mueller-Hinton agar plates
- 4-6 hour old cultures of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*
- 4 sterile cotton swabs
- Antibiotic disks
- Antibiotic disk dispenser or 70% ethanol in beakers and forceps
- Metric rulers

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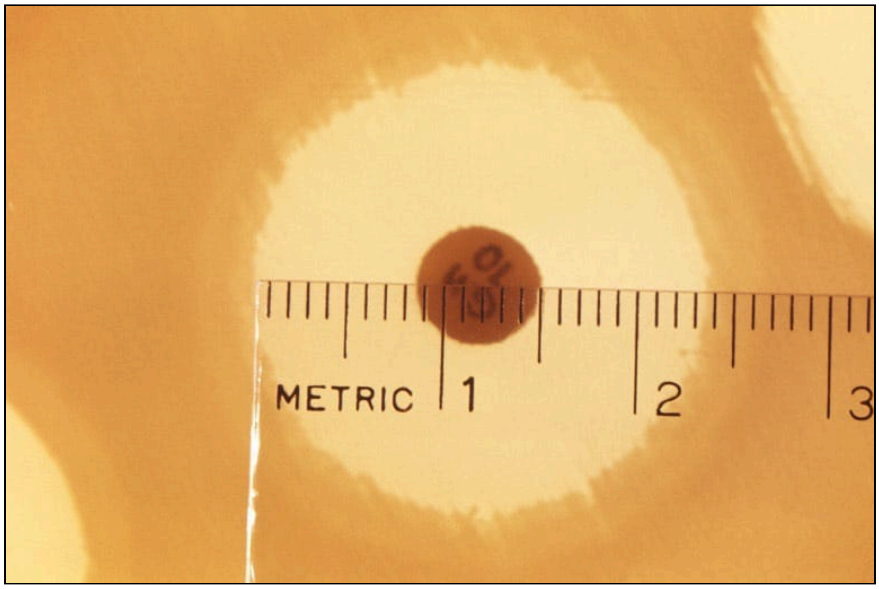
5.4.1: Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests are performed to determine the effectiveness of antimicrobial drugs in inhibiting microbial growth. This is an important test in the clinical lab, especially as microorganisms continue to become resistant to more of the antimicrobial drugs that have traditionally used to treat infections.

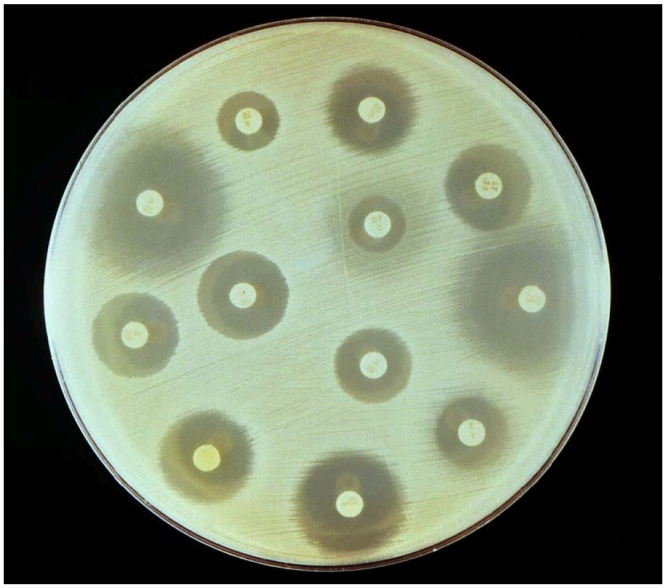
Antibiotic resistance occurs when a microorganism is not inhibited or killed by the drug. The resistance occurs as the organism acquires mechanisms to prevent the drug from working. The antimicrobial susceptibility test allows for the determination of which drugs would be potentially effective in treating an infection.

Antimicrobial sensitivity tests are performed by inoculating a plate with a lawn of the bacteria to be assessed and then placing filter discs with known concentrations of drug on the surface of the agar. After incubation, drugs that work to inhibit the growth of the bacteria produce clear zones around the disc known as ***zones of inhibition***. The antimicrobial sensitivity test must be performed using a standardized method so the results obtained are consistent across the healthcare industry. The reason the technique must be standardized is that there are several factors that may affect the size of the zone of inhibition, including the molecular weight of the drug, the type of media used for the test, and the concentration of the bacterial culture. The ***Kirby-Bauer method*** is the standardized method employed to ensure reliable results are obtained. This method makes use of a special media known as Mueller-Hinton agar, which is poured to a depth of 4 mm using 100- or 155-mm diameter plates. This is important because it affects how the drug diffuses. If they are poured to thick, then the drug diffusion rate laterally slows resulting in smaller zones of inhibition. To ensure the bacterial concentrations are consistent, they are prepared using 0.5 McFarland standards. Disks using known concentrations of the drug are utilized for the method. The plates are incubated for 16-18 hours. Following incubation, the plate is assessed for zones of inhibition and the zones are measured in millimeters. It is important to note that effectiveness cannot be determined visually. The measurements must be compared to a set of standardized values. In doing so, the measurements will indicate that the microorganism is susceptible (S), intermediate (I), or resistant(R) to the drug. If an organism is determined to be ***susceptible*** to the drug, it means that the concentration of the drug usually used is capable of inhibiting the bacteria, and there is good chance that the drug may work to treat the infection in the patient. If the result is ***intermediate***, it means that higher than normal concentrations would be needed to inhibit the bacteria. The effectiveness for using the drug to treat the patient would be uncertain, and that if used for treatment that it would likely only be effective in higher-than-normal doses. A ***resistant*** result indicates that the bacteria are resistant in vitro in the lab, and if it is resistant in the lab there is a likelihood that it would be ineffective in treating the infection in the patient.

In this lab you will perform an antimicrobial sensitivity test using the Kirby-Bauer method.



"This 1972 image depicts a Mueller-Hinton agar culture plate that had been used in an antibiotic susceptibility test (AST). Known as the Kirby-B method, each of the small, labeled discs, or wafers, contained an antimicrobial agent. This image is an enlarged view of a region of PHIL 10784 light halos surrounding each disc, also known as reaction zones, represented regions in which the bacteria on the agar's surface did not thrive, their sensitivity to the antibiotic that had been soaked into these respective discs. These tests, which measure the presence or absence of a rea zone, also take into account the pH of the medium, the concentration of the inoculum, and a number of other factors. Using this method, the se a bacterial strain has to a given antimicrobial, or combination of antimicrobials, may be determined."
Source: CDC/ Gilda L. Jones, CDC PHIL, Public domain



"This Petri dish culture plate contained an unknown growth medium, to which an inoculum of Staphylococci sp. bacteria had been applied. Atop growth medium, antibiotic-impregnated disks had been laid, which gave rise to this example of an antibiotic sensitivity test. The medium was ke pH of 7.2. The size of the inhibition zones surrounding the antibiotic-impregnated paper disks, depended on the level of sensitivity the bacteria, present in the agar, had to the antibiotic, as well as the antibiotic's ability to diffuse through the culture medium. The overall pH of the medium, case 7.2, also played an important role in influencing the results of this antimicrobial sensitivity study."
Source: CDC, CDC PHIL, Public domain

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5.4.2: Antimicrobial Sensitivity Test (Kirby-Bauer Method) Activity

Inoculation of Mueller-Hinton Agar Plates

Label three Mueller-Hinton agar plates for each of the bacteria to be assessed.

1. Turn on the Bunsen burner and aseptically obtain a cotton swab.
2. Obtain a bacterial culture. Remove the cap. Pass the mouth of the tube through the flame of the Bunsen burner.
3. Insert the swab into the tube to acquire a sample of the bacterium.
4. Pass the tube through the flame of the Bunsen burner and recap the test tube, and return the tube to the test tube rack.
5. Use the cotton swab applicator to introduce the bacterial sample to the surface of the Mueller-Hinton agar. Swab the surface multiple times rotating in the plate in between swabs. The surface of the agar should be completely covered in bacteria in order to create a lawn of growth.
6. Place the used cotton swab into a biohazard bin or temporarily into a beaker of disinfectant until they can be sterilized.
7. Turn off the burner and let the plates dry for 3-5 minutes.

Application of Antimicrobial Disks

Apply antimicrobial disks using one of the following methods:

Antibiotic Disk Dispenser

1. Load the desired antibiotic disks into the disk dispenser.
2. Remove the lid from the plate and place the dispenser over the plate.
3. Press down on the plunger to dispense the discs onto the surface of the agar.
4. Use sterile forceps to tap each disk gently to secure it to the agar.

Sterile Forceps

1. Sterilize the forceps by dipping them in a beaker of 70% of ethanol and then passing them through the flame of a Bunsen burner.
2. Pick up an antibiotic disk with the sterile forceps and place it on the surface of the agar. Tap it gently to ensure it adheres to the agar.
3. Repeat this for each antimicrobial disk to be placed on the agar plate.

******Note****** Ensure that the antimicrobial disks are at least 15 mm from the edge of the plate and that the disks are spaced at equal distances from one another.

4. Invert the plate and incubate it at 37°C for 16 - 18 hours. Repeat this process for each plate inoculated.

Measuring Zones of Inhibition

Obtain the Mueller-Hinton agar plates that were incubated.

1. Use the provided metric ruler to measure the zone of inhibition for each antimicrobial disk on each plate. Measure the diameter of the zone of inhibition across the center of the antimicrobial disk. Write your answer in the provided table.
2. Compare the measurement with the Antimicrobial Sensitivity Standardized charts on the next page for each antimicrobial disk. Record whether the data indicates susceptible (S), intermediate (I), or resistant (R).

Repeat this for each plate.

Antimicrobial Sensitivity Test Results

Bacterial Species	Antimicrobial & Abbreviation	Zone Diameter	Rating (S, I, R)	Antimicrobial & Abbreviation	Zone Diameter	Rating (S, I, R)
Staphylococcus aureus						
Escherichia coli						
Klebsiella pneumoniae						
Pseudomonas aeruginosa						

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5.4.3: Exercise #29 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Describe how a zone of inhibition should be measured when performing an antimicrobial sensitivity (Kirby-Bauer) test.
2. A bacterial strain was isolated from a patient's urine specimen and antimicrobial sensitivity test was performed. Upon observation of the results, it is noted that there is no zone of inhibition around the antibiotic erythromycin. Explain this observation.
3. When reporting the results of an antimicrobial sensitivity test the initial S, I, and R are utilized. What do each of these abbreviations mean and what does it tell us about the microorganism being tested?
4. You obtain your agar plate following incubation. You notice what appears to be a very large zone of inhibition around one of the antimicrobial disks. Can it be assumed that the drug is effectively inhibiting the bacteria on the plate? Explain your answer.

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5.5: Section Five Review

Review Purpose

This review helps you check what you learned in Section Five before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you...

- Define and distinguish the temperature groups - psychrophile, psychrotroph, mesophile, thermophile - and classify bacteria by their temperature preferences?
- Define and distinguish pH groups - acidophile, neutrophile, alkaliphile - and classify bacteria by their pH preferences?
- Define the terms used to control microbial growth - antiseptic, disinfectant, sterilant, sanitizer - and the difference between *-cidal* (killing) and *-static* (inhibiting) agents?
- Define zone of inhibition and compare the effectiveness of chemical agents in a disk-diffusion experiment?
- Explain the Kirby-Bauer sensitivity test, including the role of Mueller-Hinton agar and how the test is performed?
- Interpret a Kirby-Bauer plate to classify an organism as susceptible, intermediate, or resistant, and explain the clinical importance of the test and of antimicrobial resistance?

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5.5.1: Matching

Matching Questions

1. Match the classification on the left with the appropriate description of the conditions it prefers by placing the letter next to the correct term.

Question 1 Terms

Answer	Terms
_____	1. Psychrophile
_____	2. Psychrotroph
_____	3. Mesophile
_____	4. Thermophile
_____	5. Hyperthermophile
_____	6. Acidophile
_____	7. Neutrophile
_____	8. Alkaliphile

Question 1 Descriptions

Letter	Description
a.	optimal growth temperatures greater than 80°C
b.	optimal pH range less than pH 5.5; the *Lactobacillus* species of the normal vaginal flora are an example
c.	minimum growth temperature of 0°C or lower and a maximum around 15°C; not capable of growth above 20°C
d.	prefers alkaline conditions of pH 8 or greater; *Vibrio cholerae* is a clinically significant example
e.	optimal growth temperatures between approximately 20°C and 45°C; pathogenic microorganisms generally fall in this group
f.	tolerates growth temperatures from around 0°C - 5°C up to around 35°C
g.	prefers a neutral pH range near pH 7; most microorganisms belong to this classification

Letter	Description
h.	tolerates temperatures up to 80°C

Click to reveal answers

Answers:
1) c
2) f
3) e
4) h
5) a
6) b
7) g
8) d

2. Match the type of antimicrobial agent on the left with the appropriate description of what it does by placing the letter next to the correct term.

Question 2 Terms

Answer	Terms
_____	1. Microbicidal agent
_____	2. Microbiostatic agent
_____	3. Bactericidal agent
_____	4. Bacteriostatic agent
_____	5. Virucidal agent
_____	6. Sporicidal agent
_____	7. Antiseptic
_____	8. Disinfectant
_____	9. Sanitizer

Question 2 Descriptions

Letter	Description
a.	reduces microbial numbers on inanimate objects such as lab benches and kitchen counters

Letter	Description
b.	acts only to inhibit the growth of microorganisms
c.	specifically targets and destroys viruses
d.	acts to kill microbes
e.	reduces microbial numbers on living tissues
f.	works to inhibit the growth of bacteria
g.	destroys endospores, and is therefore also known as a sterilant
h.	used primarily in the food industry to reduce microbes to a safe level
i.	specifically works to kill bacteria

Click to reveal answers

Answers:
1) d
2) b
3) i
4) f
5) c
6) g
7) e
8) a
9) h

3. Match the Kirby-Bauer susceptibility result on the left with the appropriate description of what it means for treating a patient by placing the letter next to the correct result.

Question 3 Terms

Answer	Terms
____	1. Susceptible (S)
____	2. Intermediate (I)
____	3. Resistant (R)

Question 3 Descriptions

Letter	Description
a.	higher than normal concentrations would be needed to inhibit the bacteria, so effectiveness in the patient is uncertain
b.	the bacteria are resistant in vitro, so there is a likelihood the drug would be ineffective in treating the infection
c.	the concentration of the drug usually used is capable of inhibiting the bacteria, and there is a good chance it may work to treat the infection

Click to reveal answers

Answers:
1) c
2) a
3) b

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5.5.2: Fill-In-The-Blank

Fill-in-the-Blank Questions

1. The Kirby-Bauer method requires a special media called _____ agar.

Click to reveal answer

Answer Mueller-Hinton

2. Microorganisms that grow best in environments with less than pH 5.5 are called _____.

Click to reveal answer

Answer acidophiles

3. A clear zone showing no microbial growth around a filter disc with a drug or antimicrobial agent is a _____.

Click to reveal answer

Answer zone of inhibition

4. Bacteria that do not grow in temperatures above 20°C are called _____.

Click to reveal answer

Answer psychrophiles

5. When performing a Kirby-Bauer test, an organism is said to be _____ to an antimicrobial drug if higher than normal concentrations are needed to inhibit the microbe.

Click to reveal answer

Answer intermediate

6. Chemical agents that act only to inhibit the growth of microorganisms are _____.

Click to reveal answer

Answer microbiostatic

7. _____ are chemical agents that are used to reduce microbial numbers on living tissues.

Click to reveal answer

Answer antiseptics

8. pH is a measurement of the concentration of _____ ions in a sample or environment.

Click to reveal answer

Answer hydrogen

9. Changes in pH can interrupt _____ bonds, which may cause proteins to _____ permanently.

Click to reveal answer

Answer hydrogen; denature

10. The most resistant form of microorganism is the _____ .

Click to reveal answer

Answer endospore

11. _____ viruses are considered more resistant to chemical agents because they lack a lipid envelope.

Click to reveal answer

Answer Naked

12. Methods capable of killing all forms of microorganisms are known as _____ methods.

Click to reveal answer

Answer sterilizing

13. _____ occurs when a microorganism is not inhibited or killed by an antimicrobial drug.

Click to reveal answer

Answer Antibiotic resistance

14. The bacterial culture is swabbed repeatedly over the surface of the agar in order to create a _____ of growth.

Click to reveal answer

Answer lawn

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5.5.3: True/False

True / False Questions

1. In a Kirby-Bauer antimicrobial susceptibility test, the most effective drug can be determined by visually choosing the largest zone of inhibition.

_____ True _____ False

Click to reveal answer

Answer False

2. No bacteria can grow in liquids that are near the boiling point.

_____ True _____ False

Click to reveal answer

Answer False

3. Blood agar is used in the Kirby-Bauer test because it allows for easy diffusion of the antimicrobial agent through the agar.

_____ True _____ False

Click to reveal answer

Answer False

4. Bacteria that grow on the human body would most likely fall into the mesophile group.

_____ True _____ False

Click to reveal answer

Answer True

5. Disinfection allows for the removal of all microorganisms from the surface of an object.

_____ True _____ False

Click to reveal answer

Answer False

6. In the disk-diffusion method (Kirby-Bauer), bacteria are inoculated on a nutrient agar plate, and antibiotic disks are added 24 hours later.

_____ True _____ False

Click to reveal answer

Answer False

7. Temperatures that are lower than a microbe's optimum growth temperature tend to be microbicidal due to denaturing enzymes.

_____ True _____ False

Click to reveal answer

Answer False

8. The double helix of DNA is held together by hydrogen bonds, making it vulnerable to changes in pH.

_____ True _____ False

Click to reveal answer

Answer True

9. Changes in pH can disrupt the hydrogen ion gradient across the cell membrane that cellular respiration requires for ATP synthase to function.

_____ True _____ False

Click to reveal answer

Answer True

10. Most microorganisms are classified as neutrophiles.

_____ True _____ False

Click to reveal answer

Answer True

11. In the microbiology laboratory, a method capable of destroying endospores is assumed to have destroyed all less resistant forms of microorganisms.

_____ True _____ False

Click to reveal answer

Answer True

12. A chemical agent that is effective as a disinfectant is always safe to use as an antiseptic.

_____ True _____ False

Click to reveal answer

Answer False

13. The zone of inhibition is measured as the radius from the edge of the filter disk to the edge of the clear zone.

_____ True _____ False

Click to reveal answer

Answer False

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5.5.4: Multiple Choice / Multiple Select

Multiple Choice / Multiple-Select Questions

1. You are studying a bacterium found in the normal intestinal flora of humans and most other mammals. You have inoculated five nutrient broths, and are incubating one broth at each of the following temperatures: 5°C, 25°C, 38°C, 42°C, and 55°C. At which temperature would you predict to see the optimum growth rate?

- a) 42°C
- b) 38°C
- c) 25°C
- d) 55°C
- e) 5°C

Click to reveal answer

Answer b) 38°C
Organisms of the normal intestinal flora are mesophiles, with optimal growth temperatures between approximately 20°C and 45°C. Human body temperature is 37°C, so growth is optimal nearest that temperature.

2. Which of the following processes removes or destroys endospores?

- a) Sterilization
- b) Disinfection
- c) Antisepsis
- d) None of the above

Click to reveal answer

Answer a) Sterilization
Endospores are the most resistant form of microorganism. Methods capable of destroying endospores are known as sterilizing methods, because all forms of microorganisms have been killed.

3. What would the colony growth on a nutrient agar plate look like if you were to take a psychrophilic bacteria culture and put it in a 35°C incubator?

- a) Some growth with small colonies
- b) Large colonies in high numbers
- c) More information would be needed to make a conclusion
- d) No growth

Click to reveal answer

Answer d) No growth
Psychrophiles have a maximum growth temperature around 15°C and are not capable of growth above 20°C, so no growth would occur at 35°C.

4. Why can temperature kill microorganisms?

- a) Reduced temperatures reduce proteins, inhibiting enzymes
- b) Exposure to heat dissolves cell membranes
- c) Exposure to heat denatures proteins, inhibiting enzymes
- d) Increased temperatures disintegrate lipids

Click to reveal answer

Answer c) Exposure to heat denatures proteins, inhibiting enzymes
Proteins, many of which act as enzymes that facilitate cellular metabolism, depend on their structure to function. Heat denatures those proteins, inhibiting the enzymes and halting metabolism.

5. What are the three possible outcomes for each antibiotic and microorganism pair in a Kirby-Bauer test?

- a) Similar, reversion, indeterminate
- b) Susceptible, resistant, indeterminate
- c) Stunted, robust, intermediate
- d) Susceptible, resistant, intermediate

Click to reveal answer

Answer d) Susceptible, resistant, intermediate
The measurements indicate that the microorganism is susceptible (S), intermediate (I), or resistant (R) to the drug.

6. A *Staphylococcus* species is found to grow best at 30°C, and will not grow at temperatures below 25°C or above 38°C. Which temperature group best describes this species?

- a) Psychrophile
- b) Thermophile
- c) Psychrotroph
- d) Mesophile

Click to reveal answer

Answer d) Mesophile
Mesophiles have optimal growth temperatures between approximately 20°C and 45°C. A species growing best at 30°C within a 25°C to 38°C range falls in this group.

7. You perform a Kirby-Bauer test on *Staphylococcus aureus* and measure the zone of inhibition around a tetracycline disk as 13 mm. How would you report the susceptibility of the organism to tetracycline? (Refer to the zone of inhibition diameter standards tables.)

- a) Susceptible
- b) Intermediate
- c) Resistant

- d) Cannot be determined with the provided information

Click to reveal answer

Answer c) Resistant
For *Staphylococcus aureus*, tetracycline breakpoints are S at 19 mm or greater, I at 15 - 18 mm, and R at 14 mm or less. A 13 mm zone falls in the resistant range.

8. You perform a Kirby-Bauer test on *Staphylococcus aureus* and measure the zone of inhibition around a rifampin disk as 21 mm. How would you report the susceptibility of the organism to rifampin? (Refer to the zone of inhibition diameter standards tables.)

- a) Susceptible
- b) Intermediate
- c) Resistant
- d) Cannot be determined with the provided information

Click to reveal answer

Answer a) Susceptible
For *Staphylococcus aureus*, rifampin breakpoints are S at 20 mm or greater, I at 17 - 19 mm, and R at 16 mm or less. A 21 mm zone falls in the susceptible range.

9. You perform a Kirby-Bauer test on *Staphylococcus aureus* and measure the zone of inhibition around an erythromycin disk as 11 mm. How would you report the susceptibility of this organism to erythromycin? (Refer to the zone of inhibition diameter standards tables.)

- a) Susceptible
- b) Intermediate
- c) Resistant
- d) Cannot be determined with the provided information

Click to reveal answer

Answer c) Resistant
For *Staphylococcus aureus*, erythromycin breakpoints are S at 23 mm or greater, I at 14 - 22 mm, and R at 13 mm or less. An 11 mm zone falls in the resistant range.

10. Psychrotrophs are a particular concern for food-borne illness because they:

- a) Tolerate growth temperatures from around 0°C - 5°C, so they continue to grow in refrigerated food, yet also grow at temperatures up to around 35°C
- b) Are not capable of growth above 20°C, so they survive only in frozen food
- c) Require temperatures above 80°C, so they survive cooking
- d) Grow only at a pH less than 5.5, the pH of most preserved foods

Click to reveal answer

Answer a) Tolerate growth temperatures from around 0°C - 5°C, so they continue to grow in refrigerated food, yet also grow at temperatures up to around 35°C
Psychrotrophs tolerate growth temperatures around 0°C - 5°C up to around 35°C, meaning they can grow at refrigeration temperatures and also at temperatures found in and on the human body.

11. On the pH scale, as the concentration of hydrogen ions increases, the measurement moves toward:

- a) 0, and the sample is said to be acidic
- b) 14, and the sample is said to be acidic
- c) 0, and the sample is said to be alkaline
- d) 14, and the sample is said to be alkaline

Click to reveal answer

Answer a) 0, and the sample is said to be acidic
As hydrogen ion concentrations increase, the measurement moves toward 0 on the pH scale and is said to be acidic. When hydrogen ions decrease, the measurement moves toward 14 and is alkaline.

12. In what ways can a change in pH damage a bacterial cell? **Select all that apply.**

- a) The hydrogen bonding that holds the DNA double helix together may be disrupted
- b) Proteins may denature permanently, which can halt metabolism
- c) The hydrogen ion gradient across the cell membrane that ATP synthase requires may be affected
- d) The cell wall is converted into a capsule

Click to reveal answers

Answers: a) The hydrogen bonding that holds the DNA double helix together may be disrupted; b) Proteins may denature permanently, which can halt metabolism; c) The hydrogen ion gradient across the cell membrane that ATP synthase requires may be affected
Changes in pH can disrupt the hydrogen bonding that holds DNA together, permanently denature proteins and halt metabolism, and interfere with the gradient of hydrogen ions across the cell membrane that enables ATP synthase to produce ATP.

13. How do the *Lactobacillus* species of the normal vaginal flora inhibit the growth of yeast?

- a) They produce products that maintain a pH below 5.5, which is too low for organisms requiring a higher pH
- b) They produce a chemical agent that is virucidal
- c) They raise the pH above 8, which yeast cannot tolerate
- d) They consume the available oxygen, creating anaerobic conditions

Click to reveal answer

Answer a) They produce products that maintain a pH below 5.5, which is too low for organisms requiring a higher pH
Lactobacillus species require a pH less than 5.5 and produce products that help maintain that pH within the vagina. In doing so they inhibit the growth of microorganisms that require a higher pH, such as yeast.

14. Why are naked viruses considered more resistant to chemical agents than enveloped viruses?

- a) Enveloped viruses have a lipid membrane that can be disrupted by any chemical damaging to lipids, and naked viruses lack that envelope
- b) Naked viruses form endospores when they are exposed to chemicals
- c) Enveloped viruses are larger and therefore easier for a chemical to reach
- d) Naked viruses reproduce faster than the chemical agent can act

Click to reveal answer

Answer a) Enveloped viruses have a lipid membrane that can be disrupted by any chemical damaging to lipids, and naked viruses lack that envelope
Enveloped viruses have a lipid membrane derived from their hosts that could be disrupted by any chemical damaging to lipids. Naked viruses, lacking the lipid envelope, would be unaffected by such agents.

15. Why can some chemical agents be used as disinfectants but not as antiseptics?

- a) Some agents used solely as disinfectants are too harsh to be applied to living tissues
- b) Disinfectants are microbiostatic while antiseptics are microbicidal
- c) Antiseptics must be capable of destroying endospores
- d) Disinfectants cannot be applied to inanimate objects

Click to reveal answer

Answer a) Some agents used solely as disinfectants are too harsh to be applied to living tissues
Not all chemical agents can classify as both, because some of the agents used solely as disinfectants are too harsh to be applied to living tissues.

16. In the antiseptic and disinfectant activity, a filter disk is soaked in sterile water and placed on the agar alongside the others. What is the purpose of this disk?

- a) It serves as the control
- b) It provides the moisture the other disks need in order to diffuse
- c) It marks the center of the plate for measuring
- d) It rinses away any chemical agent that spreads too far

Click to reveal answer

Answer a) It serves as the control
A filter disk is dipped into the microcentrifuge tube containing sterile water, which serves as your control.

17. How is a lawn of growth created on the surface of an agar plate?

- a) The surface is swabbed multiple times, rotating the plate between swabs, so it is completely covered in bacteria
- b) A single streak is made down the center of the plate
- c) The plate is flooded with broth culture and the excess is poured off
- d) Four quadrants are streaked, flaming the loop between each one

Click to reveal answer

Answer a) The surface is swabbed multiple times, rotating the plate between swabs, so it is completely covered in bacteria
Use the cotton swab applicator to introduce the bacterial sample to the surface of the agar. Swab the surface multiple times, rotating the plate in between swabs. The surface of the agar should be completely covered in bacteria in order to create a lawn of growth.

18. Why must the antimicrobial sensitivity test be performed using a standardized method?

- a) So the results obtained are consistent across the healthcare industry
- b) So that only one drug needs to be tested on each plate
- c) So the plates can be incubated at room temperature
- d) So the zones can be judged by eye without measurement

Click to reveal answer

Answer a) So the results obtained are consistent across the healthcare industry
The antimicrobial sensitivity test must be performed using a standardized method so the results obtained are consistent across the healthcare industry.

19. Which factors may affect the size of the zone of inhibition? **Select all that apply.**

- a) The molecular weight of the drug
- b) The type of media used for the test
- c) The concentration of the bacterial culture
- d) The color of the filter disk

Click to reveal answers

Answers: a) The molecular weight of the drug; b) The type of media used for the test; c) The concentration of the bacterial culture
Several factors may affect the size of the zone of inhibition, including the molecular weight of the drug, the type of media used for the test, and the concentration of the bacterial culture.

20. What is the clinical purpose of performing an antimicrobial susceptibility test?

- a) To determine which drugs would be potentially effective in treating an infection

- b) To determine the temperature group to which the organism belongs
- c) To determine whether the organism is capable of forming endospores
- d) To measure the rate at which the organism reproduces

Click to reveal answer

Answer a) To determine which drugs would be potentially effective in treating an infection
The antimicrobial susceptibility test allows for the determination of which drugs would be potentially effective in treating an infection.

21. When using a spectrophotometer to analyze bacterial growth in the broth cultures, which mode and wavelength are used?

- a) Absorbance mode at 600 nm
- b) Transmittance mode at 600 nm
- c) Absorbance mode at 450 nm
- d) Transmittance mode at 450 nm

Click to reveal answer

Answer a) Absorbance mode at 600 nm
Set the mode to absorbance and the wavelength to 600 nm.

22. What is used to calibrate the spectrophotometer before samples are measured?

- a) An uninoculated medium
- b) A sample of the most turbid culture
- c) Sterile water in an open tube
- d) A cuvette left empty

Click to reveal answer

Answer a) An uninoculated medium
Calibrate the spectrophotometer using an uninoculated medium.

23. Why are the sides of the cuvette wiped with a Kim wipe before it is placed in the spectrophotometer?

- a) To ensure no skin oils or fingerprints interfere with the measurement
- b) To sterilize the outside of the cuvette
- c) To warm the sample to the temperature of the instrument
- d) To remove condensation that would dilute the sample

Click to reveal answer

Answer a) To ensure no skin oils or fingerprints interfere with the measurement
Wipe the sides of the cuvette with a Kim wipe to ensure no skin oils or fingerprints interfere with the measurement.

24. Using the visual analysis scale for assessing growth in the broth tubes, what does a recording of +++ indicate?

- a) Maximal growth, seen as maximum turbidity
- b) Moderate growth, seen as moderate turbidity
- c) Minimal growth, seen as minimal turbidity
- d) No growth

Click to reveal answer

Answer a) Maximal growth, seen as maximum turbidity
On the visual analysis scale, - is no growth, + is minimal growth (minimal turbidity), ++ is moderate growth (moderate turbidity), and +++ is maximal growth (maximum turbidity).

25. Each temperature classification group demonstrates a range. Which temperatures define that range? **Select all that apply.**

- a) A minimum temperature
- b) A maximum temperature
- c) An optimal temperature
- d) A sterilizing temperature

Click to reveal answers

Answers: a) A minimum temperature; b) A maximum temperature; c) An optimal temperature
Each of these groups demonstrates a range that has a minimum temperature, a maximum temperature, and an optimal temperature for the organism's growth.

26. *Serratia marcescens* is inoculated onto two nutrient agar slants, one incubated at 25°C and one at 37°C. What does this activity demonstrate?

- a) That temperature can affect the phenotypic expression of an organism
- b) That *Serratia marcescens* is a thermophile
- c) That nutrient agar slants are selective media
- d) That temperature has no effect on observable characteristics

Click to reveal answer

Answer a) That temperature can affect the phenotypic expression of an organism
This activity assesses the effect of temperature on phenotypic expression. The same organism is grown at two temperatures and the growth on the surface of each slant is compared.

27. A neutrophile is inoculated into a broth adjusted to pH 5.5 and incubated. What is the most likely outcome?

- a) Growth is poor or absent, because the pH is outside the range the organism prefers
- b) Growth is maximal, because acidic conditions favor all bacteria
- c) The organism is converted into an acidophile
- d) Growth is unaffected, because pH does not influence bacterial growth

Click to reveal answer

Answer a) Growth is poor or absent, because the pH is outside the range the organism prefers
Neutrophiles prefer a pH range near pH 7. Changes in pH can detrimentally affect cellular function, disrupting hydrogen bonding in DNA and proteins and halting metabolism, so a neutrophile would grow poorly at pH 5.5.

28. A proton-pump inhibiting drug reduces the acid produced in the stomach, raising the pH of the stomach contents. Would a patient taking such a drug be more or less likely to acquire an infection with *Vibrio cholerae*?

- a) More likely, because *Vibrio cholerae* grows best at a pH slightly above 8
- b) Less likely, because *Vibrio cholerae* requires a pH below 5.5
- c) More likely, because *Vibrio cholerae* is a thermophile
- d) Less likely, because raising the pH destroys all bacteria

Click to reveal answer

Answer a) More likely, because *Vibrio cholerae* grows best at a pH slightly above 8
Vibrio cholerae is an alkaliphile that grows best at a pH slightly above pH 8. Raising the pH of the stomach contents creates conditions closer to what the organism prefers.

29. When bacteria in a culture medium carry out fermentation, they produce organic acids. How could this affect further growth in that medium?

- a) The pH of the medium falls, which may inhibit or kill organisms that require a higher pH
- b) The pH of the medium rises, which favors acidophiles
- c) The pH of the medium remains unchanged, since acids are neutral
- d) The organic acids serve as a final electron acceptor, increasing growth

Click to reveal answer

Answer a) The pH of the medium falls, which may inhibit or kill organisms that require a higher pH
Organic acids increase the hydrogen ion concentration, moving the medium toward 0 on the pH scale. Because changes in pH can disrupt hydrogen bonding and denature proteins, organisms preferring a higher pH may be inhibited or killed.

30. Some bacteria produce urease, which converts urea to ammonium. How could this affect growth in the medium?

- a) The pH of the medium rises, which may inhibit organisms that require a lower pH

- b) The pH of the medium falls, which may inhibit organisms that require a higher pH
- c) The medium becomes anaerobic, favoring anaerobes
- d) The medium is sterilized, preventing all further growth

Click to reveal answer

Answer a) The pH of the medium rises, which may inhibit organisms that require a lower pH
Ammonium raises the pH of the medium, making it more alkaline. Organisms whose optimal pH is lower may then be inhibited, because changes in pH can disrupt hydrogen bonding and denature proteins.

31. Which of the following are given as examples of antiseptics? **Select all that apply.**

- a) Isopropyl alcohol
- b) Iodine
- c) Hydrogen peroxide
- d) Lysol

Click to reveal answers

Answers: a) Isopropyl alcohol; b) Iodine; c) Hydrogen peroxide
Antiseptics include substances such as isopropyl alcohol, iodine, and hydrogen peroxide. Bleach and Lysol are given as examples of disinfectants.

32. You are working in a clinical environment, helping to clean a room that had been occupied by a *Mycobacterium tuberculosis* patient. Can any cleaner available in the cleaning closet be used?

- a) No, because chemical agents do not work equally against all microorganisms; an agent effective against this organism must be selected
- b) Yes, because all disinfectants destroy all forms of microorganisms
- c) Yes, provided the cleaner is also approved as an antiseptic
- d) No, because only antiseptics may be used on inanimate surfaces

Click to reveal answer

Answer a) No, because chemical agents do not work equally against all microorganisms; an agent effective against this organism must be selected
Chemical agents do not work equally against all forms of microorganisms, because some organisms are more resistant due to their structural make up. An agent known to be effective against the organism in question must be chosen.

33. Following incubation you notice a very large zone of inhibition around one of the antimicrobial disks. Can you assume the drug is effectively inhibiting the bacteria?

- a) No, because effectiveness cannot be determined visually; the measurement must be compared to a set of standardized values
- b) Yes, because the largest zone always indicates the most effective drug

- c) Yes, provided the zone is larger than the disk itself
- d) No, because zones of inhibition indicate resistance rather than susceptibility

Click to reveal answer

Answer a) No, because effectiveness cannot be determined visually; the measurement must be compared to a set of standardized values
It is important to note that effectiveness cannot be determined visually. The measurements must be compared to a set of standardized values, which indicate whether the organism is susceptible, intermediate, or resistant.

34. A Kirby-Bauer result of resistant (R) indicates that the bacteria are resistant:

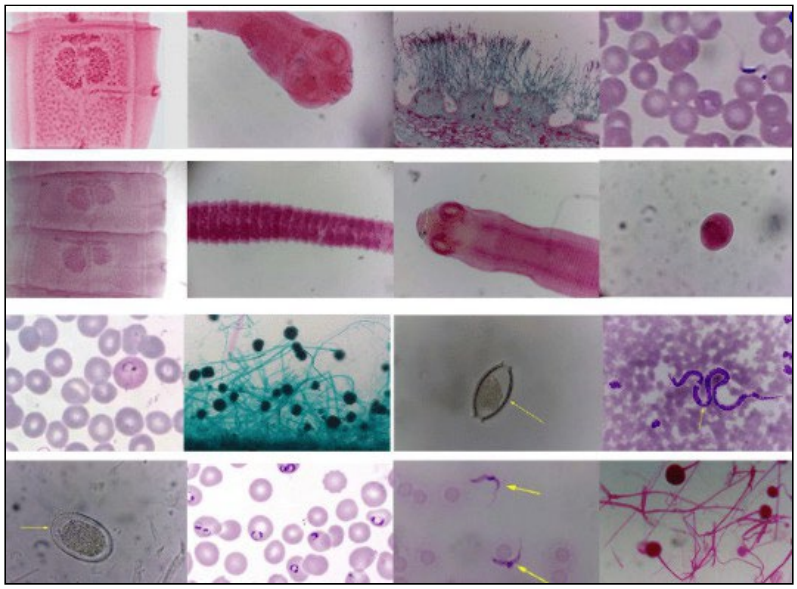
- a) In vitro in the lab, and there is a likelihood the drug would be ineffective in treating the infection
- b) Only at higher than normal drug concentrations
- c) In the patient, but not under laboratory conditions
- d) To all drugs in the same class

Click to reveal answer

Answer a) In vitro in the lab, and there is a likelihood the drug would be ineffective in treating the infection
A resistant result indicates that the bacteria are resistant in vitro in the lab, and if it is resistant in the lab there is a likelihood that it would be ineffective in treating the infection in the patient.

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6: SECTION SIX - EUKARYOTIC PATHOGENS (A SURVEY OF FUNGAL, PROTOZOAN, AND HELMINTH PATHOGENS)



Credit: Sherry Smith

In this unit you will examine some eukaryotic pathogens. The organisms to be studied belong in one of three groups: fungi, protozoans, and helminths. You will survey some of the pathogens within each group. Understand that this section is not intended to be a comprehensive study of all fungal, protozoan, and helminthic human or animal pathogens. As you study the organisms, you will identify them using microscopes and/or micrographs and will study characteristics of each, including their scientific names, common names, method of transmission, where they reside in the body, and the diseases they cause.

YouTube Video

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Beyond Bacteria: Surveying Eukaryotic Pathogens

Click on the video thumbnail or the linked title to access the video.

Credit: Yenny Mo, Josh Spradling, Sherry Smith

6.1: Exercise #30 - Fungal Pathogens

6.1.1: Fungi

6.1.2: Survey of Clinically Significant Yeasts
6.1.3: Survey of Clinically Significant Molds
6.1.4: Exercise #30 - Lab Wrap-Up

6.2: Exercise #31 - Protozoan Parasites

6.2.1: Protozoans
6.2.2: Subphylum Sarcodina (Amoeboids)
6.2.3: Subphylum Mastigophora (Flagellates)
6.2.4: Phylum Ciliophora (Ciliates)
6.2.5: Phylum Apicomplexa (Sporozoa)
6.2.6: Exercise #31 - Lab Wrap-Up

6.3: Exercise #32 - Helminth Pathogens

6.3.1: Helminths
6.3.2: Trematodes (Flukes)
6.3.3: Survey of Trematodes
6.3.4: Cestodes (Tapeworms)
6.3.5: Survey of Cestodes
6.3.6: Nematodes (Roundworms)
6.3.7: Survey of Nematodes
6.3.8: Exercise #32 - Lab Wrap-Up

6.4: Section Six Review

6.4.1: Matching
6.4.2: Fill-In-The-Blank
6.4.3: True-False
6.4.4: Multiple Choice / Multiple Select
6.4.5: Identifying Parasites

This page titled 6: Section Six - Eukaryotic Pathogens (A Survey of Fungal, Protozoan, and Helminth Pathogens) is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Sherry Smith.

6.1: Exercise #30 - Fungal Pathogens

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions.

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #8 Demonstrate basic identification protocols based on microscopic morphology of some common fungi and parasites.

Laboratory Exercise Learning Outcomes

1. Describe the morphological differences between yeast and molds.
2. Define mycosis.
3. Define and differentiate superficial, subcutaneous, and systemic mycosis.
4. Describe mechanisms by which mycoses are acquired.
5. Differentiate between fungal infections, fungal toxicosis, and fungal allergies.
6. Describe the morphology and epidemiology of important fungal pathogens and identify fungal pathogens using microscopy and micrographs.

Materials Needed:

- Microscope
- Prepared slides of fungal pathogens (*Candida albicans, Candida auris, Crytococcus neoformans, Pneumocystis jirovecii, Rhizopus spp., Trichophyton spp., Aspergillus spp. Coccidiodes immitis*)

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6.1.1: Fungi

Fungi are eukaryotic heterotrophic, eukaryotic organisms, that are abundant in most habitats/ environments. Fungi exist in two forms; yeast and molds. Yeast typically exist as globular, single- celled organisms. Molds, on the other hand demonstrate a filamentous structure, made of long cylindrical tubules known as hyphae. The hyphae may be multicellular, with walls known as septae separating the cells. These molds are known as septate molds. Aseptate molds have hyphae that are not separated by septae, and instead are compose of multi-nucleated cells.

Most fungi are saprophytes, meaning that they acquire their nutrients from decaying plants and animals. There are some however that can cause disease. Diseases caused by fungi are known as mycoses. There are three categories of mycoses; fungal infections, fungal toxicoses, and allergies.

Fungal infections are infections resulting from fungi on or in the body, and may be systemic, superficial, cutaneous/subcutaneous. ***Systemic fungal infections*** are infections in which the fungal agent has spread throughout the body involving organs of various body systems. Some examples of systemic fungal infections include pneumocystis pneumonia, coccidioidomycosis, and candidiasis. ***Superficial fungal infections*** are those involving the skin, hair, or nails. Dermaphytoses are an example of a superficial fungal infection. Fungal infections that occur in the deeper layers of the dermis and/or in the hypodermis are referred to as ***cutaneous or subcutaneous infections***. These infections are not as common as the superficial fungal infections which involve the dead cells of the integument.

Fungal toxicoses occur when toxins produced from fungi are ingested, such as the ingestion of poisonous mushrooms. Fungal allergies are hypersensitivity reactions, that usually occur in response to inhaled fungal spores. Fungal toxicoses and fungal allergies will not be part of the survey completed in this exercise.

In this exercise you will survey some clinically important yeast and molds that are known to cause fungal infections.

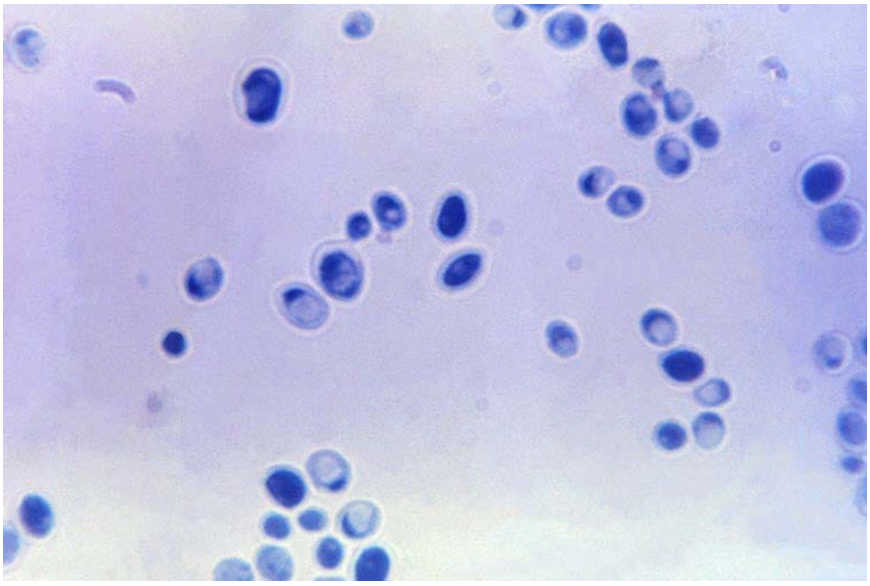
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6.1.2: Survey of Clinically Significant Yeasts

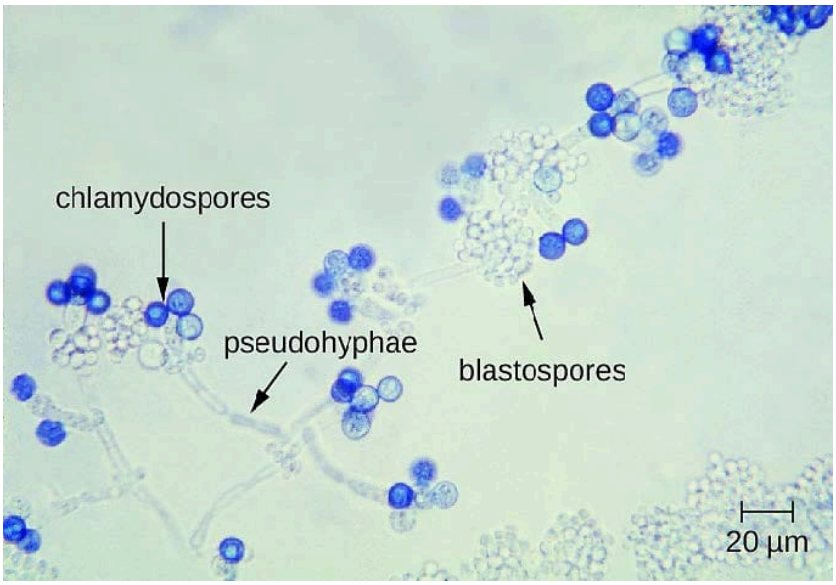
Candida *spp.*

Disease: Candidiasis Other Information:

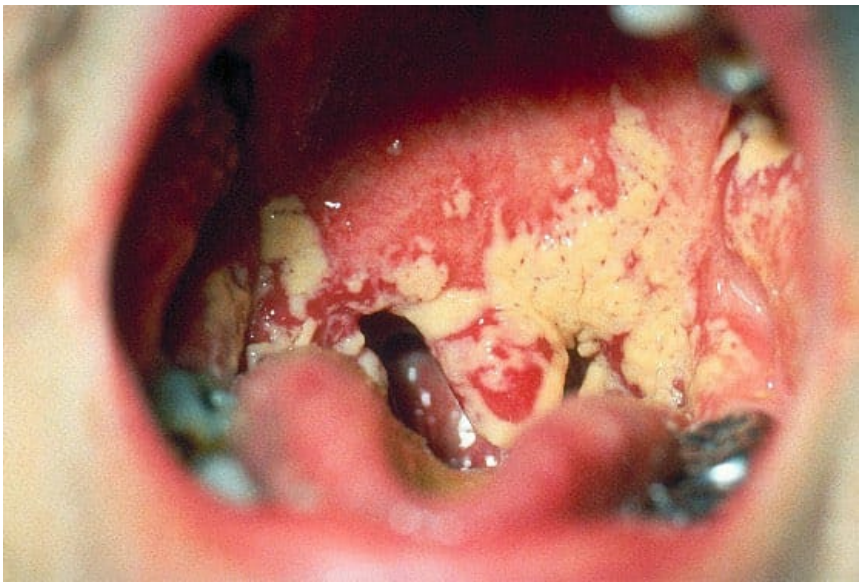
- Most common species associated with infection is *Candida albicans*
- Normally reside in the intestinal tract and mucous membranes without causing infection
- Opportunistic pathogen that produces symptoms when overgrowth occurs
- Candidiasis in the oral cavity or throat is referred to as “thrush,” while when in the vagina it is referred to as a “yeast infection”
- *Candida auris*
- Prior to ~2009 had not been reported to be able to grow at human body temperature; it has adapted to the human body temperature
- Now reported to cause severe illness in hospitalized patients; with numbers of infections increasing annually
- Does not respond to commonly used anti-fungal medications



“*Candida albicans*, magnification of 1200X.” Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain

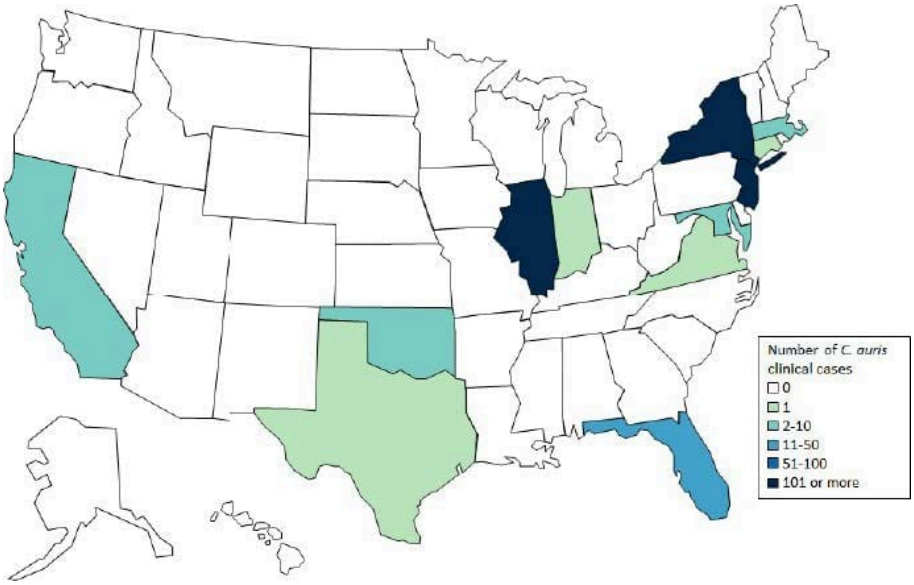


Candida albicans. Source: OpenStax CNX, Wikimedia Commons, CC BY 4.0


This image depicts a condition known as oral thrush, which manifested as a result of a *Candida albicans* infection. Note the yellowish-beige colored oral ulcers known as aphthae. Source: CDC, CDC PHIL, Public Domain



“This image depicted a frontal view of a Petri dish culture plate, which contained an unknown growth medium, that had been inoculated with a strain of *Candida auris* fungal organisms.” Source: Shawn Lockhart, CDC PHIL, Public Domain



“Candida auris cases reported prior to 2019 are categorized by the state where the specimen was collected. Most probable cases were identified when laboratories with current cases of *C. auris* reviewed past microbiology records for *C. auris*. Source: CDC, Wikimedia Commons, Public Domain

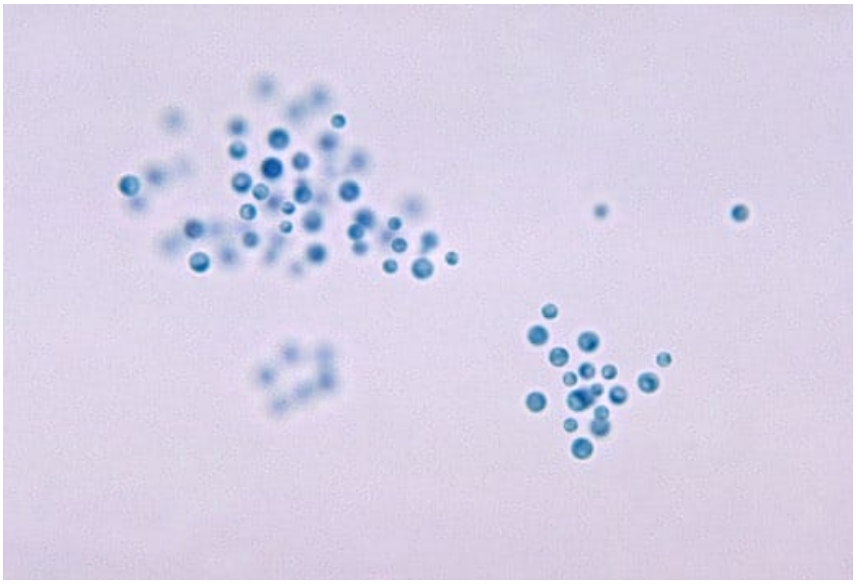
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Cryptococcus neoformans

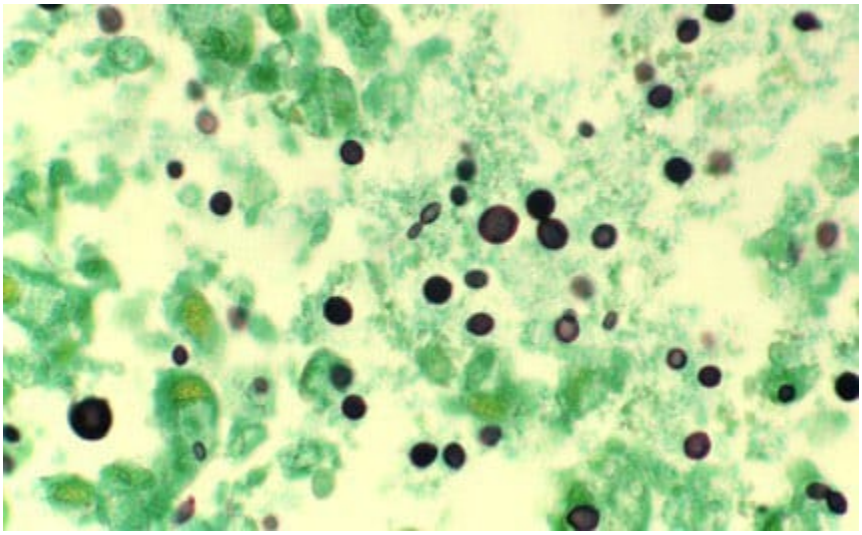
Transmission: Inhalation of fungal spores in the environment Disease: Cryptococcosis

Other Information:

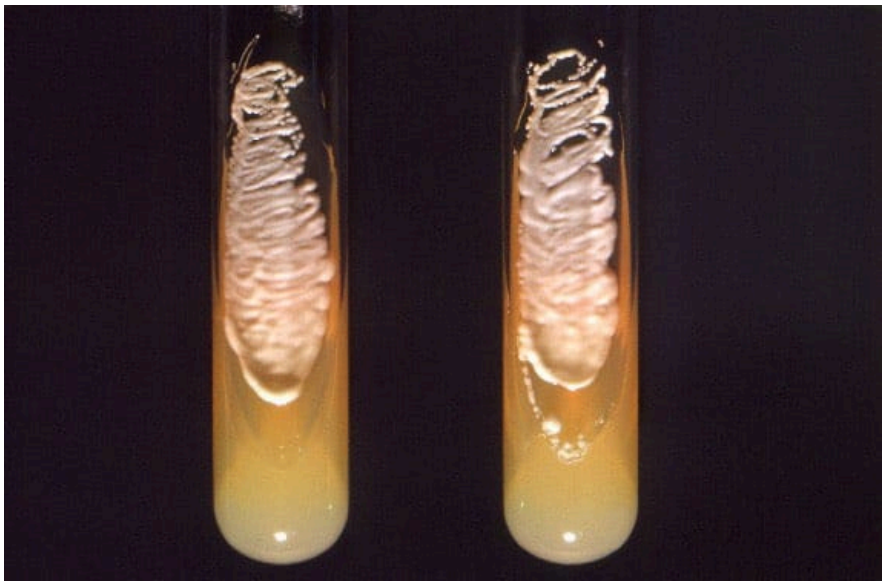
- Most infections occur in immunocompromised individuals, especially individuals with HIV/AIDS
- This fungus often found in soils where bird droppings are present
- Infection from inhalation leads to pneumonia, the fungus may spread to cause meningitis
- At one time infections were primarily associated with individuals who worked closely with poultry



“This photomicrograph revealed some of the ultrastructural morphology exhibited by the fungal organism, *Cryptococcus neoformans*, one of the causes for the infection known as cryptococcosis.” Credit: CDC/ Dr. Leanor Haley, CDC PHIL, Public Domain



“This photomicrograph of a methenamine stained lung tissue specimen, was harvested from an acquired immunodeficiency syndrome (AIDS) patient with cryptococcosis. Histopathology reveals numerous extracellular *Cryptococcus neoformans* yeast form organisms within the alveolar space. Some of the yeast organisms showed narrow base budding, and characteristic variation in size.” Source: CDC/ Dr. Edwin P. Ewing, Jr., CDC PHIL, Public Domain



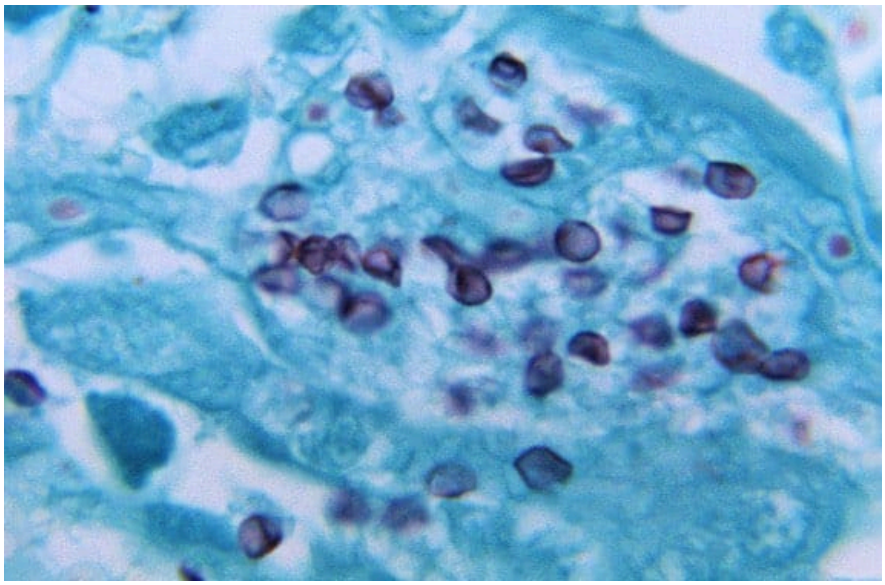
"This image depicted two test tube slant cultures, which contained an unidentified growth medium that had been inoculated with the fungal organism, *Cryptococcus neoformans*, which had been cultured from a sample, harvested from a cat. The colonial growth reflected the characteristic colonial morphology exhibited by *C. neoformans*, which included a creamy coloration, smooth surface, and mucoid appearance." (Right) Source: CDC/ Dr. Libero Ajello, CDC PHIL, Public Domain

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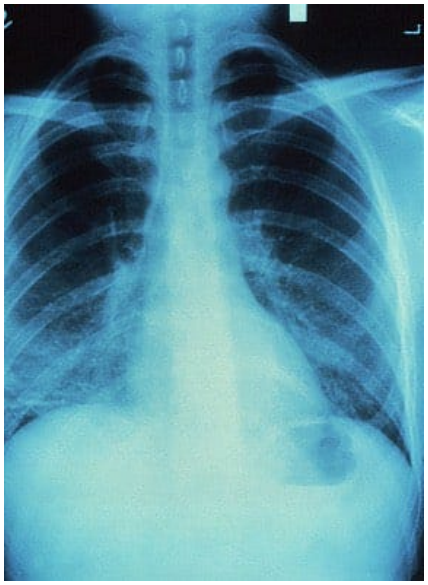
Pneumocystis jirovecii

Disease: Pneumocystic pneumonia Other Information:

- Part of normal flora
- Opportunistic pathogen
- Prior to the AIDS epidemic cases were only observes in malnourished, premature infants and debilitated elderly patients; now it is also associated with AIDS patients



“Under a magnification of 1150X, this photomicrograph of a silver stained human lung tissue specimen, revealed histopathology encountered due to a case of Pneumocystis pneumonia (PCP), caused by the fungus *Pneumocystis jirovecii*, formerly known as *P. carinii*. In this view, numerous darkly-stained cysts were plainly visible, while intracystic bodies were not visible.” Source: CDC/ Lois Norman, M.S., CDC PHIL, Public Domain



This anteroposterior (AP) chest x-ray revealed radiologic evidence of pulmonary pneumocystosis in the form of bilateral pulmonary interstitial infiltrates. This infection was due to the presence of an opportunistic fungal infection, by the fungal organism *Pneumocystis jirovecii*, formerly known as *Pneumocystis carinii*. (This image was provided by Jonathan W.M. Gold, MD, Assoc. Dir. Special Microbiology Lab, Assist. Attending Physician, Memorial Sloan-Kettering Cancer Center and Assist. Prof. of Medicine, Cornell Univ. Med. College, New York). Source: CDC/ Jonathan W.M. Gold, MD, CDC PHIL, Public Domain

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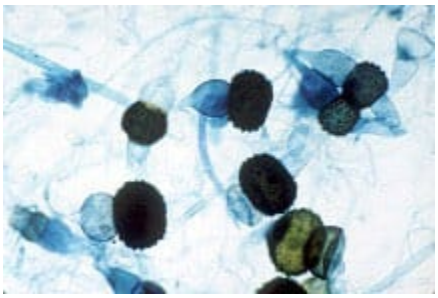
6.1.3: Survey of Clinically Significant Molds

Rhizopus *spp.*

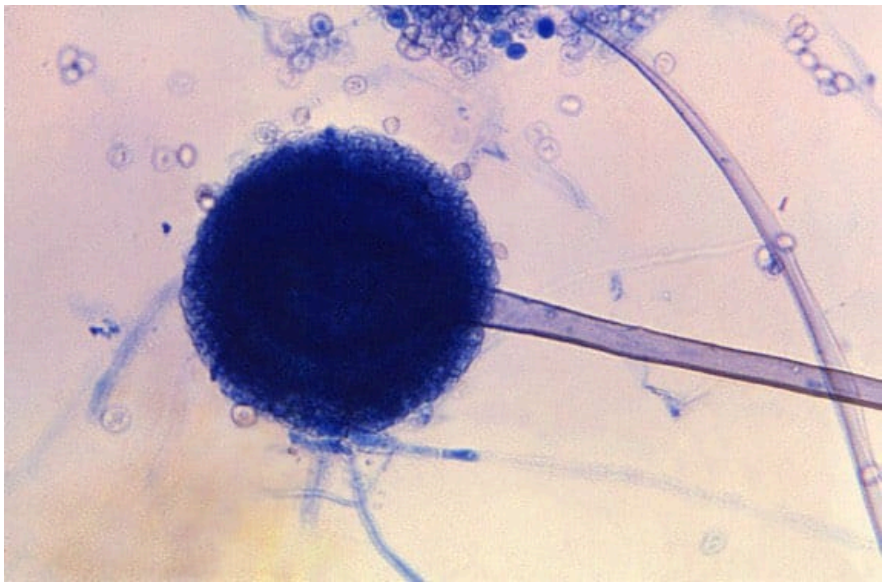
Transmission: Contact with fungal spores in the environment Disease: Zygomycosis

Other Information:

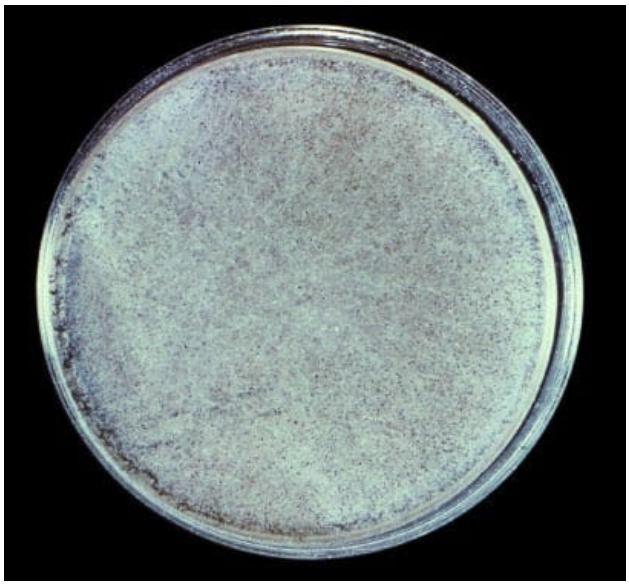
- Zygomycoses may also be known as Mucormycosis
- Serious but rare fungal infections
- May affect nearly any part of the body, but more commonly affects the sinuses and/or the lungs
- Zygomycoses are commonly seen in patients with uncontrolled diabetes, individuals who inject illegal drugs, cancer patients, and patients with weakened immune systems



"Light micrograph of a whole-mount slide of zygospores of *Rhizopus"* Source: Curtis Clark, Wikimedia Commons, CC BY 3.0



"Under a magnification of 475X, this photomicrograph revealed ultrastructural morphology exhibited by a *Rhizopus* sp. fungal organism. In this view, you can see a filamentous sporangiophore, which was topped by a globose, maturing sporangium, containing numerous reproductive spores." Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



"This photograph depicted a Petri dish culture plate, which contained an unidentified growth medium, that had been inoculated with a *Rhizopus* sp. fungal organism, and after an unknown incubation period, gave rise to this fungal colony." (Right) Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain

Trichophyton spp.

Transmission: Contact with someone who has the infection; contact with an animal (i.e. cat, dog, cows, horses, etc.) carrying the fungi; contact with contaminated surfaces (surfaces in locker rooms, public showers, etc.)

Disease: Dermaphytosis Other Information:

- Infections may be on the skin, nails, and hair
- Dermatophytes are one of the few contagious fungal infections
- Most common dermaphytosis is athlete's foot
- Dermaphytoses were formerly referred to as "ring worms" - because they may produce circular scaly patches on the skin



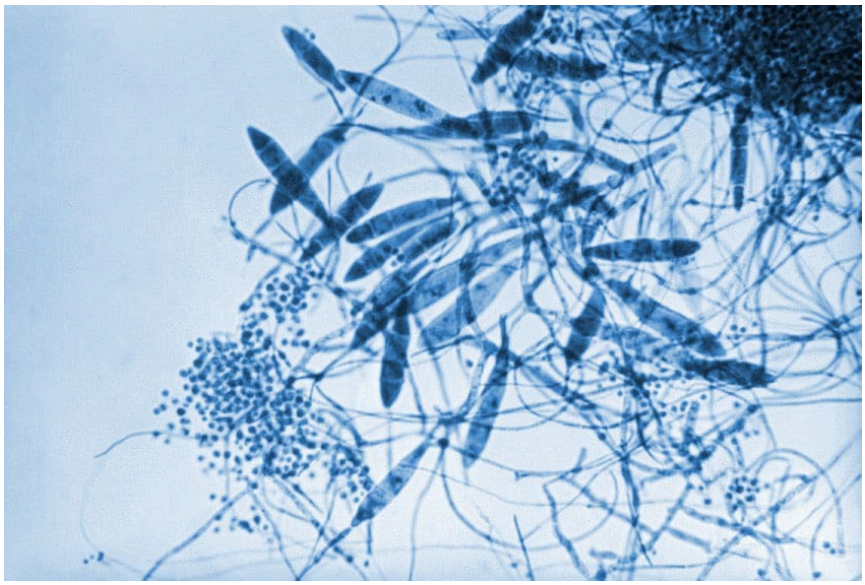
"This anterior view of a woman's chest, revealed a circular cutaneous lesion on the upper left breast, exhibiting scaly skin, and erythema. It was determined that this lesion, also referred to as tinea corporis, had been the result of a dermatophytic infection, brought on by the fungal organism, *Trichophyton mentagrophytes*." Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain (Left)



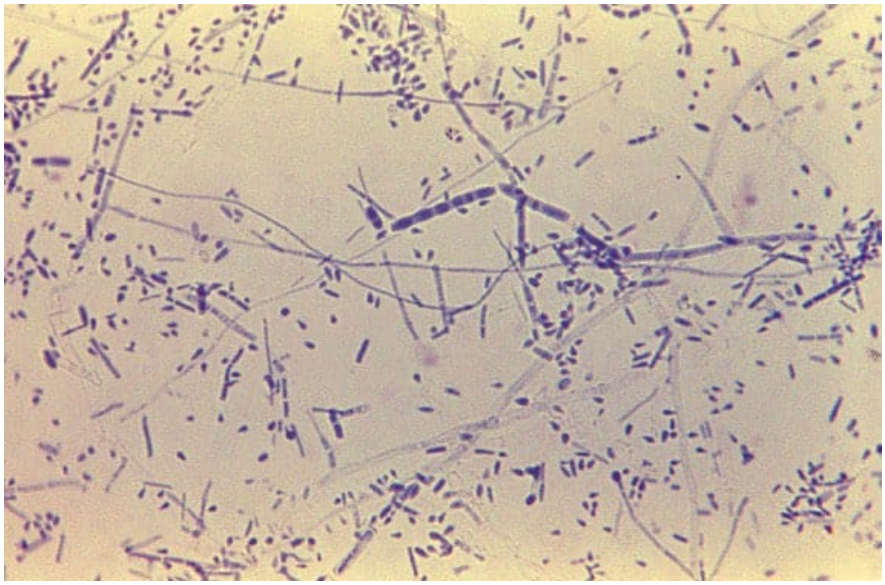
“This image depicted a left posterior-oblique view of a patient’s neck, which revealed a cutaneous scalp lesion diagnosed as a ringworm lesion, or tine capitis, caused by the dermatophytic fungal organism, *Trichophyton rubrum*. The individual was an inhabitant of New Guinea.” (Right) Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



“This image depicted a right superior-oblique view of a young female patient’s scalp, highlighting an erythematous, crusty lesion, which had resulted due to an infection known commonly as a kerion, a type ringworm, or tinea capitis, involving the formation of an abscess. The responsible dermatophytic fungal organism was identified, as *Trichophyton verrucosum*.” (Left) Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



“Under a magnification of 475X, this photomicrograph revealed some of the ultrastructural morphology exhibited by the dermatophytic fungal organism, *Trichophyton rubrum*. This view showed you a number of elongated, septate macroconidia grouped centrally, and numerous tear drop-shaped, much smaller microconidia.” Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



“Under a magnification of 475X, this photomicrograph revealed some of the ultrastructural morphology exhibited by the dermatophytic fungal organism, *Trichophyton rubrum*. This view showed you a number of elongated, septate macroconidia grouped centrally, and numerous tear drop-shaped, much smaller microconidia” Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



“Viewed from the front, this hair-soil culture plate had been inoculated with the fungal organism, *Trichophyton mentagrophytes*, which had resulted in the production of these abortive cleistothecia.” Source: CDC/ Dr. Kaplan, CDC PHIL, Public Domain



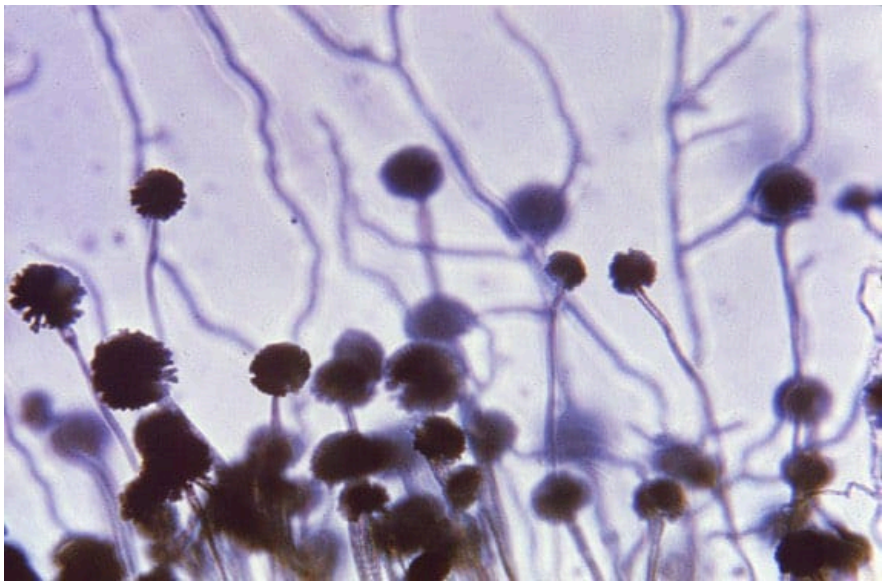
“Under a magnification of 500X, this photomicrograph revealed diaphanous, threadlike mycelia, along with numbers of chlamydospores from the fungal organism, *Trichophyton verrucosum* that had been cultured on a growth medium of Sabouraud dextrose agar, to which the vitamin nutrient, thiamine had been added.” Source: CDC/ Dr. Leanor Haley, CDC PHIL, Public Domain

Aspergillus spp.

Transmission: Inhalation of fungal spores Disease: Aspergillosis

Other Information:

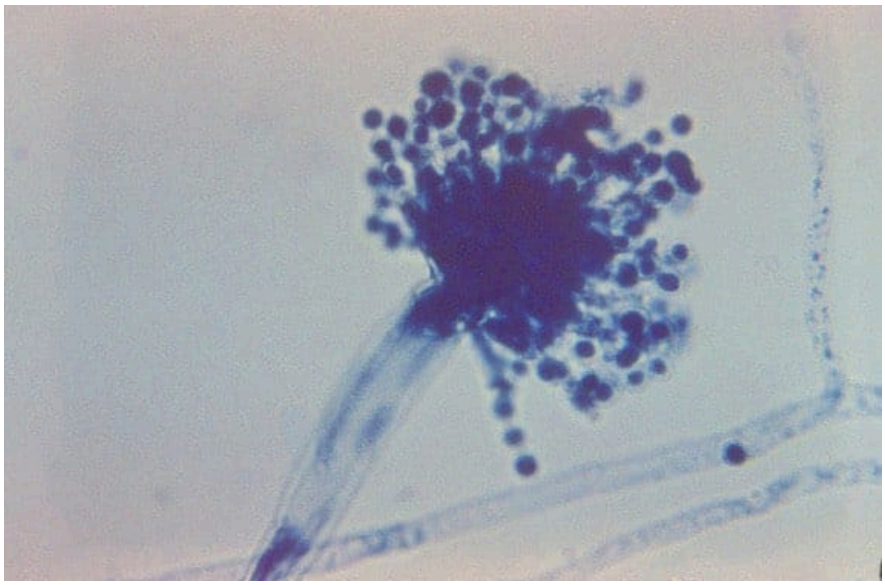
- Common mold that lives indoors and outdoors
- Most people breath in spores every day without becoming ill
- Opportunistic pathoge3n
- Most commonly only causes allergies
- Immunocompromised individuals are at a higher risk of developing more severe health conditions related to *Aspergillus*
- Aspergillosis is a growing problem for AIDS patients



“Under a magnification of 100X, this photomicrograph revealed some of the ultrastructural morphology exhibited by the fungal organism, *Aspergillus niger*. Here, you were able to see some of the organism’s septate, filamentous, hyaline hyphae, and numbers of darkly stained vesicles, also known as a radiate heads, each bearing numerous conidia.” (Left) Source: CDC/ Dr. Hilliard F. Hardin, CDC PHIL, Public Domain



“This image depicted a frontal view of a Petri dish culture plate, which had contained an unknown growth medium that had been inoculated with a fungal organism, *Aspergillus clavatus*. After having undergone a 15-day incubation period, the culture gave rise to this single, large, granular colony, which displayed an bluish-green central coloration, and a beige periphery.” (Right) Source: CDC/ Dr. Hilliard F. Hardin, CDC PHIL, Public Domain



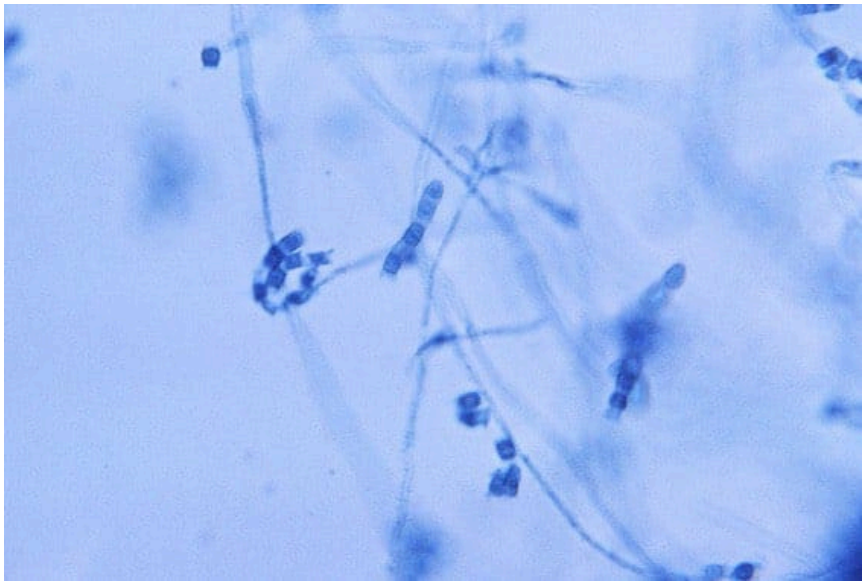
“Under a magnification of 970X, this photomicrograph revealed some of the ultrastructural morphology, exhibited by the fungal organism, *Aspergillus glaucus*. Here, you were able to see one of the organism’s conidial heads, from which chains of conidia had sprouted.” (Left) Source: CDC/ Dr. Hilliard F. Hardin, CDC PHIL, Public Domain

Coccidioides *immitis*

Transmission: Inhalation of fungal spores in the air Disease: Coccidioidomycosis

Other Information:

- Normally found in the soil of the southwestern United States and parts of Mexico
- Coccidioidomycosis is also referred to as “Valley fever” due to the organism being found in the desert soil
- Most people who breath in the spores do not get sick
- Some individuals will develop pulmonary disease; in severely compromised individuals the fungus may spread from the lungs to other organs (i.e. CNS, bones, joints)



“Under a magnification of 500X, this photomicrograph revealed the presence of thin, septate, hyaline hyphae, from which numerous, thick walled, *Coccidioides immitis* arthroconidia had evolved. The average arthrospore measures 3.0 x 4.5µm, and is barrel-shaped.” Source: CDC/ Dr. Lucille K. Georg, CDC PHIL, Public Domain



This image depicted a posterior view of a patient's torso, who had presented with a case of coccidioidomycosis , also known as valley fever, caused by the fungal organism, *Coccidioides immitis*. Source: CDC/ Dr. Brodsky, CDC PHIL, Public Domain



"After an incubation period of 14-days, this Petri dish culture plate grew a colony of *Coccidioides immitis* fungal organisms, atop an unknown growth medium." (Right) Source: CDC/ Dr. Brinkman, CDC PHIL, Public Domain

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6.1.4: Exercise #30 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Describe the structural differences between yeast and molds.
2. How do the dermatophytes differ in their mechanisms of transmission from most other fungi?
3. Superficial fungal infections are more common than cutaneous/subcutaneous fungal infections. Provide an explanation for this occurrence.
4. *Candida albicans* is part of the normal flora in health individuals. What conditions or situations might allow this fungus to become pathogenic in a patient?
5. An immunocompromised patient with AIDS presents with difficulty breathing. A fungal infection is suspected. Based on the pathogens studied in this exercise, which fungi should be considered and why?
6. *Candida auris* is considered an emerging fungal pathogen. Why is its resistance to antifungal medications especially concerning in healthcare settings?
7. This lab focuses on fungal infections but does not include fungal toxicoses. Based on what you've learned, why might fungal toxicoses be excluded from a lab focused on microscopic identifications? What makes the, different from infections in terms of diagnosis and clinical relevance?

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6.2: Exercise #31 - Protozoan Parasites

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions.

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #8 Demonstrate basic identification protocols based on microscopic morphology of some common fungi and parasites.

Laboratory Exercise Learning Outcomes

1. Describe the general characteristics of protozoans.
2. Describe the classification of protozoans based on motility, and how this classfication scheme is useful clinically.
3. Define trophozoite and cyst and differentiate between the two structures.
4. Define vector.
5. Compare and contrast biological vectors of some human pathogens.
6. Describe the morphology and epidemiology of important protozoan parasites and identify protozoan parasites using microscopy and micrographs.

Materials Needed:

- Microscope
- Prepared slides of fungal pathogens (*Entamoeba histolytica, Naegleria fowleri, Balantidium coli, Giardia lamblia, Trichomonas vaginalis, Trypanosoma brucei, Trypanosoma cruzi, Plasmodium vivax, Plasmodium falciparum, Cryptosporidium parvum*)

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6.2.1: Protozoans

Protozoans are single-celled eukaryotic microorganisms belonging to the kingdom Protista. They are heterotrophic microorganisms that are found in most habitats. Most protozoans are free-living organisms while a some serve as parasites to other organisms.

Some protozoans may exist in more than one form during their life cycle. The ***trophozoite*** form of a protozoan is the active feeding form. Some protozoans have the ability to produce cysts. ***Cysts*** are dormant forms that allow the microorganisms to withstand extremes in temperature, chemicals, lack of nutrients and/or water, etc. Additional stages are present within the life cycle of some species, making their life cycles more complex. As we progress through this exercise you will see that many of the pathogenic protozoans are transmitted to a host in the cyst form. This is often the case for those that are transmitted through fecal-oral transmission, i.e. *Giardia lambia*, *Entamoeba histolytica*, etc.

Fecal-oral transmission is only one means by which parasitic protozoans are transmitted. Another means of transmission utilized by some protozoan parasites are vectors. Vectors are arthropod animals that serve in transmitting the parasite. Vectors may be mechanical or biological. Mechanical vectors physically carry a pathogen from one source to another. Examples of mechanical vectors are house flies and cockroaches.

Biological vectors on the other hand harbor the parasite within themselves during a portion of the parasite's life cycle. Examples of biological vectors include mosquitoes and tsetse flies, which both transmit the parasites they carry as they take a blood meal from a human or animal.

There are a number of schemes that have been proposed for classifying protozoans. Protozoans are considered animal-like protists because of their ability to demonstrate motility. Protozoans may use pseudopods, flagella, or cilia to accomplish motility. One classification uses the differences in motility structures to group them. This scheme does not reflect their relatedness, but is useful clinically. Microscopic examination of the parasite assists in identifying the cause of protozoan parasitic infections. Using this scheme, one could easily work to identify the causative agent. This scheme will be used here to survey some protozoan parasites. Using motility as a means of categorizing protozoans, they may be grouped into the Phylum Sarcomastigophora, Phylum Ciliphora, and Phylum Apicomplexa. The Phylum Sarcomastigophora includes two subphyla, the Subphylum Sarcodina, which are the amoeboids that use pseudopods for motility, and the Subphylum Mastigophora, which includes protozoans that owe their motility due to the presence of flagella. Protozoans within the Phylum Ciliophora utilize cilia that cover their cell surface to accomplish motility. The Phylum Apicomplexa, are also known as sporozoa, and typically are non- motile protozoans. This will exercise will examine some parasites from each of these classifications.

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6.2.2: Subphylum Sarcodina (Amoeboids)

Entamoeba histolytica

Resides: in the cecum, appendix

Transmission: acquired by inadvertent ingestion in contaminated food or drink Disease: Causes amoebic dysentery

Other Information:

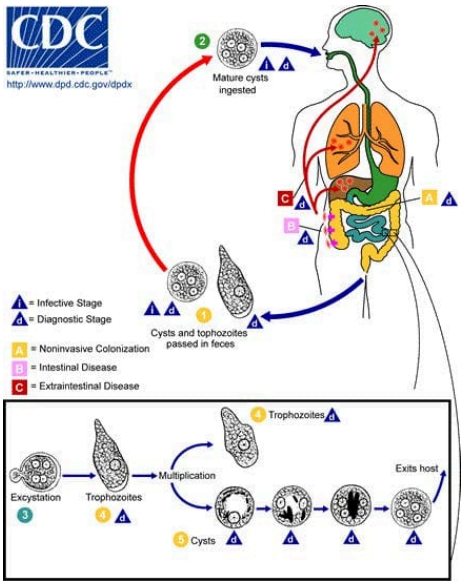
- Name translates to tissue destroying or tissue lysing
- Primary host is humans



Entamoeba *histolytica* (trophozoite) Credit: Sherry Smith



Entamoeba histolytica (cyst) Credit: Sherry Smith



Entamoeba *histolytica* life cycle. Source: DPD CDC, Wikimedia Commons, Public Domain

Naegleria fowleri

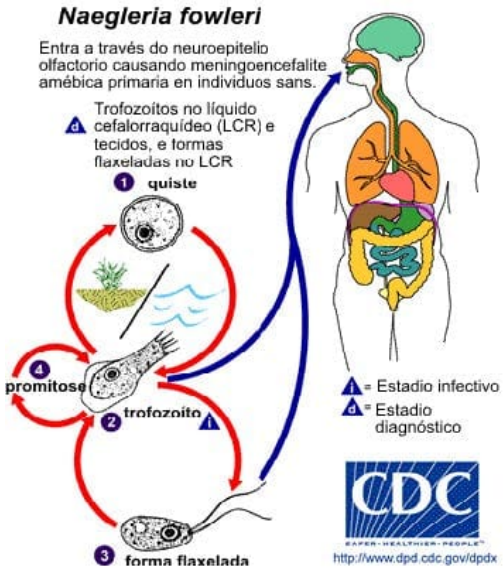
Resides: Enters through the nasal cavity then moves to the brain

Transmission: Amoeba penetrates the nasal mucosa during any type of aquatic activity; amoeba is acquired as contaminated warm freshwater is inhaled into the nasal cavity, the organism then penetrates the cribriform plate

Disease: Amoebic infection of the brain (primary amoebic meningoencephalitis (PAM)) ; almost always fatal; normally presents as flu-like symptoms (aching, fever, nausea) followed by coma and death

Other Information:

• Common name - brain-eating amoeba



Naegleria *fowleri* life cycle.
Source: Miguelferig, Wikimedia Commons, Public Domain

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6.2.3: Subphylum Mastigophora (Flagellates)

Giardia lamblia

Resides: Proliferates within the small intestine

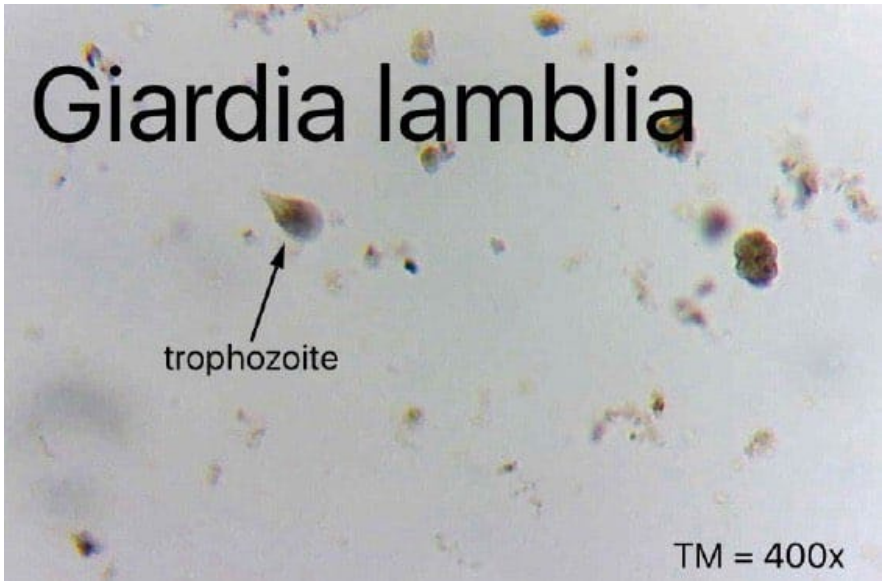
Transmission: Acquired by inadvertent ingestion of water contaminated with cysts

Disease: Causes Giardiasis (also known as beaver fever or traveler's diarrhea)

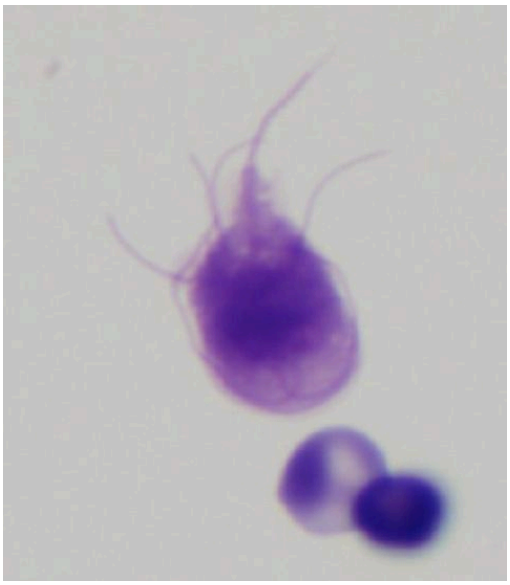
Other Information:

- Freshwater flagellate
- Beavers, nutria, and other aquatic mammals are known to be asymptomatic carriers
- Protected by an outer shell - allows the parasite to survive for long periods of time outside of the body and is highly resistant to chlorination, may be found in public pools,

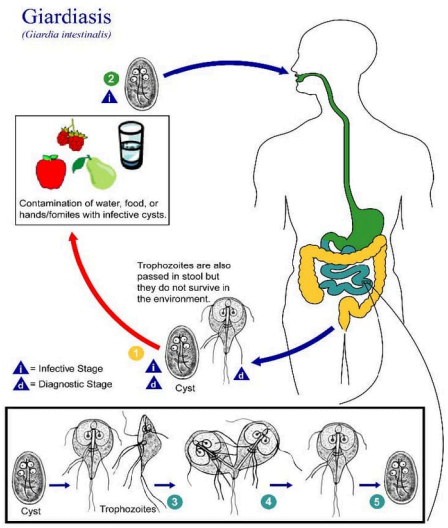
hot tubs, etc.



Giardia *lamblia* (trophozoite) Credit: Sherry Smith



Giardia lamblia seen in a cytologic preparation (cytospin of formalin from small bowel biopsy of a patient with giardia).
Source: Jerad M Gardner, MD , Wikimedia Commons, CC BY-SA 3.0,



Giardia Life Cycle Credit: PHIL, Public domain, via Wikimedia Commons

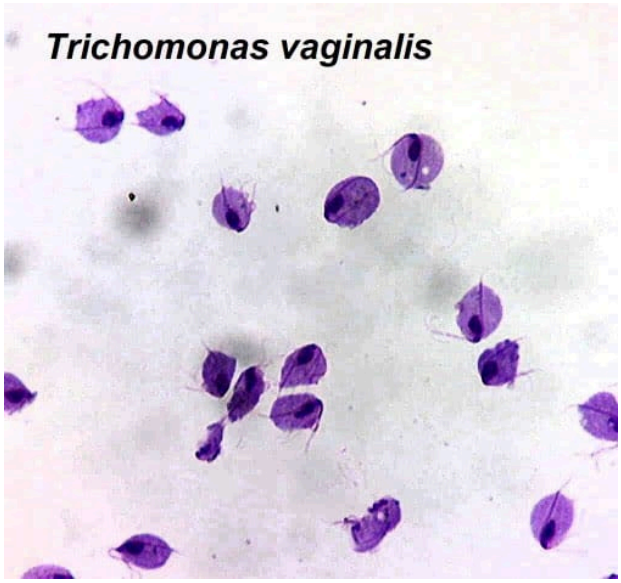
Trichomonas vaginalis

Resides: Vagina and urethra of women; urethra, prostate, and seminal vesicles of men Transmission: Sexual intercourse (lack of cyst stage requires direct transmission)

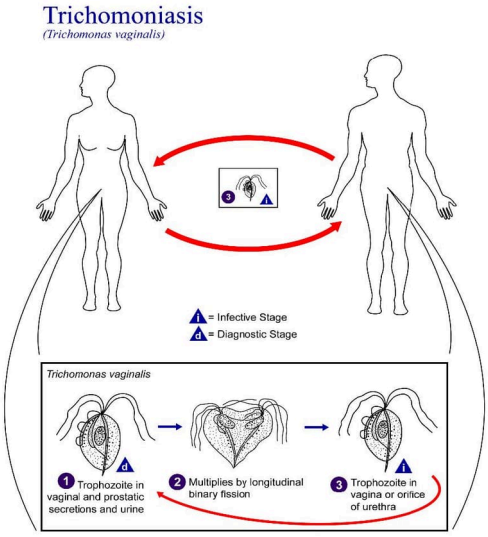
Disease: Trichomoniasis (Females - vulvitis, vaginitis, cervicitis, salpingitis; Males - urethritis, prostatitis, epididymitis))

Other Information:

- Exists only in the trophozoite form
- Worldwide distribution
- May infect both males and females, but more common in females
- Most infections are asymptomatic or mild



Trichomonas *vaginalis* (trophozoite) Credit: Sherry Smith



Trichomonas vaginalis life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

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Trypanosoma brucei

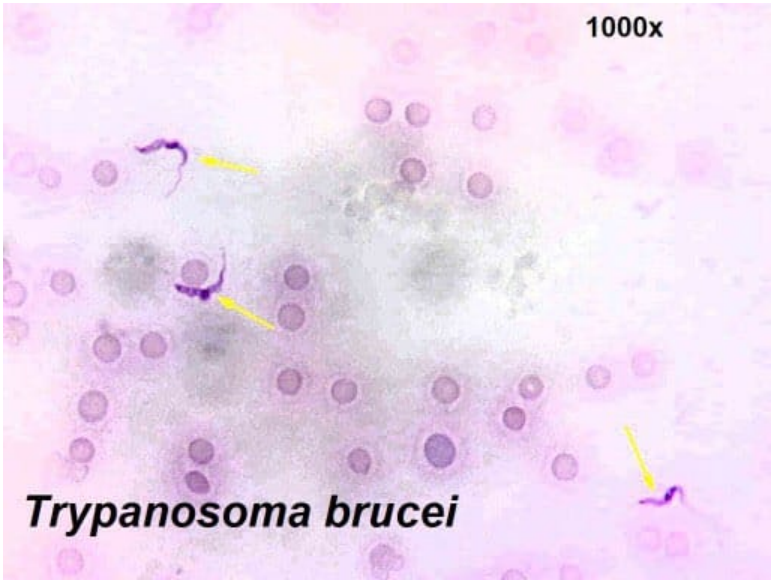
Resides: Blood

Transmission: Acquired through the bite of a tsetse fly

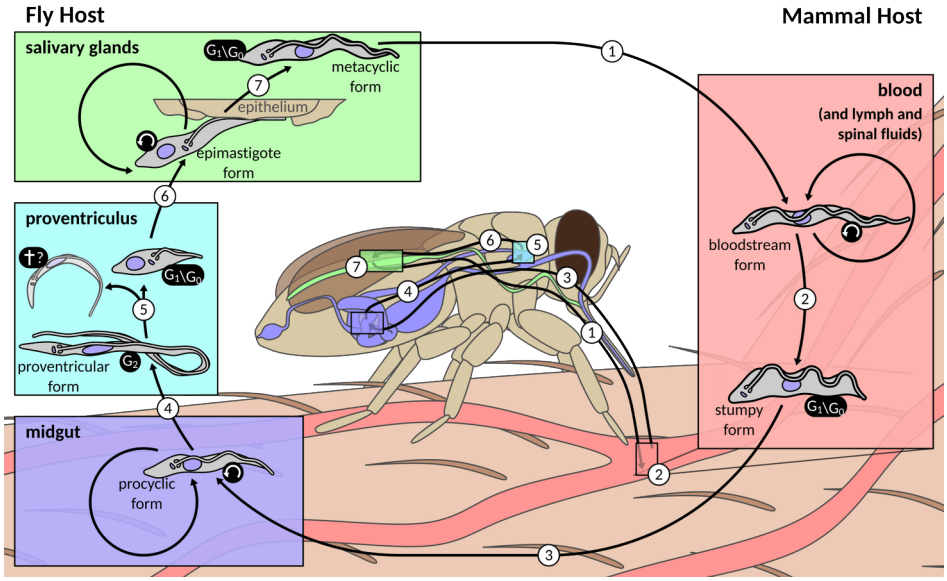
Disease: African sleeping sickness (African Trypanosomiasis)

Other Information:

- There are two variants of *Trypanosoma brucei*
- *Trypanosoma brucei gambiense* - occurs primarily in western Africa; cattle and sheep serve as reservoirs
- *Trypanosoma brucei rhodesiense* - occurs in eastern and southern Africa; several species of wild animals serve as reservoirs
- Patients with this infection generally lapse into a coma
- If left untreated is always fatal



Trypanosoma brucei trypomastigotes (yellow arrows) Credit: Sherry Smith



"The life cycle of the parasite Trypanosoma brucei which has three proliferative life cycle stages, two in the tsetse fly vector and one in the mammalian host bloodstream."
Source: Zephyris, Wikimedia Commons, CC BY 4.0

Trypanosoma cruzi

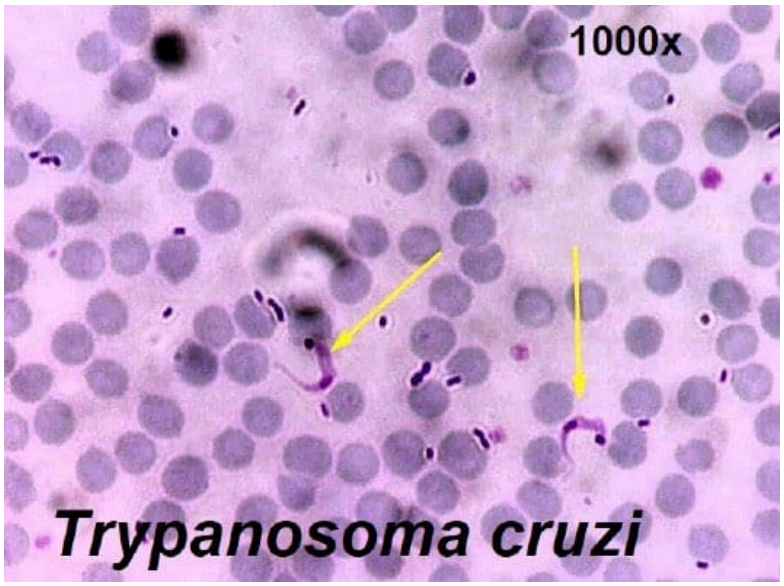
Resides: Blood, thus the parasite is known as a hemoflagellate

Transmission: Acquired through the bite of

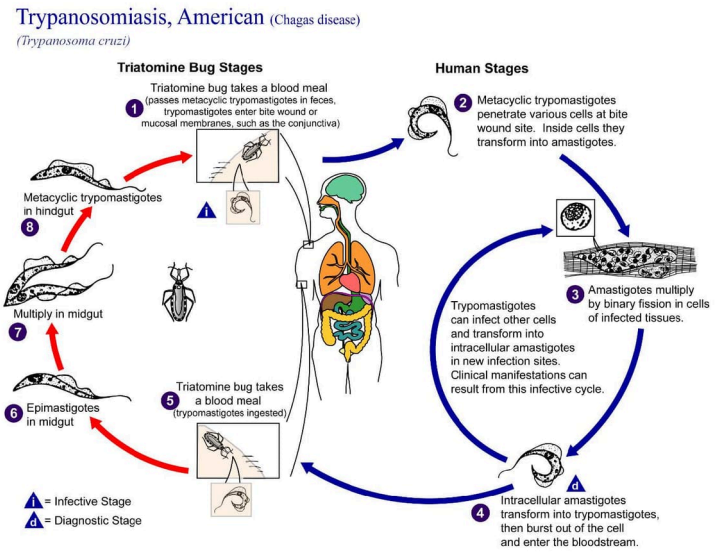
a Reduviid bug (also known as the "kissing bug") Disease: Chaga's disease

Other Information:

- Normally found in South and Central America



Trypanosoma cruzi trypomastigotes (yellow arrow) Credit: Sherry Smith



Trypanosoma cruzi life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

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6.2.4: Phylum Ciliophora (Ciliates)

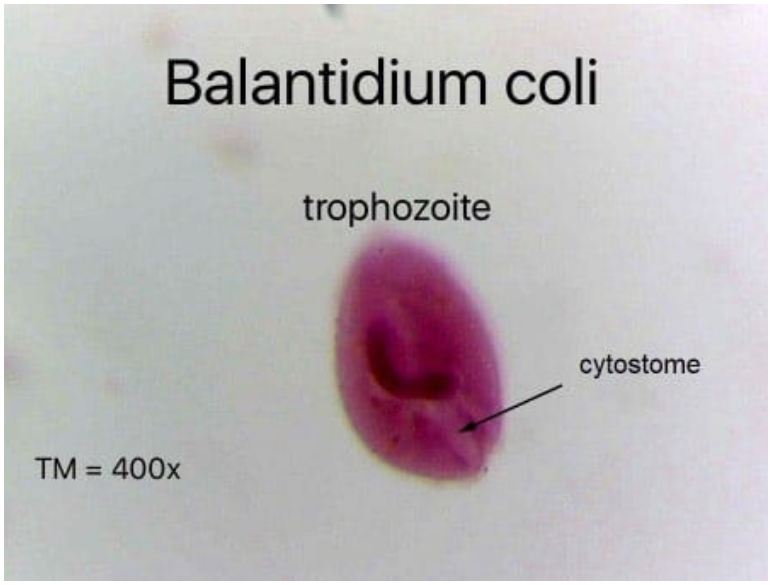
Balantidium coli

Resides: In the colon, cecum, or appendix

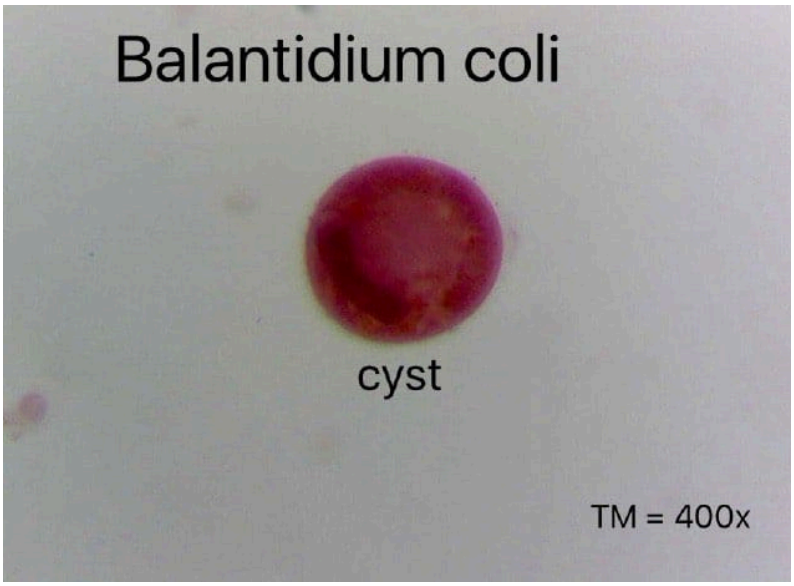
Transmission: Acquired by ingestion of food or drink contaminated with cysts

Disease: Causes Balantidiasis (also known as ciliary dysentery or Montezuma's revenge) Other Information:

• Only ciliate of human medical significance



Balantidium coli (trophozoite) Credit: Sherry Smith



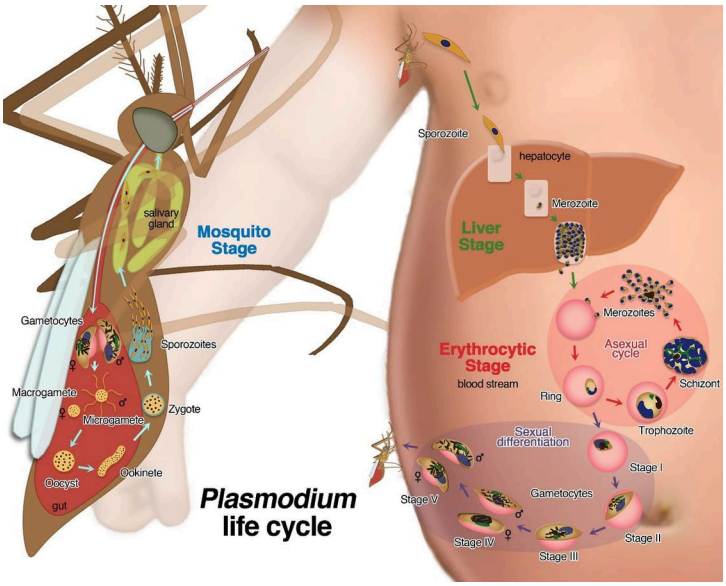
Balantidium coli (cyst) Credit: Sherry Smith

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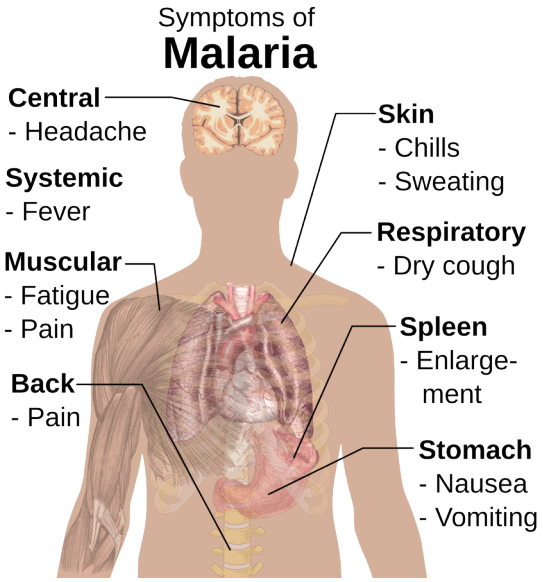
6.2.5: Phylum Apicomplexa (Sporozoa)

Plasmodium *species*

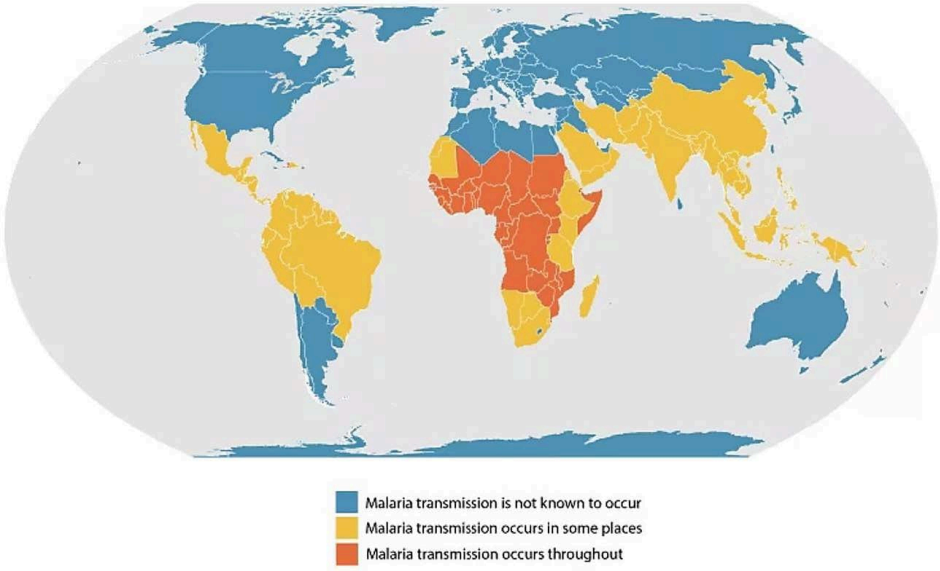
- All are agents causing malaria
- All may be found in intracellular whole blood
- Sexual phase of the parasite is carried out in the gut of the female *Anopheles* mosquito
- Asexual phase involves humans:
- In human blood sample:
- Stage 1 - look for development of a ring trophozoite
- Stage 2 - look for the development of schizonts (up to 12 tiny little merozoites may be preset within a single red blood cell); Schizonts may be found in all species of plasmodium
- Eventually the erythrocytes will lyse, releasing these agents to reinfect more red blood cells.



Plasmodium life cycle.
Credit: Le Roche Lab, UC Riverside, Wikimedia Commons, CC BY 3.0.



Source: Mikael Häggström, Wikimedia Commons, Public Domain



"Malaria distribution 2020"
Source: Julius Senegal, Wikimedia Commons, Public Domain

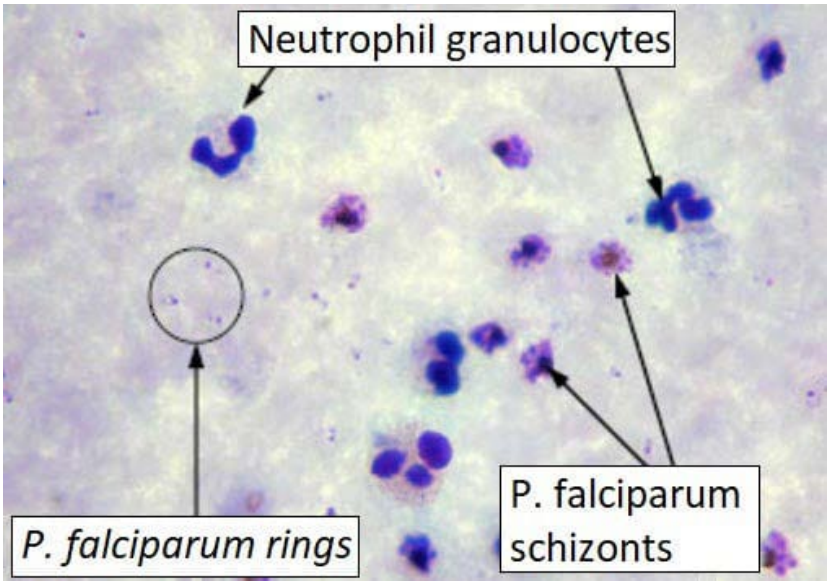
Plasmodium *falciparum*

Resides: Blood and liver cells of humans

Transmission: Acquired through bite of female *Anopheles* mosquito (only pregnant female mosquitoes take blood meals)

Disease: Malaria Other Information:

- Two or more ring trophozoites present in the red blood cell during stage 1
- Responsible for ~90% of the deaths cause by *Plasmodium* species



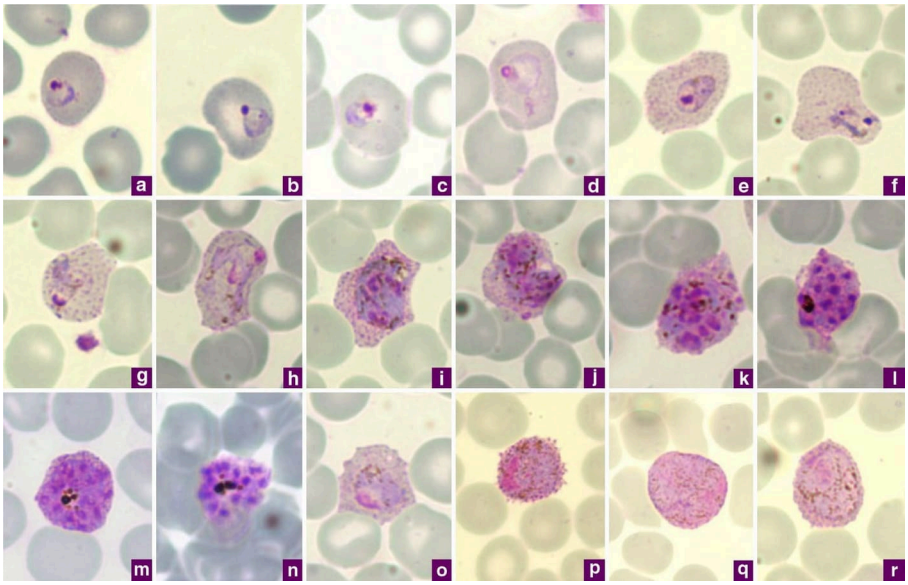
"Thick blood film with *Plasmodium falciparum* rings and schizonts."
Source: Lwin KM, Ashley EA, Proux S, Silamut K, Nosten F, McGready R, Wikimedia Commons, CC BY 2.0

Plasmodium *vivax*

Resides: Blood and liver cells of humans

Transmission: Acquired through bite of female *Anopheles* mosquito (only pregnant female mosquito's take blood meals) Disease: Malaria Other Information:

- One ring trophozoites present in the red blood cell during stage 1



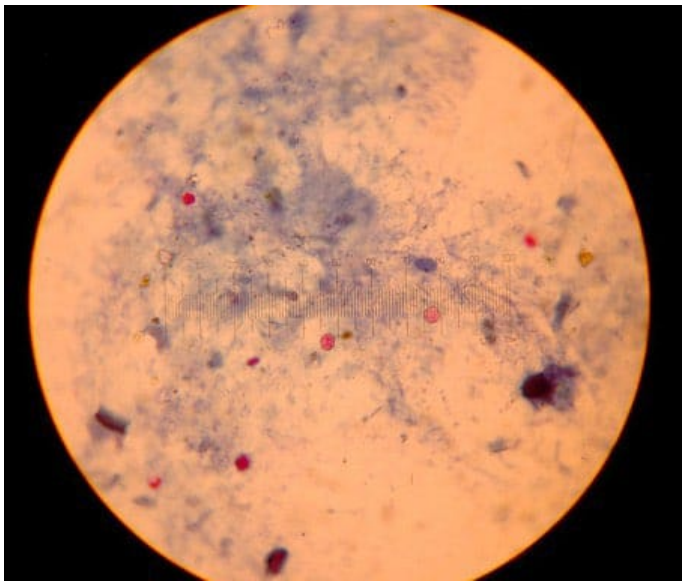
"Microphotographs of Plasmodium vivax in Giemsa-stained thin blood films collected in Singapore. a, b ring stages, c–e young trophozoites, f–h amoeboid trophozoites, i young schizont, j–l growing schizonts, m developed schizont, n mature schizont, o young gametocyte, p macrogametocyte, r, q microgametocytes."
Source: Chavatte JM, Tan SB, Snounou G, Lin RT, Wikimedia Commons, CC BY 4.0

Cryptosporidium *parvum*

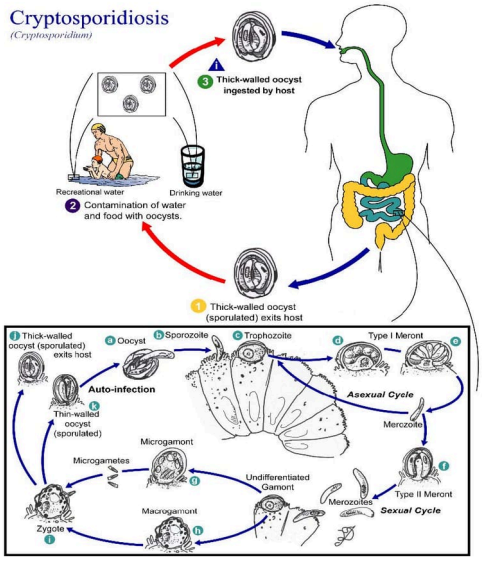
Resides: Multiplies intra-cellularly in the small intestinal epithelium Transmission: Ingestion of infective oocysts in fecal-contaminated water Disease: Cryptosporidiosis (commonly called "crypto")

Other Information:

- Water chlorination does not kill oocysts
- Infective oocyst passed in feces
- No specific host - can infect humans, cattle, horses, sheep, goats, chickens, fish, lizards, etc.
- May cause severe acute and persistent diarrhea in children less than 3 years old and immunocompromised adults
- One of leading causes of death in AIDs patients
- Worldwide distribution



Cryptosporidium *parvum*.
Source: Michael Wunderli, Wikimedia Commons, CC BY 2.0



Cryptosporidium life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

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6.2.6: Exercise #31 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. Define biological vector and list four species covered in this exercise that are transmitted by biological vectors. For each species identify its vector.
2. Describe the difference between a trophozoite and cyst.
3. The parasite *Trichomonas vaginalis* is not capable of forming a cyst. How does this effect its ability to be transmitted from one host to another?
4. *Trypanosoma brucei* and *Trypanosoma cruzi* are both flagellated protozoans that appear very similar at first glance. Examine images of these two parasites and find a way in which you might distinguish the two from one another. Describe how the two parasites can be differentiated morphologically.
5. Like the *Trypanosomes*, students often have difficulty distinguishing the Plasmodium species. If you were provided with images of the parasites in the ring stages, how could you differentiate the *Plasmodium falciparum* from *Plasmodium vivax*?
6. *Entamoeba histolytica* and *Naegleria fowleri* are parasitic amoebas. How do they differ in the mechanism of transmission?
7. Consider the protozoan parasites that infect the gastrointestinal (GI) tract (such *as Balantidium coli, Giardia lamblia,* and *Entamoeba histolytica*). What common traits do these protozoans share regarding their modes of transmission and their ability to resist environmental conditions outside the host? Explain how these traits contribute to their spread and persistence in populations.
8. A patient presents with recurring episodes of fever, chills, and sweating, along with fatigue, jaundice, and signs of anemia. Based on these symptoms and your knowledge of protozoan parasites, identify the most likely disease and its etiological agent (specific organism causing an illness). Then, explain how the parasite's movement through different tissues in the host—particularly the liver and blood—can account for the symptoms observed. Be sure to reference what is occurring at each stage of the life cycle that contributes to these clinical signs.

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6.3: Exercise #32 - Helminth Pathogens

Exercise Outcomes:

Course Laboratory Learning Outcomes

THECB - ACGM Lab Learning Outcome #1 Use and comply with laboratory safety rules, procedures, and universal precautions.

THECB - ACGM Lab Learning Outcome #2 Demonstrate proficient use of a compound light microscope.

THECB - ACGM Lab Learning Outcome #8 Demonstrate basic identification protocols based on microscopic morphology of some common fungi and parasites.

Laboratory Exercise Learning Outcomes

1. Differentiate between platyhelminths and nematodes.
2. Describe the general and physical characteristics of the typical trematode.
3. Describe the general and physical characteristics of a cestode.
4. Describe the general and physical characteristics of a nematode.
5. Define and contrast definitive hosts and intermediate hosts.
6. Describe the morphology and epidemiology of important trematode parasites and identify important trematode parasites using microscopy and micrographs.
7. Describe the morphology and epidemiology of important cestode parasites and identify important trematode parasites using microscopy and micrographs.
8. Describe the morphology and epidemiology of important nematode parasites and identify important trematode parasites using microscopy and micrographs.

Materials Needed:

- Microscope
- Prepared slides of fungal pathogens (*Clonorchis sinensis, Fasciola hepatica, Schistosoma mansoni, Schistosoma japonicum, Taenia saginata, Taenia solium, Hymenolepis nana, Dipylidium caninum, Diphyllobothrium latum, Ascaris lumbricoides, Enterobius vermicularis, Trichinella spiralis, Trichuris trichiura, Ancylostoma duodenale, Necator americanus, Wuchereria bancrofti*)

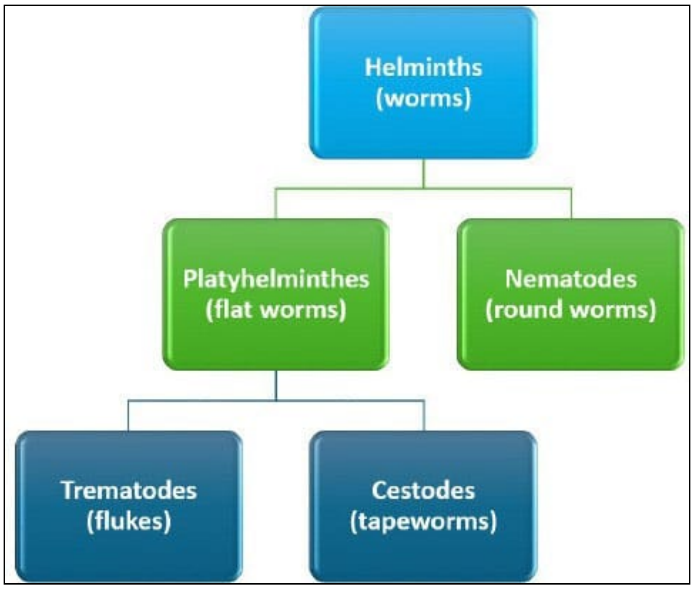
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6.3.1: Helminths

Helminths are eukaryotic organisms that belong to the kingdom animalia. Helminths like other animals have organs and organs systems. Their life cycles include an egg, larval, and adult stage. Many of the adult forms are easily observed with the naked eye. The eggs and larvae are generally microscopic. It is usually the egg or larval stage that are identified in clinical samples for diagnosis, and thus are the reason the parasitic helminths are studied in microbiology.

There are three groups of helminths that are clinically important; trematodes, cestodes, and nematodes. These three groups can be differentiated based on their body patterns, arrangement of reproductive organs, and structure of their digestive systems. Helminths share the characteristic of having complex life cycles. Most parasitic helminths will have an intermediate host and a definitive host during which they spend part of their life cycle. ***Intermediate hosts***, are hosts in which the immature (larvae) form of the parasite resides.

Definitive hosts, on the other hand harbor the mature adult worms, which are sexually reproducing.



Clinically relevant helminths. Credit: Sherry Smith

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6.3.2: Trematodes (Flukes)

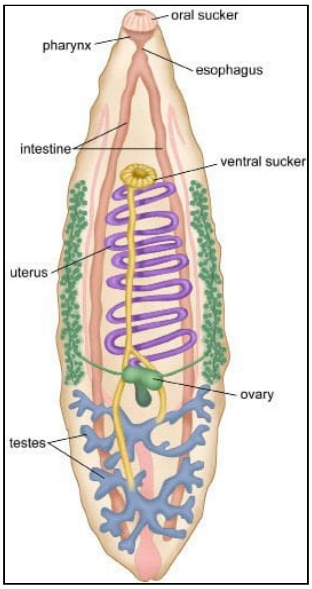
Trematodes are one type of platyhelminth (flatworms). Trematodes are commonly referred to as flukes. The typical trematode has a flat-leaf shaped body that exhibits bilateral symmetry.

They lack circulatory systems; thus, their flat body pattern is important for the diffusion of nutrients throughout the organism. Typical trematodes are also monoecious. Monoecious organisms are organisms that have male and female reproductive structures in the same individual; hermaphroditic. Trematodes are also characterized by having incomplete digestive systems. Their digestive systems are composed of an oral sucker, a pharynx, and intestines that end in blind pockets. Parasitic trematodes have structures that they utilize to aid in remaining attached to their host organism. Such structures include the oral sucker and ventral sucker (acetabulum).

Trematodes can be grouped based on the type of tissues in which they reside within their hosts. Blood trematodes reside within the blood vessels of the digestive or urinary tracts and feed on blood. Tissue trematodes live within tissues and organs such as the epithelium of the small intestine, bile ducts, gallbladder, liver, or lungs. Examples of trematodes that are considered blood flukes include *Schistosoma mansoni*, *Schistosoma japonicum*, and *Schistosoma haematobium*. Tissue flukes include species such as *Clonorchis sinensis*, *Fasciola hepatica*, and *Fasciolopsis buski*.

Most trematodes have life cycles that include an intermediate host and a definitive host. The intermediate hosts are typically freshwater snails. Definitive hosts are vertebrate animals, including humans, domestic animals, birds, fish, and others. Definitive hosts harbor the adult trematode, which reproduces sexually and releases eggs. When the eggs exit the definitive host in fecal material and are introduced to freshwater snails (the intermediate host), they enter the intermediate host and reproduce asexually. From the intermediate host the trematodes can be transmitted to a new definitive host by several different mechanisms, depending on the species of trematode involved. Some species such as *Clonorchis sinensis* and *Fasciola hepatica* are transmitted via food-borne transmission. Trematodes such as *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* are acquired via direct penetration of cercariae through the host's skin when the host is in contaminated water.

In this exercise you will survey two trematodes that demonstrate the typical body pattern and reproductive organization (*Clonorchis sinensis* and *Fasciola hepatica*), and two trematodes that do not follow the typical body pattern or reproductive organization (*Schistosoma mansoni* and *Schistosoma japonicum*).



Typical trematode (fluke) structure. Credit: Susan Decker

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6.3.3: Survey of Trematodes

Clonorchis sinensis

Resides: bile ducts of the liver

Transmission: ingestion of metacercariae in undercooked fish

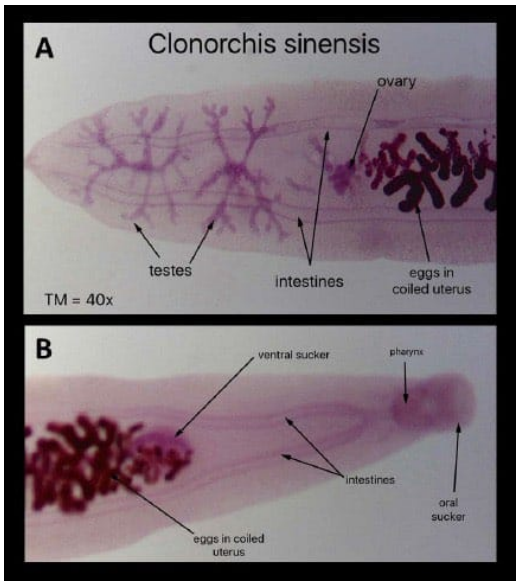
Disease: Clonorchiasis - characterized by bile duct obstruction, liver abscesses, liver enlargement, hepatic failure

Other Information:

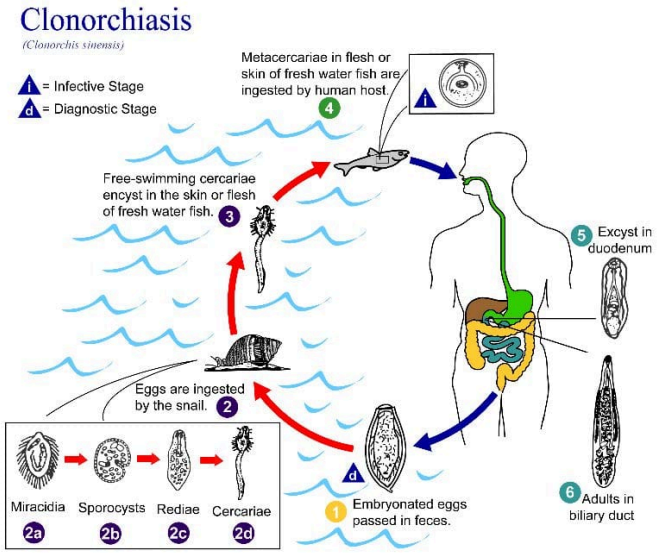
- Liver fluke
- Commonly known as the Chinese fluke of oriental liver fluke
- Monoecious
- Snail acts as intermediate host in fresh water
- Endemic to east Asia



Clonorchis sinensis. Credit: Sherry Smith



Clonorchis *sinensis*. A) posterior half of parasite B) anterior half of parasite. Credit: Sherry Smith



Clonorchis *sinensis* life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

Fasciola hepatica

Resides: bile ducts of the liver

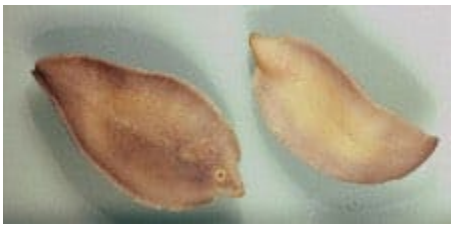
Transmission: ingestion of metacercariae in on vegetation or contaminated water

Disease: Fascioliasis (acute phase - characterized by inflammation, tissue destruction, allergic reaction; chronic phase - characterized by bile duct and gallbladder obstructions, pancreatitis

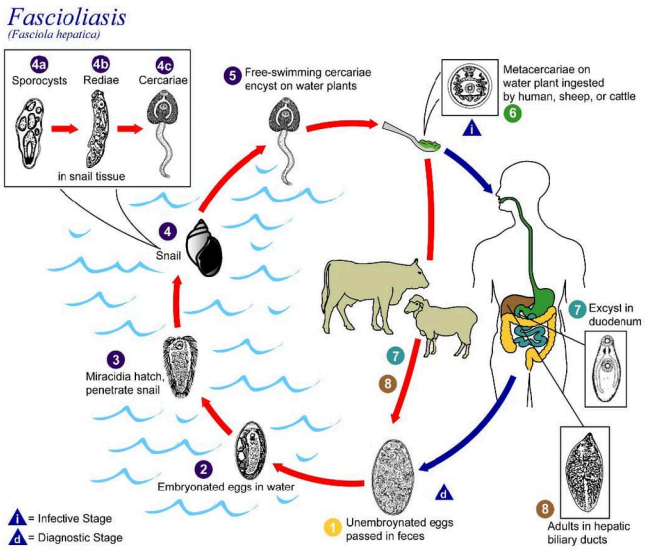
Other Information:

- Liver fluke
- Commonly known as the common liver fluke or sheep liver fluke

- Ruminants (i.e. cattle, sheep, etc.) are the typical definitive host
- Worldwide distribution - especially where sheep and cattle are raised



Fasciola hepatica adults.
Source: Alan R Walker, Wikimedia Commons, CC BY-SA 3.0



Fasciola life cycle. Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

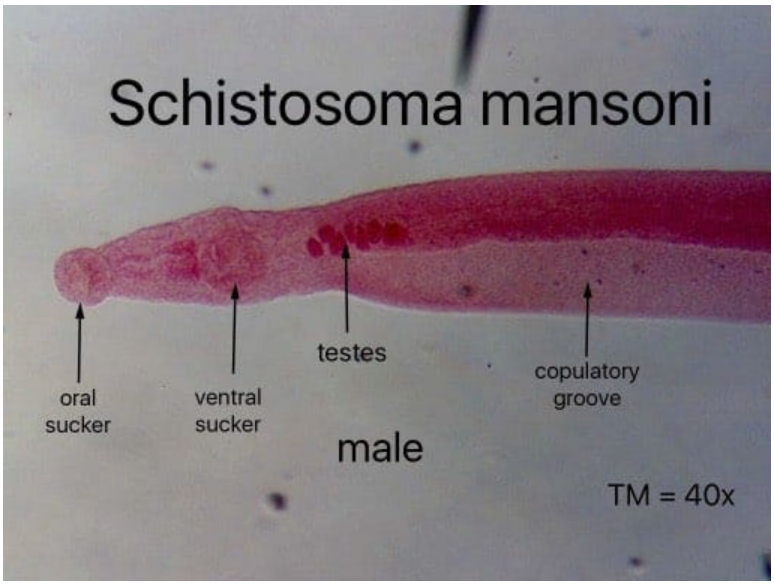
Schistosoma *mansoni*

Resides: resides within the mesenteric venules of the small intestines Transmission: cercariae in contaminated freshwater burrow through the skin

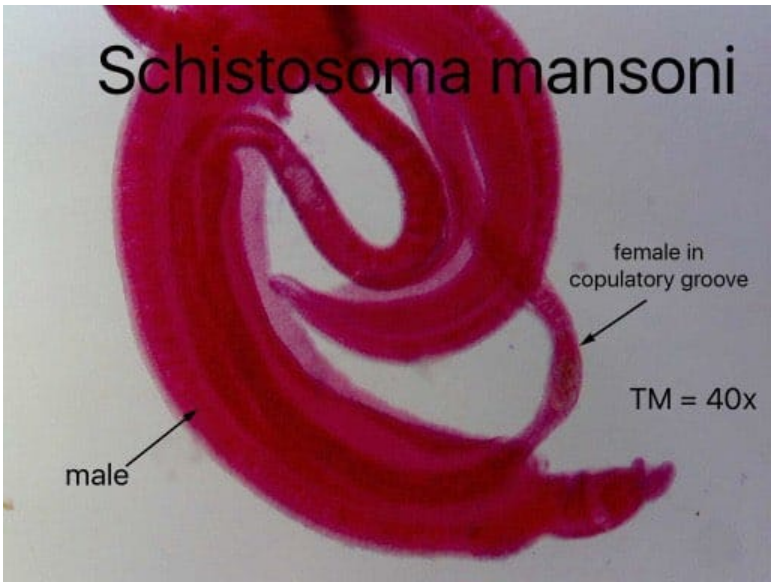
Disease: Schistosomiasis - initially causes creeping eruption of the skin with itchiness in the vicinity of the cercarial entry; fever, cough, diarrhea, liver enlargement, high blood pressure due and ascites due to liver malfunction

Other Information:

- Common name: blood fluke
- Feed on blood
- Dioecious; male and female worms mate form a pair and remain permanently together
- Do not have typical trematode body pattern; demonstrate a long cylindrical body pattern
- Primarily located within sub-Saharan Africa, South American Countries (Brazil, Venezuela, etc.), Caribbean, and the Middle East
- Infected individuals contribute to the ongoing cycle when they release parasite eggs via feces into water sources
- Snails in freshwater serve as the intermediate host; humans are the definitive host



Adult male *Schistosoma mansoni*. Credit: Sherry Smith



Light microscope image of an adult *Shistosoma mansoni* pair.
Credit:Sherry Smith



Scanning electron micro- scope image of an adult *Schistosoma mansoni* pair.
Source: Otis Historical Archives of “National Museumof Health & Medicine” (OTIS Archive 1), Wikimedia Commons, CC BY 2.0

Schistosoma *japonicum*

Resides: resides within the mesenteric venules of the small intestines

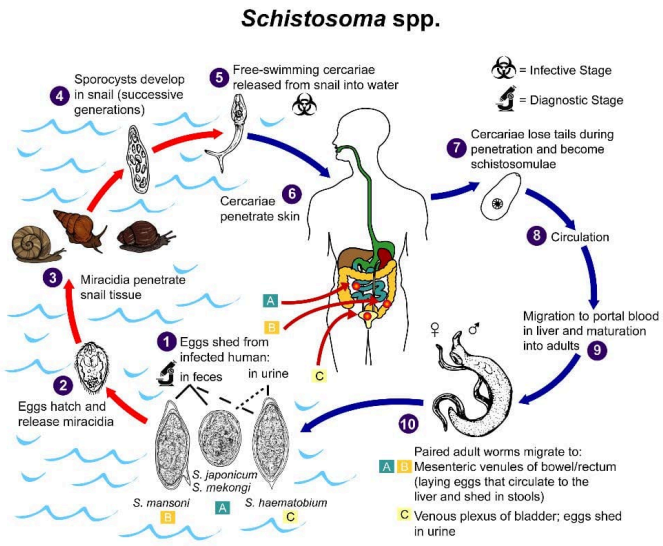
Transmission: cercariae in contaminated freshwater burrow through the skin

Disease: Schistosomiasis - initially causes creeping eruption of the skin with itchiness in the vicinity of the cercarial entry; fever, cough, diarrhea, liver enlargement, high blood pressure due and ascites due to liver malfunction

- Common name: blood fluke
- Feed on blood
- Dioecious; male and female worms mate form a pair and remain permanently together
- Do not have typical trematode body pattern; demonstrate a long cylindrical body pattern
- Primarily located within China, Philippines, and Indonesia
- Infected individuals contribute to the ongoing cycle when they release parasite eggs via feces into water sources
- Snails in freshwater serve as the intermediate host; humans are the definitive host



Adult male *Schistosoma japonicum*.
Source: グランピィ, Wikimedia Commons, CC BY-SA 4.0.



Schistosome life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain

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6.3.4: Cestodes (Tapeworms)

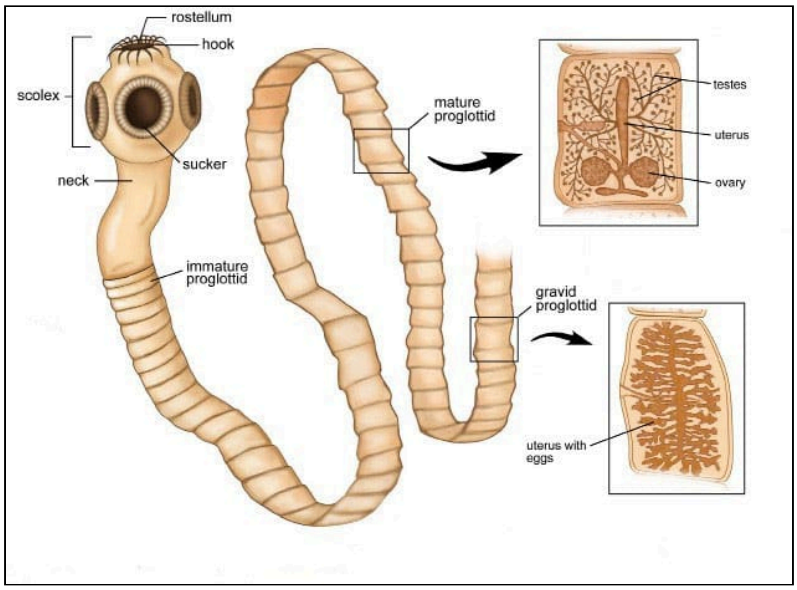
Cestodes are parasitic platyhelminths that have segmented ribbon-like bodies, they range in length from 1 mm to more than 15 m. They are commonly referred to as tapeworms. Like trematodes, they exhibit bilateral symmetry and are monoecious. Cestodes completely lack digestive systems for acquiring nutrients. Instead of ingesting nutrients, they absorb their nutrients across their cuticles. They also lack circulatory systems.

The typical adult cestode will have a head region, known as a scolex, a neck, and a body region that is referred to as a strobila. The scolex of a tapeworm is used for attachment to the host.

Thus, structures such as suckers and hooks are present. The structures present on the scolex for attachment vary fro one species to another and thus can aid in their differentiation. The neck region, contain cells that undergo mitotic divisions to produce new segments for the body portion of the parasites. The body or strobila, is composed of segments known as proglottids. Starting at the proglottid closets to the neck, proglottids are referred to as immature. These are newly formed proglottids and reproductive structures have not yet developed within the segment. As proglottids move further away from the neck they develop male and female reproductive structures; testes, ovaries, uterus, etc. Each proglottid will have both male and female sex organs and thus each segment is in itself monoecious. These proglottids can then undergo cross-fertilization from another individual) or self-fertilization. Cross-fertilization is more common. However, self-fertilization offers the parasite the ability to continue to reproduce even in the absence of a separate individual within their environment. Fertilization will result in the production of eggs. Proglottids containing eggs are known as gravid proglottids.

The adult cestode resides within its definitive host. Gravid proglottids and eggs are shed in fecal material from the host. The typical life cycle then resumes as an intermediate host ingests the eggs or gravid proglottids which develop into larval forms within the intermediate host. Cestodes may have one or more intermediate hosts. *Hymenolepis nana* is an exception to this typical life cycle and can have a single host within its life cycle.

In this exercise we will survey six of the many parasitic cestodes that exist.



Typical cestode body pattern. Credit: Susan Decker

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6.3.5: Survey of Cestodes

Taenia saginata

Resides: intestines

Transmission: ingestion of raw/undercooked beef containing cysticerci

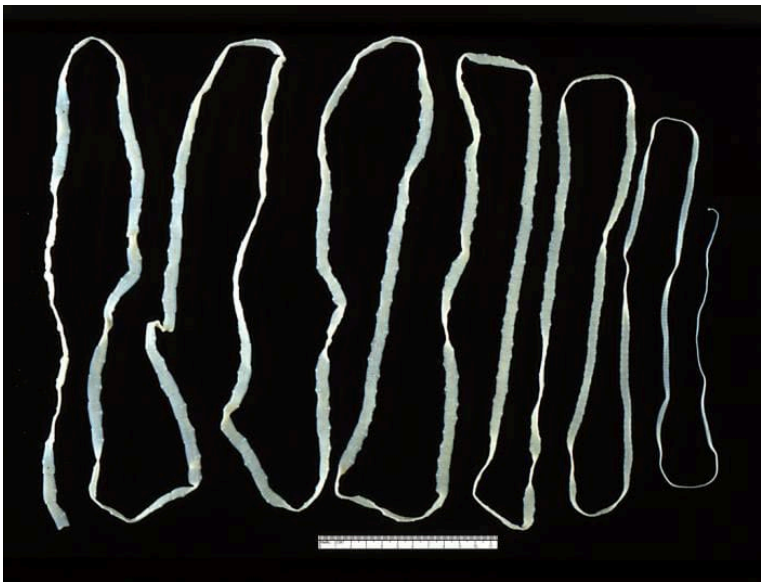
Disease: mild infections - may be asymptomatic; heavier infections - may cause diarrhea, abdominal cramping and pain, as well as vague digestive complaints; sever infections - produce body weakness, loss of weight, loss of appetite, vomiting, dizziness, and anemia; acute infections may cause colon blockage, may cause the colon to rupture or burst

Other Information:

- Common name: beef tapeworm
- One of the largest tapeworms, composed of up to 2000 proglottids, may exceed 25 ft in length
- Anchors to the lumen of the small intestine via a scolex with suckers



Taenia *saginata* scolex.
Source: CDC DPDx, Wikimedia Commons, Public Domain



Taenia *saginata* adult.
Source: CDC, CDC PHIL, Public Domain

Taenia solium

Resides: Location dependent on form acquired

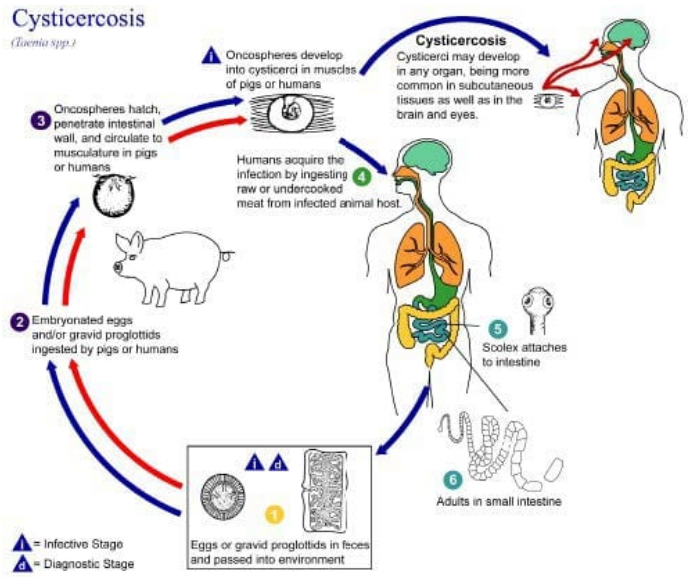
- Cysticeri ingested from raw or undercooked pork - develop into mature tapeworms that reside within the intestines
- Eggs ingested - eggs hatch and larvae penetrate the intestines and migrate throughout the body to encyst as cysticerci within the host tissues

Transmission: two different forms of the parasite can be acquired

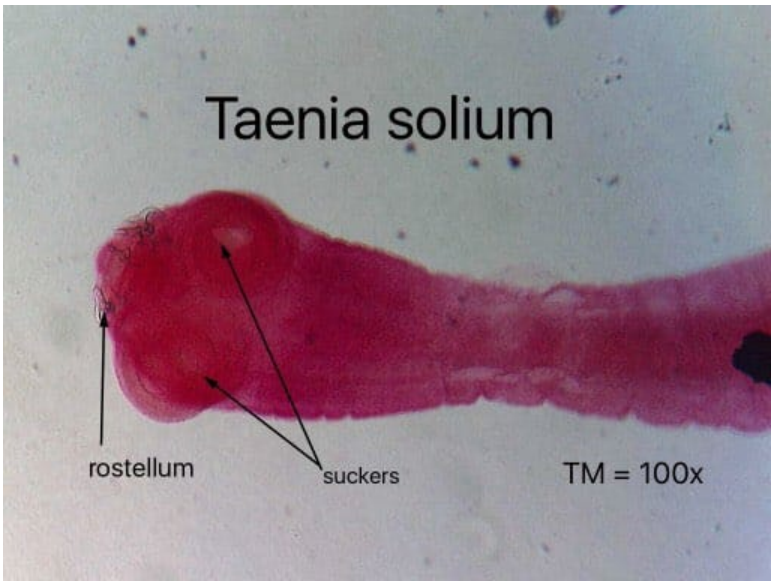
- Cysticeri ingested from raw or undercooked pork
- Eggs ingested on contaminated food or drink - due to breakdown in hygiene Disease: Different ingest forms result in different disease conditions
- Cysticeri ingested from raw or undercooked pork - develop into mature tapeworms that reside within the intestines (taeniasis) characterized by the following:
- Mild infections may be asymptomatic
- Heavier infections may cause diarrhea, abdominal cramping and pain, as well as vague digestive complaints
- Severe infections - produce body weakness, loss of weight, loss of appetite, vomiting, dizziness, anemia
- Eggs ingested - cysticerci may develop resulting in cysticercosis which is characterized by the following:
- cysticerci may develop in various organs (i.e. brain, heart muscle, eye, etc.) causing damage to the organ
- cysticerci in the brain is known as neurocysticercosis; patients may exhibit seizures, psychiatric disturbances or other neurological impairments

Other Information:

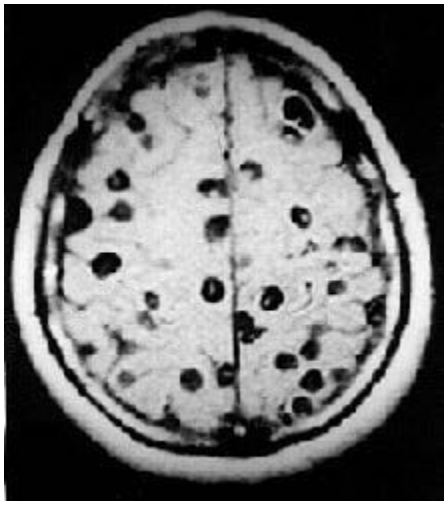
- Common name: pork tapeworm
- Patients with taeniasis may pass proglottids in their feces
- May produce 50,000 eggs per proglottid
- Scolex has an armed rostellum (suckers with hooklets) used to attach to the host's intestines
- Most dangerous of the tapeworms due to the risk of developing cysticercosis



Taenia *solium* life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain



Taenia *solium* scolex, showing suckers and armed rostellum with hooks.
Credit: Sherry Smith


"Magnetic resonance image of a patient with neurocysticercosis demonstrating multiple cysticerci within the brain."
Source: Evans C, Garcia HH, Gilman RH and Friedland JS, Wikimedia Commons, Public Domain

Hymenolepis nana

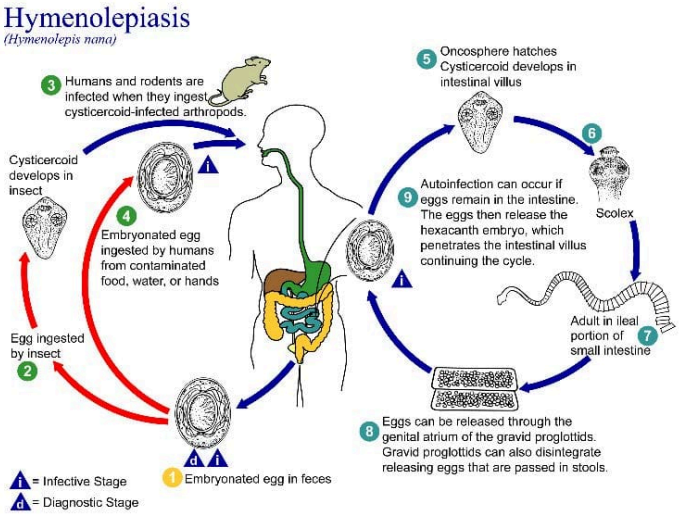
Resides: intestines

Transmission: accidental ingestion of eggs (human to human contact; fecal-oral route - fecal contamination of food or water; self inoculation - commonly especially in children; breakdown in personal hygiene)

Disease: Hymenolepiasis - asymptomatic; Heavy infections may case nausea, diarrhea, and abdominal pain, anal itching

Other Information:

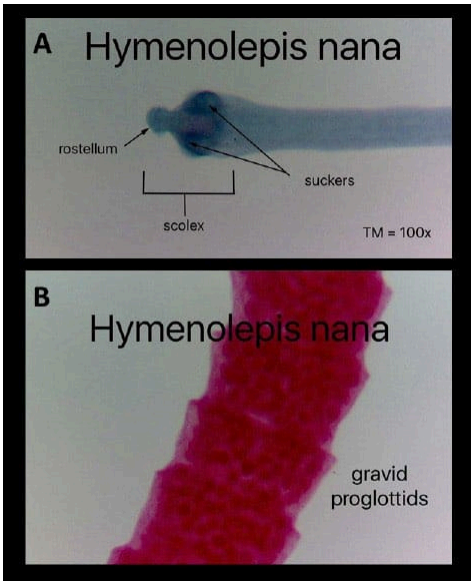
- Common name: dwarf tapeworm
- Knob-like scolex
- Normally about 150 - 200 proglottids in length
- Does not require an intermediate host
- One of the common human cestode parasites worldwide
- Life cycle unique from other tapeworms due to requirement of only one host



Hymenolepis nana life cycle.
Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain



Hymenolepis *nana* adult.
Source: Georgia Division of Public Health, Wikimedia Commons, Public Domain



Hymenolepis nana. A)Scolex B) gravid proglottid. Credit: Sherry Smith

Dipylidium *caninum*

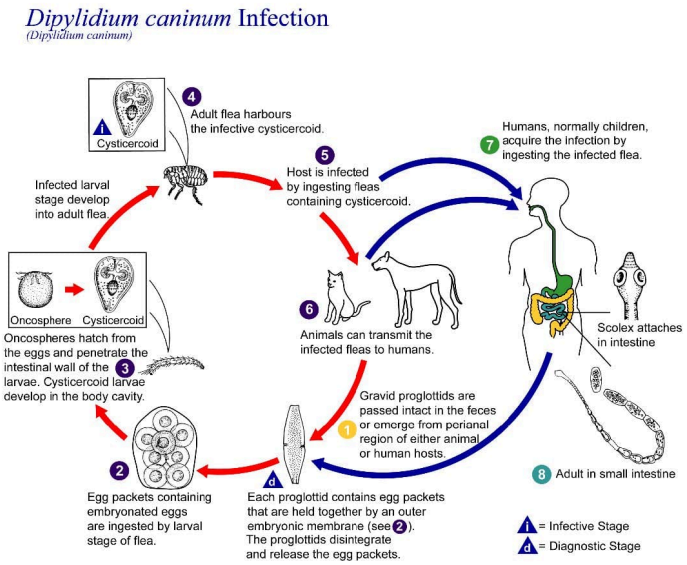
Resides: intestines

Transmission: ingestion of the flea that carries the larvae

Disease: asymptomatic or may have mild gastrointestinal disturbances; most striking in small children is passing of proglottids in feces

Other Information:

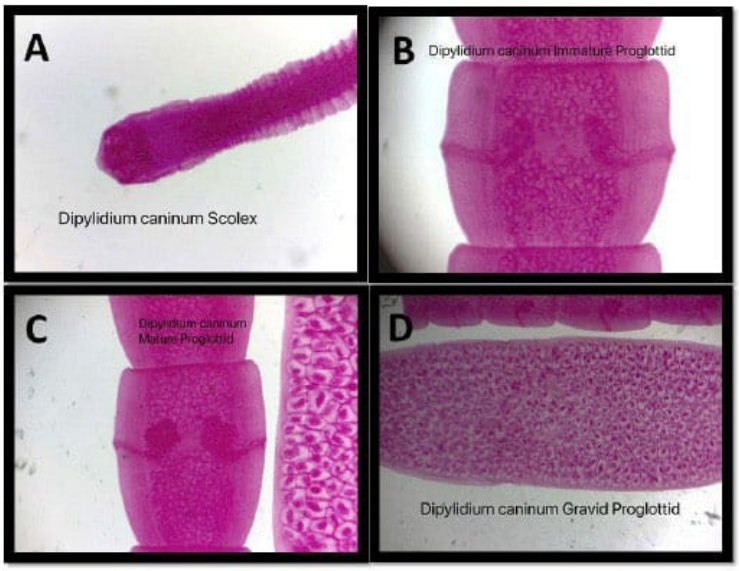
- Commonly found in dogs; may also be found in wolves, coyote, foxes, or cats
- Children are highly vulnerable to infection
- Proglottids are longer than they are wide
- Each proglottid contains 2 sets of male and female organs; the proglottids each have 2 genital pores - this is where it derives its common name



Dipylidium life cyle. Source: Alexander J. da Silva, PhD; Melanie Moser, CDC PHIL, Public Domain



Dipylidium *caninum* adult tapeworm.
Source: CDC's Division of Parasitic Diseases (DPD),
Wikimedia Commons, Public domain



Dipylidium caninum adult tapeworm sections. A) Cone-shaped scolex with suckers B) immature proglottid C) Mature proglottid D) Gravid proglottid - filled with eggs.
Credit: Sherry Smith

Diphyllobothrium *latum*

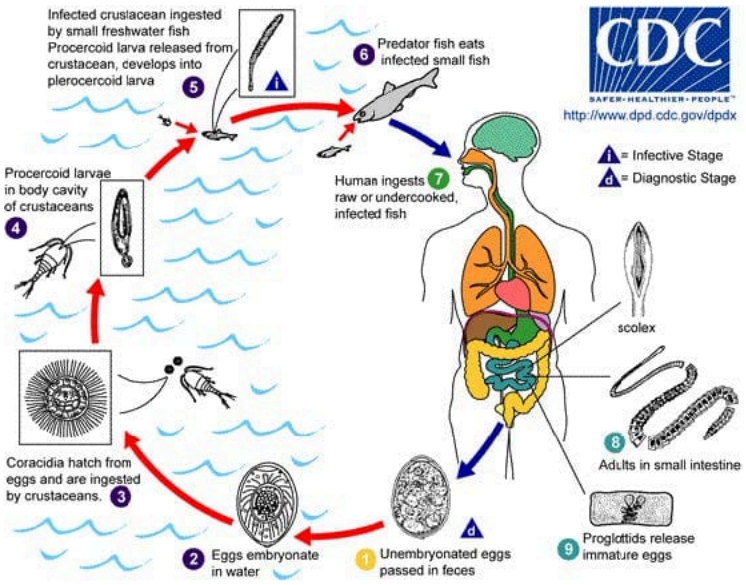
Resides: intestines

Transmission: ingestion of raw or undercooked fish

Disease: Diphyllobothriasis - most infections are asymptomatic; heavy infections - abdominal discomfort, vomiting, weight loss, vitamin B12 deficiency leading to pernicious anemia; on rare occasions result in bile obstruction or gallbladder damage due to migration of proglottids

Other Information:

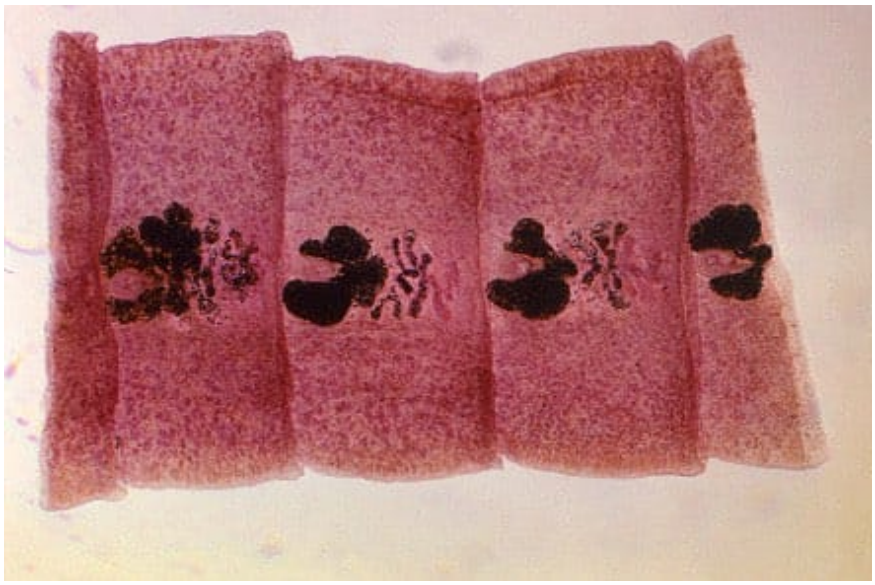
- Common name: broad fish tapeworm
- A member of the genera producing some of the longest tapeworms infecting humans; can grow up to 30 feet long
- Host specific to fish



Diphyllobothrium *latum* life cycle. Source: CDC, Wikimedia Commons, Public Domain



Diphyllobothrium *latum* scolex.
Source: CDC, Wikimedia Commons, CC BY 3.0



"This photomicrograph reveals ultrastructural details exhibited by a number of gravid proglottids of the parasitic tapeworm, *Diphyllobothrium latum*."
Source: Dr. Mae Melvin, CDC PHIL, Public Domain

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6.3.6: Nematodes (Roundworms)

Nematodes are commonly named round worms, due to their long cylindrical bodies. Their bodies exhibit bilateral symmetry and lack any segmentation. Nematodes have complete digestive tracts, composed of a mouth, pharynx, intestines, and anus. Thus, allowing both the intake of nutrients and elimination of wastes to occur through the digestive tract. They lack a circulatory system. Nematodes also differ from both trematodes and cestodes, by being dioecious. Dioecious organisms, have their male and female reproductive organs in separate individuals. So, each species has a female individual and a male individual.

Nematodes include both free-living nematodes and parasitic nematodes. Our focus in this activity is on those that act as parasites. Parasitic nematodes are classified by the location they affect into two groups; the intestinal nematodes and the tissue nematodes. These classifications are based on the location of the adult form of the parasite within the host. Examples of intestinal parasitic nematodes include *Trichuris trichiura*, *Enterobius vermicularis*, *Ascaris lumbricoides*, and the hookworms (i.e. *Ancylostoma duodenale*, *Necator americanus*, etc.). Tissue nematodes, on the other hand reside within the tissues or lymphatics of their definitive host in their adult stage. Examples of tissue parasitic nematodes include *Trichinella spiralis*, and the filarial nematodes, such as *Wuchereria bancrofti*.

Parasitic nematode life cycles may involve a direct life cycle or an indirect life cycle. Direct life cycles have only a single host in which the parasite completes its growth, reproduction, and transmission. The life cycle of the parasite *Ascaris lumbricoides* is an example of a direct life cycle. Indirect life cycles require more than one host to complete the life cycle; an intermediate host and a definitive host. The filarial parasite *Wuchereria bancrofti* is an example of a parasitic nematode that has an indirect life cycle. *Wuchereria bancrofti* utilizes the mosquito as its intermediate host and the human as its definitive host.

These differences in life cycle types and locations in which the parasites reside, result in multiple mechanisms of transmission for the parasitic nematodes. Organisms such as *Enterobius vermicularis* and *Ascaris lumbricoides*, which are both intestinal parasites with direct life cycles, use a fecal-oral route of transmission. Eggs released into the lumen of the host are passed in the fecal material. A new host acquires the parasite when they ingest food or drink contaminated with the eggs from the fecal material. The hookworms, which are also intestinal parasites with a direct life cycle employ a different transmission mechanism. Hookworms release eggs into the intestinal lumen of their host. Eggs then in fecal contaminated soil hatch to form larvae that are specialized for direct penetration through the skin of a new host. The larvae then utilize the circulatory system of their new host to migrate to the intestines. Some nematode parasites, utilize a third method, in which they are transmitted as an encysted larvae within tissue. This is the case with parasites such as *Trichinella spiralis*. *Trichinella spiralis* encysts within muscle tissue. The organism is then transmitted to a new host when they ingest raw or undercooked meat containing those cysts. A final mechanism employed by parasitic nematodes is that of transmission via a biological vector. This is observed for the transmission of the filarial parasite *Wuchereria bancrofti*, which is transmitted via the bite of an infected mosquito.

In this exercise you will survey both intestinal and tissue nematodes. You will also examine at least one species of each of the modes of transmission discussed in this introduction.

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6.3.7: Survey of Nematodes

Ascaris lumbricoides

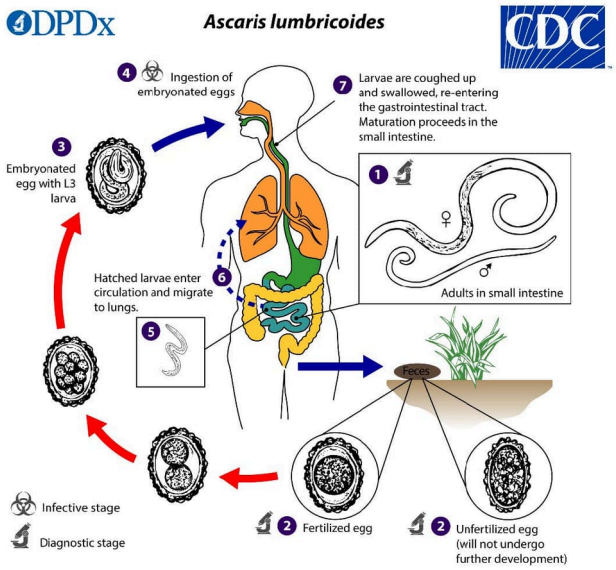
Resides: lumen of the small intestine

Transmission: ingestion of embryonated eggs in fecal contaminated food or hands contaminated with soil containing embryonated eggs

Disease: may be asymptomatic; if worm burden is high symptoms will appear and may include 1) pulmonary (cough, difficulty breathing, bloody sputum) 2) gastrointestinal complaints (abdominal pain, nausea, vomiting, intestinal obstruction) 3) heavy worm loads can retard physical and mental development in children

Other Information:

- Largest human nematode
- Responsible for the greatest number of nematode infections worldwide; most cases in the United States are located in the south east portions of the United States
- Females may produce up to 200,000 eggs daily
- Eggs may remain viable in warm moist soil for up to 15 years and are resistant to cold and chemical disinfectants



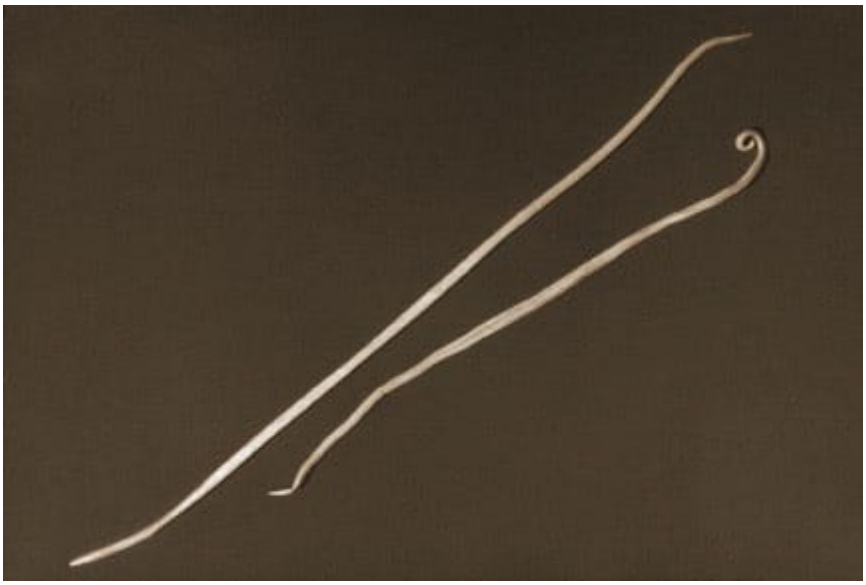
Source: DPDx CDC, Wikimedia Commons, Public Domain



This image depicts a young child, whose potbelly was due to a parasitic infection known as ascariasis, caused by a nematode, or roundworm, Ascaris lumbricoides.
Source: Dr. H. Zelman, Dr. Myron Schultz, CDC PHIL, Public Domain



“This 2007 photograph, depicted Centers for Disease Control and Prevention (CDC)/ National Center for Emerging and Zoonotic Infectious Diseases (NCZVED)/Division of Parasitic Diseases (DPDx) laboratory technician, Henry Bishop, holding a mass of Ascaris lumbricoides worms, which had been passed by a child in Kenya, Africa. This nematode parasitizes the human small intestine, and is spread from human-to-human, by the fecal-oral route. Children seemto be infected more often than adults, and though the organisms depicted here originated in Africa, the disease can be acquired in the southeastern United States as well.” (Right)
Source: Content Provider: CDC/ Henry Bishop Photo Credit: James Gathany, CDC PHIL, Public Domain
“



“Depicted in this 1960 photograph were two, Ascaris lumbricoides nematodes, i.e., roundworms. The larger of the two was the female of the species, while the normally smaller male was on the right.
Adult female worms can grow over to 12-inches in length.”
Source: CDC, CDC PHIL, Public domain

Enterobius vermicularis

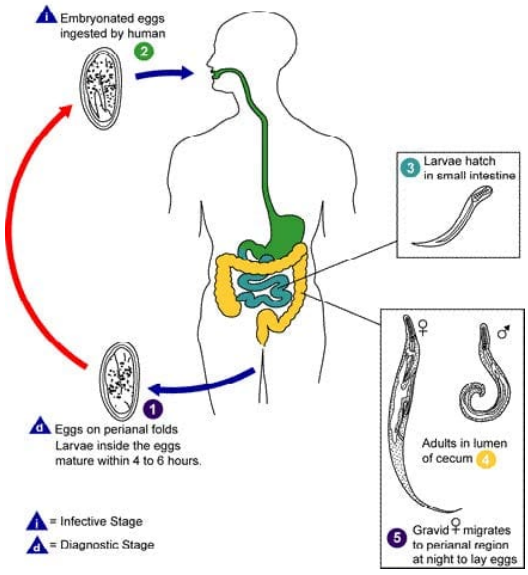
Resides: Eggs hatch in the small intestine; adults reside in the colon

Transmission: ingestion of embryonated eggs

Disease: Enterobiasis - anal itching and irritability especially at night

Other Information:

- Common name: pin worm
- Most common infection in the United States caused by a nematode
- Diagnosis often uses the “scotch tape method”



Source: CDC, Wikimedia Commons, Public Domain



Adult female Enterobius vermicularis.
Source: Daniel J. Drew, Wikimedia Commons, Public Domain



Egg of Enterobius vermicularis.
Source: Ajay Kumar Chaurasiya, Wikimedia Commons, CC BY 4.0

Trichinella spiralis

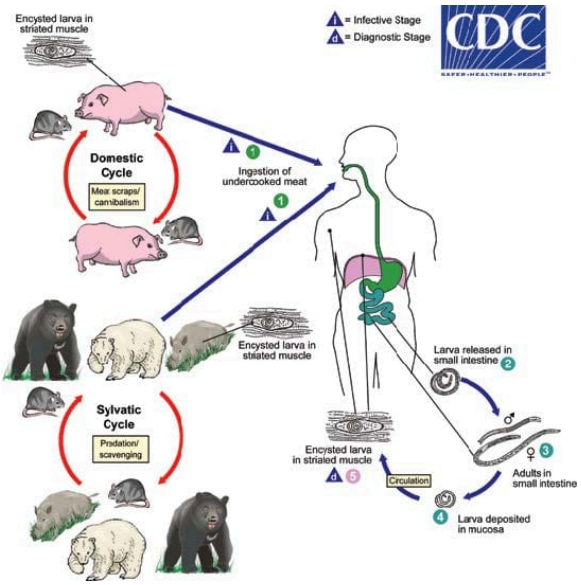
Resides: larvae encyst in striated muscles

Transmission: ingestion of undercooked pork or wild game containing cysts

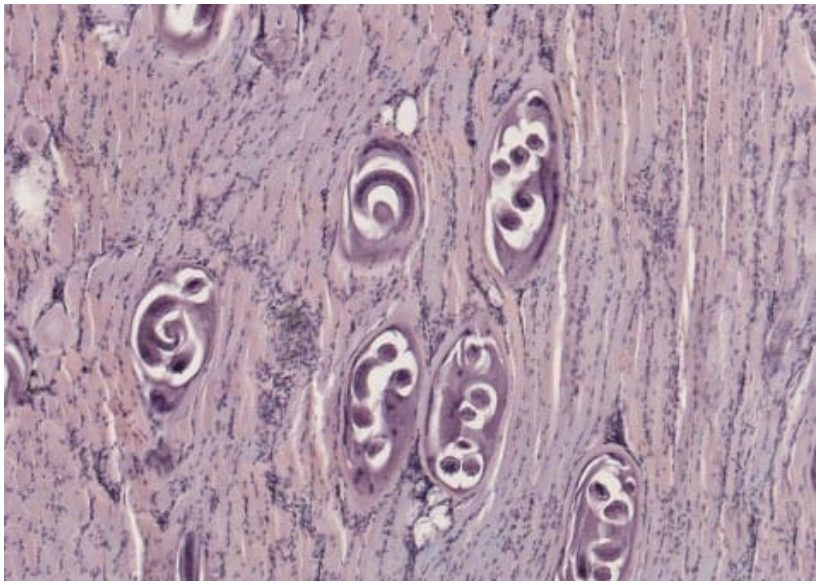
Disease: Trichinellosis

Other Information:

- Common name: pork worm
- Humans are accidental dead-end hosts



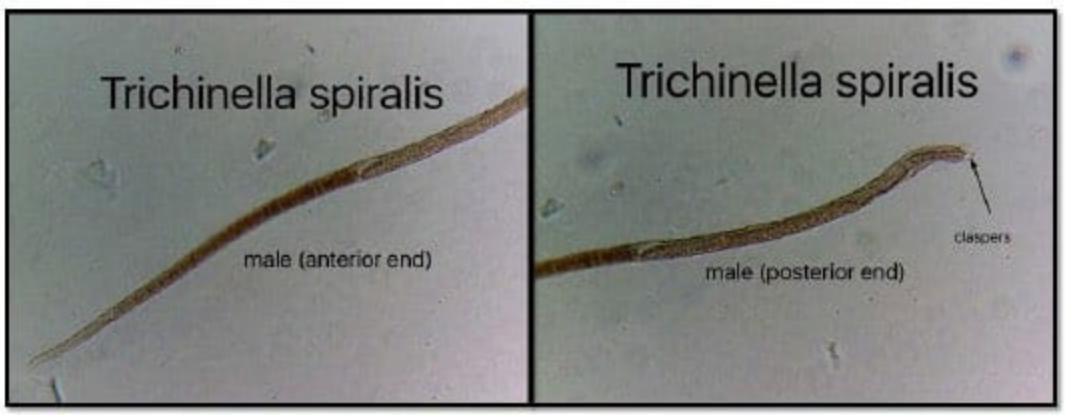
Source: CDC, Wikimedia Commons, Public Domain



Trichinella spiralis larvae encysted in muscle tissue.
Source: Daniel J. Drew, Wikimedia Commons, Public Domain



Trichinella spiralis adult female..
Source: Daniel J. Drew, Wikimedia Commons,
Public Domain



Trichinella spiralis adult male. Credit: Sherry Smith

Trichuris trichiura

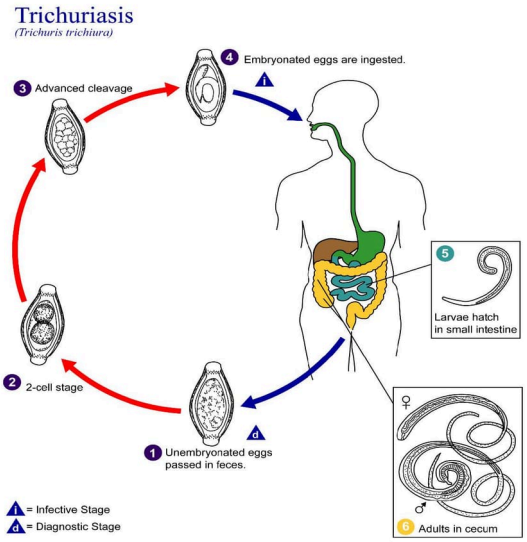
Resides: colon

Transmission: ingestion of embryonated eggs in contaminated food or drink

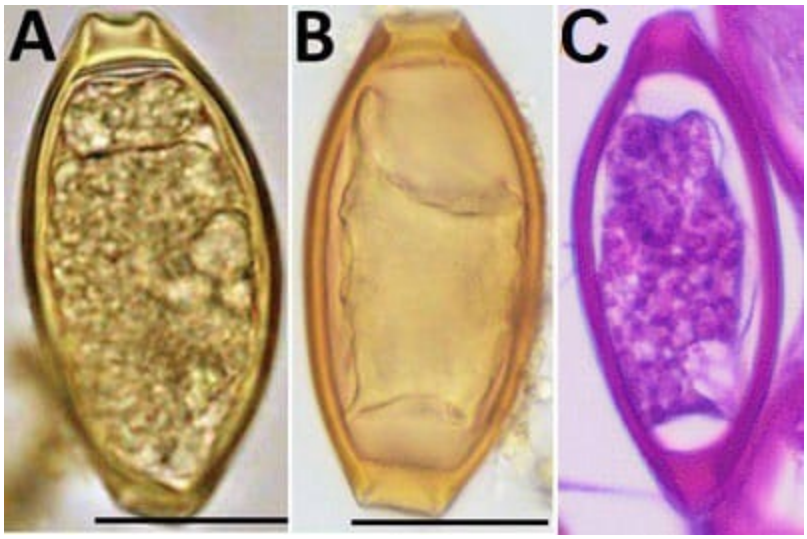
Disease: Trichuriasis

Other Information:

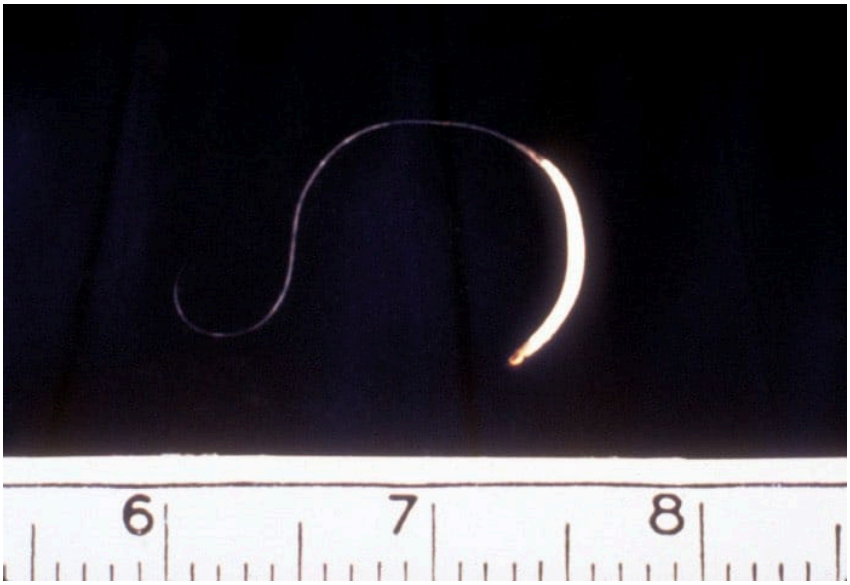
- Common name: human whip-worm
- Humans are the sole host



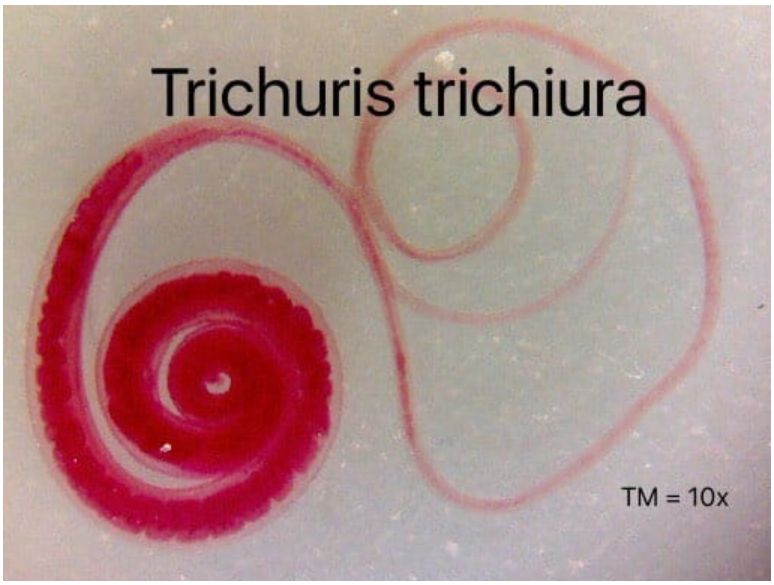
Source: CDC PHIL, Public Domain



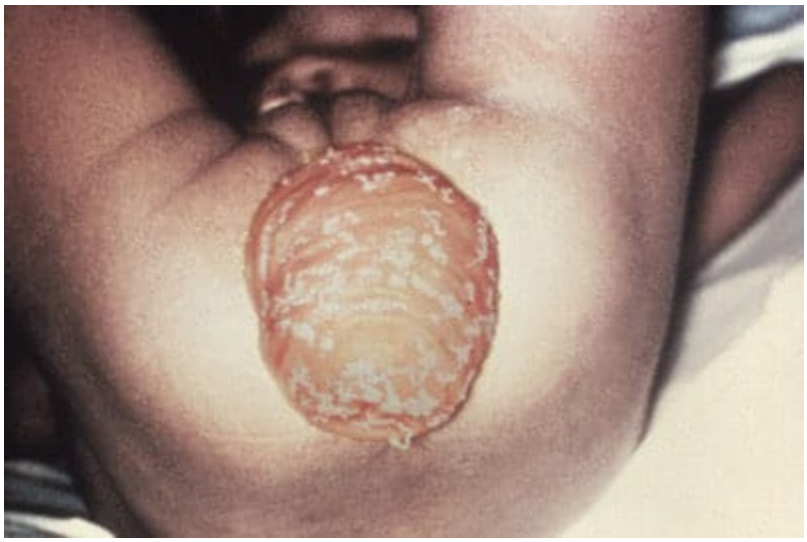
Trichuris trichiura eggs, with C showing HE stain
Source: Mikael Häggström, M.D., Wikimedia Commons, Public Domain



“This photomicrograph depicts an adult, Trichuris sp., female human whip-worm, and reveals that its size, in centimeters, is approximately 4cm.”
Source: Dr. Mae Melvin,CDC PHIL, Public Domain



Adult female *Trichuris trichiura*. Credit: Sherry Smith



"This image depicts a condition known as a rectal prolapse in a female child due to a parasitic Trichuris trichiura infestation. The rectum has lost its intestinal support due to this nematode invasion, and has emerged from the anal opening. Note the numbers of worms atop the rectal mucosal lining."
Source: CDC, CDC PHIL, Public domain

Necator americanus

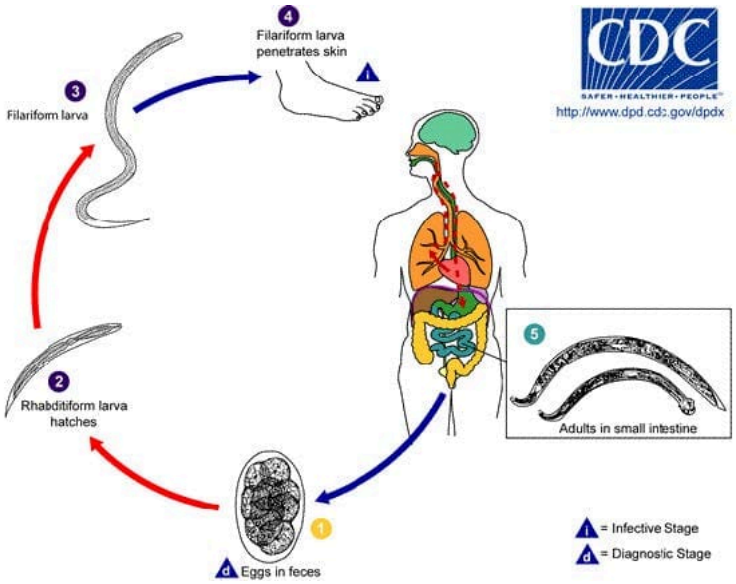
Resides: small intestine

Transmission: infective larvae penetrate intact skin

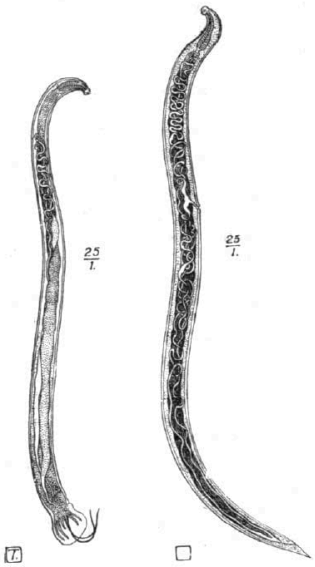
Disease: hookworm disease

Other Information:

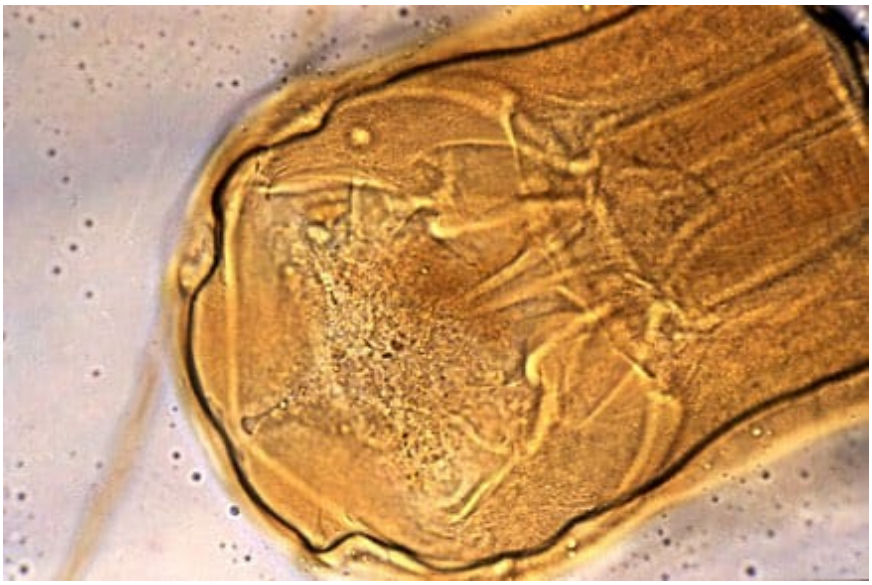
- Common name: hookworm
- Most common hookworm of North, Central, and South America



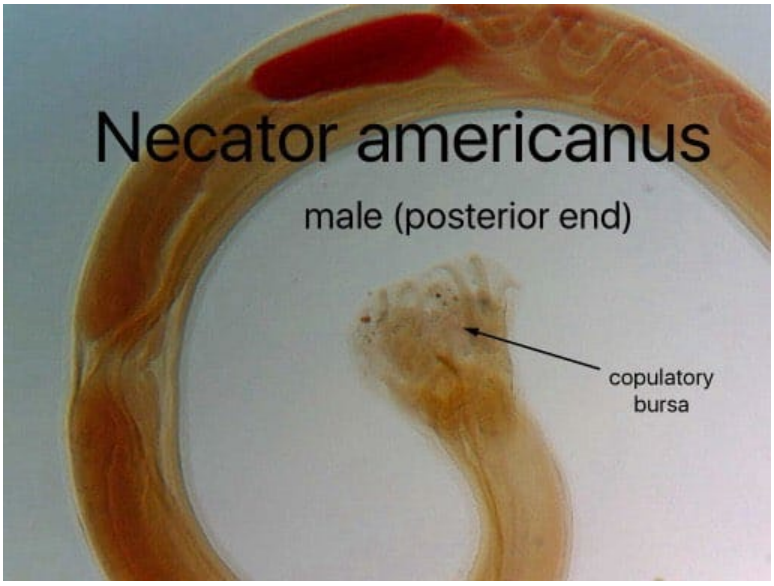
Source: DPDx - Laboratory Identification of Parasites of Public Health Concern Courtesy: Public Health Image Library, Wikimedia Commons, Public Domain



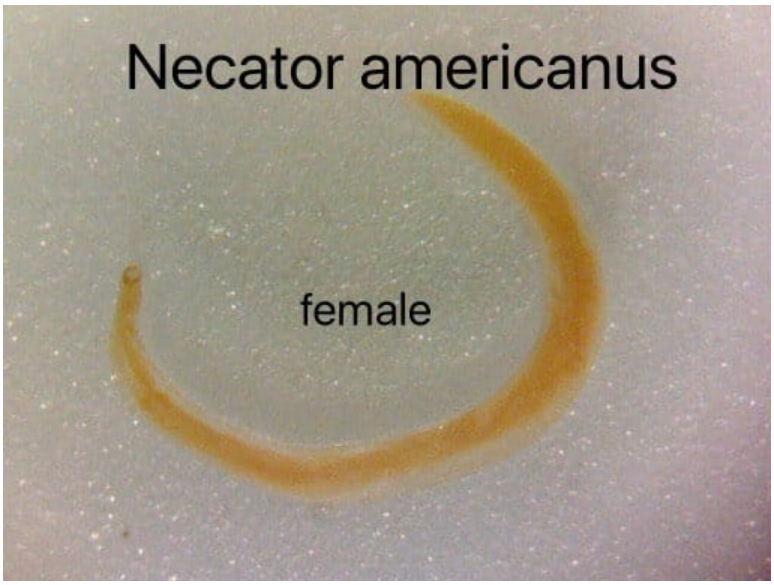
Necator americanus.
Source: Patrick Manson, Wikimedia Commons, Public Domain



“Under a magnification of 400X, this micrograph reveals the head of a hookworm, *Necator americanus,* and its mouth’s cutting plates..”
Source: Dr. Mae Melvin, CDC PHIL, Public Domain



Necator *americanus* male, showing posterior copulatory bursae, which can help distinguish male from female.
Credit: Sherry Smith



Necator americanus female. Credit: Sherry Smith

Wuchereria bancrofti

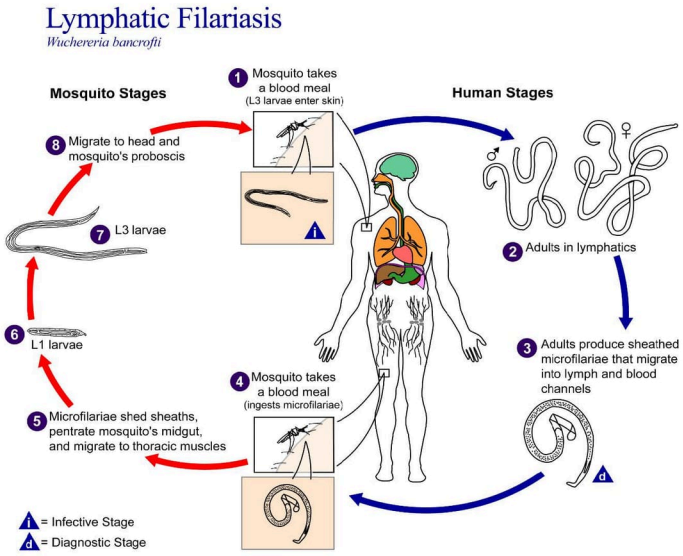
Resides: lymphatic vessels

Transmission: bite of infected female mosquito

Disease: Lymphatic filariasis

Other Information:

- Causes more than 90% of lymphatic filariasis cases
- Endemic to tropical regions



Source: Alexander J. da Silva, PhD, CDC PHIL, Public Domain



“Photographed somewhere in the country of India, this man displayed marked swelling of his lower extremities, known as filariasis, which was more pronounced on his left, and was due to the filarial parasite, *Wuchereria bancrofti*.”
Source: Dr. Myron Schultz, CDC PHIL, Public Domain



Under a magnification of 250X, this photomicrograph reveals some of the ultrastructural morphology exhibited by a *Wuchereria bancrofti* microfilaria, which was found in this thick film blood smear specimen processed using Giemsa stain.”
Source: Dr. Mae Melvin, CDC PHIL, Public domain

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6.3.8: Exercise #32 - Lab Wrap-Up

Lab Wrap-Up Questions

Answer the following questions:

1. What is the difference between a definitive host and an intermediate host?
2. What is the common name for trematode? Cestode? Nematode?
3. Differentiate between monoecious and dioecious. Provide three examples of species surveyed in the laboratory exercise that fit each definition.
4. In what ways do the schistosomes differ from a typical trematode?
5. *Taenia solium* may be acquired via ingestion of eggs in contaminated food or drink, or the parasite may be acquired via ingestion of undercooked pork. Describe how the disease conditions that result differ depending on what is ingested. Based on your information, describe how each disease could be prevented.
6. How is the transmission of *Wuchereria bancrofti* unique compared to other nematodes surveyed in this exercise?

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6.4: Section Six Review

Review Purpose

This review helps you check what you learned in Section Six before moving on. Work through each question set on the pages that follow, then use the **Click to reveal answer** option to check yourself. These questions are for practice and self-assessment—they are not graded.

Before you begin, can you...

- Describe the morphological difference between yeasts and molds, and define and differentiate superficial, subcutaneous, systemic mycoses?
- Differentiate fungal infections, fungal toxicoses, and fungal allergies, and describe how mycoses are acquired?
- Describe the general characteristics of protozoans and their classification by motility, and differentiate a trophozoite from a cyst?
- Define vector and compare the biological vectors of important human protozoan pathogens?
- Differentiate platyhelminths from nematodes, and describe the characteristics of trematodes, cestodes, and nematodes?
- Define and contrast definitive and intermediate hosts?
- Identify important fungal protozoan and helminth pathogens by their morphology and epidemiology using microscopy and micrographs?

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6.4.1: Matching

Matching Questions

1. Match the parasite to the appropriate description of its method of transmission by placing the letter next to the correct term.

Question 1 Parasites

Answer	Parasite
_____	1. *Ascaris lumbricoides*
_____	2. *Clonorchis sinensis*
_____	3. *Coccidioides immitis*
_____	4. *Cryptosporidium parvum*
_____	5. *Diphyllobothrium latum*
_____	6. *Dipylidium caninum*
_____	7. *Entamoeba histolytica*
_____	8. *Naegleria fowleri*
_____	9. *Necator americanus*
_____	10. *Plasmodium falciparum*
_____	11. *Schistosoma mansoni*
_____	12. *Taenia saginata*
_____	13. *Trichinella spiralis*
_____	14. *Trichophyton* spp.
_____	15. *Trichomonas vaginalis*
_____	16. *Trypanosoma cruzi*
_____	17. *Trypanosoma brucei*
_____	18. *Wuchereria bancrofti*

Question 1 Descriptions

Letter	Description
a.	larvae burrow through intact skin from contaminated soil
b.	ingestion of a flea carrying the larvae
c.	cercariae in freshwater burrow through skin
d.	sporozoites enter blood via bite of a female mosquito
e.	trypomastigotes enter via bite of a Reduviid bug
f.	ingestion of cysts in undercooked pork or wild game, such as bear
g.	sexual intercourse
h.	ingestion of metacercariae in undercooked fish
i.	penetration of cribriform plate after inhaling contaminated fresh water
j.	larvae introduced by bite of female mosquito
k.	trypomastigotes enter via bite of a Tsetse fly
l.	ingestion of cysts in contaminated food or drink
m.	ingestion of cysticerci in undercooked or raw beef
n.	contact with an individual, animal, or surface carrying the fungi
o.	ingestion of infective oocysts in fecal contaminated water
p.	ingestion of eggs in fecal contaminated food or from hands contaminated with soil containing eggs
q.	inhalation of fungal spores in the air
r.	ingestion of cysticerci in undercooked or raw fish

Click to reveal answers

Answers:
1) p
2) h
3) q
4) o
5) r
6) b
7) l
8) i
9) a

10) d
11) c
12) m
13) f
14) n
15) g
16) e
17) k
18) j

2. Match the parasite scientific name to the appropriate common name by placing the letter next to the correct term.

Question 2 Parasites

Answer	Parasite
_____	1. *Clonorchis sinensis*
_____	2. *Diphyllobothrium latum*
_____	3. *Dipylidium caninum*
_____	4. *Enterobius vermicularis*
_____	5. *Hymenolepis nana*
_____	6. *Naegleria fowleri*
_____	7. *Necator americanus*
_____	8. *Schistosoma mansoni*
_____	9. *Taenia saginata*
_____	10. *Taenia solium*
_____	11. *Trichinella spiralis*
_____	12. *Trichuris trichiura*

Question 2 Common Names

Letter	Common Name
a.	pork tapeworm
b.	brain eating amoeba
c.	beef tapeworm

Letter	Common Name
d.	pin worm
e.	whip worm
f.	pork worm
g.	liver fluke
h.	broad fish tapeworm
i.	blood fluke
j.	double-pored tapeworm
k.	hook worm
l.	dwarf tapeworm

Click to reveal answers

Answers:
1) g
2) h
3) j
4) d
5) l
6) b
7) k
8) i
9) c
10) a
11) f
12) e

3. Match the parasite scientific name to the appropriate disease or clinical description by placing the letter next to the correct term.

Question 3 Parasites

Answer	Parasite
_____	1. *Candida auris*
_____	2. *Clonorchis sinensis*
_____	3. *Diphyllobothrium latum*
_____	4. *Enterobius vermicularis*

Answer	Parasite
_____	5. *Naegleria fowleri*
_____	6. *Necator americanus*
_____	7. *Plasmodium falciparum*
_____	8. *Rhizopus* spp.
_____	9. *Taenia solium*
_____	10. *Trichophyton* spp.
_____	11. *Trichuris trichiura*
_____	12. *Trypanosoma brucei*
_____	13. *Trypanosoma cruzi*
_____	14. *Wuchereria bancrofti*

Question 3 Descriptions

Letter	Description
a.	cysticercosis due to ingestion of eggs
b.	Chaga's disease
c.	zygomycosis
d.	heavy infections result in abdominal pain, diarrhea, weight loss, stunted growth, and rectal prolapse
e.	lymphatic filariasis
f.	dermatophytosis
g.	bile duct obstruction, liver abscesses, liver enlargement, hepatic failure
h.	anal itching and irritability at night
i.	candidiasis
j.	abdominal discomfort, weight loss, pernicious anemia
k.	African sleeping sickness
l.	primary amoebic meningoencephalitis

Letter	Description
m.	itching at site of larval penetration, iron deficient anemia, abdominal pain
n.	malaria

Click to reveal answers

Answers:
1) i
2) g
3) j
4) h
5) l
6) m
7) n
8) c
9) a
10) f
11) d
12) k
13) b
14) e

4. Match the organism to the appropriate description of where it resides within the host by placing the letter next to the correct term.

Question 4 Organisms

Answer	Organism
____	1. *Ascaris lumbricoides*
____	2. *Balantidium coli*
____	3. *Clonorchis sinensis*
____	4. *Cryptosporidium parvum*
____	5. *Enterobius vermicularis*
____	6. *Naegleria fowleri*
____	7. *Plasmodium falciparum*
____	8. *Schistosoma mansoni*
____	9. *Trichinella spiralis*
____	10. *Trichomonas vaginalis*

Answer	Organism
_____	11. *Trypanosoma cruzi*
_____	12. *Wuchereria bancrofti*

Question 4 Descriptions

Letter	Description
a.	blood and liver cells of humans
b.	lymphatic vessels
c.	bile ducts of the liver
d.	vagina and urethra of women; urethra, prostate, and seminal vesicles of men
e.	eggs hatch in the small intestine; adults reside in the colon
f.	multiplies intra-cellularly in the small intestinal epithelium
g.	the colon, cecum, or appendix
h.	mesenteric venules of the small intestines
i.	enters through the nasal cavity, then moves to the brain
j.	the small intestine at first; larvae then encyst in muscle tissue
k.	blood, and so the parasite is known as a hemoflagellate
l.	lumen of the small intestine

Click to reveal answers

Answers:
1) l
2) g
3) c
4) f
5) e
6) i
7) a
8) h
9) j
10) d
11) k
12) b

5. Match the fungal pathogen to the disease it causes by placing the letter next to the correct term.

Question 5 Fungi

Answer	Fungus
_____	1. *Aspergillus* spp.
_____	2. *Candida* spp.
_____	3. *Coccidioides immitis*
_____	4. *Cryptococcus neoformans*
_____	5. *Pneumocystis jirovecii*
_____	6. *Rhizopus* spp.
_____	7. *Trichophyton* spp.

Question 5 Diseases

Letter	Disease
a.	cryptococcosis
b.	dermatophytosis
c.	aspergillosis
d.	zygomycosis, also known as mucormycosis
e.	candidiasis
f.	coccidioidomycosis, also referred to as “Valley fever”
g.	pneumocystic pneumonia

Click to reveal answers

Answers:
1) c
2) e
3) f
4) a
5) g
6) d
7) b

6. Match the protozoan classification to the appropriate description of how its members accomplish motility by placing the letter next to the correct term.

Question 6 Classifications

Answer	Classification
_____	1. Subphylum Sarcodina
_____	2. Subphylum Mastigophora
_____	3. Phylum Ciliophora
_____	4. Phylum Apicomplexa

Question 6 Descriptions

Letter	Description
a.	amoeboids that use pseudopods for motility
b.	utilize cilia that cover their cell surface to accomplish motility
c.	also known as sporozoa; typically non-motile protozoans
d.	owe their motility to the presence of flagella

Click to reveal answers

Answers:
1) a
2) d
3) b
4) c

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6.4.2: Fill-In-The-Blank

Fill-in-the-Blank Questions

1. _____ proglottids are filled with eggs.

Click to reveal answer

Answer Gravid

2. Trematodes are commonly referred to as _____.

Click to reveal answer

Answer flukes

3. _____ fungal infections are infections involving the skin, hair, or nails.

Click to reveal answer

Answer Superficial

4. _____ hosts are hosts that harbor mature adult worms, which are sexually reproducing.

Click to reveal answer

Answer Definitive

5. Platyhelminths that have flat, segmented ribbon-like bodies, lacking digestive tracts are known as _____.

Click to reveal answer

Answer cestodes

6. Fungi known as _____, typically exist as globular, single-celled organisms.

Click to reveal answer

Answer yeast

7. A _____ is an active feeding form of a protozoan.

Click to reveal answer

Answer trophozoite

8. _____ is the region of a cestode which is used to attach to a host.

Click to reveal answer

Answer Scolex

9. _____ are helminths with long cylindrical bodies and complete digestive tracts.

Click to reveal answer

Answer Nematodes

10. Diseases caused by fungi are known as _____.

Click to reveal answer

Answer mycoses

11. A _____ is a dormant, protective form of a protozoan.

Click to reveal answer

Answer cyst

12. _____ vectors physically carry a pathogen from one host to another.

Click to reveal answer

Answer Mechanical

13. Fungi known as _____, are filamentous, composed of long cylindrical tubes known as hyphae.

Click to reveal answer

Answer molds

14. The trypanosomes are specifically referred to as _____, because they are flagellated protozoans found within the blood.

Click to reveal answer

Answer hemoflagellates

15. _____ trematodes reside within the blood vessels of the digestive or urinary tracts, and feed on blood.

Click to reveal answer

Answer Blood

16. _____ are hosts in which the immature (larvae) form of the parasite resides.

Click to reveal answer

Answer Intermediate

17. _____ organisms have male and female reproductive structures in the same individual.

Click to reveal answer

Answer Monoecious

18. Cestodes are commonly called _____.

Click to reveal answer

Answer tapeworms

19. _____ vectors harbor the parasite within themselves during a portion of the parasite's life cycle.

Click to reveal answer

Answer Biological

20. The only ciliated protozoan parasite is _____.

Click to reveal answer

Answer *Balantidium coli*

21. _____ trematodes live within the tissues and organs, such as the epithelium of the small intestine, the liver, and the lungs.

Click to reveal answer

Answer Tissue

22. _____ organisms have their male and female reproductive organs in separate individuals.

Click to reveal answer

Answer Dioecious

23. The protozoan group that lack locomotive structures are the _____.

Click to reveal answer

Answer apicomplexan (sporozoa)

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6.4.3: True-False

True / False Questions

1. Systemic fungal infections are those involving only the skin, hair, and nails.

_____ True _____ False

Click to reveal answer

Answer False

2. *Candida* species normally reside in the intestinal tract and mucous membranes without causing infection.

_____ True _____ False

Click to reveal answer

Answer True

3. *Candida auris* does not respond to commonly used anti-fungal medications.

_____ True _____ False

Click to reveal answer

Answer True

4. *Cryptococcus neoformans* is often found in soils where bird droppings are present.

_____ True _____ False

Click to reveal answer

Answer True

5. Fungal toxicoses occur when toxins produced by fungi are ingested, such as by eating poisonous mushrooms.

_____ True _____ False

Click to reveal answer

Answer True

6. In most people, exposure to *Aspergillus* results in severe systemic disease rather than allergies.

_____ True _____ False

Click to reveal answer

Answer False

7. Protozoans are considered animal-like protists because of their ability to demonstrate motility.

_____ True _____ False

Click to reveal answer

Answer True

8. The scheme that classifies protozoans according to their motility structures reflects how closely related the organisms are to one another.

_____ True _____ False

Click to reveal answer

Answer False

9. The sexual phase of the *Plasmodium* parasite is carried out in the gut of the female *Anopheles* mosquito.

_____ True _____ False

Click to reveal answer

Answer True

10. Schizonts may be found in all species of *Plasmodium*.

_____ True _____ False

Click to reveal answer

Answer True

11. *Cryptosporidium parvum* is host specific and can infect only humans.

_____ True _____ False

Click to reveal answer

Answer False

12. *Trichomonas vaginalis* resides only in the vagina and urethra of women.

_____ True _____ False

Click to reveal answer

Answer False

13. Most parasitic helminths have both an intermediate host and a definitive host during their life cycle.

_____ True _____ False

Click to reveal answer

Answer True

14. Trematodes have complete digestive systems that end in an anus.

_____ True _____ False

Click to reveal answer

Answer False

15. Cestodes absorb their nutrients across their cuticles because they completely lack digestive systems.

_____ True _____ False

Click to reveal answer

Answer True

16. The scolex of a tapeworm contains the male and female reproductive structures.

_____ True _____ False

Click to reveal answer

Answer False

17. The proglottids closest to the neck of a tapeworm are the most mature.

_____ True _____ False

Click to reveal answer

Answer False

18. Among cestodes, cross-fertilization is more common than self-fertilization.

_____ True _____ False

Click to reveal answer

Answer True

19. The intermediate hosts of most trematodes are freshwater fish.

_____ True _____ False

Click to reveal answer

Answer False

20. Trematodes lack circulatory systems, so their flat body pattern is important for the diffusion of nutrients throughout the organism.

_____ True _____ False

Click to reveal answer

Answer True

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6.4.4: Multiple Choice / Multiple Select

Multiple Choice Questions

1. _____ are helminths that are composed of a small scolex with a long ribbon of proglottids generated by strobili.

- a) nematodes
- b) cestodes
- c) trematodes
- d) protozoans

Click to reveal answer
Answer b) cestodes

2. Which of the following statements is true of the parasite *Ascaris lumbricoides*?

- a) females and males are differentiated by a copulatory bursa on the male
- b) may encyst in muscle tissue or brain tissue causing cysticercosis if eggs are ingested
- c) females are larger than males and are capable of laying 200,000 eggs daily
- d) known to potentially cause rectal prolapse when infected with heavy worm loads
- e) is the most common nematode infection in the United States

Click to reveal answer
Answer c) females are larger than males and are capable of laying 200,000 eggs daily

3. Which of the following is a "spoon-shaped" cestode transmitted by the consumption of raw or undercooked fish?

- a) *Taenia solium*
- b) *Taenia saginata*
- c) *Hymenolepis nana*
- d) *Diphyllobothrium latum*
- e) *Dipylidium caninum*

Click to reveal answer
Answer d) *Diphyllobothrium latum*

4. A dangerous condition known as cysticercosis occurs when

- a) eggs of *Taenia solium* are ingested and they encyst in human tissue
- b) eggs of *Hymenolepis nana* are ingested and they encyst in human tissue
- c) a mosquito delivers a larval form of *Wuchereria bancrofti* and it travels to the heart
- d) *Necator americanus* larvae burrow through the skin and travel to the respiratory tract

Click to reveal answer
Answer a) eggs of *Taenia solium* are ingested and they encyst in human tissue

5. Trichinellosis, also known as trichinosis, occurs when

- a) ingested cysts mature into worms in the intestines and the larvae migrate into the blood and embed in muscles
- b) ingested cysts mature into adult worms and cause the host to suffer from malnutrition
- c) ingested cysts mature into adult tapeworms and release proglottids that force their way out of the anus
- d) ingested cysts mature into adult roundworms and release thousands of eggs as the adult releases toxins to promote severe diarrhea
- e) inhaled cysts perforate the lungs as they make their way to the intestines

Click to reveal answer

Answer a) ingested cysts mature into worms in the intestines and the larvae migrate into the blood and embed in muscles

6. Which of the following parasites is a nematode known for moving out of the body through the anus, nose, or mouth?

- a) *Necator americanus*
- b) *Trichinella spiralis*
- c) *Enterobius vermicularis*
- d) *Ascaris lumbricoides*
- e) *Schistosoma mansoni*

Click to reveal answer
Answer d) *Ascaris lumbricoides*

7. Which of the following is an intracellular parasite of erythrocytes?

- a) *Giardia lamblia*
- b) *Wuchereria bancrofti*
- c) *Trypanosoma cruzi*
- d) *Plasmodium falciparum*
- e) *Balantidium coli*

Click to reveal answer
Answer d) *Plasmodium falciparum*

8. Which of the following is an opportunistic pathogen which may cause an oral infection called thrush?

- a) *Giardia lamblia*
- b) *Rhizopus* spp.
- c) *Trichophyton* spp.
- d) *Plasmodium falciparum*
- e) *Candida albicans*

Click to reveal answer
Answer e) *Candida albicans*

9. How does a patient typically become infected with hookworms?

- a) cysts are ingested when the patient eats undercooked meat
- b) the worm penetrates the skin when the patient walks outside barefoot
- c) cysts are spread via respiratory droplets when an infected host coughs or sneezes
- d) eggs are ingested when the patient eats infected seafood
- e) cysts are spread onto fomites when an infected host does not properly wash their hands

Click to reveal answer
Answer b) the worm penetrates the skin when the patient walks outside barefoot

10. Which of the following parasites is responsible for 90% of deaths caused by a malarial parasite?

- a) *Plasmodium vivax*
- b) *Wuchereria bancrofti*
- c) *Trypanosoma cruzi*
- d) *Plasmodium falciparum*
- e) *Balantidium coli*

Click to reveal answer
Answer d) *Plasmodium falciparum*

11. Which of the following is a contagious fungal pathogen?

- a) *Candida albicans*
- b) *Rhizopus* spp.
- c) *Trichophyton* spp.
- d) *Aspergillus* spp.
- e) *Cryptococcus neoformans*

Click to reveal answer
Answer c) *Trichophyton* spp.

12. Which of the following parasites is resistant to chlorination and is a leading cause of death in AIDS patients?

- a) *Giardia lamblia*
- b) *Cryptosporidium parvum*
- c) *Balantidium coli*
- d) *Plasmodium falciparum*
- e) *Naegleria fowleri*

Click to reveal answer
Answer b) *Cryptosporidium parvum*

13. Beavers, nutria, and other aquatic mammals are known to be asymptomatic carriers of which of the following parasites?

- a) *Giardia lamblia*
- b) *Cryptosporidium parvum*
- c) *Balantidium coli*
- d) *Plasmodium falciparum*
- e) *Naegleria fowleri*

Click to reveal answer
Answer a) *Giardia lamblia*

14. Which of the following is a protozoan parasite that lacks a cyst structure and thus is transmitted only via direct transmission?

- a) *Balantidium coli*
- b) *Plasmodium falciparum*
- c) *Cryptosporidium parvum*
- d) *Trichomonas vaginalis*
- e) *Naegleria fowleri*

Click to reveal answer
Answer d) *Trichomonas vaginalis*

15. Which of the following is an intracellular parasite of the intestinal cells?

- a) *Giardia lamblia*
- b) *Trichomonas vaginalis*
- c) *Plasmodium falciparum*
- d) *Balantidium coli*
- e) *Cryptosporidium parvum*

Click to reveal answer
Answer e) *Cryptosporidium parvum*

16. The deposition of eggs in the perianal area is characteristic of which of the following?

- a) *Wuchereria bancrofti*
- b) *Ascaris lumbricoides*
- c) *Necator americanus*

- d) *Enterobius vermicularis*
- e) *Trichuris trichiura*

Click to reveal answer
Answer d) *Enterobius vermicularis*

17. Fungal infections that occur in the deeper layers of the dermis and/or in the hypodermis are referred to as

- a) systemic infections
- b) superficial infections
- c) fungal toxicoses
- d) cutaneous or subcutaneous infections

Click to reveal answer
Answer d) cutaneous or subcutaneous infections

18. Fungal allergies are best described as

- a) infections that spread throughout the body, involving organs of various body systems
- b) reactions to toxins produced by fungi that have been ingested
- c) infections involving the skin, hair, and nails
- d) hypersensitivity reactions that usually occur in response to inhaled fungal spores

Click to reveal answer
Answer d) hypersensitivity reactions that usually occur in response to inhaled fungal spores

19. What changed about *Candida auris* around 2009 that made it clinically significant?

- a) it acquired the ability to form endospores
- b) it became transmissible through the bite of an arthropod vector
- c) it lost its resistance to anti-fungal medications
- d) it adapted to grow at human body temperature, having not previously been reported to do so

Click to reveal answer
Answer d) it adapted to grow at human body temperature, having not previously been reported to do so

20. Infection with *Cryptococcus neoformans* begins with inhalation and leads to pneumonia. Where may the fungus spread from there?

- a) to the liver, causing hepatic failure
- b) to the meninges, causing meningitis
- c) to the lymphatic vessels, causing filariasis
- d) to the small intestine, causing diarrhea

Click to reveal answer
Answer b) to the meninges, causing meningitis

21. Which statement best describes *Pneumocystis jirovecii*?

- a) it is a contagious dermatophyte spread by direct contact
- b) it is part of the normal flora and acts as an opportunistic pathogen
- c) it is acquired by inhaling spores found in desert soil
- d) it is transmitted by the bite of a female mosquito

Click to reveal answer
Answer b) it is part of the normal flora and acts as an opportunistic pathogen

22. Coccidioidomycosis is also referred to as “Valley fever.” Why?

- a) the infection produces a fever that rises and falls in a valley pattern
- b) the fungus was first isolated from a patient living in a river valley
- c) the organism grows only in valleys at low elevation
- d) the organism is normally found in the soil of the southwestern United States and parts of Mexico

Click to reveal answer
Answer d) the organism is normally found in the soil of the southwestern United States and parts of Mexico

23. Zygomycoses, caused by *Rhizopus* species, most commonly affect which parts of the body?

- a) the skin, hair, and nails
- b) the sinuses and/or the lungs
- c) the bile ducts of the liver
- d) the lymphatic vessels

Click to reveal answer
Answer b) the sinuses and/or the lungs

24. What are vectors, as the term is used in the transmission of parasitic protozoans?

- a) arthropod animals that serve in transmitting the parasite
- b) dormant forms that allow the parasite to withstand extremes in temperature
- c) the active feeding form of a protozoan
- d) freshwater snails that harbor the immature form of the parasite

Click to reveal answer
Answer a) arthropod animals that serve in transmitting the parasite

25. *Giardia lamblia* is protected by an outer shell. What does this allow?

- a) it allows the parasite to burrow through intact skin
- b) it allows the parasite to survive inside red blood cells
- c) it allows the parasite to attach to the intestinal wall with suckers
- d) it allows the parasite to survive for long periods outside the body and makes it highly resistant to chlorination

Click to reveal answer
Answer d) it allows the parasite to survive for long periods outside the body and makes it highly resistant to chlorination

26. Giardiasis is also known by which other names?

- a) ciliary dysentery or Montezuma's revenge
- b) Valley fever
- c) beaver fever or traveler's diarrhea
- d) Chaga's disease

Click to reveal answer
Answer c) beaver fever or traveler's diarrhea

27. Balantidiasis, caused by *Balantidium coli*, is also known as

- a) beaver fever or traveler's diarrhea
- b) ciliary dysentery or Montezuma's revenge
- c) African sleeping sickness
- d) primary amoebic meningoencephalitis

Click to reveal answer
Answer b) ciliary dysentery or Montezuma's revenge

28. When examining a human blood sample for *Plasmodium*, what should be looked for at Stage 1?

- a) the development of a ring trophozoite
- b) the development of schizonts containing merozoites
- c) encysted larvae within muscle tissue
- d) gravid proglottids filled with eggs

Click to reveal answer
Answer a) the development of a ring trophozoite

29. *Entamoeba histolytica* resides in which location within the host?

- a) the cecum and appendix
- b) the bile ducts of the liver
- c) the lymphatic vessels
- d) the mesenteric venules of the small intestine

Click to reveal answer
Answer a) the cecum and appendix

30. On what basis are the three clinically important groups of helminths differentiated from one another?

- a) the temperature at which they grow best
- b) the type of vector that transmits them
- c) the pH of the host tissue in which they reside
- d) their body patterns, the arrangement of their reproductive organs, and the structure of their digestive systems

Click to reveal answer
Answer d) their body patterns, the arrangement of their reproductive organs, and the structure of their digestive systems

31. Which structures do parasitic trematodes use to remain attached to their host?

- a) a scolex bearing suckers and hooks
- b) cilia covering the cell surface
- c) a copulatory bursa
- d) the oral sucker and the ventral sucker, also called the acetabulum

Click to reveal answer
Answer d) the oral sucker and the ventral sucker, also called the acetabulum

32. Which of the following is considered a blood fluke?

- a) *Clonorchis sinensis*
- b) *Schistosoma mansoni*
- c) *Fasciola hepatica*
- d) *Fasciolopsis buski*

Click to reveal answer
Answer b) *Schistosoma mansoni*

33. What occurs in the neck region of a cestode?

- a) cells undergo mitotic divisions to produce new segments for the body of the parasite
- b) eggs are released into the intestinal lumen of the host
- c) nutrients are absorbed across the cuticle
- d) attachment to the host is accomplished using suckers and hooks

Click to reveal answer
Answer a) cells undergo mitotic divisions to produce new segments for the body of the parasite

34. Nematodes are characterized by which of the following?

- a) long cylindrical bodies and complete digestive tracts
- b) flat leaf-shaped bodies and incomplete digestive tracts
- c) segmented ribbon-like bodies and no digestive tract
- d) single-celled bodies with flagella for motility

Click to reveal answer
Answer a) long cylindrical bodies and complete digestive tracts

35. For *Taenia solium*, the location in which the parasite resides depends on

- a) the form that was acquired, since ingesting cysticerci and ingesting eggs lead to different outcomes
- b) the temperature of the host tissue
- c) whether the host is a definitive or an intermediate host
- d) the number of proglottids the worm has produced

Click to reveal answer
Answer a) the form that was acquired, since ingesting cysticerci and ingesting eggs lead to different outcomes

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6.4.5: Identifying Parasites

Parasite Identification Questions

Identify the eukaryotic pathogen seen in each image and answer the associated questions.



Eukaryotic pathogen identification image 1. Credit: Sherry Smith

1. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Balantidium coli*

2. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer Balantidiasis, also known as ciliary dysentery or Montezuma's revenge

3. How is the organism seen in the image acquired?

Click to reveal answer

Answer ingestion of cysts in contaminated food or drink



Eukaryotic pathogen identification image 2. Credit: Sherry Smith

4. What is the scientific name of the parasite seen in the image?

Click to reveal answer

> **Answer** *Clonorchis sinensis*

5. What disease is caused by the organism seen in the image?

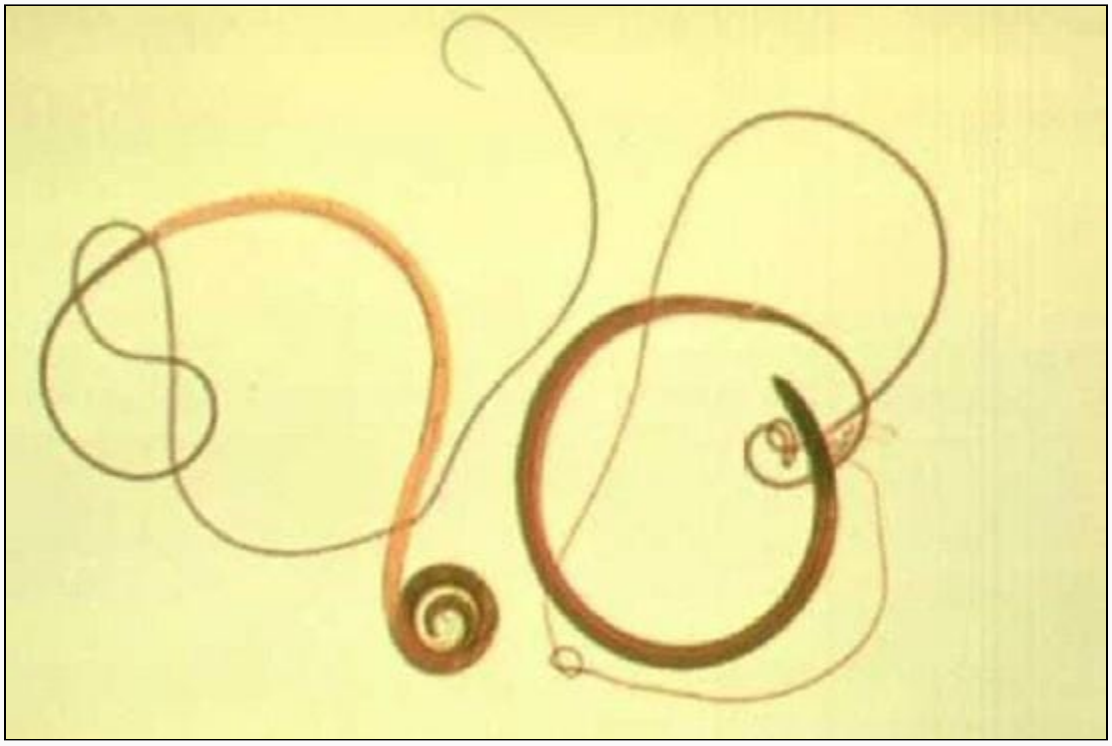
Click to reveal answer

> **Answer** clonorchiasis — characterized by bile duct obstruction, liver abscesses, liver enlargement, and hepatic failure

6. How is the organism seen in the image acquired?

Click to reveal answer

> **Answer** ingestion of metacercariae in undercooked fish



Eukaryotic pathogen identification image 3. Credit: Sherry Smith

7. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Trichuris trichiura*

8. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer trichuriasis — light infections are generally asymptomatic; heavy infections may cause abdominal pain, diarrhea, weight loss, stunted growth, and rectal prolapse

9. How is the organism seen in the image acquired?

Click to reveal answer

Answer embryonated eggs are ingested in contaminated food or drink



Eukaryotic pathogen identification image 4. Credit: Sherry Smith

10. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Enterobius vermicularis*

11. What disease is caused by the organism seen in the image?

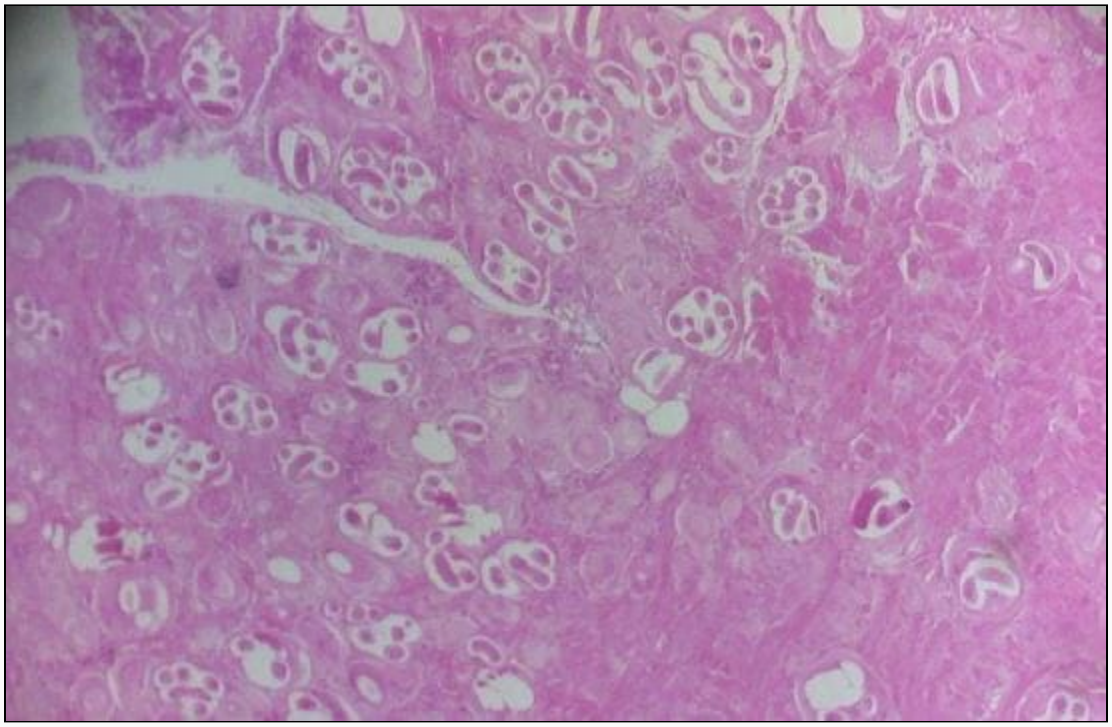
Click to reveal answer

Answer Enterobiasis — cardinal signs include anal itching and irritability at night

12. How is the organism seen in the image acquired?

Click to reveal answer

Answer ingestion of embryonated eggs, which may occur through self-infection or person-to-person transmission through contaminated clothing, bed linens, etc.



Eukaryotic pathogen identification image 5. Credit: Sherry Smith

13. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Trichinella spiralis*

14. What disease is caused by the organism seen in the image?

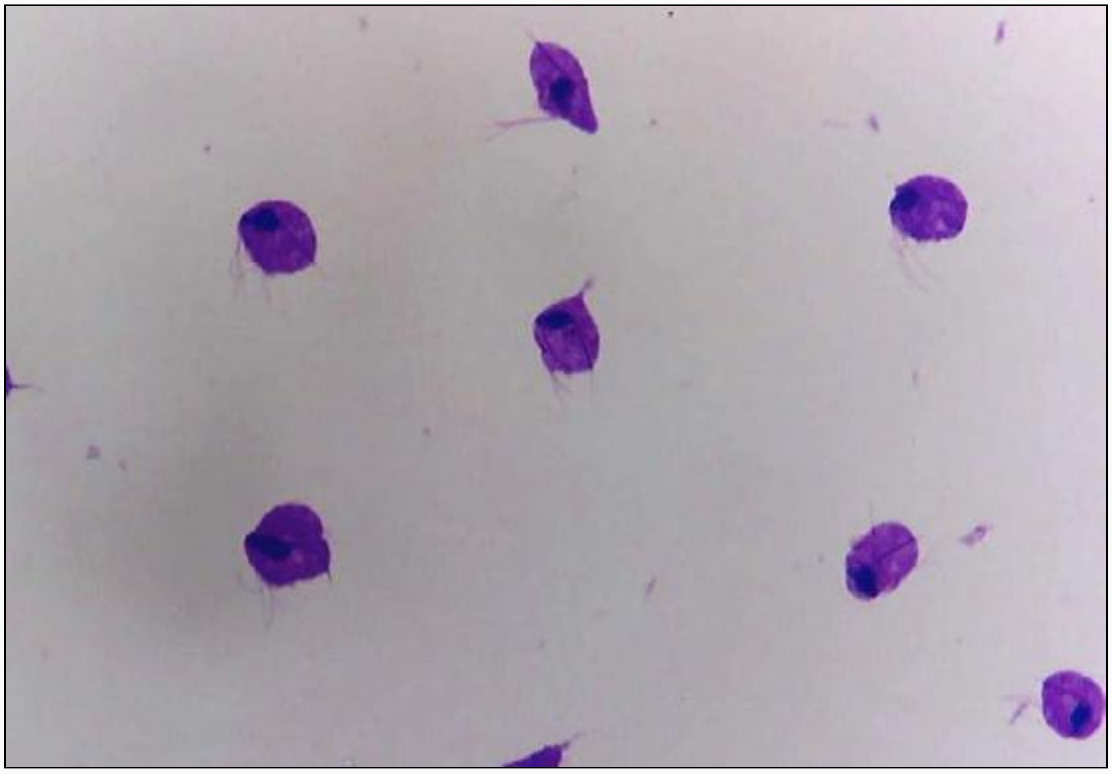
Click to reveal answer

Answer Trichinellosis — fever, diarrhea, abdominal cramping, facial edema, muscle pain, and weakness

15. How is the organism seen in the image acquired?

Click to reveal answer

Answer ingestion of meat containing cysts, usually undercooked pork, but it can also be transmitted by undercooked wild game such as bear



Eukaryotic pathogen identification image 6. Credit: Sherry Smith

16. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Trichomonas vaginalis*

17. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer trichomoniasis — females may experience vulvitis, vaginitis, cervicitis, and salpingitis; males may experience urethritis, prostatitis, and epididymitis

18. How is the organism seen in the image acquired?

Click to reveal answer

Answer sexual intercourse



Eukaryotic pathogen identification image 7. Credit: Sherry Smith

19. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Trypanosoma cruzi*

20. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer Chaga's disease

21. How is the organism seen in the image acquired?

Click to reveal answer

Answer bite of a Reduviid bug



Eukaryotic pathogen identification image 8. Credit: Sherry Smith

22. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Dipylidium caninum*

23. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer infection may be asymptomatic or may cause mild gastrointestinal disturbances, including passage of proglottids in feces

24. How is the organism seen in the image acquired?

Click to reveal answer

Answer ingestion of the flea that carries the larvae



Eukaryotic pathogen identification image 9. Credit: Sherry Smith

25. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Rhizopus* spp.

26. What disease is caused by the organism seen in the image?

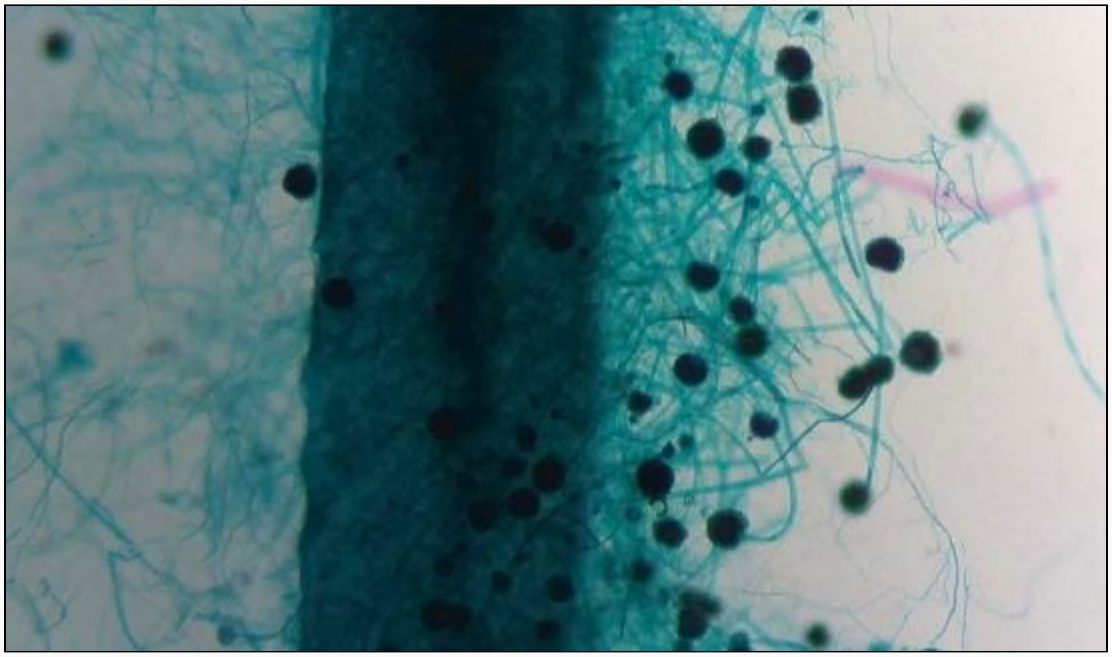
Click to reveal answer

Answer Zygomycosis, also known as mucormycosis — a serious but rare fungal infection that may affect nearly any part of the body, but more commonly affects the sinuses and/or the lungs

27. How is the organism seen in the image acquired?

Click to reveal answer

Answer contact with fungal spores in the environment



Eukaryotic pathogen identification image 10. Credit: Sherry Smith

28. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Aspergillus* spp.

29. What disease is caused by the organism seen in the image?

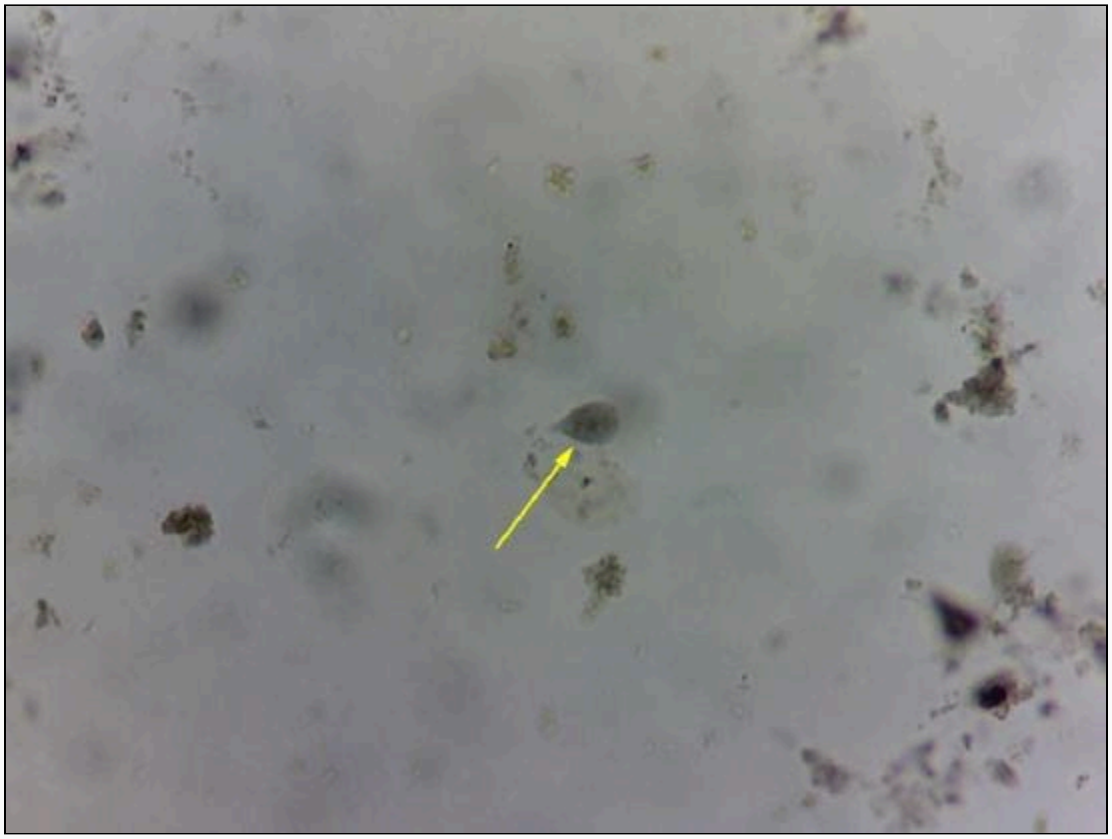
Click to reveal answer

Answer Aspergillosis — most commonly causes only allergies, though immunocompromised individuals are at higher risk of developing more severe conditions

30. How is the organism seen in the image acquired?

Click to reveal answer

Answer inhalation of fungal spores



Eukaryotic pathogen identification image 11. Credit: Sherry Smith

31. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Giardia lamblia*

32. What disease is caused by the organism seen in the image?

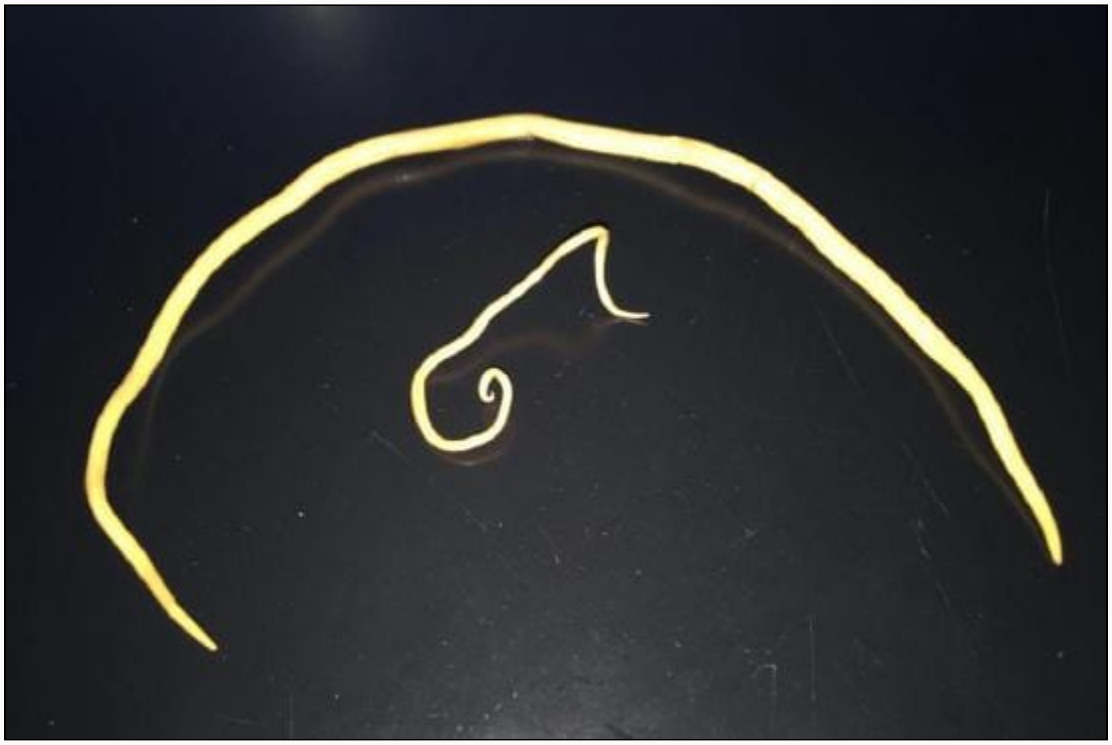
Click to reveal answer

Answer Giardiasis, also known as beaver fever or traveler's diarrhea

33. How is the organism seen in the image acquired?

Click to reveal answer

Answer inadvertent ingestion of water contaminated with cysts



Eukaryotic pathogen identification image 12. Credit: Sherry Smith

34. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Ascaris lumbricoides*

35. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer ascariasis — may be asymptomatic; if the worm burden is high, symptoms may include pulmonary complaints (cough, difficulty breathing, bloody sputum), gastrointestinal complaints (abdominal pain, nausea, vomiting, intestinal obstruction), and retarded physical and mental development in children

36. How is the organism seen in the image acquired?

Click to reveal answer

Answer ingestion of embryonated eggs in fecal contaminated food, or from hands contaminated with soil containing embryonated eggs



Eukaryotic pathogen identification image 13. Credit: Sherry Smith

37. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Schistosoma mansoni*

38. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer Schistosomiasis — initially causes a creeping eruption of the skin with itchiness near the site of cercarial entry, followed by fever, cough, diarrhea, liver enlargement, and high blood pressure and ascites due to liver malfunction

39. How is the organism seen in the image acquired?

Click to reveal answer

Answer cercariae in contaminated freshwater burrow through the skin



Eukaryotic pathogen identification image 14. Credit: Sherry Smith

40. What is the scientific name of the parasite seen in the image?

Click to reveal answer

Answer *Hymenolepis nana*

41. What disease is caused by the organism seen in the image?

Click to reveal answer

Answer Hymenolepiasis — usually asymptomatic; heavy infections may cause nausea, diarrhea, abdominal pain, and anal itching

42. How is the organism seen in the image acquired?

Click to reveal answer

Answer accidental ingestion of eggs, through human to human contact, the fecal-oral route, or self inoculation

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GLOSSARY

Example and Directions

Words (or words that have the same definition)	The definition is case sensitive	(Optional) Image to display with the definition [Not displayed in Glossary, only in pop-up on pages]	(Optional) Caption for Image	(Optional) External or Internal Link	(Optional) Source for Definition
(Eg. "Genetic, Hereditary, DNA ...")	(Eg. "Relating to genes or heredity")		The infamous double helix	https://bio.libretexts.org/	CC-BY-SA; Delmar Larsen

Use this glossary to look up the definitions for bolded terms in the laboratory manual.

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z

A

Acid dye - See “Acidic dye”

Acid-fast - bacterial cells that contain mycolic acid in their cell walls and retain the primary stain, carbolfuchsin, during the acid-fast staining process

Acid-fast stain - microbiological stain used to detect the presence of mycolic acid in the cell walls of bacteria

Acidic dye - negatively charged dyes (also known as anionic dyes); examples include nigrosin and India ink

Acidophile - microorganisms that prefer to grow in acidic environments with pH 5.5 or less

Aerobe - microorganisms that require oxygen to grow because they use oxygen as a final electron acceptor during cellular respiration

Agar - a polysaccharide derived from red algae that is used to solidify microbiological media

Alkaliphile - microorganisms that prefer to grow in alkaline environments with pH 8 or greater

Alkalophile - See “Alkaliphile”

Alpha-hemolysis - partial breakdown of red blood cells on blood agar that produces a greenish discoloration around bacterial growth

Amylase - an enzyme that hydrolyzes starch into smaller sugars

Anaerobe - a microorganism that grows without oxygen by using fermentation or anaerobic respiration

Anaerobic media - microbiological media that are made to restrict the presence of oxygen and are used to grow bacteria that require low oxygen or an absence of oxygen

Antimicrobial - any substance that inhibits or kills microorganisms

Antimicrobial agent - See “Antimicrobial”

Antimicrobial resistance - the ability of a microorganism to survive or continue growing in the presence of an antimicrobial agent designed to inhibit or kill it

Antimicrobial sensitivity test - a laboratory test used to determine whether an antimicrobial drug effectively inhibits a microorganism; also known as the Kirby-Bauer method

Antiseptic - a chemical agent used to reduce microbial numbers on living tissue

Arrangement - See “Cellular arrangement”

Aseptic technique - procedures used to reduce microbial numbers thereby preventing the spread of microbes

Aseptic transfer - the process of moving microorganisms from one culture medium to another culture medium without introducing contaminants to the culture

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B

Bacilli - See “Bacillus”

Bacillus - a rod-shaped bacterium

Bactericidal - describes a chemical agent that kills bacteria

Bacteriostatic - describes a chemical agent that inhibits the growth of bacteria

Basic dye - positively charged dyes (also known as cationic dyes); examples include crystal violet, methylene blue, and safranin

Beta-hemolysis - complete lysis of red blood cells on blood agar due to the action of enzymes known as hemolysins

Biological vector - a living organism in which a parasite develops or reproduces before being transmitted to a host

Biosafety level - one of four levels of safety practices, equipment, and facility requirements used when working with microorganisms; higher levels require greater protective measures

Blood agar - See “Blood agar plate”

Blood agar plate - a nutrient medium containing blood; usually used to grow fastidious bacteria or to assess their hemolysis capability

Broth - See “Liquid media”

BSL - See “Biosafety level”

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C

Capsule - an organized layer of glycocalyx surrounding some bacterial cells; it is usually composed of polysaccharides but may be composed of polypeptides

Capsule stain - a staining procedure used to detect bacterial capsules; an acidic dye stains the background, a basic dye stains the cell, and the capsule remains visible as a clear halo

Carbohydrate fermentation test - a biochemical test performed to determine if an organism can ferment sugars

Carbol fuchsin - See “Carbolfuchsin”

Carbolfuchsin - a dark red, lipid-soluble dye used as the primary stain in the acid-fast staining procedure

Casease - an enzyme that hydrolyzes the milk protein casein

Casease test - a biochemical test performed to determine if a bacterium is able to produce casease and thereby hydrolyze the milk protein casein

Catalase - an enzyme that converts hydrogen peroxide to water and gaseous oxygen

Catalase test - a biochemical test that is performed to detect the ability of bacteria to produce the enzyme catalase; used to differentiate Staphylococci and Micrococci from Streptococci and Enterococci

Cellular arrangement - the manner in which cells are organized following cell division; it is determined by how they remain attached following cell division and the plane(s) in which they divide

Cellular morphology - See "Morphology"

Cestode - parasitic flatworms (Platyhelminthes) that have a flat ribbon-like body pattern, lack digestive tracts, and are monoecious

CFU - See "Colony forming unit"

Chemical fixation - a form of fixation that uses chemicals to preserve cell shape in preparation for staining

Citrate test - a biochemical test performed to determine whether a bacterium can use citrate as its sole carbon source

Coagulase - an exoenzyme that permits some bacteria to clot blood

Coagulase test - a biochemical test that is performed to detect the presence of the exoenzyme coagulase; differentiates Staphylococcus aureus from other species of Staphylococcus

Cocci - See "Coccus"

Coccus - bacteria that are spherical in shape

Colony - a visible mass of microorganisms growing on solid medium that usually develops from a single cell or colony-forming unit

Colony forming unit - the unit used in microbial enumeration; because some microbes naturally form arrangements, a single colony may arise from several genetically identical cells attached to one another rather than from a single cell

Complex media - microbiological media in which the exact chemical composition is unknown due to the addition of yeast, plant, or animal products

Compound light microscope - See "Compound microscope"

Compound microscope - a microscope that uses two sets of lenses, the ocular lens and an objective lens, to magnify a specimen

Contaminant - See "Contamination"

Contamination - the accidental introduction of unwanted microorganisms to a sample

Counterstain - See "Secondary stain"

Counting chamber - a specialized microscope slide that has an etched grid and holds a precise quantity of sample allowing for accurate enumeration of a sample

Crystal violet - stain used as the primary stain in the Gram stain procedure

Culture medium - See "Growth medium"

Cyst - the dormant, resistant form of some protozoa that allows them to withstand unfavorable conditions such as temperature extremes or a lack of water or nutrients

▲ Back to A–Z

D

Decolorizer - a chemical used in a differential staining procedure to selectively remove the primary stain from certain cells/structures while leaving others stained

Decolorizing agent - See “Decolorizer”

Defined media - microbiological media in which the exact chemical quantities and composition are known

Definitive host - a host in which a parasite reaches sexual maturity and reproduces sexually

Differential media - microbiological media that contain ingredients that cause distinct types of bacteria to grow differently on the same media

Differential stain - a staining procedure that allows an observer to distinguish cells or cellular structures based on differences in staining

Differential staining - See “Differential stain”

Dilution factor - the ratio of the final volume of the diluted sample to the volume of the original sample used to prepare the dilution

Dioecious - an organism in which the male and female reproductive organs exist in separate individuals

Direct microscopic count - a microbiological enumeration method in which a counting chamber (Petroff-Hausser counting chamber) and a microscope are used to count the number of microorganisms in a sample

Disinfectant - a chemical agent used to reduce microbial numbers on inanimate objects, such as laboratory benches, kitchen counters, and stethoscopes

Disk diffusion test - See “Antimicrobial sensitivity test”

DMC - See “Direct microscopic count”

Durham tube - a small glass tube inverted inside a larger test tube to trap gas produced by bacteria during tests such as carbohydrate fermentation and nitrate reduction

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E

Endospore - a metabolically inactive, resistant structure produced within certain bacterial cells to protect a copy of the genetic material during harsh environmental conditions

Endospore stain - a staining procedure used to detect bacterial endospores; steam heat helps drive the primary stain into the resistant endospore

Enriched media - microbiological media that contain special ingredients such as blood or serum to support the growth of fastidious organisms

Enteric bacteria - See “Enterobacteriaceae”

Enterobacteria - See “Enterobacteriaceae”

Enterobacteriaceae - family of gram-negative bacteria that include species such as Escherichia coli, Klebsiella spp., Salmonella spp., Enterobacter spp. etc.; some of these bacteria reside in the intestines of humans or animals thus being referred to as “enteric bacteria”

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F

Facultative halophile - a microorganism that can tolerate high salt concentrations but does not require a high salt concentration for growth

Fastidious bacteria - bacteria requiring additional nutrients and growth factors, such as amino acids, lipids, or other specialized nutrients

Fermentation - a metabolic process that produces ATP by substrate-level phosphorylation and uses an organic molecule as the final electron acceptor

Fixation - process performed to attach cells to a microscope slide and maintain cell shape

Flatworm - See “Platyhelminth”

Fluke - See “Trematode”

Fungal allergy - hypersensitivity reaction, usually to fungal spores

Fungal toxicosis - a condition that occurs when toxins produced by fungi are ingested

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G

Gamma-hemolysis - the absence of red blood cell lysis on blood agar

Glycocalyx - a sticky outer layer composed of carbohydrates, proteins, or a combination of both that surrounds some bacterial cells; may form an organized capsule or a loosely attached slime layer

Gram stain - staining procedure used to differentiate cells based on their structural differences in cell walls; cells are differentiated as gram-positive or gram-negative

Gram's iodine - mordant used in the Gram stain that acts to bind to the primary stain crystal violet forming an insoluble complex that can become trapped within a cell

Gram-negative - bacterial cells that have thin peptidoglycan cell walls with outer membranes

Gram-positive - bacterial cells with thick peptidoglycan cell walls

Growth media - See “Growth medium”

Growth medium - source of nutrients for microbial growth in the laboratory; may exist in three physical forms liquid, semi-solid, or solid

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H

Halophile - microorganisms that thrive in environments with high salt concentrations

Heat fixation - fixation process in which an air-dried smear on a microscope slide is passed back and forth through the flame of a bunsen burner to attach the cells to the slide in order to prevent them from being washed away in the staining process

Helminth - biological term for a parasitic worm; helminths include cestodes, trematodes, and nematodes

Hemocytometer - See “Counting chamber”

Hemolysin - an enzyme produced by some microorganisms that causes the destruction of red blood cells

Hemolysis - the breakdown of erythrocytes (red blood cells)

Hypha - a long, branching filament that forms the body of fungi known as molds

Hyphae - See “Hypha”

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I

IMViC - See “IMViC tests”

IMViC tests - a panel of biochemical tests used to differentiate members of the Enterobacteriaceae family; includes the indole, methyl red, voges-proskauer test, and the citrate test

Indole test - a biochemical test that is performed to assess a bacterium's ability to produce the enzyme tryptophanase

Inoculate - See “Inoculation”

Inoculating loop - inoculating tool with a small loop at the end; used for transferring bacteria

Inoculating needle - inoculating tool with a straight tip that is used to stab media to introduce microbes to the media

Inoculation - the process of introducing microorganisms into or onto a culture medium

Inoculum - a sample of microorganisms introduced into or onton a culture mediumm to begin a new culture

Intermediate - in the antimicrobial susceptibility test - intermediate means that higher than normal concentrations of the drug would be needed to inhibit the microorganism

Intermediate host - a host in which an immature or asexual stage of a parasite develops

Iodine - See “Gram's iodine”

Isolation - the process of separating a microorganism from a mixed population to obtain a pure culture containing only one type of microorganism

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J

No current entries under “J”.

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K

Kirby-Bauer method - See “Antimicrobial sensitivity test”

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L

Larvae - an immature developmental stage of some animal, including helminths, that occur between the egg and adult stages of development

Liquid media - also known as broths; physical form of media that contain nutrients dissolved in water, does not contain agar

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M

MacConkey agar - a selective and differential medium that is used to select for gram-negative bacteria and differentiate them based on their ability to ferment lactose

Malachite green - stain used as the primary stain in the endospore staining procedure

Mannitol salt agar - a selective and differential medium that is used to select for salt-tolerant species and then differentiate them based on mannitol fermentation

Media - See “Growth medium”

Medium - See “Growth medium”

Mesophile - microorganisms that are adapted to growth temperatures between approximately 20°C - 45°C

Methyl red test - a biochemical test performed to identify if a bacterium can produce acid-end products as a result of the mixed-acid fermentation of glucose

Microbe - See “Microorganism”

Microbicidal - chemicals that work to kill microorganisms

Microbiostatic - chemicals that work to inhibit the growth of microorganisms

Microbistatic - See “Microbiostatic”

Microorganism - organisms too small to be seen with the unaided eye; also known as microbes

Mixed culture - a microbiological sample containing two or more species of microorganisms

Mold - form of fungi that have a filamentous structure, made of long cylindrical tubules known as hyphae

Monoecious - an organism that has both male and female reproductive organs within the same individual

Mordant - chemical agent that helps a stain bind to a specimen

Morphology - the study of form and structure; in microbiology it is focused on the shape and size of microbial cells

Motility - in microbiology - it refers to the ability of a microorganism to move due to the presence of flagella or other motility structures (i.e. pseudopods)

MR test - See “Methyl red test”

MSA - See “Mannitol salt agar”

Mueller-Hinton agar - specialized agar that is used for antimicrobial susceptibility test

Mycelium - the vegetative portion of a fungus that consists of thread-like hyphae

Mycolic acid - wax found embedded in the thick cell walls of bacteria in the genera Mycobacteria and Nocardia

Mycoses - See “Mycosis”

Mycosis - a disease caused by fungi

Mycotoxicosis - See “Fungal toxicosis”

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N

Negative stain - a staining technique that uses an acidic dye to color the background rather than the microbial cells, allowing cell size, shape, and arrangement to be observed with minimal distortion

Negative staining - See “Negative stain”

Nematode - a cylindrical, unsegmented roundworm with a complete digestive tract containing both a mouth and an anus; nematodes are typically dioecious, meaning they have separate male and female individuals, and some species are parasitic

Neutrophile - microorganisms that prefer to grow at a neutral pH range near pH 7

Nitrate reduction test - a biochemical test that is performed to determine if a bacterium can reduce nitrate using the enzyme nitrate reductase

Non-acid-fast - bacteria that lack mycolic acids in their cell walls

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O

Objective lens - lenses on the compound microscope that serve as the primary source of magnification

Ocular lens - the lens of a compound microscope that the observer looks through; typically provides 10× magnification

Oil immersion lens - See “Oil immersion objective”

Oil immersion objective - a high-power objective lens designed for use with immersion oil, which reduces light refraction and improves image resolution

Oil objective lens - See “Oil immersion objective”

Ova - the eggs or egg stages of parasitic helminths

Oxidase test - a biochemical test performed to determine if a bacterium contains the enzyme cytochrome c oxidase

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P

Pathogen - a microorganism capable of causing disease

Peptidoglycan - a polysaccharide unique to bacterial cell walls that is composed of alternating sugars linked by short peptide chains and provides the cell with shape and structural support

Petroff-Hausser chamber - See “Counting chamber”

Phenol red - a pH indicator that is commonly used in biochemical tests to detect changes in pH; it appears red at neutral pH, yellow in acidic conditions (below pH 6.8), and bright pink (fuchsia) in alkaline conditions (above pH 8.2)

Platyhelminth - a worm belonging to the phylum Platyhelminthes that has a flattened body; medically important parasitic platyhelminths include trematodes (flukes) and cestodes (tapeworms), and most are monoecious

Pleomorphic - bacteria that vary in their shape and size with no one consistent pattern in shape

Primary stain - the initial dye applied during a differential staining protocol

Proglottid - an individual body segment of a cestode that contains male and female reproductive structures

Protozoa - See “Protozoan”

Protozoan - microscopic, single-celled eukaryotic organisms lacking cell walls that may be free-living or parasitic

Psychrophile - a cold-loving microorganism that can grow at 0°C or lower, has an optimum growth temperature near 15°C, and generally cannot grow above 20°C

Psychrotroph - a microorganism that can grow at refrigeration temperatures but has an optimum growth temperature between approximately 20°C and 30°C

Pure culture - culture containing a single species or strain of microorganism

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Q

No current entries under “Q”.

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R

Resistant - in the antimicrobial susceptibility test - resistant means that the microorganism is not inhibited by the drug

Rod - See “Bacillus”

Roundworm - See “Nematode”

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S

Safranin - a basic stain used as the counterstain in both the Gram stain and endospore stain procedures

Salt-tolerant - See “Facultative halophile”

Sanitizer - chemical agents used primarily in the food industry to reduce microbes to a safe level

Scolex - the anterior attachment structure of a cestode that bears suckers, hooks, or grooves used to attach the worm to the host’s intestinal wall

Secondary stain - a stain applied after the decolorizing step of a differential staining procedure to stain cells/structures that were left colorless following the decolorizing step

Selective media - microbiological media that contain ingredients that act to encourage the growth of some types of microbes while inhibiting others

Semi-solid media - physical form of media containing 0.5% or less agar; commonly used for motility tests

Serial dilution - a stepwise dilution process in which a known volume of a sample is transferred into a sterile diluent, and then a known volume of each dilution is transferred into the next container; each successive container is a dilution of the previous one

SIM - See “SIM medium test”

SIM medium test - a biochemical test used to detect the ability of a bacterium to produce hydrogen sulfide gas (S), the ability to produce the enzyme tryptophanase (I), and the ability to demonstrate motility (M)

Simple stain - a staining procedure in which a single stain is used to provide contrast to cells to allow the observer to visualize their cellular morphology (size, shape, arrangement)

Simple staining - See “Simple stain”

Slant - solid culture medium that has hardened at an angle in a test tube to provide an area for microbial growth; commonly used to maintain and store microbial cultures

Smear - a thin film of bacterial sample placed on the surface of a microscope slide

Solid media - physical form of media that contain 1.5% - 2% agar; placed in petri plates or in test tubes

Spirilla - See “Spirillum”

Spirillum - spiral shaped bacteria with rigid cell walls

Spirochete - spiral shaped bacteria with flexible cell walls

Spore - See “Endospore”

Standard plate count - See “Viable plate count”

Staphylo- arrangement - an arrangement produced when bacteria divide in multiple planes and remain attached in clusters; cocci with this arrangement are described as staphylococci

Staphylococcus arrangement - See “Staphylo- arrangement”

Starch hydrolysis test - a biochemical test performed to determine if a bacterium produces amylase and therefore is capable of hydrolyzing starch

Sterilant - chemical agents that are used to completely destroy or eliminate all forms of microorganisms on a surface

Sterile technique - See “Aseptic technique”

Streak plate - See “Streak plate method”

Streak plate method - a microbiological technique that is used to isolate bacteria in pure colonies by diluting the sample over an agar surface

Strepto- arrangement - an arrangement produced when bacteria divide in a single plane and remain attached in chains; rod-shaped cells form streptobacilli, and cocci form streptococci

Streptobacillus - See “Strepto- arrangement”

Streptococcus arrangement - See “Strepto- arrangement”

Subcutaneous mycosis - fungal infections that occur in the deeper layers of the dermis and/or in the hypodermis

Superficial mycosis - fungal infections that involve the skin, hair, or nails

Susceptible - in an antimicrobial susceptibility test, describes a microorganism that is inhibited by the concentration of a drug normally used for treatment

Synthetic media - See “Defined media”

Systemic mycosis - fungal infection in which the fungal agent has spread throughout the body involving organs of various body systems

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T

Thermophile - a microorganism adapted to growth at high temperatures, typically between approximately 45°C and 80°C

Total magnification - the magnification that results from the use of the ocular lens and an objective lens when using the compound microscope

Transport media - microbiological media that are specialized for transporting microbes in the clinical setting; contain buffers and salts to maintain the organism without allowing it to multiply

Trematode - a class of parasitic flatworms in the phylum Platyhelminthes; most have leaf-shaped bodies, incomplete digestive tracts, and are monoecious, while schistosomes are elongated and dioecious

Triple sugar iron agar test - a biochemical test performed to assess the ability of a bacterium to ferment sugars and produce hydrogen sulfide gas

Trophozoite - the active feeding form of a protozoan

Tryptophanase - an enzyme that converts the amino acid tryptophan into indole, pyruvic acid, and ammonia

TSI - See “Triple sugar iron agar test”

Turbidity - cloudiness in a liquid culture medium caused by the growth of microorganisms suspended throughout the medium

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U

Urease - an enzyme that hydrolyzes urea to release ammonia

Urease test - a biochemical test that is performed to determine if a bacterium is capable of producing the enzyme urease

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V

Vector - a living organism that transmits a pathogen from one host to another

Vegetative cell - an actively metabolizing cell

Viable plate count - a microbiological enumeration method used to estimate the number of living microorganisms in a sample by serially diluting the sample, plating it, and counting the colonies that develop

Vibrio - a bacterium with a curved rod shape that may appear comma-shaped

Virulence - the ability of a pathogen to infect and cause disease in a host organism

Voges-Proskauer test - a biochemical test performed to identify the ability of a bacterium to produce acetoin from the catabolism of glucose

VP test - See “Voges-Proskauer test”

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W

No current entries under “W”.

▲ Back to A–Z

X

No current entries under “X”.

▲ Back to A–Z

Y

Yeast - a globular, single-celled form of fungus

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Z

Ziehl-Neelsen stain - See “Acid-fast stain”

Zone of inhibition - a clear area surrounding a filter disk containing an antiseptic, disinfectant, or antibiotic where microbial growth has been inhibited

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