Athabasca University

Faculty of Science and Technology

BIOL 207

Introductory Biology Laboratory

Laboratory Manual 2024

BIOL 207

2024

This lab manual was compiled by Roland Treu, Carmen Allen, Erin Clark, Rob Carmichael and Elaine Birkigt.

BIOL 207 Introductory Biology Lab

Introduction

Welcome to *BIOL 207: Biology Laboratory*. You will experience 5 days of hands-on biology. The emphasis of the laboratory part is to provide you with basic biology lab skills and to observe the diversity of organisms. Microscopy skills will be one of the more important learning outcomes of this lab as well as the ability to carry out basic biological lab procedures. With due emphasis on lab safety, you will also train your observational skills as well as the documentation of biological experiments and objects through drawings and photographs. You will also learn how to summarize your results in the form of a lab report.

Expectations

Academic expectations

Students who participate in the lab part of BIOL 207 are expected to have an adequate theoretical background in introductory biology. You would normally have acquired that background by having passed BIOL 204 and BIOL 207. Students who are still enrolled in BIOL 207 but have advanced far in the course (as evidenced by having submitted at least one or two assignments) may attend the lab but are expected become familiar with the relevant chapters in the textbook as preparation for the lab.
Students are expected to attend all lab sessions from Monday to Friday and to participate in all activities. Some lab activities are carried out in groups.
Students who for some reason are unable to attend a particular lab session on short notice are expected to contact the Lab Development Specialist **in advance** via email**:** *fst_success@athabascau.ca*

Required reading before attending the lab: You are required to read the general part of this lab manual, up to p.9. In addition, it is advisable to watch the pig dissection video at least once before you attend the respective lab:
https://www.youtube.com/watch?v=1oM1TUMqN54
During the lab: read through the respective labs the evening before.

Lab Times

Laboratory sessions will be held between 9:00 a.m. sharp and 12:00 noon, and between 1:00 p.m. and 5:00 p.m., Monday to Friday. Occasionally we will need to modify lunch times, but there will always be a one-hour lunch break. You should spend the last 10 minutes in the afternoon cleaning up your workspace.

What to bring

Essential:

- Pencils and erasers for drawings

Recommended:

- Your textbook (highly recommended if you haven't finished the theory parts of BIOL 204 and BIOL 207); most of you will have the e-text version
- A laptop
- A padlock if you have valuables that need to be stored
- A computer flash drive (if you want to save some of your own microscope pictures; please note however: AU will retain the copyright of all pictures)
- A lab coat (we provide one if you don't have your own)

Lab safety

Athabasca University is committed to a safe lab and work environment. You will obtain general safety instructions at the beginning of the week and specific ones before each relevant lab session. Closed toed shoes are required for all lab activities.

The following are minimum safety requirements at all times:

- Familiarity with fire exits and procedures
- Familiarity with location of first aid kits, fire extinguishers, safety showers and eyewash stations
- Absolutely no food and drink in the labs
- No alcohol and drugs in the laboratory, no smoking in the building
- Lab coats must be worn in the lab area at all times
- Lab coats must be taken off when leaving the lab area
- Hands must be washed before leaving the lab area

Lab marking

Your lab grade will consist of a participation mark, your lab notes and drawings, a continuous skill evaluation and the lab report.

Participation mark (10%)

You will receive a full participation mark if you punctually attend all lab sessions and show a reasonable amount of interest in the lab activities.

Lab notes and drawings (20%)

The lab manual (lab book) will include your drawings and the notes that you take and the results that you obtain during the labs. Some labs also have a set of questions that you need to answer. At the end of the lab you will leave your lab book with the course instructor for marking (to be returned). See also *Guidelines for lab book writing* below.

Lab report (35%)

The lab report will have to be sent in three weeks after the end of the lab via moodle or email attachment. The exact deadline will be given during the lab. *Late reports will have five percent deducted from the mark obtained*. You will learn on Day 5 how to write lab reports, although these skills have been covered in BIOL 204. An average lab report written with font size 12 would be between 10 and 15 pages long (including illustrations).

Continuous skill evaluation (35%)

You will be evaluated every day for the skills that you learn in the lab. This will constitute your continuous skill evaluation. Your continuous evaluation consists of the following components, each one graded out of possible 10 marks: Microscope skills, Gram stain, streak plates, mitosis/meiosis, cheese curd, pig dissection, protist diversity, cleanliness of workspace.

Lab grade total

The BIOL207 lab grade counts 25% towards your final course grade.

Guidelines for lab book writing

All lab activities must be recorded in the lab manual. Lab book writing, i.e. recording of results, is a crucial skill that all future lab scientists and lab technical staff will have to learn and master over time. A good lab book should be an exact and permanent reference for yourself and your co-workers. The more details you include, the better.

Start by referring to the number of the lab and its title as well as the date and the time when you started the lab (e.g. Lab XIX Enzymes, February 32, 1865, 1:00 PM).

For experimental labs note down all results. It is fine to use photographs, sketches, printouts, etc. but they must be accompanied by a brief explanation of what they represent. Sharing the results and copying for a whole group is encouraged for the experimental labs (but not for the observational labs).
The purpose of a lab book is the exact and precise recording of your results, while the interpretation and explanation of your experimental results is not expected in a lab book.

For the diversity (observational) labs, write down the organisms that you observed in the displays and in your microscope. Use the lowest taxonomic category that is possible (ideally the species) and include the phylum for all of them (e.g.: *Apis mellifica*, honeybee, Arthropoda). Whenever possible, include a short note with the characters that identify an organism as belonging to a particular phylum. Include sketches and drawings of selected organisms as time allows. If applicable, include microscope information in your lab book.

Lab skills, where applicable, are described in the lab book by giving a very brief description of the skills you learned in a particular lab (e.g. loading of an electrophoresis gel, using of a spectrophotometer). If they involve a scientific instrument include the name and model of the instrument.

Please note: Lab books are often not very neat, but your writing must be legible.
Please note: you may include any notes that you take during the mini lectures or from the textbook, however, these notes are not considered

when grading your lab book. *Lab books are ideally written during your lab sessions and not propped up after hours*.

Drawings should be large (at least half a letter size page) and include your name (if on a separate page), a title (that clearly identifies the organism and/or its parts and the total magnification, type of microscope and the date) and labeled with as many details as possible. A minimum of 2 drawings must be submitted, from two different kingdoms.
Please note: drawings are different from sketches and require careful observation of the organism under study.
Do not copy drawings from books; rather you should sharpen your skills to draw from nature.

Overview of the labs

DAY		AM	PM
1	MONDAY	Welcome. Lab safety. Introduction. Microscopy.	Bacteria. Microbiology.
2	TUESDAY	Diversity of Protista (algae, protozoans). Mitosis/meiosis	Enzymes.
3	WEDNESDAY	Molecular biology. PCR, Electrophoresis. Forensic lab.	Plant diversity. Cheese making.
4	THURSDAY	Animal diversity.	Pig dissection.
5	FRIDAY	Diversity of fungi.	Analysis of results. Discussion. How to do lab reports. Lab evaluations. End of lab.

Please note: Time estimates for the individual labs are only approximations. For the biodiversity labs we may occasionally have modifications depending on organisms available.

You are expected to read through the respective labs on a particular day the evening before.

Day 1 Lab I Microscopy

(total time to complete = 3h)

Objectives

After finishing this lab you should be able to

- confidently use a light microscope
- confidently use a dissecting microscope
- explain which type of microscope would be used for various applications
- describe the components of a microscope and define their function
- prepare wet mounts for the compound microscope and observe them at the highest magnification (with immersion oil)

Preparation

Read handouts.

Introduction

In this first lab you should start to become familiar with microscopes, both dissecting and compound microscopes. While this first lab will be the only one that deals exclusively with microscopes, you will still use microscopes on most of the following days. You will learn how to make your own slides (wet mounts) and what objects require dissecting or compound microscopes. Familiarity and dexterity with microscopes is one of the most important learning outcomes for this BIOL 207 lab.

Procedures

- ✓ Select one or more of the permanent slides that your instructors provide. These slides are for practice only and we will not explore the structures that you observe at this time. Rather, you will use the permanent slides to become familiar with the basic procedures of handling a compound microscope.

- ✓ **Before** you turn on the light switch of the microscope, ensure that the brightness control is at its lowest level. After switching the light on, increase the brightness to a moderate level. Adjust as required. (It is a good practice to turn the brightness control back to its lowest level before switching the light off.)

- ✓ On the stage below the objective lens, insert the slide by moving the stage clip aside.

- ✓ With the slide securely placed, select the objective lens with the lowest magnification by turning the objective revolver. Move the objective lens with the lowest magnification above your light source. This should become a permanent habit; you should always start observing slides with the lowest magnification!

- ✓ Familiarize yourself with the stage controls that move the slide up and down and left and right. Move the centre of the slide above the light source.

- ✓ Now look through the two ocular lenses and adjust their distance by gently pushing back and forth until they fit your personal eye distance. Ensure that you see one big circle only.

- ✓ Focusing: While looking through the oculars, use the coarse adjustment knob and then the fine adjustment knob. Move them up and down until the object on the slide is in clear focus.

- ✓ After focusing, observe the object by moving the stage controls left and right and up and down.

- ✓ You can now increase the magnification by moving to the next higher objective lens. Observe again and move to the next higher objective lens. Repeat these steps until you reach the 40x objective magnification. (With a 10x magnification ocular lens, this will give you a total magnification of 10x40 = 400x).

- ✓ Select at least one more permanent slide and repeat all of the above steps until you are familiar with these basic procedures of the compound microscope.

- ✓ Before you use the oil immersion objective (100x), watch your instructors' demonstration of the procedure: After selecting a desired location on the slide, using the 40x objective lens, carefully move the objective revolver into a halfway position towards the 100x objective. Before you move the 100x objective into position, place a small drop of immersion oil in the centre of the slide. Now carefully move the 100x objective into its position

and watch as the immersion oil connects the objective lens with the slide. Use **only** the fine adjustment knob to focus, otherwise you could damage the slide and the objective lens.

- ✓ Once you are done with a permanent slide, wipe off the oil from the slide with tissue paper and lens cleaning solution.

- ✓ In the next step which requires some skills, prepare a wet mount from one of the pond water cultures. Your instructor will demonstrate the procedure: Using a small disposable pipet, take a small (!) drop of pond water onto the centre of your slide. Carefully place a cover slip on top (with forceps or by holding the coverslip on 2 opposite edges), ensuring that there are no air bubbles and no excessive amounts of water. As usual, start with the lowest magnification and move upwards to the higher objectives. Select an interesting organism; it will be easier if you start with organisms that don't move or move slowly. Then move to the 100x objective, adding immersion oil in the final step. You may have to practice a few times, but once you have a good wet mount, call your instructor for a skills evaluation.

- ✓ At the end of the day, clean your 100x objective with lens cleaning solution. For the objective lenses, use only lens paper.

When you work with a compound microscope, remember that you

- should see only one compound circle with both eyes. If you see two different circles, adjust the distance between the two oculars and/or the distance between your eyes and the oculars)
- select your object of interest with a lower magnification, only then you proceed to a higher magnification
- have clean objective lenses
- use ONLY lens paper (and lens cleaning solution) to clean lenses
- use immersion oil for the highest magnification (100x objective) *only*

The most common mistake in microscopy is the preparation of wet mounts with excessive amounts of material (aim for a size << $1mm^2$). The smaller your object is, the more you will recognize in the microscope.

Skills Evaluation

Prepare a wet amount from one of the pond water jars. Once you have a good slide, call your instructor for a skills evaluation. A good slide will have no air bubbles and a clearly visible organism under 1000x magnification.

Day 1 Lab II Microbiological techniques

(total time to complete = 4h)

Preparation

Read the chapter on prokaryotes (Bacteria, Archaea) in the textbook.

Objectives

After finishing this lab, you should be able to

- define the safety issues around working with micro-organisms.
- use several aseptic techniques for working with bacteria.
- streak plates to isolate colonies.
- stain bacteria using the Gram Stain.
- identify different colonial morphologies.
- describe a biochemical test that is crucial in the identification of bacteria

Procedures

1. Microbiological safety (time to complete = 10 minutes)

Even simple matters like hand washing and surface decontamination are crucial to success in microbiology experiments, as the number one source for contaminating organisms are from the lab worker and their immediate surrounding environment.

Safety consideration: All microbiological waste material must be disposed of in the marked waste containers. Do NOT use regular trash cans for microbiological material. Your instructors will point out where the microbiological waste containers are located.

2. Surface decontamination and environmental swabs

(time to complete = 10 minutes)

- Select a suitable surface decontaminating germicide (e.g. ethanol 70%, Lysol).

- Pour/Squirt sufficient liquid onto the lab bench surface so that the entire surface will be in contact with disinfectant.

- Use a paper towel to spread the liquid over all points of the

surface.

- Once spread out, allow the disinfectant the appropriate amount of contact time and depending on the choice of germicide, allow for evaporation and dissipation.

- Use a sterile swab to wipe over a large amount of the recently decontaminated bench area, and then inoculate a Nutrient Agar plate, as demonstrated by the instructor.

- Use two more plates to produce environmental swabs (try to be creative and innovative: e.g. toilet seats, parts of your body, but use discretion!)

- Incubate the plates at 37 C for 24-48 hours and record your results for growth or no growth in the table below (to be evaluated on Day 5 or earlier).

Environmental Swabs					
	Date and time exposed	Date and time collected	Incubation time (hours)	Location description	Growth + or –; Notes on colonies observed (if any)
Plate 1					
Plate 2					
Plate 3					

3. Aseptic techniques: handling broths

(time to complete = 25 min)

Microbiologists frequently use liquid cultures for bacteria. It is therefore crucial that liquid cultures always remain contaminant free. In this exercise you will transfer sterile liquid broth into sterile test tubes. If you were successful, your liquid broth control will remain free of contaminants after incubation.

- Obtain 3 sterile test tubes with nutrient broth (NB).
- Label the test tubes Tube 1-3.
- Following the safety instructions provided by your lab instructor, light your Bunsen burner.
- Sterilize an inoculating loop and use it to transfer a small volume of broth from tube 1 to tube 2.
- Sterilize an inoculating loop and use it to transfer a small volume of broth from tube 2 to tube 3.
- One or two students or the instructor may deliberately contaminate their tubes to produce a positive result (contamination)
- Incubate the tubes for 48 h at 37°C and record your results for growth or no growth in your lab notebook (Day 4 or 5).

Sterile broths				
	Date and time transferred	Date and time evaluated	Incubation time in hours	Growth of contamination + or –; any other notes
Tube 1				
Tube 2				
Tube 3				

4. Aseptic techniques: streak plating

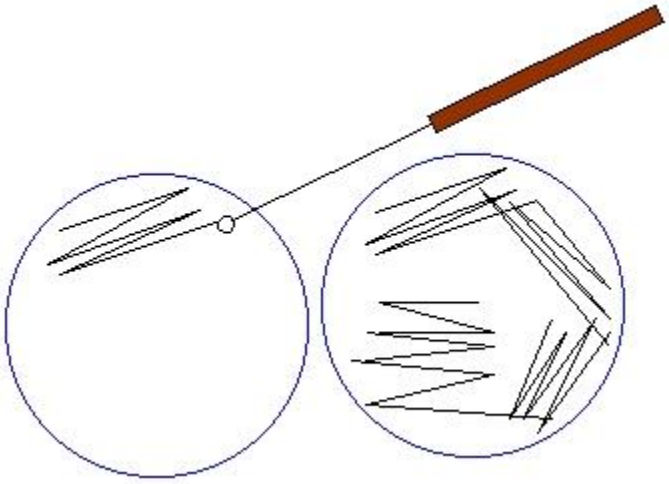

Fig. 2.1 How to streak plates.
Attribution: Drawn by Theresa Knott
CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=438234

The streak plate method, if properly performed, is probably the most practical and useful method for obtaining discrete colonies and pure cultures. In this lab you should learn to streak a loopful of a bacterial suspension (several million cells) onto an agar surface, in such a way that single cells will be deposited in some areas and will then grow into isolated colonies. Also, you will learn how to make a NA subculture of *E. coli*.

- Obtain two sterile Nutrient Agar (NA) plates. Please note that in microbiology (bacteriology) Petri dishes are stored upside down, with the lid at the bottom.
- Also obtain a NB culture of *E. coli*, and a NA plate culture of *E. coli*.
- Look closely at the 2 sterile NA plates to make sure they are indeed sterile and have no growth on them. Discard any plate with growth into the waste biohazard bag for petri dishes.
- Label your NA plates, writing on the side that has the agar (underside)
- Start your Bunsen burner, flame your loop and obtain a loopful of the *E. coli* broth to streak onto the new NA plate.
- Gently touch the surface instead of gouging.
- Re-heat the loop between the streaks.
- Repeat the streaking procedure with your 2nd NA plate, but this time use the provided *E. coli* NA plate culture as your inoculum source (i.e. make a subculture). Incubate both plates at 37 C for at

least 24 hours, agar side up. Record your observations into your lab notebook. Show your plates to the lab instructor for a skills evaluation on Day 5 (or earlier).

Streak plates				
	Date and time produced	Date and time evaluated	Incubation time in hours	Observations: are there isolated colonies?
Plate 1				
Plate 2				

5. Gram staining of Bacteria

(time to complete = 90 min)

Preparation of smears

- Setup and light a Bunsen burner as demonstrated.
- Using a marker pen, draw a circle (about 1 cm diameter) near the centre of a slide. Flame a loop, as demonstrated, and transfer a sample of the liquid culture of the mixed culture sample (*E. coli* + *S. epidermidis*) of bacteria to the surface within the circle and spread evenly.
- Let the smears dry. This may be hastened by very gently warming the slide in a Bunsen burner.

Heat fixation

- Once the slide has dried, the smear must be heat fixed.
- Using forceps, quickly pass the slide through the flame two or three times as demonstrated by your instructor.
- The slide is now ready for staining.

Gram staining fixed smears

- Flood the upper (cell side) surface of the slide with crystal violet and let stand for 1 minute.
- Gently wash with a water bottle to remove all free crystal violet stain. Drain off the excess water.
- Flood the upper (cell side) surface of the slide with Gram's Iodine and let stand for 1 minute.
- Again, gently wash with water and drain off the excess water.
- Gently add drops of decolourizing solution until the runoff is no longer purple. The length of washing is dependent on the thickness of the smear applied to the slide.
- Wash immediately but gently with water and drain off the excess.
- Flood the upper (cell side) surface of the slide with Safranin (counterstain) and let stand for 1 minute.
- Wash gently with water and drain off the excess and allow the slide to air dry before viewing.

Observing Gram-stained smears

- Observe the stained preparation under oil immersion (use only with 100x objective) and record your results.
- One of the organisms is Gram positive (purple) and one is Gram negative (pink).
- Repeat the procedure if you have not been successful in preparing good Gram stains.
- Show your lab instructor your Gram stain (with 100X objective) for evaluation.
- Using your micrometer, determine the size of the bacterial cells you observe.
- In your lab notebook below, describe the microscopic morphologies you see, and produce a sketch of a representative field for each smear. Note the magnifications in each case.

Gram stains				
	Date and time produced	Cell size in µm using micrometer	Magnification	Description of morphology; include a sketch
Smear 1				
Smear 2				

6. Colonial Morphologies (time to complete = 10 min)

- Obtain 3 Nutrient Agar (NA) plate cultures: one of *E. coli*, *S. epidermidis*, and one of *B. subtilis*.
- Using a dissection scope, observe and record the colonial morphologies of each organism. Refer to Fig. 2.2 for the correct technical terms to use for describing colonies. Note the results in your lab book.

Colonial morphologies		
	Date and time evaluated	Description of colonial morphology
E. coli		
S. epidermidis		
B. subtilis		

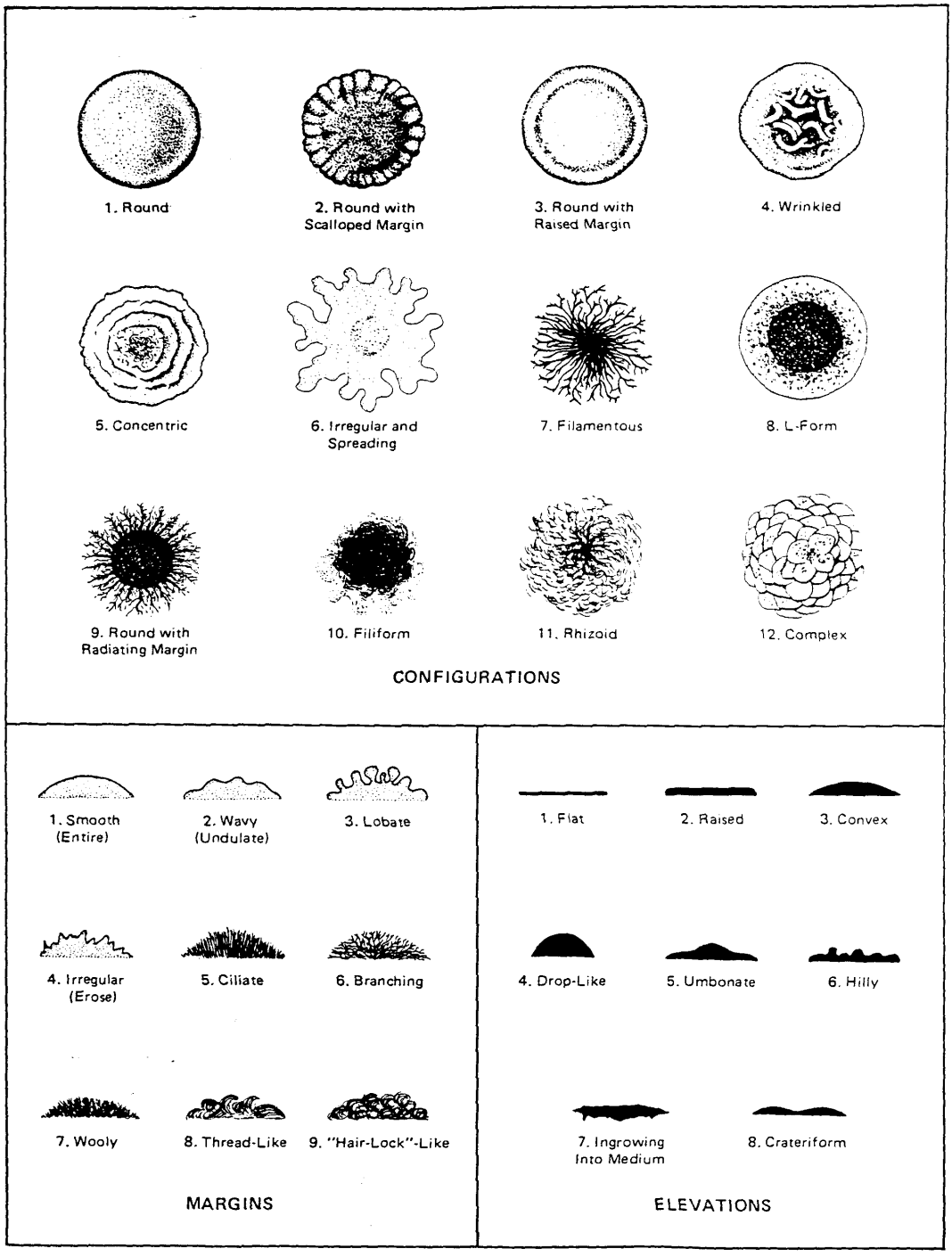

Fig. 2.2 Bacterial colony shape

adapted from Conn HJ and Jennison, eds., Manual of Microbiological Methods, Mc Graw-Hill, 1957

6. Biochemical Tests

(time to complete = 10 min)

Microbiologists use numerous tests for identifying bacteria. Apart from the gram stain, various biochemical tests are crucial for the closer identification of microorganisms. In this enzyme test we evaluate the presence of catalase by adding H_2O_2 to some bacterial cultures. In the presence of catalase, H_2O_2 is degraded into water and oxygen (bubbles!).

- Obtain 3 Nutrient Agar (NA) plate cultures: one of *E. coli*, *S. epidermidis*, and one of *B. subtilis* (from previous activity).
- Open the plates and pipet several drops of Hydrogen Peroxide solution onto several colonies. Record your observations in your lab notebook below.

Catalase reaction		
	Observations	Catalase + or -
E. coli		
S. epidermidis		
B. subtilis		

Skills Evaluation

Lab II has two skills evaluations. You will be evaluated on the streak plates (are there isolated colonies?) and the quality of the gram stain.

Day 2 Lab III Protist diversity

(total time to complete = 2h)

Objectives

After finishing this lab you should be able to

- recognize protists as an artificial group with various levels of organization
- recognize major phyla and groups of protozoans and algae

Preparation

Read the protist chapter in your textbook.

Introduction

In this lab you will look at various fresh and preserved materials of protists. There is no good definition of a protist, other than being a diverse, unrelated assemblage of organisms that taxonomists could not place anywhere else. Nevertheless, some groups within the Protista are well defined, e.g. the phyla red algae (Rhodophyta) and green algae (Chlorophyta). Protozoans ("animal-like"), another artificial group contain important parasites, most notoriously *Plasmodium*, the malaria parasite. Some organisms that were traditionally included in the fungi (*Physarum*, or other slime moulds and *Saprolegnia*, an oomycete) are also placed in this group because, although they have fungal characters, they are not related to the true fungi.

Note: The availability of fresh material (organisms) will differ from lab to lab. Some of the live organisms mentioned below may not be available in a particular year.

Procedures

This lab will be another opportunity to practice your microscope skills as many protists are unicellular.
When you study the live, preserved and microscopic material it is important that you are aware which phylum or the group you are looking at. If necessary, refer to your textbook, so that you develop your skills of recognizing important phyla of protists.

Make sure that you look at all the live material first. There will be more than you can handle in the pond water cultures. Indicate the organisms observed in the table below, together with the other required

information.

As a minimum you should study representatives of the following phyla and enter them into the provided table:

- Protozoans as live material: try to distinguish flagellates, amoebae and ciliates.
- Green algae (Phylum Chlorophyta, they are the direct ancestors of plants)
- Red algae (Phylum Rhodophyta); a mainly tropical, marine group with some large representatives; you have used cell wall material of red algae as agar agar. They have red phycobilins as accessory pigments.
- Brown algae (Phylum Phaeophyta): marine algae, temperate, up to 20 m long.
- Diatoms (Phylum Bacillariophyta): you should see both live and preserved specimens; they have silica as cell wall material and constitute one of the major elements of phytoplankton in both freshwater and marine environments.
- *Plasmodium*: every biology student must know about the malaria parasite Phylum Apicomplexa); view the provided slides (do NOT confuse the genus name with the name for the structures found in the lifecycle of slime moulds!)
- Make a wet mount of fresh *Saprolegnia* (if available). Phylum Oophyta. It grows on dead insects in water (white fuzz).
- Observe *Physarum* cultures as plasmodia (Phylum Myxomycota) on a petri dish under the dissecting scope. Alternatively, dried material of fruiting bodies.

Make at least one drawing of a microscopic organism in the space provided below. In addition, make quick sketches as time allows.

Organism observed (*Scientific name*)	PHYLUM	**Notes:** Include one or more of the following: L = Live material; M = observed under compound microscope (include highest total magnification used, e.g. M1000x); DM = dissection microscope (include magnification); P = preserved material; any other observations
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Skills Evaluation

Find one of the three (informal) groups of the protozoans and show them to your instructor. Identify whether they are ciliates, flagellates or amoeba.

Lab questions:

1. Indicate which of the organisms that you observed today contains chlorophyll. Simply note the numbers from your table (e.g. 2,4,7,8)

2. What is the difference between *Plasmodium* and a plasmodium?

EMPTY PAGE FOR ONE LARGE DRAWING
Make at least one large drawing. Drawings must include: Date, scientific name, phylum, microscope type and magnification, as well as extensive labeling.

EMPTY PAGE FOR ONE LARGE DRAWING
Make at least one large drawing. Drawings must include: Date, scientific name, phylum, microscope type and magnification, as well as extensive labeling.

EMPTY PAGE FOR SKETCHES

Sketches are drawn quickly, they can be small, but must have the name of the organism as well as the phylum. Be selective, you are not expected to sketch every organism that you have observed.

EMPTY PAGE FOR SKETCHES

Sketches are drawn quickly, they can be small, but must have the name of the organism as well as the phylum. Be selective, you are not expected to sketch every organism that you have observed.

Day 2 Lab IV Mitosis and meiosis

(total time to complete = 1h)

Objectives

After finishing this lab you should be able to

- recognize the various phases of the cell cycle and specifically mitosis under a microscope
- recognize the various stages of meiosis under a microscope
- differentiate between these two major types of cell divisions

Preparation

Revise the chapters in your textbook on mitosis and meiosis if necessary.

Introduction

All eukaryotes have a type of cell division called *mitosis*. It ensures that the resulting daughter cells have identical genetic information. Microscopically, the various phases of mitosis can be recognized by the degree of condensation in the chromosomes. With a little practice you will comfortably differentiate between interphase, prophase, metaphase, anaphase and telophase.
Meiosis is a form of cell division that only occurs in certain phases of an organism's lifecycle and only in generative cells; it results in four haploid gamete cells and produces gametes that are different from each other. You will likewise look at some of these stages under the microscope.

Procedures

Look at the various stages of mitosis and meiosis that you find on the prepared slides. Make sure you understand the fundamental difference between these two types of cell division. Ensure that you have seen all the major stages of mitosis and meiosis. Use a book or the provided posters to learn how the stages look in the microscope. Consult your instructors if you need help.

> Skills Evaluation
>
> You will be asked to identify various stages of mitosis in the microscope.

Day 2 Lab V Enzymes

(total time to complete = 4h)

Objectives

After finishing this lab you should be able to

- determine the influence of various parameters (temperature, pH, enzyme concentration, substrate concentration) on the reaction rate of an enzyme
- confidently use a spectrophotometer for measuring absorbance

Preparation

Read the chapter on enzymes in your textbook.

Introduction

Enzymes are crucial catalysts of all life processes, and their proper functioning is required at all times to keep cells alive. Like animals, fungi use enzymes to degrade their food into smaller molecules that can be absorbed. While animals carry out food degradation in their digestive tracts, fungi secrete enzymes into the surrounding environment and later absorb the products of this digestion.
In this lab we are looking at a group of enzymes called cellulases and more specifically cellobiases. These enzymes break down cellulose which is critical to maintain ecosystem functioning. However, we are specifically interested in the potential of cellobiase to produce biofuel from cellulose rich substrates.

The focus of this lab will be the influence of several parameters on enzyme reaction rates. We will look at how temperature, pH, enzyme and substrate concentration influence the rate of reaction for cellobiase.

We use a colorimetric assay and an artificial substrate (*p*-Nitrophenyl glucopyranoside) to determine the enzymatic activity of cellobiase. The substrate is broken down by the enzyme into glucose and *p*-nitrophenol. The latter turns yellow when bases are added (our "stop solution" contains a base!).

The more substrate is broken down into p-nitrophenol, the darker the colour of the product (after adding base). We will be measuring the colour intensity with a spectrophotometer. Therefore, apart from

learning about the basics of enzyme function and the practical application for using biofuels, you will also learn the practical skills of using a spectrophotometer.

Procedures

How to use automatic pipettes

At the beginning, your lab instructors will demonstrate how to use automatic pipettes (sometimes called pipettors) and their disposable pipet tips. You will be given the opportunity to practice pipetting.

Before we start our enzymatic reactions, you will need to familiarize yourself with the spectrophotometer ("spec" in short). It measures the amount of light absorption when light is passing through a liquid filled cuvette. Distilled water has an absorbance of 0, and the more pigment is in solution, the higher the absorbance. Familiarize yourself with the 2 different scales, absorbance, and transmittance.

How to use the spectrophotometer

1. Turn the instrument on and allow 15 minutes for warm-up.

2. Set the desired wavelength (410 nm).

3. Set the filter lever for the appropriate wavelength.

4. Make sure that the compartment is empty and closed. Adjust to 0% transmittance (for digital specs, make sure you push the appropriate button) using the left knob.

5. Fill a clean cuvette with water and push it into the cell. Place the cell into the compartment and close the lid.

6. Adjust the display to 0 % absorbance with the right knob (for digital specs, make sure you push the appropriate button).

7. Always close the compartment before taking the reading. You are now ready to take your readings after removing the original blank.

8. If you change the wavelength or after long periods of inactivity, start with Step 4 again.

Fig. 3: Conical tube, microcentrifuge tube, cuvette (left to right)

Measuring the standards

In order to create a standard curve, you will determine the absorbance of 5 standard solutions, S1 to S5.

They contain the following given amounts of *p*-Nitrophenol. Fill in the absorbance that you measured for each standard in the table below:

Standard	Amount of *p*-Nitrophenol in nmol	Absorbance at 410 nm
S1	0	
S2	12.5	
S3	25	
S4	50	
S5	100	

1 nmol = 1 nanomole = 10^{-9} mol

By plotting your absorbance at 410 nm on the y-axis against the respective amounts of p-Nitrophenol in nanomoles (x-axis) you will create a standard curve. Using that standard curve in the following activities, you will be able to determine from your spec readings how many nanomoles of product a particular sample contains.

NOTE: Activities 1-5 are visualized in the Quick Guide on pp. E1-E9

Activity 1. Determination of initial reaction rate with and without enzyme.

Refer to the Quick Guide.
At the end of this activity 1 you will measure the absorption of your 7 cuvettes and populate the table below. Then proceed to the next activity. Make sure to take detailed notes in your lab book.

Cuvette #	Time (minutes)	Amount of p-Nitrophenol (nmol)	Absorbance (410nm)
Start			
1			
2			
4			
6			
8			
End (Control)			

Additional notes:

Analysis of Activity 1:
a) initial rate of product formation in the presence of enzyme: amount of product (in nanomole) against time in minutes (x-axis).
We use the *initial* rate (where the graph is linear) because towards the end, the substrate becomes more and more depleted.
b) initial rate of product formation (nmol/min) in the absence of enzyme.

Activity 2. Effect of temperature on the enzyme reaction rate

Refer to the Quick Guide.
At the end of activity 2 you will measure the absorption of your 3 cuvettes and proceed to the next activity. Make sure to take detailed notes in your lab book.

Temperature (C)	Absorbance (410nm)	Amount of p-Nitrophenol (nmol)	Initial reaction rate (nmol/min)

Additional notes:

Analysis of Activity 2: Initial reaction rate (nanomoles per minute) at different temperatures. (assuming an amount of 0 nmol at time 0)

Activity 3. Effect of pH on the enzyme reaction rate

Refer to the Quick Guide.
At the end of this activity 3 you will measure the absorption of your 3 cuvettes and proceed to the next activity. Make sure to take detailed notes in your lab book.

pH	Absorbance (410nm)	Amount of p-Nitrophenol (nmol)	Initial reaction rate (nmol/min

Additional notes:

Analysis of Activity 3: Initial reaction rate (nanomoles per minute) at different pH values. (assuming an amount of 0 nmol at time 0)

Activity 4. Effect of enzyme concentration on the reaction rate.

Refer to the Quick Guide.
At the end of this activity 4 you will measure the absorption of your 6 cuvettes and proceed to the next activity. Make sure to take detailed notes in your lab book.

Cuvette #	Time (min)	Absorbance (410nm)	Amount of p-Nitrophenol (nmol)
L1			
L2			
L8			
H1			
H2			
H8			

Analysis of Activity 4: amount of product vs time for the 2 enzyme concentrations (in the same graph, for easy comparison).

Activity 5. Effect of substrate concentration on the reaction rate.

Refer to the Quick Guide.
At the end of this activity 5 you will measure the absorption of your 6 cuvettes. Make sure to take detailed notes in your lab book.

Cuvette #	Time (min)	Absorbance (410nm)	Amount of p-Nitrophenol (nmol)

Additional notes:

Analysis of Activity 5: amount of product versus time for both substrate concentrations (in the same graph, for easy comparison).

Skills Evaluation

There is no skills evaluation for this lab, but it is eligible for a lab report.

Day 3 Labs VI and VII Molecular biology/forensics

(total time to complete = 3h)

Objectives

After finishing these two labs you should be able to

- describe the role of PCR in DNA technology
- describe the principles of electrophoresis
- confidently use electrophoresis for separating DNA and other macromolecules
- describe the use of DNA technology, in particular PCR, in forensic science

Preparation

DNA Technologies chapter in your textbook, in particular the sections about PCR, electrophoresis, and STR.

Introduction

Labs 3-A and 3-B will simulate a crime scene investigation. You will receive DNA from a "crime scene" and compare it with DNA from various "crime suspects". Your task will be to find out whether one or more of the suspects have DNA that matches with the DNA from the crime scene.

During this investigation you will learn about some important techniques used in molecular biology and, by extension, in forensic science. PCR (polymerase chain reaction) has become a standard method in molecular labs, and it involves the synthesis of large amounts of DNA (through a process called amplification) from a tiny amount of a DNA template that was previously isolated from the environment. PCR uses specific DNA primers that bracket a particular target sequence and therefore only the target sequence will be amplified. The process consists of multiple repeats of a sequence of heat denaturation, annealing of primers and extension. Due to the exponential nature of the PCR process, millions of copies of the target sequences are produced.

Electrophoresis is widely used to separate either DNA or proteins. The method is based on the principle that in an electric current, large molecules move faster or slower through an agarose gel depending on both their size and their charge. Smaller molecules pass easier through the agarose gel, while molecules with multiple negative charges are

moving quicker than those with fewer. The combination of these two forces acting on a molecule makes it possible to obtain a high level of resolution.

The differences between the alleles that we investigate are based on a different number of short tandem repeats (STRs) which contribute to different allele sizes. They are located in non-coding regions of the DNA.

Procedures

Safety considerations

There are three possible safety risks for this lab: The electrophoresis apparatus uses a high voltage and should not be tinkered with while the electrophoresis is running. Avoid wet lab benches. Secondly, gloves and safety glasses must be worn for all staining procedures and for preparing the gels. Also, proper waste disposal procedures must be followed for all used stains.

Lab 3-A. Setting up PCR reactions

Familiarize yourself with the items that you find on your workstation. Make sure that your group has a unique number or designation.

Ice bath with 6 tubes: 5 tubes of DNA (suspects A to D, crime scene DNA) as well as a tube with Master Mix and Primer (MMP). Master Mix contains Taq Polymerase, nucleoside triphosphates, ions and buffer. Primers have previously been added to the Master Mix by the instructors.

In addition, you will find five PCR tubes and 5 adapters, a marker pen, a 20 µl micropipet and a rack of micropipet tips.

Label your racks with your group number (or designation).

- Label your PCR tubes as follows: CS (= crime scene), A (= DNA of suspect A), B, C, D. Add your group number or designation (e.g. “C2” is suspect C DNA of Group 2).
- Make sure to keep everything on ice for the following procedures
- Change pipet tips for each step

- To each of the five PCR tubes, add 20 µl of the corresponding DNA from the microcentrifuge tube (example: add 20 µl of suspect B DNA to the PCR tube labelled “B”; immediately eject pipet tip).

- Now add 20 µl of MMP mix to each of the five PCR tubes. Mix the contents by gently pipetting up and down. **Again, change pipet tips after each step!** Close the cap of your PCR tube after mixing.

- Move your capped PCR tubes into their adapters on ice.

- Move the PCR tubes to the thermal cycler. Your instructor will have programmed the unit. The PCR reactions will take about 40 minutes. During this time, you can move to Lab 3-B.

Lab 3-B. Electrophoresis

1. Casting an agarose gel:

- Agarose gels are produced by suspending and heating 3% agarose in electrophoresis buffer (3 g in 100 mL buffer). This will be demonstrated for you first.

- Prepare the gel apparatus to cast a gel. Place the gel casting tray in the chamber with an 8-well comb inserted at one end (sitting in grooves in the tray). Seal the open ends of the tray with the black wedge-shaped casting gates.

- Slowly pour about 40 mL of the liquid gel into the gel tray (use gloves for heat protection). Avoid introducing air bubbles into the gel. Let the gel cool at least 20 minutes.

- Carefully remove the comb and casting gates. Pour a couple of mL of electrophoresis buffer on the gel if it will not be used immediately.

2. Loading and running the gel

Before you begin, ensure that the gel is in the correct position with the wells towards the black electrode.

- Fill the electrophoresis chamber with enough buffer to completely cover the gel.

- Obtain your five PCR tubes that have completed the thermal cycling process. Place each PCR tube in a tube adaptor on a rack.

- Add 10 µl of Orange G loading dye (LD) to each of the five PCR tubes. Mix and eject tip.

- First practice loading the gel with one of the extra gels; use loading dye for the practice run.

- Using a separate tip for each sample, load the indicated volume of each sample into 6 wells of the gel:

  Lane 1: ***Allele ladder*** (size standard), 20 µl
  Lane 2: **CS** 20 µl
  Lane 3: Suspect **A** 20 µl
  Lane 4: Suspect **B** 20 µl
  Lane 5: Suspect **C** 20 µl
  Lane 6: Suspect **D** 20 µl

- Making sure that there are no spills, place the lid on the electrophoresis chamber and connect the jacks on the lid to the jacks on the power supply with red to red and black to black. Switch on the power supply and turn it to 300 V. You have now started your electrophoresis.

- After about 5 minutes observe the progress of the loading dye. The dye front should be moving towards the bottom of the gel. When the loading dye has migrated to the end (approx. 20 min), turn the power down and switch the unit off. The power unit must be completely off and unplugged before you can open it.

3. Visualization of DNA fragments

- Carefully remove the gel and place it in a small tray with 1x staining solution. Stain overnight with gentle agitation.

- Take a photograph of the gel. Instructors will assist.

Your notes and observations:

Make a sketch of your gel or insert a photograph below:

Analysis

The allele ladder is based on the BXP007 locus and it contains all 8 possible alleles of that locus (corresponding to the bands that you see). The size of the alleles in base pairs is as follows: 1500, 1000, 700, 500, 400, 300, 200, 100 base pairs. By visual comparison with the bands in the ladder you will be able to describe the genotypes of your suspects.

Note that the different alleles are the result of a different number of STRs. Each individual has a combination of two alleles. For convenience, we use single- and double-digit numbers to describe the individual genotypes (divide the number of bp by 100). E.g., if suspect F has a band with 1000 bp and one with 300 bp, we designate the genotype as "10-3".

For the lab report (if applicable): your lab report must demonstrate that you understand DNA Technologies and the principles upon which they are based. Also, include the ethical implications of these technologies. See link below of a sample article.

https://www.nature.com/scitable/topicpage/forensics-dna-fingerprinting-and-codis-736/

Skills Evaluation

There will be no skills evaluation. However, Labs VI and VII (combined) are eligible for a lab report.

Day 3 Lab VIII Plant diversity

(total time to complete = 2.5h)

Objectives

After finishing this lab, you should be able to

- recognize the major groups of plants
- distinguish vascular and non-vascular plants
- describe how ferns and related groups differ from angiosperms
- identify monocots, dicots and gymnosperms
- recognize an evolutionary trend towards a reduced gametophyte generation

Preparation

Read the plant chapter in your textbook.

Introduction

Our final lab will cover the phylum plants. Plants are crucial for the functioning of ecosystems (more than animals), because they continuously capture energy from the sunlight, and it is this energy that maintains ecosystems and life on earth. We will look at representatives of non-vascular plants, the mosses and liverworts, first. Ferns and related groups are the remnants of groups that have peaked in the Mesozoic but whose remains are still used by humans in the form of fossil fuels. Among the seed-bearing plants, conifers are the major group of gymnosperms and cover huge areas of the Canadian boreal forest. Most plants, however, are either monocots or dicots and you should become familiar with the differences between them.
Unlike most animals, plants have two distinct generations, the gametophyte and the sporophyte, but you will see an evolutionary trend to reduce the gametophyte stage.

Procedures

Note: Winter labs will only have a limited amount of fresh material.

Observe the material on display and make some sketches (or one drawing).

1. Mosses and liverworts

- Observe the mosses and liverworts which are the only group of

non-vascular plants. Start with the live material and move on to the slides.
- Make sure you can distinguish the two generations. Also differentiate mosses from liverworts.

2. Ferns and horsetails, clubmosses

- Find the spore bearing structures. Look at slides of the gametophyte stage.

3. Gymnosperms

- Look at the live display material and study male and female cones. Locate the seeds.

4. Angiosperms

- Learn to distinguish monocots and dicots.
- Look at various pollen material. Make photographs.

Skills Evaluation

There is no skills evaluation for this lab.

Organism observed (*Scientific name*)	Kingdom PLANTAE LW = liver wort; M = moss; F = fern; E = Equisetum (horse tail); L = Lycopods (club moss); G = gymnosperm; M = monocot; D = dicot	**Notes:** Include one or more of the following: L = Live material; M = observed under compound microscope (include highest total magnification used, e.g. M1000x); DM = dissection microscope (include magnification); P = preserved material; any other observations
1		
2		
3		
4		
5		
6		
7		
8		
9		

EMPTY PAGE FOR ONE LARGE DRAWING
Drawings must include: Date, scientific name, phylum, microscope type and magnification, as well as extensive labeling.

EMPTY PAGE FOR SKETCHES

Sketches are drawn quickly, they can be small, but must have the name of the organism as well as the phylum (or a reference to the table above). Be selective, you are not expected to sketch every organism that you have observed.

Day 3 Lab IX Cheese making

Objectives

After finishing this lab, you should be able to

- Evaluate the process of making cheese curd from milk
- Define the role of enzymes in food technology
- Describe the ninhydrin test for amino acids

Preparation

None required

Introduction

Milk is an emulsion and consists of water, fats, proteins (casein and whey protein), lactose, vitamins, and minerals. It is the precursor of many other food products (yoghurt, butter, butter milk, etc.) but in this lab we will focus on its protein components, particularly casein. We will use rennet, a mixture of several enzymes, including rennin. The latter is a proteolytic enzyme that coagulates the milk, resulting in two components, the liquid whey, and the solid curd.
We will then test the resulting cheese curd for the presence of amino acids after digesting it with a protease. Ninhydrin is a test reagent for amino acids and a positive reaction is indicated by a purple colour change (after heating) if amino acids are present.

Procedures

Chemical safety considerations: Wear gloves and safety glasses in the final part of the lab when you work with ninhydrin!

This lab consists of two parts. In the first part we will produce the cheese curd from milk by adding rennet:

1. Crush a ½ rennet tablet and place in a 400 ml beaker.
2. Pour 200 ml of whole milk into the other 400 ml beaker.
3. Place the beaker with the milk on a hot plate and heat to 40 ˚C stirring constantly. Avoid burning the milk!
4. Remove from heat, pour the hot milk over the rennet tablet, and stir for 2 minutes, then allow the milk to sit on the lab bench undisturbed for 5 minutes or until the milk coagulates. The remaining liquid is whey.
5. Attach the cheesecloth to the empty beaker using the rubber

band, make a depression to hold the curd. After the curd has formed, break it up with the stir rod and pour it through the cheesecloth.

6. Gather up the cheese cloth and squeeze out as much water as possible. Wash the curd carefully, for at least 2 minutes.
7. Spread out the cheesecloth and allow the curd to dry for 5 to 10 minutes. **At that stage, present the cheese curd to the instructor for your skills evaluation.**
8. Dispose of the whey in the sink.

We have now obtained the curd.

In the second part of the lab, we will do an amino acid assay of our cheese curd after exposing the curd to the enzyme protease.
For the assay we use ninhydrin which is an indicator that turns purple in the presence of amino acids.

1. Label one 250 ml beaker “Enzyme” and the other one “Control”.
2. Label one large test tube “Enzyme” and the other one “Control”.
3. Split the cheese curd equally between the 2 beakers.
4. Add 100 ml of water to each beaker and stir vigorously to break up the curd.
5. Add 1 g protease to the beaker “Enzyme” and stir. Also stir the control with a clean stir rod.
6. Allow the mixtures to sit undisturbed for 10 minutes.
7. Place the cheesecloth over the funnels and filter 5 ml of the liquid from the beakers into the respective test tubes.

   Perform the final steps only under the fume hood. Do not remove items from the fume hood. Use gloves and safety glasses for these final steps.

8. Using the disposable pipet attached to the ninhydrin bottle, add 1.0 ml of the indicator ninhydrin to each test tube (by filling the attached and gently mix by tapping the sides of the test tubes.
9. Place the test tubes in a boiling water bath in the fume hood for 10 minutes.
10. Under the fumehood, remove the test tubes and record the color changes.
11. Dispose of the curd in the garbage and the contents of the test tube in the provided waste container for ninhydrin.

Skills Evaluation

Your product (cheese curd) will be evaluated.

Skills Evaluation
Your product (cheese curd successfully obtained) will be evaluated.

Day 4 Lab X Animal diversity

(total time to complete = 3 h)

Objectives

After finishing this lab you should be able to

- develop an overview of the diversity of animals
- recognize insects as the largest and most diverse group of organisms
- recognize major phyla of animals
- confidently use your compound microscope

Preparation

Read the chapters on animal diversity in your textbook.

Introduction

In these two labs you will look at various fresh and display materials of animals with an emphasis on invertebrates (Lab X) and vertebrates (Lab XI). Animals show various levels of organization in their body plans and in these two labs you will look at the most important phyla.

Procedures

In Lab X we will have an emphasis on insects and other invertebrates. Make drawings and continue to develop your microscope skills (where applicable).

When you study the live, preserved, and microscopic material it is important that you are aware which phylum of animals you are looking at. Refer to your textbook if necessary, so that you develop your skills of recognizing important phyla and their levels of organization. Make sure that you look at all the live material.

As a minimum you should study representatives of the following phyla:

Phylum Porifera	Sponges	No symmetry; low level of cell specialization; regeneration from disintegrated animals
Phylum Cnidaria	Cnidarians	Radial symmetry, medusa or polyp body plan; diploblastic
Phylum Platyhelminthes	Flatworms	Bilateral symmetry, triploblastic, incomplete digestive system, without coelom, predators, (but flukes and tapeworms parasites)
Phylum Rotifera	Rotifers	Microscopic animals, pseudocoelom
Phylum Annelida	Annelids	Segmented worms, coelom, metanephridia, closed circulatory system
Phylum Mollusca	Mollusks	Soft body with hard shell, true coelom, open circulatory system, body plan head, foot, visceral mass, mantle
Phylum Nematoda	Roundworms	Pseudocoelom, parasites or predators, important soil organisms
Phylum Arthropoda	Arthropods	Segmented and with chitin exoskeleton, jointed appendages, open circulatory system highest diversity of all organisms
Phylum Echinodermata	Echinoderms	Radial symmetry (secondary!), endoskeleton, water vascular system and tube feet, no head, regeneration
Phylum Chordata	Chordates	Notochord, dorsal hollow nerve cord, gill slits and tail; vertebrates include mammals, birds, reptiles, amphibians, bony fish and cartilage fish

While each of these phyla represents a distinct level of organization, it must be emphasized that there are large differences in diversity. Be mindful that most species of organisms (at least 80%) are insects. Therefore make sure that you study this group and its lifecycle thoroughly during Lab 4-A.

Organism observed (*Scientific name*)	PHYLUM (refer to Table above). If you have the phylum Arthropoda, also include the class	**Notes:** Include the symmetry of the animal observed. B = bilateral symmetry; r =radial; S if the animal is segmented;
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		

Skills Evaluation

There is no skills evaluation for this lab.

Lab questions:

1. Name three properties that only insects have (and no other arthropods!)

2. Why are echinoderms not included in the Radiata (major group of phyla with radial symmetry)?

EMPTY PAGE FOR ONE LARGE DRAWING

Make at least one large drawing. Drawings must include: Date, scientific name, phylum, microscope type and magnification, as well as extensive labeling.

EMPTY PAGE FOR SKETCHES

Sketches are drawn quickly, they can be small, but must have the name of the organism as well as the phylum. Be selective, you are not expected to sketch every organism that you have observed.

Day 4 Lab XI Fetal pig dissection

(total time to complete = 4h)

Objectives

After finishing this lab, you should be able to

- recognize various parts and organs of the internal anatomy of a pig with an emphasis on the digestive tract

Preparation

Watch the video on pig dissection before you start with this lab.
https://www.youtube.com/watch?v=1oM1TUMqN54

Introduction

We will look at fetal pigs as representatives of vertebrates. You should be aware that the internal organs of this fellow mammal are very similar to those of humans.
Note: The fetal pigs we are using for this lab originate from the meat industry and were not killed for the purpose of dissections.

Procedures

We follow the procedure of the pig dissection video. Our emphasis will be on the digestive system.

Skills Evaluation

You will be asked about the position of several major organs of the digestive system and their function. You will have to identify organs that relate to the digestive system that are pointed out to you.

Day 5 Lab XII Diversity of fungi (Mycology)

(total time to complete = 3.5h)

Objectives

After finishing this lab you should be able to

- recognize the representatives of some major fungal phyla
- characterize fungal mycelium, hyphae and spores under the microscope and differentiate fungi from other organisms

Preparation

Read the Chapter on Fungi in your textbook.

Introduction

The 1.5 million species of the Kingdom Fungi are among the most important organisms, and they play a crucial role as decomposers of lignin (only higher fungi are able to degrade this component of wood) and as pivotal components of mycorrhizal symbioses (many plants could not grow without their mycorrhizal fungi). Penicillin, beer, wine and bread are all derived from fungi. Mushrooms are eaten by gourmets and some fungi can clean up toxic wastes. Most fungi grow as a mycelium while some exist as single celled yeasts.

Fungi are good objects for microscopic studies. We will look at five major groups of fungi and you should be able to distinguish them either at the microscopic or the macroscopic level.

Procedures

Fungi are good objects to practice your microscope skills. At the same time, you will see representatives of major fungal groups. You should draw and photograph as many objects as possible.

We will also work with dissection microscopes for larger structures. Your instructors will explain how to use the dissection microscopes.

Note: Winter labs will have less fresh material than summer labs.

1. Basidiomycota

- Observe living mycelium (demonstration: jar or bag with mycelium).

- Observe fungal mycelium by preparing a wet mount from the basidiomycete cultures provided. Using forceps, take a small piece (appr. 1mm^3) of agar with immersed mycelium from the margin of a fungal colony on the petri dish onto your slide. Add Congo Red or a similar stain to increase the contrast and carefully squeeze your cover slip with a pencil until there is a thin film of agar below the covers slip. Use the highest magnification with oil immersion (AFTER moving through the lower magnification lenses). Look for mycelium, hyphae and septa (cross walls). Avoid large air bubbles under the cover slip .

- Observe fresh material of fruitbodies of basidiomycetes (e.g. *Agaricus, Russula, Lactarius,* polypores) under the dissecting microscope. Look especially for the spore producing hymenium which can be in the form of gills, pores, spines or completely smooth. The hymenium is usually on the lower side of the fruitbodies. Use dried material if no fresh fruitbodies are available. Make a drawing or several sketches.

- Observe the prepared spore prints.

- Observe spores in the microscope by transferring a small (!) piece of gill (or pore) onto a slide (use fungi with pigmented spores, such as *Leccinum* or *Agaricus*). Your instructors will help. Soak in KOH (5%) or Melzer's reagent (only for *Russula* or *Lactarius*) for a minute to soften the material, then add a cover slip and carefully squeeze. Tap on the cover slip repeatedly with a wooden pencil until the piece is spreading into a thin film. Observe the spores under the highest magnification. This will work both with fresh and dried material.

- In addition, view the permanent slides of rust fungi and other basidiomycetes. View other demonstration material as provided.

2. Ascomycota

- Observe fresh (if available) or dried material of fruitbodies of ascomycetes (e.g. *Peziza* or *Morchella*) under the dissecting microscope.
- View sclerotia of *Claviceps*.
- Using the compound microscope, observe ascospores and asci (singular: ascus) on the permanent slides of *Peziza*.
- Make a wet mount from the prepared yeast suspension. Look for budding yeast cells. Use the 1000x magnification and immersion oil.

3. Lichenomycota (lichens)

- Observe dried material of various lichen species under the dissecting microscope.
- Observe permanent slides of lichens (*Physcia*) with the compound microscope. Differentiate the fungal from the algal structures.

4. Deuteromycota (imperfect fungi)

- Observe fresh material (plates or mouldy food) of moulds (*Penicillium* and others) under the dissecting scope.
- Observe permanent slides of *Penicillium*; use 1000x magnification.

5. Zygomycota

- Make a wet mount from the cultures of *Phycomyces* (alternatively other zygomycetes). Under low magnification (up to 400x), observe the sporangia and sporangiophores. Then move to the highest magnification (immersion oil!) and observe the spores.
- Observe permanent slides of *Rhizopus*. View the zygospores under the compound microscope.

Skills Evaluation

There will be no skills evaluation for this lab.

Organism observed (*Scientific name*)	Kingdom FUNGI PHYLUM	**Notes:** Include one or more of the following: L = Live material; M = observed under compound microscope (include highest total magnification used, e.g. M1000x); DM = dissection microscope (include magnification); P = preserved material; any other observations
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

EMPTY PAGE FOR ONE LARGE DRAWING
Drawings must include: Date, scientific name, phylum, microscope type and magnification, as well as extensive labeling.

EMPTY PAGE FOR SKETCHES

Sketches are drawn quickly, they can be small, but must have the name of the organism as well as the phylum (or a reference to the table above). Be selective, you are not expected to sketch every organism that you have observed.

Day 5 Lab XIII Evaluation of previous experiments, Discussion of lab results.

How to do lab reports

(total time to complete = 2h)

In this last section of the course, you will be instructed on how you prepare your lab report which counts for 35% of your lab grade.

Please remember that the lab report should be written like a scientific article and you are strongly encouraged to read a few scientific publications to get an idea about scientific style. Before you write the lab report, read the section on Academic Integrity below.

Academic Integrity

Students are expected to adhere to the principles of academic integrity and avoid plagiarism when *submitting lab reports*. Plagiarism is defined in AU's undergraduate calendar (http://calendar.athabascau.ca/undergrad/page11_02_new.php#plagiarism) as follows:

Plagiarism involves submitting or presenting work in a course as if that work were the student's own, when, in fact, it was not. Often plagiarism exists when:

1. the work submitted was done in whole or in part, by an individual other than the person submitting the work
2. the whole or parts of a work are taken from another source without reference to the original author, publication, journal or Internet source
3. the whole or parts of the coursework submitted lacks citations even though a list of sources is provided
4. the coursework has been copied in whole or in part from an individual, a textbook, a solution manual, the Internet or any other source
5. when paid or professional editors are used inappropriately.

Students are encouraged to contact the individual to whom their coursework is being submitted to discuss their plan on the use of an editor prior to submission of their coursework.

In the context of this lab, students are encouraged to cooperate and to share raw data, photographs and results with their peers. However, the lab reports must be written by each student on their own. Failure to do so would result in a **0 grade for the 207 lab component**. Also note that submitted lab reports will be compared with online "student help sites" for a plagiarism check. Lab reports that are plagiarized from online sources will incur a grade of 0 for the entire lab component.

Citation style for the lab reports

Students are expected to use ONLY CSE style in the Name-Year format (NOT the citation-sequence nor the Citation-Name format). For more details, consult the link to CSE style on the library website: https://libguides.athabascau.ca/citationguide/othercitationstyles

Remember: you will have to use CSE style, using the Name-Year system for citations.

Marking Guide

Here is our marking guide, with some hints for writing a successful lab report.

Note: Use caution with the sample lab reports posted on the world wide web. None of these posted lab reports would receive an A grade in BIOL 207.

1. Title (3 marks)

- The title must be descriptive and in one sentence, include all relevant information about the experiment. Try to write in a sentence what you did.

Example of a good title:

> "Indirect measurement of net photosynthesis in Coleus by evaluating buoyancy of floating leaf disks"

Example of a title that is not satisfactory:

> "Lab 2 Osmosis"

2. Abstract (15 marks)

- Typically, not longer than a paragraph (avoid lengthy introductory statements and discussions)
- **Must briefly state the main results; avoid vague and general**

statements

- No citations should be included
- Should state a brief research hypothesis and indicate if results support hypothesis or not

3. Introduction (15 marks)

- Give a general background of the topic and its relevance.
- Must include a minimum of two different citations
- Citations must be included within the same sentence (statement) that they refer to, rather than at the end of a paragraph.
- Provide a clear research hypothesis, but only at the end of the introduction.

Example for a research hypothesis:

> "For our study it was hypothesized that a 30-minute exposure to a 50% watery THC solution would result in the revitalization of some of the brain cells in culture."

4. Material and Methods (7 marks)

- Avoid recipe style and use past tense: Don't write "Add 15 g of sugar" but rather "15 g of sugar were added."
- Use SI units and the metric system. Inches, pounds and miles are not acceptable.

5. Results (15 marks)

- Must include a narrative. Write in clear sentences what your results were and include illustrations (photos) from the labs; if appropriate, include tables.
- Tables and figures (photos or other illustrations) should be included where appropriate.
- Tables and figures must be successively numbered and have a legend (complete descriptive title); your narrative must mention all figures and tables.

Example:

> "Fig. 3. Test tube showing precipitation of red pigment 10 minutes after adding the solvent."

Example:

> "Table 5. Number of *Drosophila m*. individuals caught after 24 hours in traps with various baits."

- No discussion of results.

- If appropriate, graphs should be included as figures.

6. Discussion (20 marks)

- In a discussion, you compare the results you obtained with results that you find in the literature (use citations in CSE style) and then give an interpretation. Obviously, you need to do some searching to find literature that covers the same or similar topics as you did in your experiment. The literature may or may not confirm your results, if not, try to come up with an explanation for why they are different.
- Discussions are thorough and are typically the longest part of a lab report. Discuss all your results in detail first. *In the final part* of the discussion, you can include the "bigger picture" by pointing out broader applications.
- Your discussion must include a minimum of four citations.
- Indicate if your experimental results confirmed your hypothesis. If they don't, come up with possible explanations why they didn't.
- Results must be explained, not just repeated. Why is it important to understand this information? What are possible sources of experimental error? (if appropriate).
- Whenever appropriate, refer to tables and figures from the Results section.

7. Citations and references (15 marks)

- Note the difference between citations and references (sometimes referred to as "in-text citations" and "end references)."
- Citations are in the text, written in the Name-Year system and follow CSE style. Don't include page numbers and author initials in the citations.

Examples:

> "Hamilton and Dolittle (2001) reported the enzyme as having an optimum temperature."
> "...there is no agreement on the mechanism of pigment interaction (Corner et al. 1995; Smith 2000)."

- References are detailed, in alphabetical sequence, placed at the end of the lab report, and must adhere to the format given in the CSE resource.
- Out of 15 points total, you will receive 5 points if citations and references are formally correct (i.e., follow CSE style, citations match references, etc.). You will receive an additional 10 points depending on the quality of your references: books and journals

incur three points each; web resources and course material carry one point each.

- Do not use direct quotations in science writing. For example, the sentence below is not acceptable scientific writing:

According to Smith and Webster (2003) "all Cactaceae can be categorized as phylogenetically advanced…"

Instead, you could write:

> Some authors consider the Cactaceae as a phylogenetically advanced family (Smith and Webster 2003)…

8. Language, grammar, style (10 marks)

- Use well-written English sentences with correct grammar and style.
- Use the past tense.
- Latin names of species (scientific names) must be italicized, e.g., *Homo sapiens* rather than Homo sapiens.
- Science writing abhors the words *proof* or *to prove* as they do not conform to the scientific method and the scientific way of thinking. Instead of writing "the results prove that…" you may write "the results provide strong evidence that…"

Acknowledgements:

The enzyme lab and the forensic lab are adapted with modifications from the Biorad Forensic Kit and the Biorad Biofuel kit.
Fig. 1 is adapted from Conn HJ and Jennison, eds., Manual of Microbiological Methods, Mc Graw-Hill, 1957