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Tell me, what's lurking in your nose? Using flowcharts and oral laboratory reports to assess learners' knowledge

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Abstract

Isolation of bacteria present in the human may be used to teach students basic microbial techniques, such as colony isolation, Gram staining and rapid enzymatic tests, to identify major groups of bacteria. In our second-year microbiology laboratory, results from this exercise are used to produce one of the laboratory reports students complete during the semester. As part of an effort to use a variety of assessment tools to accommodate different learning styles, as well as teach different communication skills, we have replaced the traditional pre-lab quiz with a flowchart activity and the written report with an oral report for this lab. Implementing oral reports requires careful planning at the beginning of the semester to fit within schedule constraints, particularly in the case of courses with a large number of students and/or multiple sections. Clear instructions for the students and careful rubric design for assessment reduces potential resistance from the parties involved. We have included a set of instructions for student use for the for the lab, flowcharts, and oral report, and another set of instructions for those activities for the instructor and/or teaching assistant. Additionally, examples of student work and a marking rubric can be found in the appendices.

Keywords: Microbiology, Teaching Tools and Techniques

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INTRODUCTION

This lab exercise consists of having students create flowcharts to prepare for the laboratory exercises and use basic microbiology lab techniques; summative assessment is done as an oral report.

Pre-lab flowcharts

Flowcharts can be a useful evaluation tool to assess student preparedness for laboratory exercises in any discipline from Botany to Microbiology. For the lab to function safely and efficiently, students must be able to demonstrate that they have done the requisite readings and have reviewed the protocols for the lab experiment(s). For example, for this lab exercise, it is important that students understand basic microbiology safety rules, know how to use proper aseptic techniques, and understand good microbiological practices to minimize risk and prevent the spread of contaminated materials. Good practices include no drinking and eating in the lab, always wearing a lab coat and close toed shoes, covering open wounds, no bookbags and jackets on lab benches, and avoiding touching eyes and mouth. Students should be prepared and understand the steps and timing involved in conducting a T-streak, heat fixing and staining samples.

Many instructors choose to have a pre-lab assessment, often in the form of a quiz, to make students accountable for this preparation. However, the preparation and marking of quizzes take a lot of instructor's/TA's time and may use up precious wet lab time. In addition, the time spent by students preparing for quizzes is often passive, students may read the protocol but may not challenge themselves to verify if they understand and can apply what is written. The quizzes themselves reward memorization, not synthesis and higher-level thinking and are not authentic tasks. Finally, the quiz is often stressful, may not allow capture of the “real” knowledge of diverse learners, and can be a negative start to the lab.

We have both moved to having students prepare a flowchart of the planned experiment in lieu of a pre-lab quiz. Flowcharts are a simple way for students to visually demonstrate an understanding of the lab experiment protocol. Examples of student flowcharts are included in Appendix B, and we encourage readers to also read work by Burnette (2020), as cited in the reference section. In short, the benefit of having students sketch out the steps for the lab activities is that it allows them to: 1) engage in an authentic task, as the flowchart will be useful to them during the lab, 2) maximizes student reflection, higher level thinking, and recall of the protocol, and 3) allows us to quickly determine student preparedness and give immediate feedback to correct any misconceptions before the lab begins. Burnette (2020) also noted that when students created pre-lab flowcharts, they were better able to help each other with procedural questions, and that they used time in lab more efficiently, finishing earlier.

We have chosen to have a small grade associated with the pre-lab flowcharts. As students come in, the teaching assistant or instructor checks their flowchart. As this is the weekly routine, students improve quickly. They report preferring the preparation of flowcharts to taking quizzes, even if it takes them a little more time at first than studying would.

Nose culture lab

We do the nose culture lab described here with second year students, but the lab could be done with first year or upper-level students as well. No CL2 containment laboratory is required for this exercise, since unknown isolated cultures are rated as RG1 organisms (risk group 1). Therefore, it is considered safe to conduct most procedures on a benchtop in a regular lab, under aseptic conditions. Organisms isolated from the nose pose minor risk to the student population. Potential RG2 (risk group 2) cultures that may be isolated include Beta hemolytic *Staphylococcus aureus* and Beta hemolytic *Streptococcus pyogenes.* All bacterial cultures and contaminated materials/waste (such as isolated samples on agar plates, and soiled swabs and inoculating loops) must be disposed of properly, and appropriate biosafety practices applied to contain and minimize exposure to hazardous materials.

Blood agar plates should be ordered a few weeks ahead of time to allow for timely delivery. Blood agar plates are inoculated by students at least 48-72h prior to their scheduled lab, which only requires a few minutes of their time. Identification of nose isolates is completed during scheduled lab day. The lab exercise is scheduled for 3 hours but many students can complete the lab within 2 hours. The nose lab is scheduled later in the course when students have already learned aseptic techniques, the T-streak method, making a bacterial smear and Gram staining.

Post-lab oral laboratory report

Traditional written laboratory reports are limited in helping instructors capture student’s depth of understanding of the material and can result in a lot of effort from the student without necessarily helping them to engage actively with the findings (Carmel *et al.* 2019). Oral lab reports allow instructors to ask follow-up questions to determine what students really understand and to what depth (Burrows *et al.* 2021). Hambrecht (2003) notes that students also learn more in preparing for oral reports, as the accountability of a face-to-face assessment motivates them to think about the material at a higher level to feel ready to answer questions. During the discussion, oral lab reports also allow instructors to quickly give feedback and correct misconceptions. They allow students to practice oral skills and can provide an alternate mode of representation of knowledge that can foster a more inclusive classroom/lab experience for diverse learners. We have included the table that students fill out during the lab to prepare for the oral lab report (Appendix C), as well as the oral laboratory report rubric used to assess students (Appendix D). Please see Burrows *et al.* (2019) for other examples of oral lab report rubrics.

STUDENT OUTLINE

Objectives

Create a flowchart to use as a guide for your experiment (see guidelines in Appendix A)
Determine presence of hemolysins and catalase enzyme, and Gram stain result for each isolate
Attempt to identify 2 bacterial nose Isolates using Blood Agar for growth medium

Introduction

The upper respiratory tract harbours a wide variety of microorganisms, and today you will attempt to isolate and identify bacteria from your own nose. Gram-positive bacteria are the most abundant bacteria found, but there are some Gram-negative genera as well. In addition to colony morphology and cell morphology, the catalase test and oxidase test are two rapid tests that aid in further identification of nose isolates.

Catalase is an enzyme that degrades hydrogen peroxide into water and oxygen $2H_2O_2 \rightarrow 2H_2O + O_2$
Hydrogen peroxide is toxic to bacterial cells, but it is also a common by-product of metabolic reactions that take place in the presence of water and oxygen. Therefore, most organisms that can survive in an atmosphere containing oxygen produce enzymes to degrade peroxide. *Streptococcus* and *Lactobacillus* are the most frequently encountered exceptions to this rule. Neither of these two genera produces catalase, nor is the enzyme catalase produced by anaerobic bacteria. In this exercise, you will practice several methods of evaluating microbial catalase production as well as aseptic procedure.

1. Place 2 drops of 3% hydrogen peroxide on opposite ends of a microscope slide.
2. Use a sterile toothpick to transfer a colony of *E. coli* from the TSA plate to one of the drops. Report and describe the catalase reaction. Discard the toothpick in biohazard bag.
3. Using a new sterile toothpick, add a colony of *Enterococcus faecalis* to the second drop of hydrogen peroxide. Report and describe the catalase reaction.
4. Discard the glass slide and toothpick in appropriate biohazard containers and disinfect your bench.

Hemolysins

Hemolysins (Greek *haima:* blood, and *lysis:* dissolution) are exotoxins that can lyse red blood cells. Two kinds of hemolysins were identified from bacteria grown on blood agar plates. Alpha (α) hemolysins partially break down hemoglobin and produce a greenish ring around colonies. Beta (β) hemolysins also lyse blood cells but completely break down hemoglobin and leave a clear ring around colonies. Streptococci and staphylococci produce different hemolysins that are helpful in identifying them in lab cultures. Bacteria that can produce hemolysins can grow better than those that do not produce these enzymes. Bacteria that do not produce hemolysins and show no hemolysis on blood agar plates are sometimes referred to as gamma (γ) hemolytic or non-hemolytic.

Staphylococcus

The genus *Staphylococcus* is composed of both pathogenic and non-pathogenic forms. They are Gram-positive cocci that occur most commonly as grape-like clusters of spherical cells. They can survive outside of the body for extended periods of time. Many staphylococci are indigenous to skin surface and mucous membranes of the upper respiratory tract. Breaks in the skin and mucous linings may serve as portals of entry into underlying tissues, with the possibility of infection by virulent strains.

The three major species are: *S. aureus, S. saprophyticus,* and *S. epidermidis*. Strains of the last two species are generally avirulent, however if a suitable portal of entry is provided, *S. epidermidis* may be the etiological agent for skin lesions and endocarditis. *S. saprophyticus* has been implicated in some urinary tract infections.

The most common habitats of *S. aureus* are the upper respiratory tract (especially the nose and throat) and the surface of the skin. Many healthy people are carriers, and in most cases, resident staphylococci do not cause disease. Serious staphylococcal infections occur when the resistance of the host is low because of hormonal changes, debilitating illness, wounds, or any treatment that compromises the immune system. Pathogenic strains of *S. aureus* are often responsible for the formation of abscesses (localized pus-producing lesions). These occur on the skin, resulting in boils, carbuncles, acne and impetigo.

Extensive use of antibiotics has resulted in the natural selection of resistant strains of *S. aureus* and *S. epidermidis*. Hospitalacquired (nosocomial) infections with antibiotic-resistant staphylococci often occur in patients whose resistance is lowered due to other diseases, surgical procedures, or drug therapy. Patients often acquire staphylococci from hospital personnel who are asymptomatic carriers of drug-resistant strains. Therefore, appropriate antimicrobial drug therapy for *S. aureus* infections is a problem. Prevention of staphylococcal infections is problematic because most individuals are asymptomatic carriers, and some diseases, such as acne, can be transmitted by simple contact with contaminated fingers.

When grown on blood agar, hemolysis patterns are useful in identification. *S. aureus* demonstrates **β hemolysis** on blood agar. *S. aureus* is also capable of producing a heat-stable enterotoxin that is associated with food poisoning. A second toxin, TSST-1, is involved in the induction of staphylococcal toxic shock syndrome in tampon-using females.

Streptococcus

The genus *Streptococcus* is also comprised of a wide variety of both pathogenic and non-pathogenic (commensal) Gram positive cocci. They are typically facultative anaerobes and are found to inhabit a wide range of hosts, including humans, horses, pigs and cows. Within these hosts, streptococci are often found to colonize the mucosal surfaces of the mouth, nostrils, and pharynx. However, in certain circumstances, usually under disease conditions, they may also inhabit the skin, heart, or muscle tissue.

Pathogenic streptococci include *S. pyogenes, S. pneumoniae*, and *S. faecalis*. Among the pathogenic **β hemolytic** streptococci, *S. pyogenes*, or group A streptococcus, has been implicated as the etiologic agent of acute pharyngitis ("strep throat"), impetigo, rheumatic fever, scarlet fever, glomerulonephritis, and invasive fasciitis.

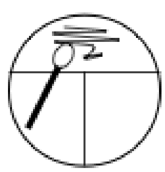
S. pneumoniae are nearly always α-hemolytic but have been shown to exhibit ß-hemolysis during anaerobic incubation. In contrast, most oral streptococci and enterococci are non-hemolytic and thus considered gamma types.

Methods and Data Collection (use aseptic technique)

Nose swab and T-streak

This portion of the experiment is to be done by each student on your own time 24h-72h prior to your scheduled lab period and will only take 5 minutes! A message will be sent as a reminder.

1. Dip a sterile swab into saline then obtain a nose sample by gently rolling the moistened swab in the opening of both nostrils. This may tickle.
2. Inoculate the blood agar plate by rolling and swabbing a small area on the top portion of each plate as shown below.



3. Then using a sterile wire loop, use the T-streak method to obtain isolated colonies. Begin by streaking over the swabbed area several times, then flame your loop and proceed with the T-streak.
4. Place at the front bench and all blood agar plates will be incubated in the candle jar at 37°C for 24-48 hours. This is to promote B-hemolysis visualization under semi-anaerobic conditions.

Identification (complete during your regular lab period)

5. Following incubation, you will try to identify **two isolated colonies** with different appearances.
6. Look for hemolytic and non-hemolytic colonies on your blood agar plate (see example plate/photo at front of the lab). If there are several colonies of this description on the plate, record colony morphology (this includes hemolysis).
   If you are unsure which to identify, pick the predominate colonies that are also well isolated to allow for further tests.
7. Determine if your isolates produce the catalase enzyme.
8. Gram stain each isolate. Do not forget to **add a small drop of water to your microscope slide to make a smear.**
9. Record **all** colony morphology (from agar plate) and cell morphology descriptions (from microscope slide) and summarize the data into one table in your assignment. Gram results (positive or negative) are included in cell morphology.

MATERIALS

Blood agar plates may be ordered (10 per pack) from suppliers such as Fischer Scientific, VWR, Cole Palmer, Hardy Diagnostics etc., and must be kept refrigerated until lab day. Do not store them for longer than 2 months. Sterile water or saline can be purchased or prepared in the lab and aliquoted into glass tubes or microcentrifuge tubes for students to dampen their sterile swabs (also purchased from Fisher, VWR or the drugstore). Wire loops may be used to prepare T-streaks, but to avoid using gas/flame for student plate set up day, plastic pre-sterilized loops can be purchased individually wrapped or in larger packages. Used swabs and loops must be disposed of appropriately (decontaminated or autoclaved). A candle jar is handy to promote the growth of fastidious organisms and to better demonstrate hemolysis on blood agar plates. Metal candle jars can be purchased, or substitutes such as glass cookie jars, metal stock pots and desiccator jars work just as well when tape or parafilm is used along the opening of the jar/pot lid to prevent the entry of O_2.

NOTES FOR THE INSTRUCTOR

A note regarding the use of oral laboratory reports: We have used oral lab reports in a variety of formats. They can be done individually or with groups (randomizing who answers what question), be short (5 min) or more in-depth (30 min), focus on just one or two aspects of a lab or be an oral equivalent of a full lab report with all sections addressed, questions and/or rubric can either be shared with students or not, and finally, the oral report can either be done during lab time or out of lab as appointments. There is a lot of flexibility with how you could use this assessment tool.

In addition, students might at first be apprehensive about the idea of an oral assessment (as addressed in Burrows *et al.* 2021), however, their initial uncertainty can be assuaged by having them do a practice run with their lab partners, having the first oral report be low stakes (a small grade) and potentially giving students the questions and/or rubric for their first experience with this form of assessment.

CITED REFERENCES

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About the Authors

Hélène d’Entremont started working at Acadia University as a microbiology technician in 1994 and has been an instructor since 2000 teaching Microbial Biodiversity, Cell & Molecular Biology, and more recently, Applied & Environmental Microbiology laboratories.

Jennifer Kershaw has been teaching botany courses and labs at Acadia for 6 years.

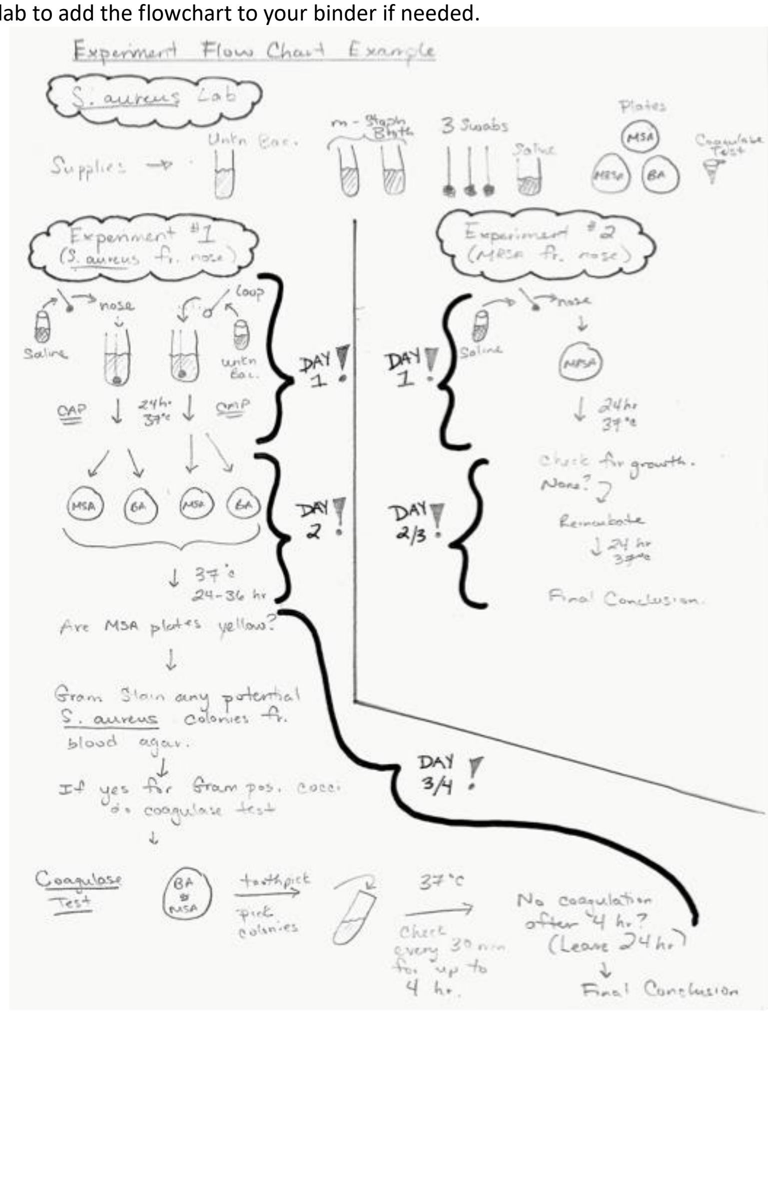
APPENDIX A – FLOWCHART GUIDELINES FOR STUDENTS

Pre-Lab Assignment | Experiment Flowchart

Experiment Flowcharts are a way of assessing whether you have come to the lab prepared, (I.e., have read the manual & have a good general idea of what will happen during the lab period).

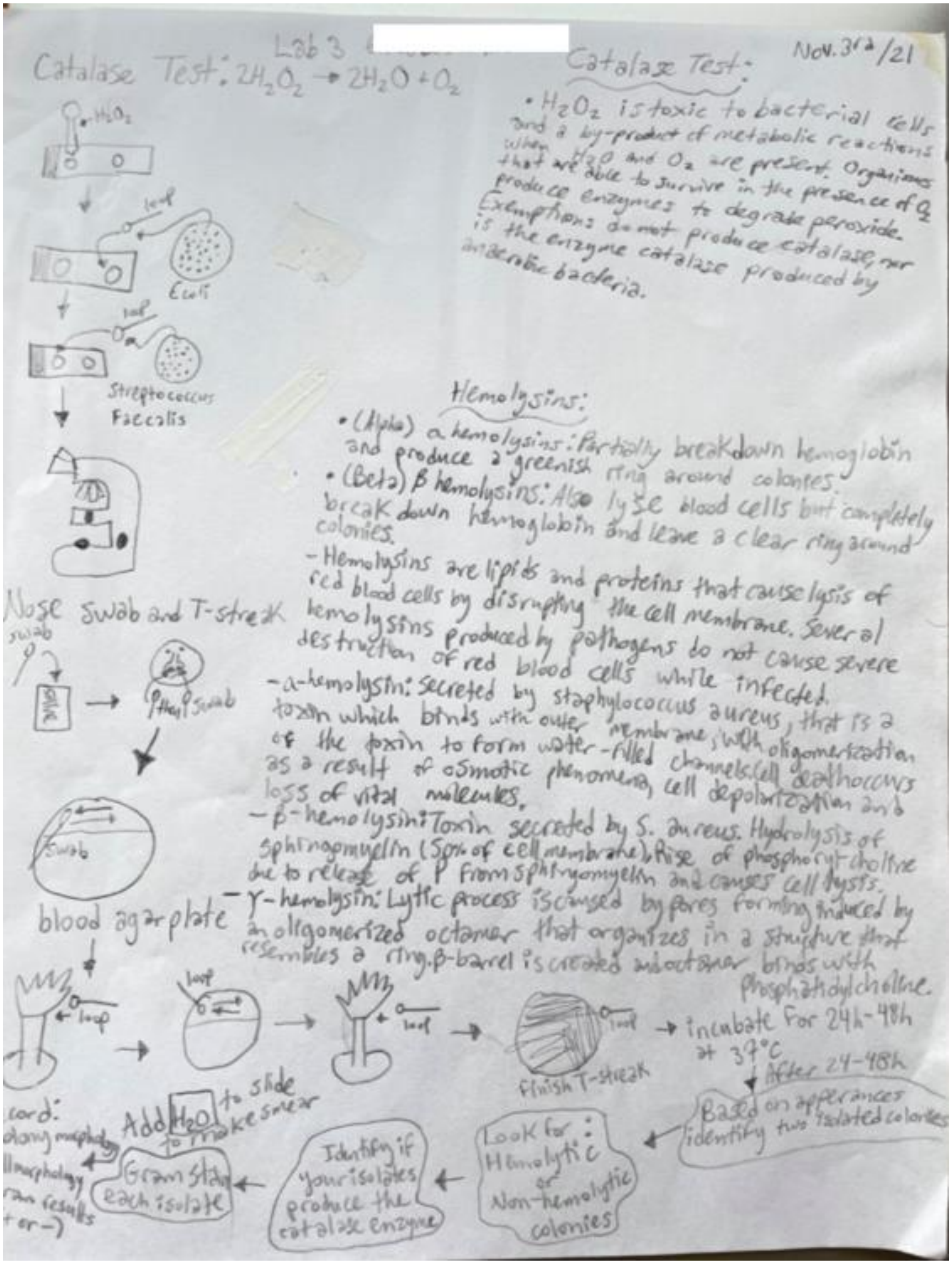
General Guidelines

1. See the following page for an example of what is meant by an 'Experiment Flowchart.'
2. Your 'Experiment Flowchart' must meet the following objectives:
   a. Provide evidence that you have read the lab protocol for that day.
   b. Indicate that you know the general string of events that are to occur on each day. I.e., during a lab period and any/all comeback times.
   c. Include visuals of the materials used during the lab. E.g., hand-drawings, photos from the web, etc. You can get creative with this!
3. Your 'Experiment Flowchart' must also:
   a. Include a clear step-by-step order of events. These can be generalized; there is no need to re-copy everything in the manual (see attached example).
   b. Contain the timing of steps & any/all incubation periods/temperatures.
   c. Alert the reader of any important observations to make during the experiment(s).
4. We do recognize that not everyone is an artist, however, assignments still need to be legible and organized. If we cannot understand what you have written, then points will be deducted. Sideline: Computer generated flow chart will also be acceptable. E.g., using Paintbrush, PowerPoint, etc. However, they must be printed off and meet the page limit requirements.
5. There will be a max page limit of 2 letter-sized sheets (8 ½ by 11 inches each). You can also use both sides. A 3-hole punch will be available to use in the lab to add the flowchart to your binder if needed.

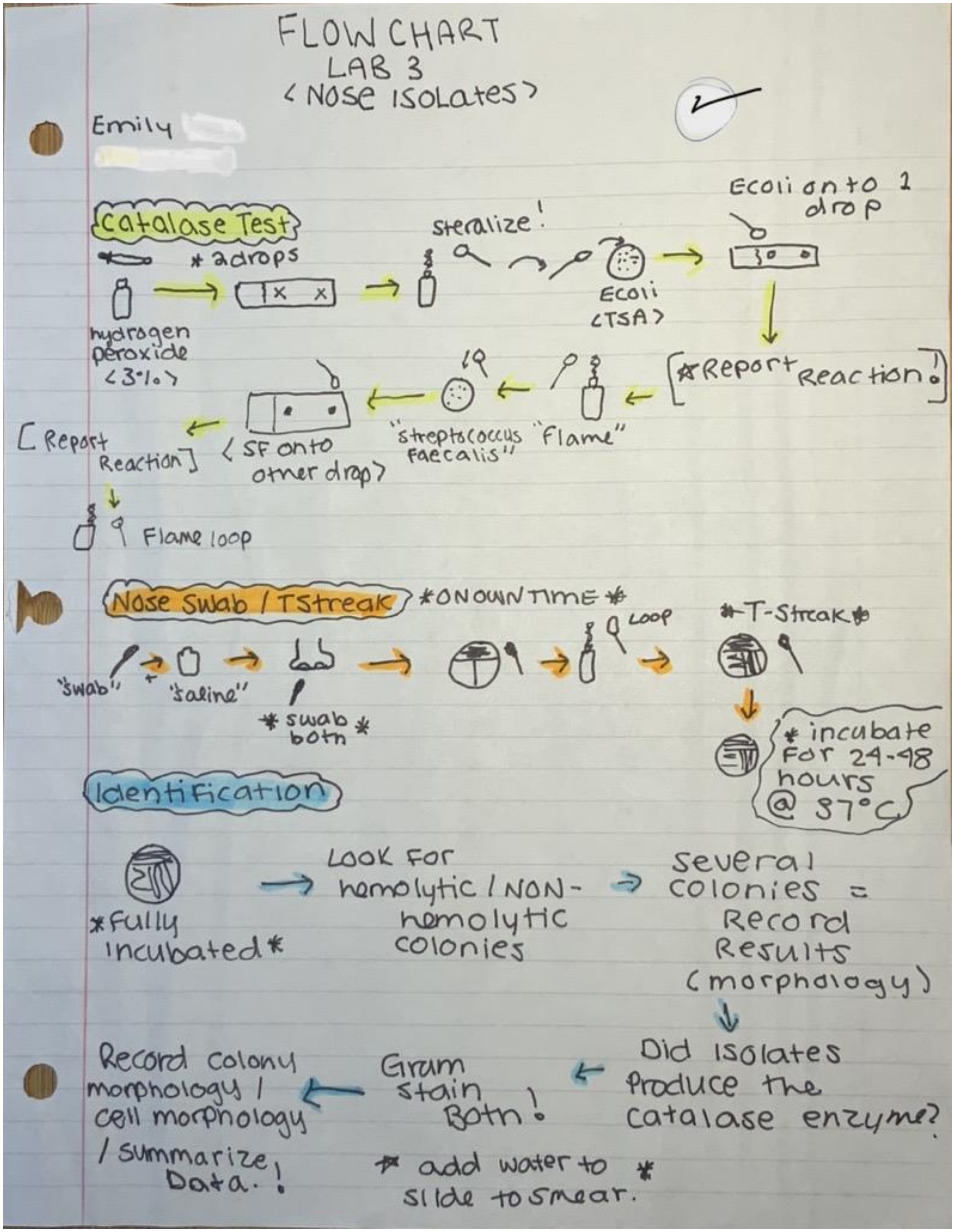


APPENDIX B – SAMPLE FLOWCHARTS

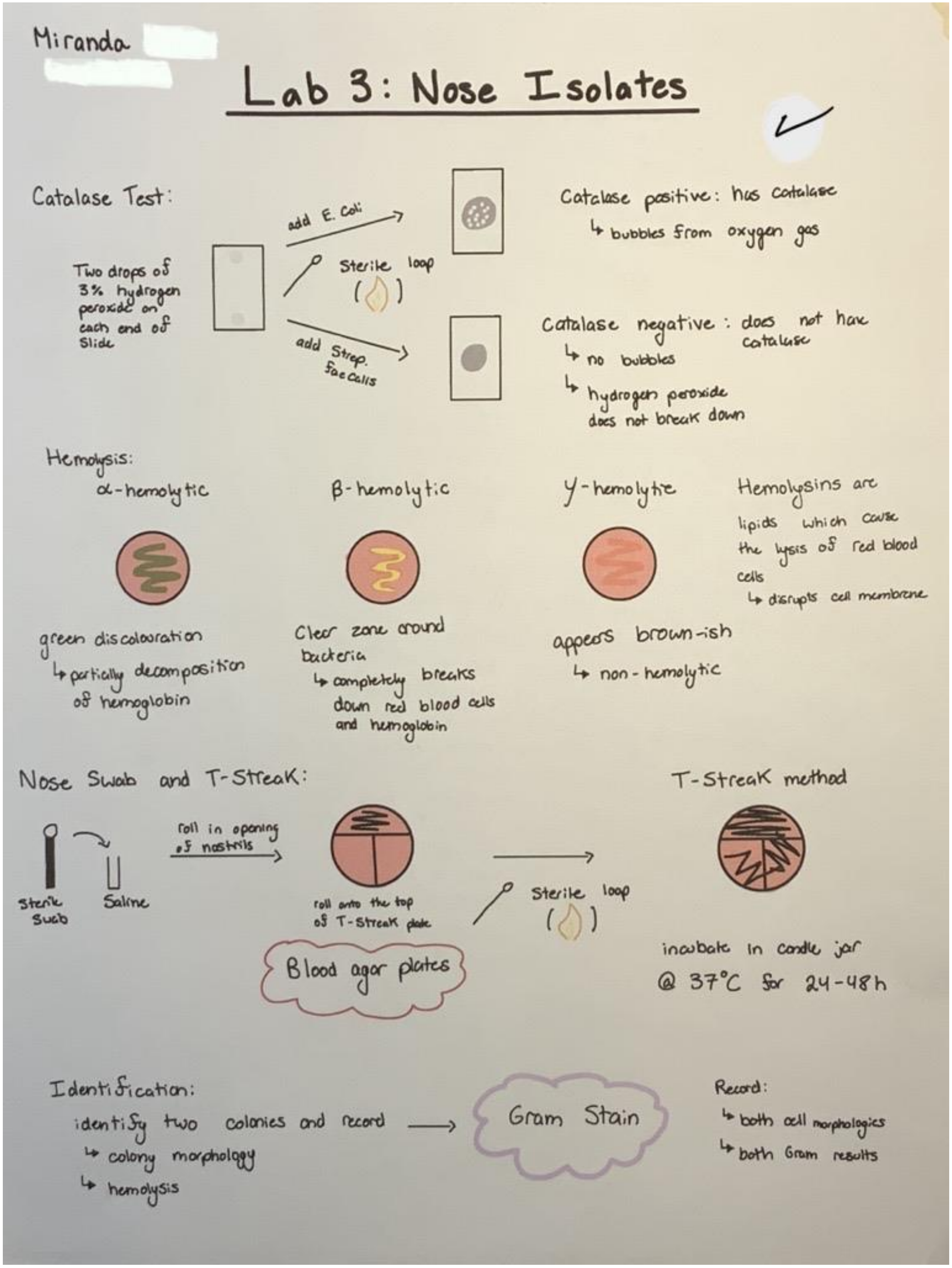
The following is an example of a poor flowchart; it has too much text. This student would be encouraged to remove the text for next time, and to add more to the drawing (using small labels for timing, quantity, temperature, reagent name, etc.):



The following is an example of a good flowchart. It is clear and concise. It illustrates all the main steps and includes short, informative labels for timing, quantities, temperature, reagent name, etc.:



This is also a good flowchart; the student chose to draw it free hand on their I-pad so it has a more polished look, but the previous example draw on paper is also excellent. We want students to have choice, and be focused on utility and accuracy, not how polished the flowchart looks:



APPENDIX C – STUDENT ORAL LAB REPORT PREPARATION TABLE

Table 1.

Characteristics	Colony 1	Colony 2
Colony morphology		
Cell morphology		
Catalase test		

Rather than writing up a lab report, you will be tested during a 5-minute oral assessment on your regular lab day during the week of October 22-24. You will be shown your Blood Agar plate, or a picture of it and will be asked various questions regarding your chosen isolated colonies. The following is to help guide your responses.
Create a **table,** which includes:

- colony morphology of each isolate (include hemolysis)
- cell morphology descriptions for both (Gram, shape, arrangement)
- produces or does not produce catalase enzyme

Think about

- why you chose those particular isolated colonies
- how you identified them
- are you confident in your ID or could they be another organism?
- errors, difficulties, or unexpected results?
- Additional characteristics or facts regarding these isolates (e.g. origin, pathogenicity, antibiotic resistance, issues in health care, elderly, infants etc.)

APPENDIX D – ORAL LAB REPORT RUBRICS FOR TAs and INSTRUCTOR

TA Rubric for Oral Report (Part 1)

PLATE # _______ NAME:_____________________

1. How many colonies did you identify? (circle one) 1 2
2. How did you choose your isolates? (check) isolated ☐ hemolysis ☐
3. Describe the colony morphology of each isolate (circle all that apply)

#1.	**hemolysis**	Beta	Alpha	Gamma
	colour	Yes	No	
	shape/elevation	Yes	No	
#2.	**hemolysis**	Beta	Alpha	Gamma
	colour	Yes	No	
	shape/elevation	Yes	No	

4. Describe the cell morphology of each isolate (circle all that apply)

#1.	**Gram**	Positive	Negative		
	shape	Cocci	Rods	Other	
	arrangement	Clusters	Chains	Random	Other____
#2.	**Gram**	Positive	Negative		
	shape	Cocci	Rods	Other	
	arrangement	Clusters	Chains	Random	Other____

5. Catalase test result (circle) #1. positive negative
   #2. positive negative

6. Please state the full **Genus** and **species** name of each identified colony. Take your time. (circle all that apply)

Colony #1. Colony #2.

Genus	**Species**	**Genus**	**Species**
Staphylococcus	*aureus*	*Staphylococcus*	*aureus*
Streptococcus	*epidermidis*	*Streptococcus*	*epidermidis*
Corynebacterium (aka diphtheroids)	*pyogenes*	*Corynebacterium* (aka diphtheroids)	*pyogenes*
Moraxella	Viridans group *sanguis, sanguinis mutans, mitis, oralis*	*Moraxella*	Viridans group *sanguis, sanguinis mutans, mitis, oralis*
Haemophilus	*pneumoniae*	*Haemophilus*	*pneumoniae*
Neisseria	*parainfluenzae*	*Neisseria*	*parainfluenzae*
Lactobacillus	*catarrhalis*	*Lactobacillus*	*catarrhalis*
Proteus	*spp*	*Proteus*	*spp*
Other	Other	Other	Other

Proper pronunciation (½ for partial) 1. Yes No 2. Yes No

Instructor Rubric for Oral Reports (Part 2)

A: You Identified 2 isolates, what were they?

Correct pronunciation	1. Yes	No
	2. Yes	No

B: How did you choose your isolates?

Hemolysis, isolated, within streak zone etc.

C: How did you know you didn't have….?

Difference between staph and strep, Gram +/-, within a Genus (morphology)

D: Tell me a little more about…?

Students were asked to look up "extra information" about their ID, can cause illness under certain circumstances, also found in…, etc.

E: Did you prefer an oral report over a written one?