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Assessment of Nursing Student Learning Outcomes in Traditional and Online Modalities for a Microbiology Laboratory Course

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COVID-19 has created the need for teaching labs to adopt online modalities. Online options for lab-based courses, particularly in a college of nursing, often levies concern for equal learning outcomes between the traditional, on-campus and at-home, online versions. This study reflects on the data generated from student performance between a pilot online lab section and four traditional in-person sections of Introduction to Microbiology to gauge overall completion of course learning objectives. This course is a requirement for University of Vermont students enrolled in the nursing program, and the instructors' goal was to ensure an equivalent learning experience for the web-based students who did not partake in the hands-on laboratory experiments. This reflective study reports the end of course grades, frequent comments and critiques on assignments, and course evaluations between the two modalities. Students also completed the Microbiology Concept Inventory from the American Society for Microbiology Curriculum Guidelines at the beginning and end of the semester to evaluate improvement in microbiology knowledge and problem solving. The instructors conclude there were no significant differences in student performance between modalities. The successful implementation of the online option provides strong support for retaining web-based laboratory experiences in microbiology in future semesters.

Keywords: online modality, microbiology for nursing, virtual laboratory, evaluating learning objectives, Gram stain

Link To Supplemental Materials: https://doi.org/10.37590/able.v42.sup58

Link To Original Poster File: https://doi.org/10.37590/able.v42.poster58

Introduction

Virtual or "distance" learning is becoming increasingly popular in postsecondary education. Indeed, in the fall of 2019, 36% of all college students in the US enrolled in at least one online course, and 15% of students had an entirely online semester (NCES, 2021). The COVID-19 pandemic created an even larger need for online learning options in 2020. While virtual courses offer convenience of instruction and accessibility, online options for lab-based

courses, particularly in a college of nursing, often levy concerns for equal learning outcomes between the traditional and online versions. This study reflects on the data generated from student performance between a pilot online lab section and four traditional in-person sections of Introduction to Microbiology to gauge overall completion of course learning objectives. The Microbiology in Nursing and Allied Health (MINAH) Undergraduate Curriculum Committee has responded strongly on maintaining microbiology curriculum for undergraduate nursing programs (Norman-McKay *et al*., 2018). However, some in-person lab skills may not be relevant for successful nursing careers. A large-scale survey from registered nurses revealed that traditional lab skills such as microscopy and Gram staining were ranked lowest in both personal interest and career relevance (Durrant *et al*., 2017). In the fall of 2020, the University of Vermont Department of Microbiology and Molecular Genetics launched a pilot version of a lab-based microbiology course for nursing majors, entirely online. Each week, the online students would conduct virtual exercises that were as similar to the in-person activities as possible (Table 1). While some weeks allowed the students to conduct virtual labs using publicly accessible materials, other weeks required using data collected from the in-person students or reading microbiology case studies online. Following the end of the semester, we performed a retrospective analysis on earned grades from weekly laboratory exercises ("study questions"), projects on infection control plans and case studies, and lecture exams to assess differences on learning outcomes between traditional and online laboratory modalities.

Table 1. Lab Schedule for Online Students.

Week	Activity	Method of Instruction
1	Ubiquity and Aseptic Technique	Images of results from in-person lab
2	Microscopy	Virtual lab
3	Gram Stain	Virtual lab
4	Disease Triangle	Live discussion
5	Control of Growth	Images of results from in-person lab
6	Culturing Microbes	Images of results from in-person lab
7	Normal Flora	Images of results from in-person lab
8	Respiratory Infections	Case studies
9	Skin and Wound Infections	Case studies
10	Diagnostic Microbiology	Virtual lab
11	Systemic and CNS Infections	Case studies
12	GU and GI Infections	Case studies

Data Collection

Grades were recorded and exported from Blackboard for statistical analyses between lab modalities for study questions, infection control plans, case study projects, and overall lab and course grades. The number of "online" students was 27. The number of "on-campus" students was 111, pooled from four laboratory sections.

Statistical Analysis and Data Presentation

Statistical significance was calculated via two-way ANOVA followed by Šidák's multiple comparisons test for analyzing differences among lab study questions. Student's unpaired t test was used for analyzing differences among infection control plans, case study projects, and final grades. All statistical analyses were performed using GraphPad Prism 9 software. Data is presented as the mean +/- standard error of the mean in red. (ns = not significant $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$)

Presentation of an MMG 065 Exercise Adapted for the Online Modality

To provide context for how a traditional laboratory exercise can be implemented for a virtual lab section, we have provided the student handout for the week 3 exercise, "Gram Staining". The in-person students received a similar exercise document with modified instructions and study questions to reflect the physical lab environment (Supplemental Material).

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Student Outline

Exercise 3: Gram Staining

Objectives

Introduce the student to procedures for Gram staining smears
Reinforce understanding basic cellular characteristics of bacteria
Prepare a smear properly from broth and solid cultures
List reagents, functions, and steps of a Gram stain
Evaluate Gram stain reaction quality and troubleshoot causes of Gram staining problems
Describe the Gram stain reaction, cell shape, and arrangement of common bacterial species
Interpret unknown slides for Gram stain reaction, cell morphology, and arrangement

Introduction

The Gram staining method, named for Danish bacteriologist Hans Christian Gram, is a differential stain. It is one of the most important staining techniques in microbiology and is almost always the first test performed for the identification of bacteria. The Gram stain differentiates between two major cell wall types. The microorganisms that are stained by Gram's method are commonly classified as Gram positive (purple) or Gram negative (pink.) The difference in the staining properties of bacterial cells reflects the difference in cell wall composition, most importantly, the amount of peptidoglycan present. Peptidoglycan, found only in bacteria, is a high molecular weight repeating carbohydrate polymer linked by amino acid bridges, which forms the structural backbone of the bacterial cell wall.

The Gram-positive cell wall consists of a thick sheath of peptidoglycan with tightly bound acidic polysaccharides, including teichoic acid and lipoteichoic acid. Gram positive bacteria are those bacterial species with cell walls containing relatively large amounts of peptidoglycan and no lipopolysaccharide.

The Gram-negative cell wall consists of an outer membrane containing lipopolysaccharide (LPS), a thin shell of peptidoglycan, periplasmic space, and an inner membrane. Gram negative bacteria are those bacterial species with cell walls containing lipopolysaccharide and small amounts of peptidoglycan. The cell wall for Gram negative microorganisms has a higher lipid content compared to Gram positive cells. Gram negative bacteria do not retain crystal violet. Rather, these stain pink or red following decolorization with alcohol and subsequent application of safranin, the counterstain. Gram variable refers to Gram positive cells that sometimes stain Gram negative, as seen with clinical samples.

Patient samples that are smeared and Gram stained are referred to as Primary Gram Smears (PGS). This initial step guides identification and work up of the sample. The smear from a patient source, will often appear very thick due to the extra protein present in the form of mucus and host cells. The method of transferring patient sample material to a glass slide will vary greatly depending on the sample type and is beyond the scope of this exercise.
Components of the Gram stain:

The primary stain, crystal violet, enters both Gram positive and Gram-negative cells, staining them deep purple. This basic dye diffuses throughout the bacterium and is held in both the bound and unbound states. The mordant, Gram's Iodine, is added next. A mordant makes the staining solution stain more intensely. Wherever the mordant meets the basic dye, a water insoluble lake is formed and is composed of a stable crystal violet-iodine complex (CV-I) within the cell. The lake is only moderately soluble in low molecular weight alcohols and acetone. Next is decolorization, which is the treatment of the stained cells with alcohol or acetone. This step removes the lipids in the walls of the Gram-negative bacteria causing them to become porous. The CV-I complex leaks from the cells due to the increased permeability, and the cells become colorless. These colorless cells take up the safranin (or counterstain) and appear pink or red. These cells are called Gram negative. The high peptidoglycan content in Gram positive cell walls responds to the alcohol or acetone treatment by shrinking. This traps the CV-I complex in the Gram-positive cells causing these cells to remain purple even after counterstaining with safranin.

Table 1: Components of the Gram Stain.

Reagent	Reagent color	Function	Cell color after application
Cystal violet	Purple	Primary stain	All cells purple
Gram's iodide	Yellow/orange	Mordant; forms complexes with crystal violet	All cells purple

Acetone or ethanol	Colorless	Decolorizer: dissolves lipid in the outer membrane of Gram negative cells Dehydrates the cell wall of Gram positives, “trapping the stain”	Gram positives purple Gram negatives colorless
Safranin	Red	Counterstain: stains decolorized bacteria	Gram positives purple Gram negatives pink

Observing and Evaluating Gram-Stained Smears

A Gram-stained smear should appear only lightly colored to the naked eye. A good slide is evenly stained, and the bacteria are spread thinly enough that you can identify individual cells. The bacteria should not be in clumps, as this will alter the amount of stain retained in that area. The 10X (low power) objective should be used to focus in on a region of the smear. Remember to use your coarse adjustment first, then your fine adjustment to focus on your specimen. You may choose to view the smear at 40X (high power). Use only the fine adjustment at this magnification. Now you should be able to see cells, but probably cannot make out their shape. Moving to 100X will allow you to completely evaluate the cells on your smear. If you feel comfortable using your scope, you may wish to skip from 10X straight to 100X (oil immersion) to observe the bacterial cells. Recall that oil is necessary when viewing specimens with the 100X lens to increase resolution. These colors are hard to differentiate at first and you should find an area of your slide in which the cells are not too densely packed in order to observe them. Once stained, bacterial cells can be observed for Gram stain reaction, size, cellular morphology, and spatial arrangement. Arrangements of cells are best observed from broth cultures because the emulsification process disrupts the natural arrangement from colonies "picked" from solid media.

Note: Some individuals who are Red/Green colorblind may find it difficult to perceive the pink/red appearance of Gram-negative cells. In this case, Bismarck Brown may be substituted for Safranin as a counterstain. With Bismarck Brown, Gram negative cells will appear a very light brown.

Methods and Data Collection

Part A: Accessing the online platform

Visit the Virtual Interactive Biology Laboratory from Michigan State University: http://learn.chm.msu.edu/vibl/content/gramstain.html.

Part B: Practice Gram Staining the Correct Way

1. Heat-fix the slide: click on the Bunsen burner, pass the slide gently two or three times (1-2 seconds total) through the flame.
2. Flood the slide with crystal violet for 1 minute.
3. Rinse with H2O.
4. Flood the slide with iodine for 1 minute.
5. Rinse with H2O.
6. Decolorize with alcohol for 5-10 seconds.
7. Rinse with H2O.
8. Flood the slide with safranin for 1 minute.
9. Rinse with H2O.
10. View slide under the microscope

Part C: Practice Gram Staining with Mistakes

Mistake 1: Follow the above protocol but do NOT heat fix the slide.
Mistake 2: Follow the above protocol but heat fix the slide for 10 seconds.
Mistake 3: Follow the above protocol but skip adding the iodine.
Mistake 4: Follow the above protocol but skip adding the alcohol.

Study Questions

1. Practicing Gram staining the correct way: Once you have obtained your perfect Gram stain, describe the two species of bacteria on your slide. (It does not matter which species you call #1 or #2.)

Species #1

Gram Reaction:
Cellular Morphology:
Spatial Arrangement:

Species #2
Gram Reaction:
Cellular Morphology:
Spatial Arrangement:

2. Mistake 1: Describe what your slide looks like at the end of the protocol, and explain why this happened.

3. Mistake 2: Describe what your slide looks like at the end of the protocol, and explain why this happened.

4. Mistake 3: Describe what your slide looks like at the end of the protocol, and explain why this happened.

5. Mistake 4: Describe what your slide looks like at the end of the protocol, and explain why this happened. Note: You should include the word “underdecolorized” or “overdecolorized” in your answer.

Discussion

It is important to remember the following things when preparing Gram stains. As Gram positive cultures age, the cell walls tend to become naturally more porous, allowing the CV-I complex to be extracted with alcohol or acetone. This can cause them to appear red when counterstained. To avoid this problem, always use young cultures (16 to 24 hours) to obtain accurate Gram stain results. Gram positive cells grown under acidic conditions can also lose their ability to retain the CV-I complex, resulting in erroneous Gram reactions. To avoid this problem, use cells grown in a neutral medium for Gram staining. For proper decolorization, the smear should be a thin, uniform film. Thick smears will make it very difficult to discern useful information about the cells.

Decolorization is the most common place for error in the Gram stain procedure. It is easy to over decolorize a slide. When a slide is over decolorized, cells that are normally Gram positive will appear Gram negative. This results in what is called a false Gram negative. Students may overcompensate and, as a result, they may under decolorize their smears. When under decolorization occurs, cells that are normally Gram negative will appear Gram positive resulting in a false Gram positive.

While the Gram stain is differential only for bacteria, some yeasts and fungi may retain the color of the crystal violet and are often referred to as Gram positive. *Candida albicans*, for example, is sometimes misinterpreted as a Gram-positive coccus. Animal cells cannot retain the CV-I complex but retain the counterstain, so they appear uniformly Gram negative when viewed microscopically. For this reason, evaluation of clinical specimens requires a great deal of practice; it can be difficult to identify bacterial cells among the background of pink-stained host cells and mucus. Staining requires specific slide preparation for viewing microorganisms.

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An English translation is in Brock, T.D. 1999. Milestones in Microbiology 1546–1940 (2 ed.). ASM Press. pp. 215–218. ISBN 1-55581-142-6.

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Materials

A computer with Internet access to use the Virtual Interactive Biology Laboratory from Michigan State University (Arvidson *et al*., 2021): http://learn.chm.msu.edu/vibl/content/gramstain.html

Notes for the Instructor

As expected with any virtual activity, there were a few students for whom online content was inaccessible. Instructors should be prepared for this circumstance and may wish to provide students with screen captures of final microscope images for each study question (Appendix A). Providing this failsafe option greatly alleviated student stress after being unable to access the materials.

The authors recommend the following setup for this activity: teaching assistants should fully familiarize themselves with the virtual module and review basics of the Gram stain; students should be fully prepared by reading the activity handout and attempting to load the module prior to the assignment deadline.

Post-hoc analyses were conducted on student laboratory and final course grades to assess learning outcomes between nursing majors in the traditional, on-campus format compared to the online option.

Analysis from the weekly study questions revealed a consistent similarity in earned grade average for each activity (Figure 1). There were exceptions to the activities, in which online students performed slightly better in activities 3 and 6, while in-person students earned higher grades on activities 7 and 10.

There were no significant differences in the average grade earned for the infection control plan or case study projects (Figures 2 and 3). Encouragingly, there was a consistent trend between modalities for students to perform better on the case study project compared to the earlier performed infection control plan. This may reflect an equal propensity for students to accept feedback from the infection control plan and apply it to the subsequent case study project, regardless of having an in-person environment.

Importantly, we detected no significant differences between cohorts in the final average lab grade (Figure 4), lecture exam grades (Figure 5), or the final overall course grade (Figure 6).

In conclusion, we found that there were no significant differences in assessment outcomes regardless of modality. The pilot course proved to be a viable alternative to an in-person experience. Content developed here can be used to augment future iterations of the in-person course. In the future, we will evaluate the results of a Microbiology Concept Inventory administered before and after the course to validate our conclusions. End-of-course evaluations will be reviewed prior o launching any future online microbiology course to assess student perception of learning relative to course modality.

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About the Authors

Cole Davidson has been a Ph.D. student in the Cellular, Molecular, and Biomedical Sciences

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Karin Hodge is an instructor in the Department of Microbiology and Molecular Genetics, where she creates curricula and trains and supervises teaching assistants in curriculum delivery.

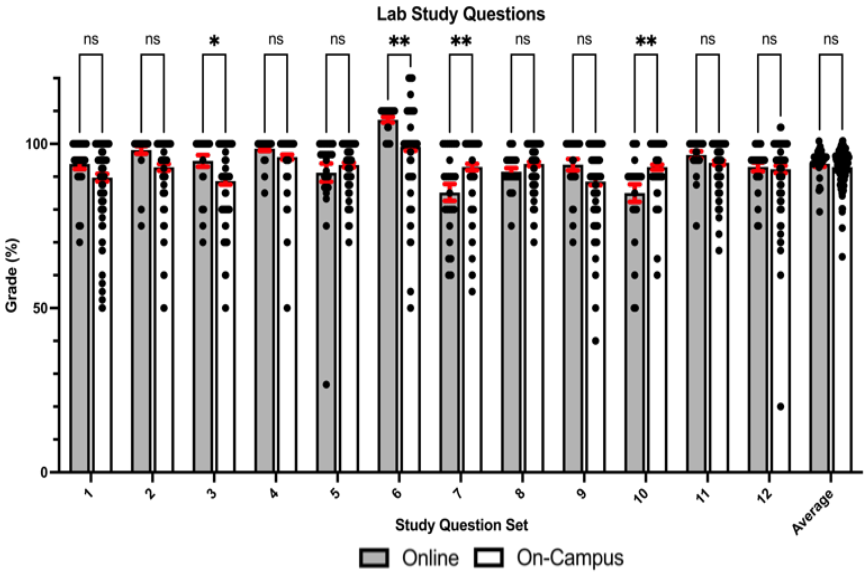


Figure 1. Grades earned from the lab study questions between cohorts. Grades for each laboratory exercise were recorded and analyzed for statistical significance between teaching modalities. Two-way ANOVA followed by Šidák's multiple comparisons test was performed. Data is presented as the mean +/- standard error of the mean in red. (ns = not significant $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$)

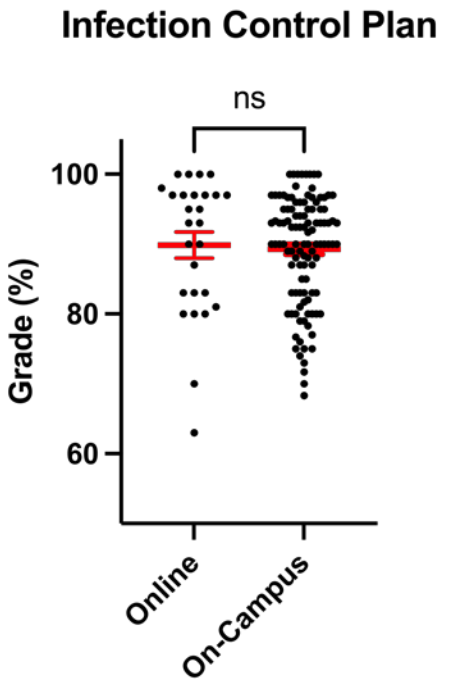


Figure 2. Grades earned from the infection control plans between cohorts. Grades were recorded and analyzed for statistical significance between teaching modalities. Student's unpaired t test was performed, and data is presented as the mean +/- standard error of the mean in red. (ns = not significant p ≥0.05)

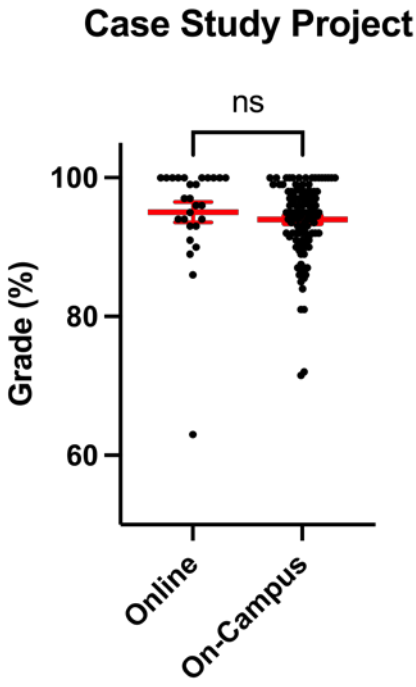
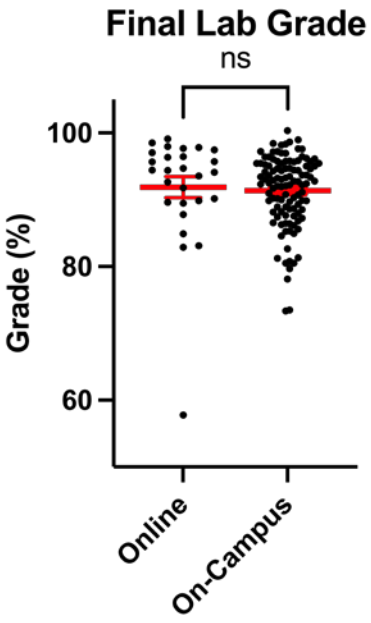


Figure 3. Grades earned from the case study projects plan between cohorts. Grades were recorded and analyzed for statistical significance between teaching modalities. Student's unpaired t test was performed, and data is presented as the mean +/- standard error of the mean in red. (ns = not significant p ≥0.05)



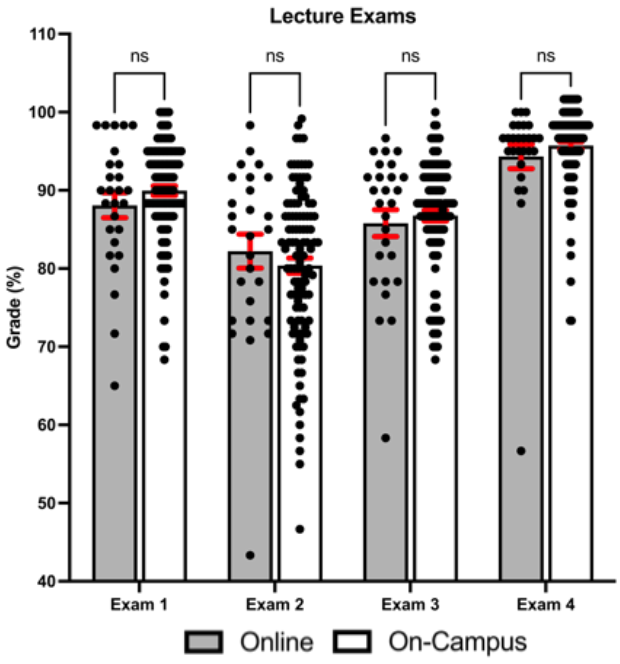


Figure 4. Final lab grades earned between cohorts. The final grade consisted of weekly study questions (40%), the infection control plan (40%), and the case study project (20%). Grades were recorded and analyzed for statistical significance between teaching modalities. Student's unpaired t test was performed, and data is presented as the mean +/- standard error of the mean in red. (ns = not significant p ≥0.05)

Figure 5. Grades earned on each lecture exam between cohorts. Grades were recorded and analyzed for statistical significance between teaching modalities. Student's unpaired t test was performed, and data is presented as the mean +/- standard error of the mean in red. (ns = not significant p ≥0.05)

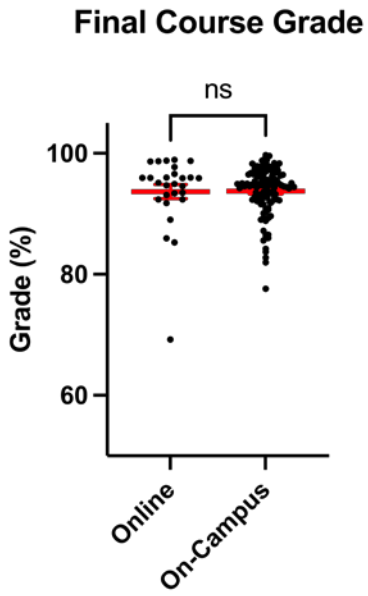


Figure 6. Final course grades earned between cohorts. The final grade consisted of homework assignments (15%), quizzes (10%), discussions (10%), exams (40%), and the overall laboratory grade (25%). Grades were recorded and analyzed for statistical significance between teaching modalities. Student's unpaired t test was performed, and data is presented as the mean +/- standard error of the mean in red. (ns = not significant p ≥0.05)

Appendix A

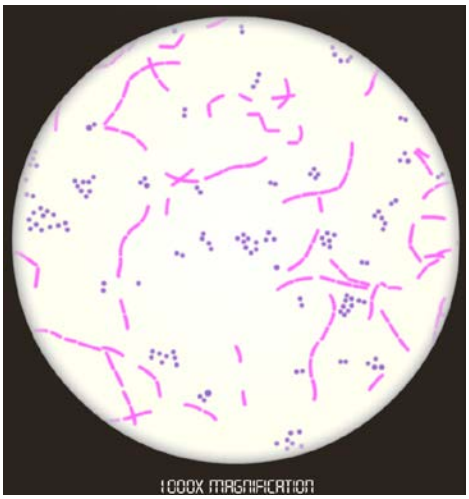


Figure 7. Image of *Staphylococcus aureus* and *Escherichia coli* properly Gram stained.

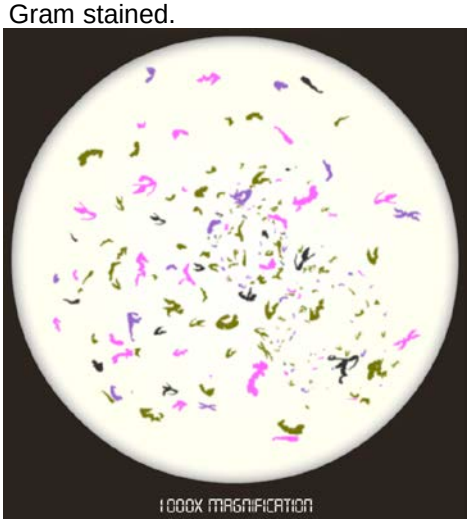


Figure 9. Mistake 2.

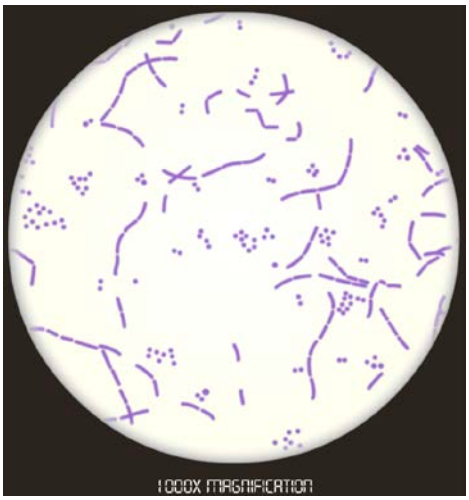


Figure 11. Mistake 4.

Figure 8. Mistake 1.

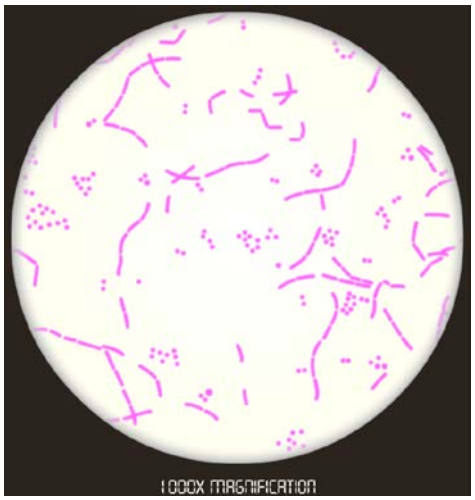


Figure 10. Mistake 3.

Images were captured from:

Arvidson C, Chen J, Rhodes B, Guibord M, Spurbeck R, Foster D. 2021. Virtual interactive biology laboratory. Michigan State University Board of Trustees. East Lansing, MI 4882. Online module.

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