

Lesson Title: How Many Bacteria Are On The Contaminated Lettuce?

Created by: Sky Reece, Ph.D., Beaufort Community College

Major Sections	Content
Lesson Overview	<p>Overall Purpose: Using an integrated approach to teach students the practical application of mathematics in the biology field; preparing our students for their mathematical skills in higher-level science courses or in biotech field jobs.</p> <p>Estimated Timeframe: 3 hours 1st lab + 1-hour 2nd lab</p> <ul style="list-style-type: none"> • During 2 hours of 1st lab, the instructor will teach students basic math concepts and lab techniques to dilute a sample and count a plate • In the last hour of 1st lab, students will perform the experiment • In the 1st hour of the 2nd lab, students will count plates and calculate the results <p>Courses for Implementation: Microbiology General Biology Biotechnology Pre-Calculus Algebra</p> <p>Format: Seated, hybrid</p> <p>Key Terms: Bacteria, scientific notation, serial dilution, standard plate count</p> <p>Standards/Skills Addressed: Reading, Math Academic: scientific notation, concentration calculation, metric system Technical: serial dilution Employability: interpersonal skills, critical thinking skills, problem-solving skills Industry (if applicable): cGMP, GLP</p> <p>Learner Outcomes/Student Learning Objectives: (Learners will be able to)</p> <ul style="list-style-type: none"> • Use scientific notation as a tool in representing, calculating, and solving expressions with dilution • Understand the metric system and convert from μL to mL

Major Sections	Content
	<ul style="list-style-type: none"> • Calculate the concentration of a solution and serial dilutions • Aseptically perform a serial dilution • Perform a standard plate count to determine the bacterial density of a stock sample
Equipment/Materials	<p>List of Materials/Equipment/Texts: Bunsen burner Bacterial incubator Micropipettes Sterile tubes Sterile water Nutrient agar (NA) plates Ethanol jar Glass rod Hand-tally counter 24-hour broth culture of <i>E. coli</i></p> <p>Safety Precautions: <i>E. coli</i> is a biosafety level (BSL) 1 microorganism; therefore, follow BSL1 lab safety. Please wear proper personal protection (PPE): safety glasses, disposable gloves, and lab coats.</p> <p>Cleanup Instructions: After plate counting, dispose of the bacterial culture plates in an appropriate autoclave container.</p>
Discussion	<p>Industry/Real-world Scenario: We sometimes hear/read on the News that the <i>E. coli</i> outbreak is linked to packaged salads. Those outbreaks cause consumers to be ill or lead to death in the worst scenario. Scientists in the food industry constantly conduct different microbiological experiments to ensure the foods are safe for consumers. One of the crucial experiments is to quantify the microbe present in foods.</p> <ul style="list-style-type: none"> • CDC warns consumers of romaine lettuce E. coli contamination • Wisconsin residents sickened by contaminated romaine lettuce

Major Sections	Content
	<p>Microbes are tiny. Therefore it presents a challenge for scientists to count them. Scientists rarely count all the microbes in a sample they collected. Instead, they usually count the microbes in a known volume to establish their density and then report in the standard density units of microbes/mL. Although a milliliter is a relatively small volume, a sample can still have over 10^7 microbes/mL, which is still impossible to count. To avoid counting these vast numbers of microbes, scientists can count a small fraction of a milliliter volume by performing serial dilution on the sample.</p>
Instructional Strategies	<p>Proposed Teaching Strategies:</p> <p>Bloom's: Remember: Define scientific notation, metric system, standard plate count method, countable, TNTC, and TFTC Understanding: Explain concentration Apply: Use scientific notation and metric system to calculate concentration and serial dilutions Analyze: Compare calculations with each other Evaluate: Evaluate the experiment results by performing a standard plate count following 24/48 hours of culture Create: Write a lab report</p> <p>REACT: Relating: cold brew coffee concentrate from grocery stores needs to be diluted – the concept of dilution Watch the two videos from the Industry/Real-world Scenario and discuss product recalls from the FDA or CDC Experiencing: students use food color dye to prepare different dilutions Apply: bacterial dilution to a suitable concentration for counting Cooperating: students work as groups to perform this experiment Transferring: scientific notation, serial dilution, standard plate count</p>
Activities/Lesson Procedure	<p>Activity Preparation: Instructor: Prepare 24-hour broth culture of <i>E. coli</i></p> <p>Learner: Before students come to the lab, they should watch this video to review scientific notation and metric system Pre-lab videos:</p> <ul style="list-style-type: none"> • Introduction to scientific notation from Khan Academy • Introduction to the metric system • Introduction to the Metric System: Volume

Major Sections	Content
	<p>Activity Steps/Lesson Procedure:</p> <ul style="list-style-type: none"> • Review and teach mathematic concepts <ul style="list-style-type: none"> ○ Scientific notation ○ Metric system: liter (L), milliliter (mL), microliter (μL) ○ Calculate the concentration of a solution ○ Serial dilution: how it works ○ Using serial dilution to count bacteria • Teach standard plate count <ul style="list-style-type: none"> ○ Use the practice problems to help students link the concepts between standard plate count and serial dilutions • Students will be assigned three students per group to work <ul style="list-style-type: none"> ○ Each group will be given 24-hour broth culture of <i>E. Coli</i> ○ Students will prepare 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions of the original culture ○ Students will transfer 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions to four pre-labeled plates, respectively, and spread the plates using the spread plate technique. ○ After 24 to 48 hours of incubation, students will examine the plates, determine the countable plates, and then count them. <p>Expected Results/Learner Products: Each group of students should have eight nutrient agar plates grown with <i>E. Coli</i>. Students will count the plate during the second lab and calculate the original cell density. Some groups won't have countable plates. Students will have to explain what has gone wrong and explain the possible pitfalls during their experiments.</p> <p>Extension Options: If none of the groups has countable plates, we will go over each group's potential mistakes and maybe a re-do of the experiment.</p>
Faculty Resources	<p>Background Material: Scientific notation Lab manual: Standard Plate Count Serial dilution: how it works Using serial dilution to count bacteria</p>

Major Sections	Content
	<p>Handouts and Supplemental Materials: Practice Problems for using serial dilution to count bacteria Standard Plate Count Worksheet (*Download the full book pdf and use lab 9: worksheet)</p>
<p>Assessment</p>	<p>How will students demonstrate what they have learned? If a group of students has successfully prepared countable plates, accurately counted the plate, and correctly calculated the original cell density, this group has succeeded in their experiments. At the end of the semester, students will perform another four weeks project, which will involve the standard plate count. The end-semester project will serve as a good way to test students' understanding of this experiment, and they will put those mathematics skills to use too.</p> <p>Learner Products/Assessment Tools or Processes:</p> <ul style="list-style-type: none"> • Observations Students will use their 2nd lab to make observations about their plates. They will decide if the plate is countable (colony numbers: 30-300). If the plate contains fewer than 30 colonies, it is TFTC (too few to count). If the plate includes more than 300 colonies, it is TNTC (too numerous to count). They will record their results on the Standard Plate Count worksheet. • Discussion participation Students will discuss what has gone wrong during their experiment and provide explanations. • Laboratory reports Students will write a lab report about the standard plate count lab. Their lab report will detail each step of serial dilution calculation. They will document their discussion about what went wrong during their experiment and their explanations.