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Chicken Wing Microbiology: A Lesson in Food Safety and Microbiological Technique

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Abstract: In the first week of this three-lab series, students wash store-bought chicken wings in sterile saline, serially dilute the resulting bacterial suspension, and inoculate agar plates. In the following week, students count the colonies and calculate the number of colony-forming units per ml of wash fluid (typically around 10^6 cfu/ml). They design experiments to test treatments that might reduce the microbial contamination of chicken wings, and they collect the data during the third week. Instructor notes include safety reminders, suggestions for incubating plates, and ideas for possible assignments.

Keywords: aseptic technique, serial dilution, plate count, experimental design

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Introduction

In this series of three labs, groups consisting of 3-4 students pretend that they have been hired by the Wing Nut Chicken Wing Company of Arkansas to measure the level of microbial contamination of raw, store-bought chicken wings. During the first week, students learn aseptic technique, wash bacteria from chicken wings, make serial dilutions, and plate samples on tryptic soy agar to set up their initial counts. During the second week, they count colonies on the first week's plates, calculate the number of colony-forming units per milliliter of wash fluid, and try to devise a method of treating the wings to eliminate the bacteria. They then design and carry out their own experiments, and collect the data in the third week. During the third week, they can also do Gram stains on selected colonies (if desired).

The labs are appropriate for beginning-level biology students in majors or nonmajors courses. The first week's activities take about 1.5-2 hours to complete. The second week is the longest, requiring most of a 3-hour lab. The third week's activities should take less than an hour. The first lab could be omitted for advanced students already familiar with aseptic technique.

The first two labs require quite a bit of preparation, especially to pour Petri dishes and to dispense and sterilize test tubes containing saline solution. Instructors must also prepare materials for students to use in their independent experiments during the second week. The third week requires virtually no preparation.

Student Outline

WASHINGTON, May 2, 2001--Thorn Apple Valley, Inc., a Forrest City, Ark., meat and poultry plant, was sentenced April 10 in federal court for violating the Poultry Products Inspection Act, the U.S. Department of Agriculture's Food Safety and Inspection Service announced today.

Thorn Apple Valley, Inc. was sentenced on one felony count of distributing chicken and turkey frankfurters that were adulterated with *Listeria monocytogenes*, a foodborne pathogen. The U.S. District Court for the Eastern District of Arkansas ordered the plant to pay a \$50,000 fine and a special assessment fee of \$400.

This action is the result of an investigation by FSIS compliance officials. FSIS is responsible for ensuring that meat, poultry, and egg products are safe, wholesome, and properly labeled.

Source: <http://www.fsis.usda.gov/OA/news/2001/thorn.htm>

The above article represents an actual news item about a poultry processing plant that was fined for distributing poultry products that were contaminated with harmful bacteria. You have no doubt heard of other instances in which severe illness, or even death, resulted when consumers ate contaminated meat, poultry, or other foods.

You have been hired by the Wing Nut Chicken Wing Company of Arkansas to measure the number of microbes contaminating their raw chicken wings. They also want you to experimentally test a potential method of reducing the microbial contamination of their wings. The executives of this

fictional company are concerned about the safety of their food products, and they do not want to be liable for the spread of foodborne bacteria such as *Salmonella*.

To achieve your aims, your team must first learn to measure the approximate bacterial population on a chicken wing. Next, your group will choose a decontamination method, then design and carry out your own experiment to determine how effective your chosen method is.

Specifically, the next three lab sessions will go like this:

- During the first week, you will learn how to measure the microbial contamination of the wings. To do this, you will apply bacteria that you wash from store-bought chicken wings to a bacterial growth medium, or food source, called tryptic soy agar.
- During the second week, you will count your bacteria from the first week, then design and carry out an experiment to test a possible method for killing the microbes on chicken wings.
- During the third week, you will collect and analyze your decontamination data. Each student must use the data collected during the third week to write a short report to the Wing Nut Chicken Wing Company describing your findings.

Warning: take good notes on the exact procedures your group uses throughout this lab! You will NOT remember the details if you don't write them down! Include every detail, including all instances where you think you MIGHT have messed up (don't worry, you wouldn't be the first to make a mistake, so don't be afraid to document it). You'll need these details to write up your experimental design, interpret your data, and write your report. Remember, no detail is too small to write in your notes, because you never know what you'll need later.

Background

The following pages contain important information about the rationale behind two important microbiological techniques (plate counts and serial dilutions), how to work safely and effectively with microorganisms, and food preservation.

If microorganisms are too small to see with the naked eye, how do we count them?

Microbiologists use several methods to count bacteria; in this lab, we will use a technique called the **plate count**. If we spread a sample containing bacteria on a nutritious growth medium in a petri dish (the "plate"), each living bacterial cell should begin to grow and divide. After several days of growth, bacterial colonies will become visible with the naked eye. If you know how much of the sample you spread, you can calculate the number of "colony-forming units" (CFUs) per milliliter of the sample.

In this lab, we will wash store-bought chicken wings with sterile saline solution, then count the bacteria in the wash fluid. Unfortunately, it is very likely that the wash fluid will have so many bacteria that no matter how tiny the volume we plate, we will end up with way too many colonies on the plate. They will all crowd together and it will be impossible to get an accurate count.

The solution to this problem is called the **serial dilution**: a series of 10-fold dilutions of the original wash fluid. The objective is to find an optimal dilution that results in a density of bacteria that can be accurately counted (plates with *between 30 and 300 colonies* are considered to be "countable"). Since we won't know how many colonies are present until next week, we need to plate many different dilutions and see which produce(s) countable plates.

Details of these procedures appear in a subsequent section of this lab.

Aseptic technique

Microbiologists use aseptic, or sterile, technique when working with microorganisms. Aseptic literally means “germ-free,” so **aseptic technique** means not contaminating a culture, growth medium, or your surroundings with unwanted microorganisms.

Aseptic technique means starting with sterile (germ-free) materials. In this lab, for example, you’ll begin with sterile plastic baggies, test tubes, saline solution, and pipettes. Aseptic technique also means not contaminating your sterile starting materials with microorganisms. In other words, in this lab you are interested in counting bacteria on a chicken wing, so you don’t want to contaminate your plates with bacteria from your skin or hair or breath or the lab room.

Here are a few simple **rules to follow** when using aseptic, or sterile, technique:

- Inspect your petri dishes before you begin to make sure they are not already colonized by bacteria – contaminated plates are a common problem and have ruined many an experiment.
- Wash your hands before you begin and after you finish.
- Wipe down your bench with Lysol before you begin and after you finish.
- Only open sterile containers such as capped test tubes and petri dishes for a few moments at a time, and only when they are being used.
- Sterile surfaces such as pipettes should never touch any other surface, such as the lab bench or your fingers. You shouldn’t breathe on any sterile surface either.

Food preservation

During the second week of this lab, you will have the opportunity to design and carry out an experiment to determine the effectiveness of one or more treatments for decontaminating store-bought chicken wings. The following information might give you some ideas of what you might like to test.

Food preservation can involve either killing microorganisms in the food, or temporarily retarding their growth. Common food preservation methods include:

- heating
- freezing or refrigeration
- drying or smoking
- treating with a variety of chemical preservatives such as salt, nitrates, nitrites, sulfite, vitamin C (ascorbic acid), organic acids (e.g. vinegar = acetic acid), and even sugar if present in high enough concentrations. Get in the habit of checking food labels and you’ll see how common preservatives are in commercial foods!
- irradiation

Besides cooking and preservatives, you probably also know about other ways to kill microbes, such as applying bleach, Lysol, alcohol, hydrogen peroxide, etc. Of course, not all of the above are suitable for food treatment, or available for your experiments.

Notes on safety

You will handle raw chicken, possibly contaminated with human pathogens, in this lab. You will also work with open flames. For your safety, and for the safety of your friends and roommates, please:

- Wear gloves whenever you handle raw chicken.
- Dispose of the chicken, and anything that touches raw chicken, as directed by your TA.
- Whether or not you touched chicken, wash your hands thoroughly before you leave the lab.

- Because each group will use open flames and highly flammable alcohol, please tie back long hair, restrain loose clothing, wear closed shoes, and please, please, please do not horse around. Your TA will throw you out if your behavior in this lab endangers your fellow students in any way.

First Week Procedures

During the first week of this three-week series, you will learn the serial dilution and plate count techniques mentioned previously. Read all the instructions before you begin. Each group will do the counts for TWO wings!

****Remember to wear gloves whenever you handle the wings****

****Check with your TA for any last-minute changes to the following procedures****

****Inspect your plates before you begin; ask your TA to replace any contaminated plates you find****

1. Use a wax pencil to label your test tubes and the bottoms of your petri dishes before you begin (figure 1). Remember, to maintain aseptic conditions, keep the test tubes and petri dishes closed as you label them. Double-check your labels!

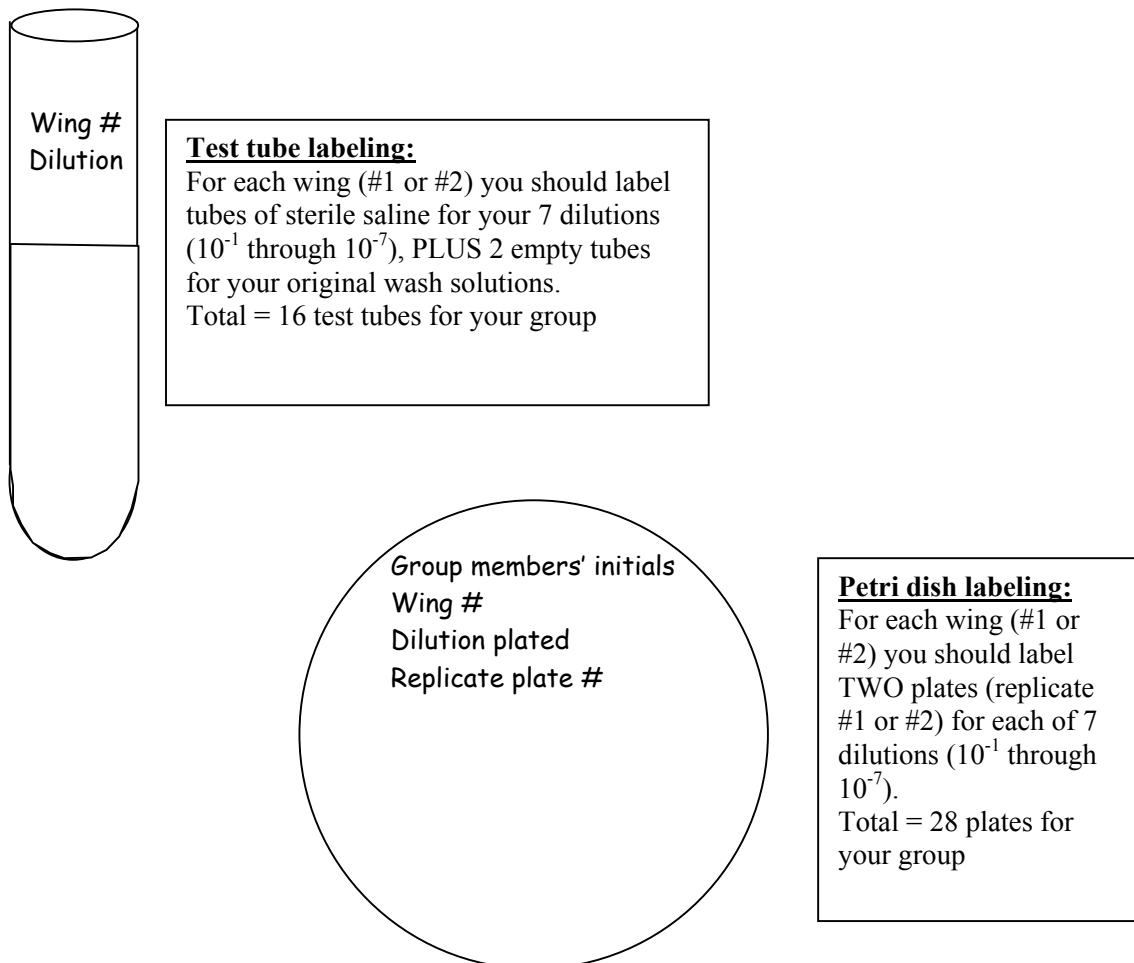


Figure 1. How to label your test tubes and Petri dishes

2. Obtain two raw, fresh, store-bought chicken wings for your group. Aseptically place each wing in a separate resealable plastic sandwich baggie.
3. Aseptically add 10 ml of sterile saline solution (0.85% NaCl) to each bag.
4. Seal each bag while pressing out most of the air.
5. Carefully wash the wings in the saline solution by *gently* massaging the bags for 5 min.
6. For each bag, use a sterile plastic 1-piece pipette to aseptically transfer about 2-3 ml of the liquid in the bag to a labeled empty sterile test tube.
7. Now you are ready to prepare your set of serial dilutions of the chicken wash fluid (see figure 2).

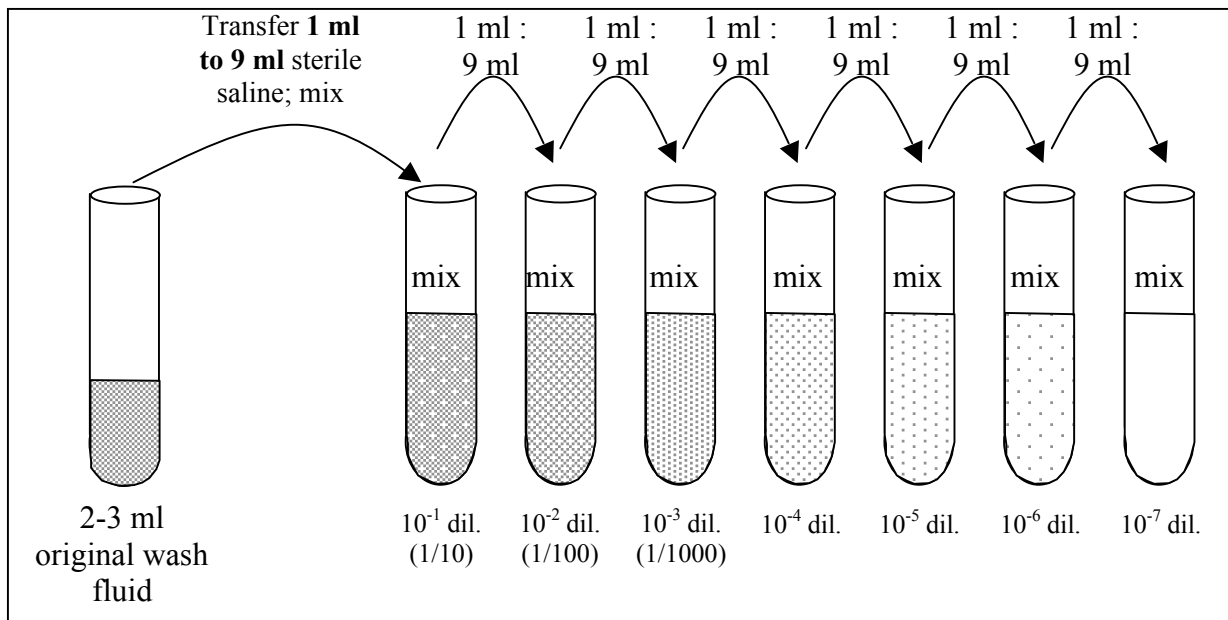


Figure 2: How to prepare a serial dilution

*****Remember -- use a fresh, sterile pipette for each transfer -- dispose of used pipettes immediately in a beaker of disinfectant -- and mix each dilution as you make it!*****

- a. With a sterile plastic 1-piece pipette, aseptically transfer 1.0 ml from the test tube containing the original wash fluid to the test tube labeled “ 10^{-1} .” Dispose of the used pipette immediately in a beaker of disinfectant. The wash fluid has now been diluted 10 times, to 10^{-1} . Mix this tube.
 - b. With a fresh sterile plastic 1-piece pipette, transfer 1 ml of the 10^{-1} dilution to the tube labeled “ 10^{-2} .” Mix this tube.
 - c. Continue in this way through the 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} dilutions by successively adding 1.0 ml portions to tubes containing 9.0 ml of sterile saline solution. Mix each tube immediately after adding 1 ml dilution to it.
8. Now you will plate duplicate samples from each of your dilutions. Beginning with your most dilute tube, use a fresh, sterile 1-ml pipette to add 0.1 ml to duplicate petri dishes containing tryptic soy agar (that is, you’ll make two identical plates for each dilution, or 14 plates total for each wing).

Check your pipette carefully! You'll need a pipette pump and a sterile 1-ml pipette. Make sure you are using 1/10 of a 1-ml pipette, and remember to use a fresh pipette each time you sample from a different tube. Also, mix each tube before you withdraw your sample!

9. Immediately after adding the 0.1 ml of solution to the agar, use a **hockey stick** (a bent glass rod) to gently spread the solution over the surface of the agar. Here's how:
 - a. Sterilize the hockey stick: Take the hockey stick out of the alcohol bath and swipe the flattened end briefly through an open flame, just long enough to catch the alcohol on fire. Allow the flame to go out by itself. Don't blow on it, otherwise you'll introduce new microbes and/or fail to kill those that are already there.
**** Don't hold the burning hockey stick over the alcohol bath, or flaming drops of alcohol will ignite the bath. If this happens, do not panic. Simply place a lid over the alcohol bath, and the flame will extinguish itself. ****
 - b. Spread your sample on the agar: Immediately after the alcohol has burned off of the hockey stick, lift the lid of the Petri dish slightly. Briefly cool your hockey stick by lightly pressing the flattened end to a sterile area of the agar. Next, spread your sample droplet on the agar by using one hand to lightly drag the hockey stick back and forth over the droplet, while the other hand rotates the plate (your TA will demonstrate). Press hard enough to ensure even distribution of microbes across the plate, but not so hard that you tear the agar surface.
 - c. Remove the hockey stick from the plate and replace the lid. Return the hockey stick to the alcohol bath.
10. Repeat steps 8 and 9 for each tube in your serial dilution. Remember, work from MOST dilute to LEAST dilute.
11. After you are done, invert your plates (lid-side-down). Your TA will incubate them at room temperature for 2-3 days to give colonies time to form, then refrigerate them until the next lab session.

Second Week Procedures

One week ago, your group plated serial dilutions of saline solution that you used to wash the bacteria off the surface of a fresh, raw, store-bought chicken wing. For about 48 hours after you plated the bacteria, your TA incubated the inverted plates at room temperature to allow the colonies to develop. Your TA then moved the plates to the refrigerator to stop the bacterial growth.

In today's lab, you will do the following:

- Count the colonies on your plates, and use your data to calculate the number of bacteria (technically, "**colony-forming units**," or CFUs) present in 1 ml of chicken wash fluid.
- Design an experiment to test one or more ways to decontaminate chicken wings.
- Apply your chosen treatment(s), then create and plate serial dilutions from your treated wings. Next week, you will collect the data you need to write your report to the Wing Nut Chicken Wing Company.

Activity 1: Calculating CFUs per milliliter of wash fluid

1. Use a marking pen and a helper (and a dissecting microscope on low power if necessary) to mark and count the bacterial colonies on all plates with 300 or fewer colonies. Plates with more than 300 colonies do not produce reliable data and are therefore recorded as “TNTC” (too numerous to count). Record your data in the table provided (see Appendix).
2. Use your raw data to decide which dilution produced valid counts. Remember, only plates with between 30 and 300 colonies are valid. For each wing, choose the dilution that best fits this criterion of validity:

Wing 1:

Wing 2:

3. For each wing, calculate the average number of colonies on the two replicate plates for the dilution that best met the criterion of validity (30-300 colonies):

Wing 1 average:

Wing 2 average:

4. Use the following formula to calculate the original cell density (colony-forming units, or CFU) per milliliter of wash fluid:

$$\text{CFU/ml wash fluid} = \frac{\text{average \# of colonies}}{(\text{ml plated}) \times (\text{dilution factor})}$$

Here's a sample calculation:

number of colonies on replicate plates: 46 and 54; average = 50

ml plated = 0.1

dilution factor for plate = 10^{-6}

$$\text{CFU/ml wash fluid} = 50 / [(0.1)(10^{-6})] = 500,000,000 = 5 \times 10^8 \text{ CFU/ml}$$

Now you do a practice problem:

If you plated 0.1 ml per dilution, and your counts for two replicate plates are 116 and 152, and the dilution factor for those plates was 10^{-4} , how many CFU per ml of original wash fluid? (answer is on next page)

Answer to practice problem on previous page:

$$\begin{aligned} \text{Plate average} &= (116 + 152) / 2 = 134 \text{ CFU} \\ 134 / [(0.1) \times (10^{-4})] &= 13,400,000 = 1.3 \times 10^7 \text{ CFU/ml} \end{aligned}$$

Now that you're proficient, calculate the CFU per ml original wash fluid for both your chicken wings:

Wing 1:

Wing 2:

Note that several types of mistakes can produce screwy results in a serial dilution experiment. Be specific in describing the sorts of results you might see if:

- one of the tubes in your serial dilution was heavily contaminated with bacteria *before* you even began to make the dilution series. (Does it matter which tube in the dilution series is the contaminated one?)
- you forget to use a fresh pipette at each step as you make the serial dilution.
- you do not notice that one of your plates is contaminated before you spread your chicken wing fluid on it.
- you are impatient and blow out the flame on the hockey stick before all the bacteria are burned off.
- you forget to mix each dilution tube before taking a sample (either as you make the dilution series or as you plate the bacteria).
- you grab your pipettes from the working tip instead of the top.
- you decide to save time by unwrapping all your pipettes at once and laying them out on the lab bench before you begin your experiment.
- you create your serial dilutions by adding 0.1 ml to 9 ml sterile saline (instead of adding 1 ml to 9 ml sterile saline).

Activity 2: Experimental design

Design an experiment to test a method of killing the bacteria on chicken wings (don't worry about whether the wings will still be edible after the treatment is complete). Your TA will tell you the maximum number of wings you can use in your experiment, but you must use at least 2 wings per treatment. Your chosen method may include cooking the wings in boiling water for as long as you choose, or exposing them to various chemical solutions (or even applying a combination of heat and chemical treatments). Here are a few guidelines:

- If you choose to cook wings: after you have boiled the wings for the specified time, use sterile forceps to remove them aseptically from the boiling water, cool them for at least 15 minutes in a sterile empty beaker, place them in a plastic sandwich bag, and process them as you did during week 1.
- If you choose a chemical treatment, place the wing in the sandwich bag with 10 ml of the test solution and wash for the specified time. Remove the wing from the bag while making sure most of the liquid remains in the bag, and transfer the wing to a new bag.

During next week's lab, you will turn in a description of your group's experimental design. Before you begin to carry out your experiment, make sure your TA has explicitly told you that all the components of your group's design are OK. This is your group's responsibility – if your experiment is flawed (e.g. lacks proper controls or replication), your TA will deduct points when he or she grades your experimental design.

- Briefly explain the basic idea of your experiment and explicitly state your hypothesis:
- Describe your dependent variable:
- Describe your independent variable:
- Explain why you think this independent variable will affect your dependent variable:
- Describe your control(s):
- Describe your replication or sample size:
- Describe your methods:
- What materials will you require?
- Design a table to collect your data:
- Predictions: what results would support your hypothesis? What results would suggest your hypothesis is false?

Once a TA has approved your hypothesis and methods, carry out the experiment. Before you leave, you must have applied the treatment(s), created the serial dilution series, plated the dilutions, inverted the plates, and placed them in the crate for incubation. Refer to the procedures described in Part 1 to make sure you correctly construct your serial dilutions and inoculate your plates!

Third Week Procedures

Last week, you set up and carried out an experiment to determine whether your group's chosen treatment eliminates bacteria from chicken wings. This week, you will collect the data needed to submit your final report (due in lab next week) to the Wing Nut Chicken Wing Company.

Activity 1: Data collection

Collect your data and calculate your CFU/ml for each treatment, in just the same way as you did during the second lab period. Be sure to make a note if you see anything unusual about your plates. Data tables are provided in the Appendix.

Activity 2: Data interpretation

Answer the following questions before you write your report to the Wing Nut Chicken Wing Company:

1. Look back at the hypothesis or question you posed last week. Look at the graphs or tables of your data. Do your results support your hypothesis or prove it false? Explain your answer, using your data for support.
2. If your results did not correspond to the prediction you made, explain how your results are different from your expectations and why this might have occurred.
3. Describe how your data are supported by information from other sources (e.g. textbooks or other lab teams working on the same problem).
4. If you had any problems with the procedure or questionable results, explain how they might have influenced your conclusion.
5. If you had an opportunity to repeat and extend this experiment to make your results more convincing, what would you do?
6. Summarize the conclusion you have drawn from your results.

Materials and Equipment List

Item	Week 1 (2 wings per group)	Week 2 (4 wings per group)	Week 3
Squirt bottles of Lysol or other disinfectant	several per classroom	several per classroom	several per classroom
Raw chicken wings	2 per group	4 per group	--
Latex or vinyl gloves	1 or more pairs per student	1 or more pairs per student	--
Wax pencil or waterproof pen for labeling test tubes and plates	1 per group	1 per group	--
1-quart freezer-type zipper baggies; must be thick to prevent leaks while students massage chicken wings	2 per group	4 (or more) per group, depending on treatment	--
Capped test tubes containing 10 ml sterile saline solution (0.85% NaCl); for dispensing into baggie containing chicken wing	2 per group	4 per group	--
Empty, sterile, capped test tubes; for receiving chicken wing wash fluid	2 per group	4 per group	--
Capped test tubes containing 9 ml sterile saline solution (0.85% NaCl); for making serial dilutions	14 per group	28 per group	--
Sterile 1-piece 1-ml transfer pipettes for making serial dilutions (Fisher 13-711-20)	20 per group	40 per group	--
Plates of tryptic soy agar	28 per group	40 per group	--
Sterile 1-ml serological pipettes with 0.1-ml gradations for inoculating plates (we use Fisher 13-678-12B)	14 per group	20-28 per group, depending on number of dilutions to be inoculated	--
Enamel pan containing Lysol or other disinfectant; for receiving "used" pipettes	1 per group	1 per group	--
Glass (or disposable) hockey stick	1 per group	1 per group	--
Jar of 95% ethanol for sterilizing glass hockey stick	1 per group	1 per group	--

Materials and Equipment List (continued)

Item	Week 1 (2 wings per group)	Week 2 (4 wings per group)	Week 3
Alcohol lamp (or other source of fire) for flaming hockey sticks	1 per group	1 per group	--
Fine-tipped Sharpie pens for tallying colonies	--	1 or more per group	1 or more per group
Dissecting microscope for confirming colony growth (optional)	--	1 per group	1 per group
Options for independent experiments: hot water, sterile water, acids, bases, vitamins, hydrogen peroxide, bleach, soap, meat tenderizers, salt, microwave oven, pre-frozen chicken wings ...	--	As needed	--

Notes for the Instructor**Safety and demonstrations**

Since bacteria and fire are involved in this lab, it is important to point out safety procedures. Students should tie back long hair, know what to do in case of an alcohol fire, wash their hands frequently, and use Lysol to clean the lab benches both before and after the lab.

If students are unfamiliar with aseptic technique, instructors may need to demonstrate the correct way to use a “hockey stick” to spread a plate. It is also worth emphasizing the importance of *not* blowing out a flaming hockey stick, not touching the “business end” of a sterile pipette to the skin or lab bench, and other fine points of sterile technique. Students also must remember to keep good notes on all procedures, including those that they know (or suspect) they did wrong. Good notes come in very handy when students try to interpret the data they collect during week 3.

Student-designed experiments

The types of treatments that students typically propose include boiling, freezing, and soaking in fluids such as lemon juice, marinades, vinegar, acid (e.g. HCl), salt water, and soda pop. At the ABLE conference, participants suggested hot sauce, Coke vs. diet Coke, second hand smoke, exposure to UV radiation vs. fluorescent light, soy sauce, garlic, mustard, and honey. It is also possible to test items other than chicken wings, including food from a buffet line, pre-washed baby carrots, frozen vegetables, raisins, fruits, alfalfa sprouts, and unwashed spinach.

Incubating plates

Tryptic soy agar plates smell awful when bacteria are growing on them! We incubate crates of inoculated plates in a hood for about 48 hours to give the colonies time to grow, then move them to a

cold room until the next time the class meets. Refrigerating the plates for the last five days keeps the colonies from overgrowing the plates.

On omitting week 1

Although the first week's activities could be omitted, especially for advanced students, we do not recommend that instructors skip them for students who are unfamiliar with sterile technique. Our students (freshman through senior non-science majors) often make mistakes in creating serial dilutions and spreading their plates. They learn from these errors once they see their results during week 2; as a result, the vast majority of groups get usable results from their independent experiments.

Student evaluations

After each lab, we ask each student to fill out a brief evaluation form that, in part, asks for an overall rating on a scale of 1 (incredibly boring) to 10 (incredibly interesting). Over all semesters (fall 2001 to fall 2005), the first chicken wing lab has received an average rating of 7.9, and the second one has received an average of 7.1. The third week has an average rating of 6.4, but that number does not have as much meaning as for weeks 1 and 2 because we combine week 3 with another lab, so the two labs are rated together. For comparison, our overall average for all labs is 6.9 (range 5.8 - 9.4).

Assignments

We have our students turn in two assignments over the three-week course of the lab. After week 2, they turn in a summary of the group's experimental design, including the hypothesis, dependent variable, independent variable, explanation of why the independent variable should affect the dependent variable, control(s), replication/sample size, predictions, explanation of methods, and list of things that might have gone wrong as the group carried out the experiment. After the students collect the data during week 3, they write short reports over their experiments.

An alternative is to have students design their experiments during the first week of the lab, after they have inoculated their plates. A three-hour lab has ample time for students to form groups and design an experiment by the end of class. The advantage is that students can be more creative because they are not constrained by what we happen to have available in the lab. We tried this for the first time in fall 2005 and were pleased overall, but we found that students did not take seriously the feedback that the teaching assistants gave. Next time, we plan to try again but we will incorporate peer review, similar to the experimental design exercise we do in lecture (Hoefnagels, 2003).

Acknowledgments

The procedures for this lab originally came from the following article; the author has kindly given us permission to publish the modified lab in this volume:

Deutch, C. E. 2001. Microbial contamination of chicken wings: an open-ended laboratory project. *The American Biology Teacher* 63 (4): 262-266.

Other procedures for this lab were adapted from the following sources:

Cappuccino, J. G. and N. Sherman. 1999. *Microbiology: A Laboratory Manual*. Fifth edition. Addison Wesley Longman, Inc., Menlo Park, California. pp. 119-124.

Pierce, B. E. and M. J. Leboffe. 1999. *Exercises for the microbiology laboratory*. Morton Publishing Co., Englewood, Colorado. p. 13.

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Hoefnagels, M. H. and Rippel, S. A. 2003. Using superstitions and sayings to teach experimental design in beginning and advanced biology classes. *The American Biology Teacher* 65(4): 263-268.

About the Authors

Mariëlle Hoefnagels is an assistant professor at the University of Oklahoma, where she has worked since 1997. She teaches introductory biology for non-majors, introductory microbiology, mycology, and a capstone course in zoology. She is also the co-author of a college-level general biology textbook (*Life*), and she has won several teaching awards at the University of Oklahoma. She earned her Ph.D. in botany and plant pathology from Oregon State University.

Mark Walvoord is a Student Services Manager at UNC-Chapel Hill, where he helps with undergraduate and graduate recruitment, advising, graduation, and departmental administration. He also enjoys keeping his ties to the University of Oklahoma, where he has worked since 2003, through teaching an online biology class (fall 2005). He earned his B.S. in Biology from Oklahoma Baptist University in 1998 and received his M.S. in Zoology from the University of Oklahoma in 2002. He hopes to start a doctoral program soon using his research interests in ecology, conservation, and herpetology, and he plans to continue to learn more about technology effectiveness in the classroom as he seeks his goal of becoming a teaching faculty member.

Appendix A: Plate Count Data Sheet

Treatment:				Treatment:			
Tube dil.	Plate (replicate)	ml plated	# of colonies	Tube dil.	Plate (replicate)	ml plated	# of colonies

$$\text{CFU/ml wash fluid} = \frac{\text{average \# of colonies}}{(\text{ml plated}) \times (\text{dilution factor})}$$

Notes: