Bacterial Plating Lab C. Kohn, Waterford, WI
*To be paired with the* [*Minnesota Easy Culture System*](http://qmps.vet.cornell.edu/Services/minnesotaculturemanual.pdf) *materials and directions.*

Partner Names: Hour Date:

Date Assignment is due: Why late? Score: + ✓ -
 Day of Week Date If your project was late, describe why

**Objective**: to investigate the implications of bacteriology on veterinary medicine through a simulated pathogen analysis.

**Overview**: The U.S. is the second largest pork producing country in the world, after China. In 2009, 10% of the world’s production was produced in the U.S. The total number of farms with hogs in the U.S. has been steadily decreasing. The number of farms with hogs decreased by 94% in 45 years. During this same time frame, the average number of hogs per farm has increased from 48 to 1,064 head, an approximate 95% increase. Because of the huge increase in the number of pigs per farm, it is essential that a producer reduces biological risks created by potential pathogens and by the diseases they can cause.

Pathogens can be spread from animal to animal, animal to human, or human to animal through a variety of transmission routes. Animals or humans can acquire disease causing agents by breathing the same air, through oral contact (e.g. by eating the same feed or drinking the same water), direct contact, by clothing and utensils, or by vectors (such as mice or insects). Many pathogens can survive for extended periods of time in dust or organic matter. This survival time is specific for each pathogen and dependent on many factors including temperature, light exposure, humidity, and environmental pH.[[1]](#footnote-1)

In this activity, you will be given two samples from infected animals. To test for a gram-negative or gram-positive bacterial pathogens, we will use the Minnesota Easy Culture System agar plates. An agar plate is simply a petri dish with agar – a nutrient substance on which we can grow bacteria in order to identify it. The MECS plates are divided into sections and each section is treated in such a way that only specific kinds of bacteria can grow on it. For example, if you only see bacteria growing in the Gram-Positive portion of the petri dish, you would know that you have a gram-positive bacterial pathogen.

# Directions

1. Sanitize your lab bench or work area. You can wipe down the area with an alcohol solution. Spraying Lysol and wiping with a clean paper towel would also work fine.
2. Acquire a sample of bacteria from your instructor. Record the sample you have here:
3. See your instructor to acquire your MECS Easy Culture Plate. This is a petri dish that only allows gram negative growth on one side and gram positive growth on the other side.
4. Acquire cotton swabs from you instructor. Avoid touching the plate or cotton end of the sterile swab as this will result in contamination, which will give inaccurate findings.
5. Place a sterile cotton swab end in the bacterial sample for approximately 10 seconds until swab becomes completely saturated. Rotate the swab in the sample to ensure it is fully saturated.
6. Swab half of your bi-plate, spreading the sample evenly over the surface of the agar.
7. Re-dip the swab into the sample for 10 seconds until it is saturated.
8. Swab the second half of your bi-plate, spreading the sample evenly over the surface of the agar.
9. Close your dish and label it with permanent marker (or masking tape and pen or pencil).
	1. Mark your dish with your class’s hour, date, and last names.
10. Put your petri dish face-down (or upside down) into your incubator.
	1. If the dish is upside down (with the lid on the bottom),
	it is less likely to be contaminated by environmental
	bacteria in the air (the bacteria cannot “fall upwards”).
11. Allow your dishes to incubate at least 24 hours.

*Note: more time will be needed if you don’t have an incubator.*

1. After 24 hours, remove the dish and inspect your findings.

Growth on the darker half indicates the presence of gram positive
bacteria while growth on the lighter half indicates gram negative growth.

 *Image: https://ahdc.vet.cornell.edu/sects/QMPS/Services/minnesotaculturemanual.pdf*

# Questions

1. Which sample did you have?
2. As best as you can, draw the results from your plate
after you took it out of the incubator. Label the sides

so that your instructor knows which is gram negative
and which is gram positive.
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1. Do you think your sample contained harmful bacteria?

Explain:
2. Was your sample gram negative or gram positive?
3. Would an infection caused by this pathogen respond to antibiotics? Explain:
4. Which is harder to treat, a gram negative or gram positive bacterial infection?

Why?
5. Which are more likely to be associated with your sample – exotoxins or endotoxins?
6. How would the effects of an endotoxin differ from those of an exotoxin?
7. How could this particular pathogen cause the death of an animal? Explain by describing the steps that lead to death by septic shock and explain how and why death occurs as a result of septic shock.
8. How does a bacterial infection differ from a viral infection in regards to…

	1. How the pathogen harms the animal:
	2. How the infection is treated:
9. Bacteria and viruses are not the only pathogens that can harm animals. Summarize the most important information a producer would need to know about each of the following pathogens:

Fungi:

Protozoa:

Helminths:

Prions:

Instructor Notes:

MECS Agar bi-plates (MacConkey Agar Plates, $1.25) can be ordered from the QMPS Western Laboratory, FAX – 585-243-1713. Visit <https://ahdc.vet.cornell.edu/sects/QMPS/Services/LabSupplyPrices.cfm> for prices and ordering information.

Bacterial samples can be acquired from Nasco (enasco.com). For this lab, the following has been used:

Gram Positive Sample: Bacillus Subtilis (In Broth Culture). Price: $8.00. Product Number: LM00284M

Gram Negative Sample: Enterobacter aerogenes. Price: $8.00. Product Number: LM00913M

**Order these materials at least two weeks in advance!**

Bacterial samples are mixed with tapwater and stored in vials. For a class of six groups, prepare 3 of each sample and provide them at random to student groups (diagnosing whether it is gram positive or gram negative depends on not knowing in advance which is which; you may want to label them with color so that you as the instructor know).

If possible, provide a plate and sample for every two students in order to increase the student involvement and exposure to plating. For a class of 24, this would mean 12 vials bacteria (6 of each kind) and 12 MECS plates.

**Samples do need to incubate at least 24 hours, so be sure to stagger this lab protocol over two days or more.**

If you choose to include microscopy and cell staining as part of this lab, a good protocol can be found at <http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab6/lab6.html> .

1. Source: http://www.cfsph.iastate.edu/pdf/swine-biological-risk-management [↑](#footnote-ref-1)