Guide for Instructors

1. Materials Needed for Lab Module

1.1 Materials List with suggested retailers

Material	Supplier	Item #
S17-1 *E. coli* strain*	The Coli Genetic Stock Center – Yale	CGSC# 8175
LB agar plates	Carolina Biological	216620
LB agar liquid	Carolina Biological	216650
Sterile Swabs	Fisher Science Education	22-029-488
Micro centrifuge Tubes - Sterile	Carolina Biological	215236
12 mm Culture tubes - sterile	Fisher Science Education	14-956-3D
Sticks - sterile	Fisher Science Education	22-029-641
Ethanol	Carolina Biological	861261
Nalidixic acid disks	Fisher Science Education	CT0031B
Blank Disks	Fisher Science Education	CT0998B
37°C Incubator		

Note: Similar items can be substituted. Item numbers are provided as a reference but there is no need to use the exact items listed.
*This strain can also be obtained through the ATCC website (Item # 47055) or from the Brown Lab (email brownpb@missouri.edu to request the strain).

- This lab was also tested with *E. coli* strains DH5α and SM10, and both performed similarly to S17. Strain K12 did not work under these lab conditions.

1.2 LB media recipe for plates or liquid

If you have the ingredients below and the ability to sterilize them, it may be cheaper to make instead of buy LB plates and liquid. You will need to calculate the amount of media to make based on the number of students you will have performing the lab. (One plate is about 25 ml of LB agar).

- Materials (500 mL or about 20 plates): ● Tryptone powder (5 g) ● Yeast extract (2.5 g) ● NaCl (5 g) ● Water (500 mL) ● Agar (7.5 g)
- Protocol for LB plates: 1. Add the tryptone (5 g), yeast extract (2.5 g), and NaCl (5 g) into a beaker containing 400 mL of water. 2. Use a magnetic spinner or shake to dissolve. 3. Add 100 mL water and further mix until

dissolved. 4. Pour into a 1L bottle and sterilize (for autoclaves use 121°C, 15 psi for 15-20 minutes). 5. Allow to cool until you can comfortably touch the bottle, then pour warm mixture into petri dishes. For LB liquid, do not add the agar and dispense the liquid into storage bottles before sterilization.

1.3 Ordering *E. coli*

Typically, when ordering a bacterial strain, it will come in a small tube filled with agar. This tube of *E. coli* can be stored in the fridge for 1-2 weeks; however, the students should pick their colonies from a freshly struck plate. For example, from the original tube, an LB agar plate will need to be struck for isolated colonies. Once the streak plate has been made, the plate needs to be incubated for about 24 hours at 37°C. This plate should be used within 1-2 days after colonies have grown. If the *E. coli* colonies are used for the lab are older than 1-2 days, mutations may accumulate, potentially affecting the ZOIs of the starting population.

2. Tips for Running the Lab Exercise

2.1 Set up a station to ethanol sterilize tweezers and put on disks

Each station should have a cup with ethanol, a lighter, tweezers, blank disks, and antibiotic disks. The disks come stacked in a small plastic tube. We found it best to distribute the blank and antibiotics disks into empty sterile petri dishes before the lab so students have an easier time grabbing the disks with the tweezers.

Students should come to the station with their agar plate after they have swabbed the plate with bacteria. Once at the station, they dip the tweezers into the ethanol, shake off any excess ethanol, and use the lighter to flame sterilize the end of the tweezers. Once sterilized, the students grab one blank disk and place it on their plate. Flame sterilize the tweezers again, then grab one antibiotic disk and place it on the plate. Finally, flame sterilize the tweezers once at the end.

- The antibiotic disks should always be kept in the refrigerator, and can be taken out just before the lab period, and distributed into petri dishes.

2.2 How to incubate agar plates

Once students have swabbed their plate and added the disks, the plate needs to be incubated right side up in the 37°C incubator (The lid of the plate should be on top.) Once the plates have been incubated for about 24 hours, they should be stored in the fridge upside down to prevent moisture from accumulating on the plates. (In the fridge, the lid of the plate should face down, and the side of the plate that has agar in it should be on top.)

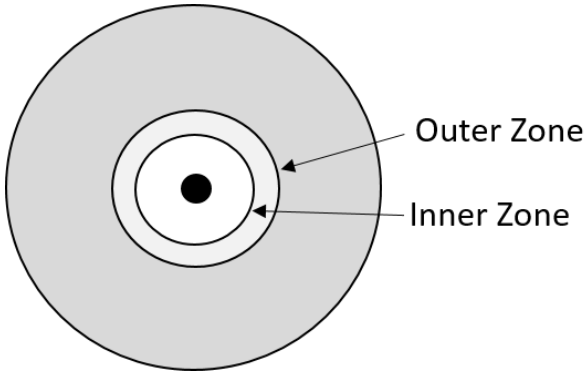
- If some of the plates do not have a lawn of growth after 24hours, incubate the plates 1 more night, then put them in the fridge.
- Optional: If your classroom has parafilm, wrap the plates with parafilm before incubating them.

2.3 How to measure the ZOI

When measuring the ZOI, do not take the lid off the plates. Instead, measure from bottom side of the plate.

2.4 ZOI has multiple rings

When measuring the ZOI, make sure student's look carefully at the zone, because sometimes there is a fainter zone inside of the original zone where the bacteria have begun to grow toward the disk. If there is an inner, fainter zone have the students measure this zone, and not the bigger zone.



2.5 How to dispose of waste

- The swabs, sticks, and micro centrifuge tubes can be thrown in the trash.
- The test tubes with liquid *E. coli* should have 10% bleach added to them for 12-24 hours, then the liquid can be poured down the sink, and the tubes thrown in the trash (or autoclaved/pressure cooked, see below).
- The plates of *E. coli* can be wrapped with parafilm or plastic wrap and thrown in the trash (or autoclaved/pressure cooked, see below).
- If you have access to a pressure cooker or autoclave; the plates, empty test tubes, microfuge tubes, pipet tips, swabs and sticks can be pressure cooked on high for 20-30 minutes or autoclaved at 121°C and 15 psi for 20-30 minutes. Then throw the waste in the trash.

2.5 Adapting the lab for other class schedules

- The subculture step on day 2 that is carried out by the teacher can be done more than 1 day. For example, if day 1 of the lab was done on a Friday, then the "day 2" subculture step could be carried out over the weekend and Monday. When the class met again on a Tuesday, students would proceed with "day 3" of the lab.
- The plates from day 1 and day 5 of the lab can be kept in the fridge for up to 1 week before data analysis, if needed, to accommodate different class schedules.

3. Troubleshooting the Lab Exercise

3.1 Plates are contaminated

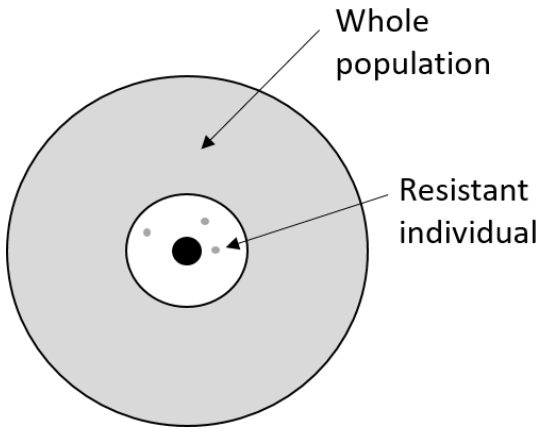
If a student's plate was contaminated with a different bacterium other than *E. coli* then the lawn of bacteria on the plate may be a different color, and this may have affected the outcome of their lab. Still have the students measure their ZOI, but make sure they make a note that their plate was contaminated so their group and the class can consider this when interpreting their data.

3.2 Disk moved or fallen off

In some cases, the blank or antibiotic disk may move or fall off during incubation or moving from the incubator to the fridge. If this happen most likely the ZOI will be much smaller or non-existent. If this occurs, have that group team up with another group to measure and record the ZOI.

3.3 Difference between a resistant population and resistant individuals

The lawn of bacteria on the plate represents a population of bacteria. Thus, when the zone gets smaller on the day 5 plate this indicates that there has been a shift in the level of resistance at the population level. If a single individual became resistant, but the entire population did not change, then you would only see a single resistant colony that arose from that one individual, and not a reduction in the zone size.



3.4 No Lawn

If students had no bacterial growth on their plate, have them team up with another group to measure and record the ZOI so that they can still participate in the lab exercise.

3.5 Outliers – no change in ZOI, or ZOI started as resistant

In rare cases, some students saw no change in the ZOI or the ZOI started off as resistant instead of susceptible. If this occurs, it should be emphasized to these students to compare their data to the class room data and determine if their results are within the normal range or if they have an outlier in the data. Outliers can be cause by many factors including contamination, human error, or natural variation.

4. Claim Evidence Reasoning Example[a]

Claim: Model 2 best explains how antibiotics become useless because it illustrates that a population of bacteria evolve antibiotic resistance through natural selection.	
Evidence: 1. On average, the ZOI from day 1 of the lab after antibiotic exposure indicated that the bacteria were susceptible to nalidixic acid.	**Reasoning (Analysis + Synthesis + Evaluation):** 1. This illustrates that bacteria **do not gain antibiotic resistance after they are exposed to antibiotics**, and allows us **to rule out model 1**, which shows that on day 1, after antibiotic exposure the bacteria are resistant.
2. Not all the ZOI on day 1 were the same, there was initial variation in the starting population.	2. Model 1 shows the starting population as having the same level of resistance, which is not what we saw in the lab. The zones from the classroom data on day 1 varied, which shows that **a population of bacteria has initial variation**. In model 2 the starting population has initial variation.
3. On average, after allowing the bacteria from day 1 to grow, the ending population on day 5 was resistant to nalidixic acid.	3. This shows that over time a population of bacteria can acquire antibiotic resistance, model 2 illustrates that **during growth bacteria can gain random mutations** that make individuals resistant to antibiotics, and these individuals spread through the population (via inheritance from mother to daughter cell and lateral gene transfer) as the bacteria grow.
4. Some students had zones that were intermediate to niladic acid on day 5. Conclusion: Only model 2 correctly shows that if a population of bacteria has variation in their starting population, and that population is given time to grow and acquire random mutations, then when you expose this population to antibiotics you can select for those individuals that have acquired antibiotic resistance. If this selection event occurs, then you have a population of bacteria that has evolved through natural selection to be antibiotic resistant.	4. This illustrates that depending on the make-up of your starting population and when and what type of mutations are acquired you may end up with an intermediate population of bacteria on day 5. Only model 2 accounts for an intermediate level of resistance arising.

[a]This example was put together using evidence from the lab only. Evidence from other areas of this module are also acceptable.