Why don't antibiotics work like they used to? **Name: _____ Period: _____**

Investigative Question: How do antibiotics affect bacteria when they are put together?

To be able to make a prediction on our investigative question, we will need to understand:
1. **How antibiotics travel in a petri plate?**
2. **How would it look for antibiotics to travel in the petri plate?**

Pre-Investigation: Dyes in Agar

Time: 00:00:00

Draw what you initial observations.

Make a prediction about what the plate will look like in 10 minutes:

Was your prediction supported by the evidence? Why/Why Not?

Time: 00:10:00

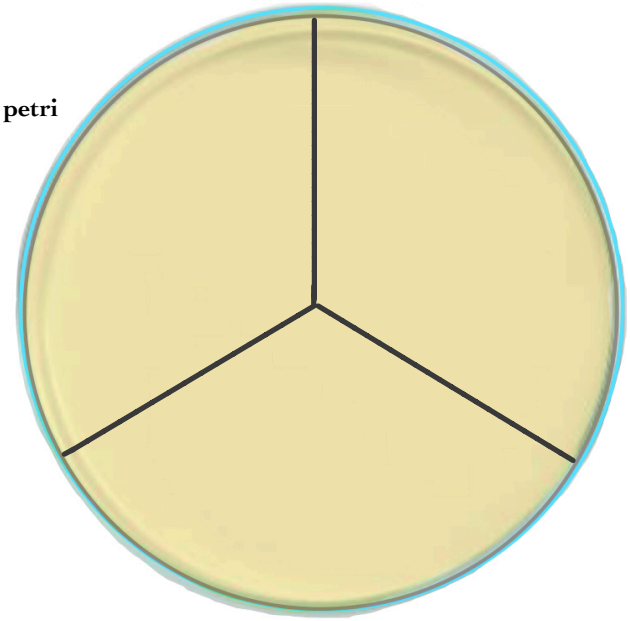
Draw your final observations.

For safety reasons, we will be going over how to set up this lab, but students will not actually be completing the experiment.

Experimental Set-Up:

1. **Obtain a petri plate of nutrient agar.**
2. **Label the petri plate with a sharpie near the edge. Divide it into 3 sections. It should have your group letter, today's date and each of the following concentrations: 1 mg, 3 mg, or 5 mg per section.**
3. **Carefully open a package, dip into a beaker of E. Coli and plate the bacteria in the normal zig-zag pattern, in 3 directions, rotating 90 degrees after each zig-zag pattern.**
4. **Using forceps, transfer one 1 mg antibiotic soaked filter paper disc and place it in the center of the 1mg section of your petri plate.**
5. **Repeat for step #4 for the 3 mg and 5 mg section of the petri plate.**
6. **Seal the petri plate in a ziplock bag and place in the incubator.**

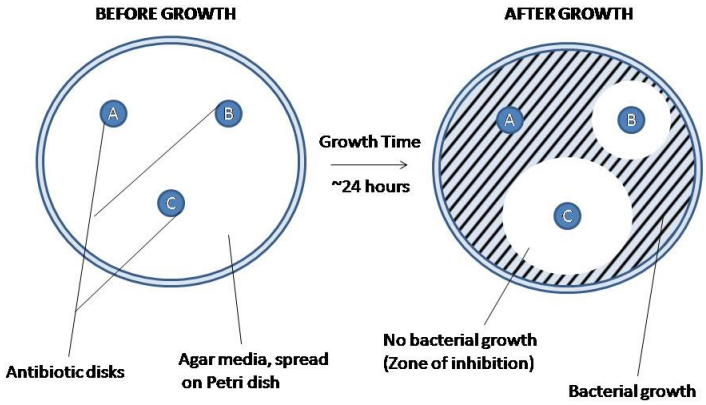
Based on the above experimental set up, draw and label what you think the petri Plate should look like before it is placed in the Ziploc Bag.



Preparing to Interpret Results

1. Looking at the graphic to the right, what do you think the **The Zone of Inhibition is?**

2. What do you think affects the size of the **Zone of Inhibition?**

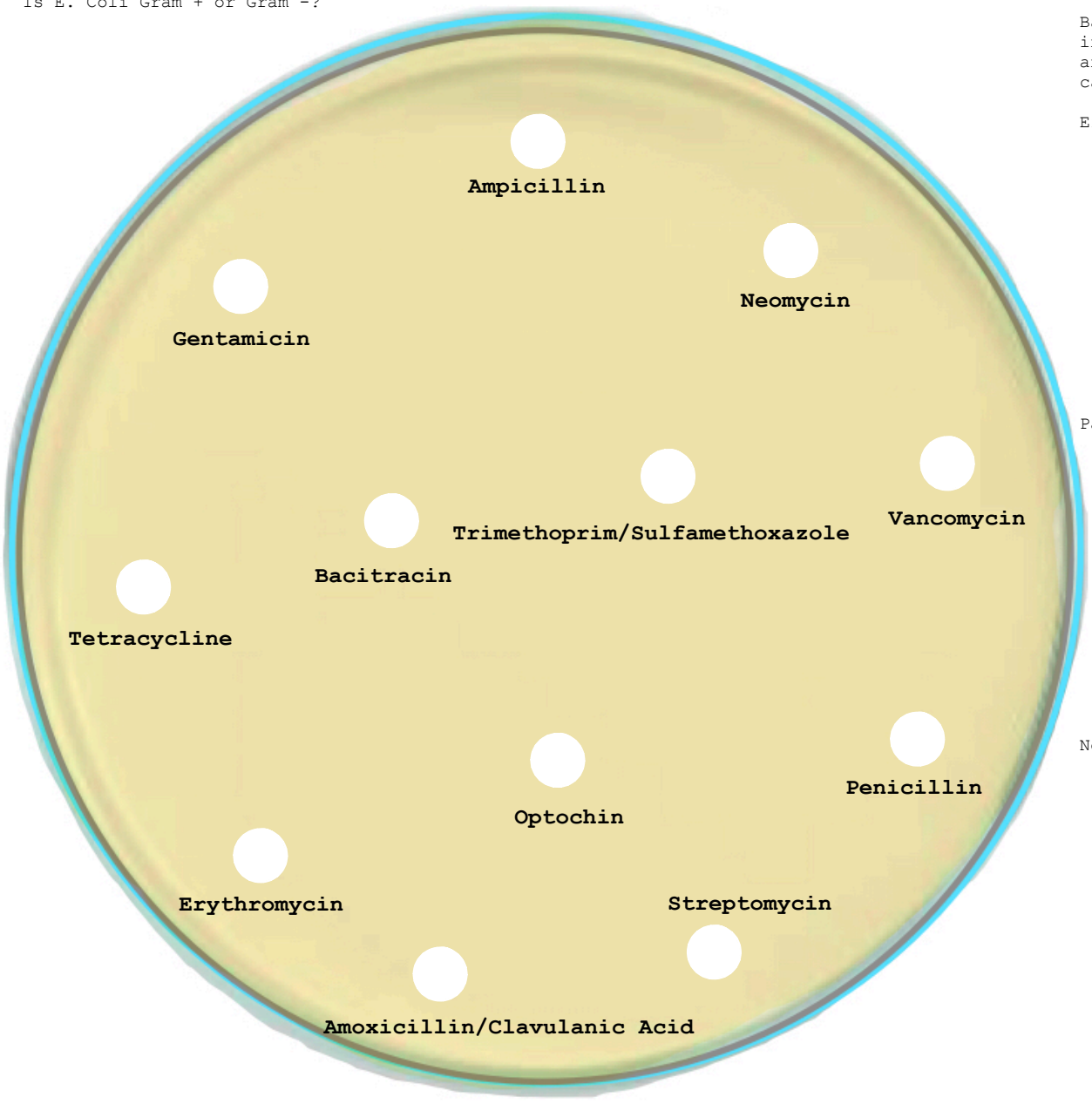


Kirby-Bauer Test Time Lapse with E. Coli **https://youtu.be/-L4MeZBtvXM**

Your teacher will pause the video, sketch the zone of inhibition for E. Coli for the following antibiotics.

Is E. Coli Gram + or Gram -?

E. Coli



Based on the zone of inhibition, rank the antibiotics into three categories:

Effective:

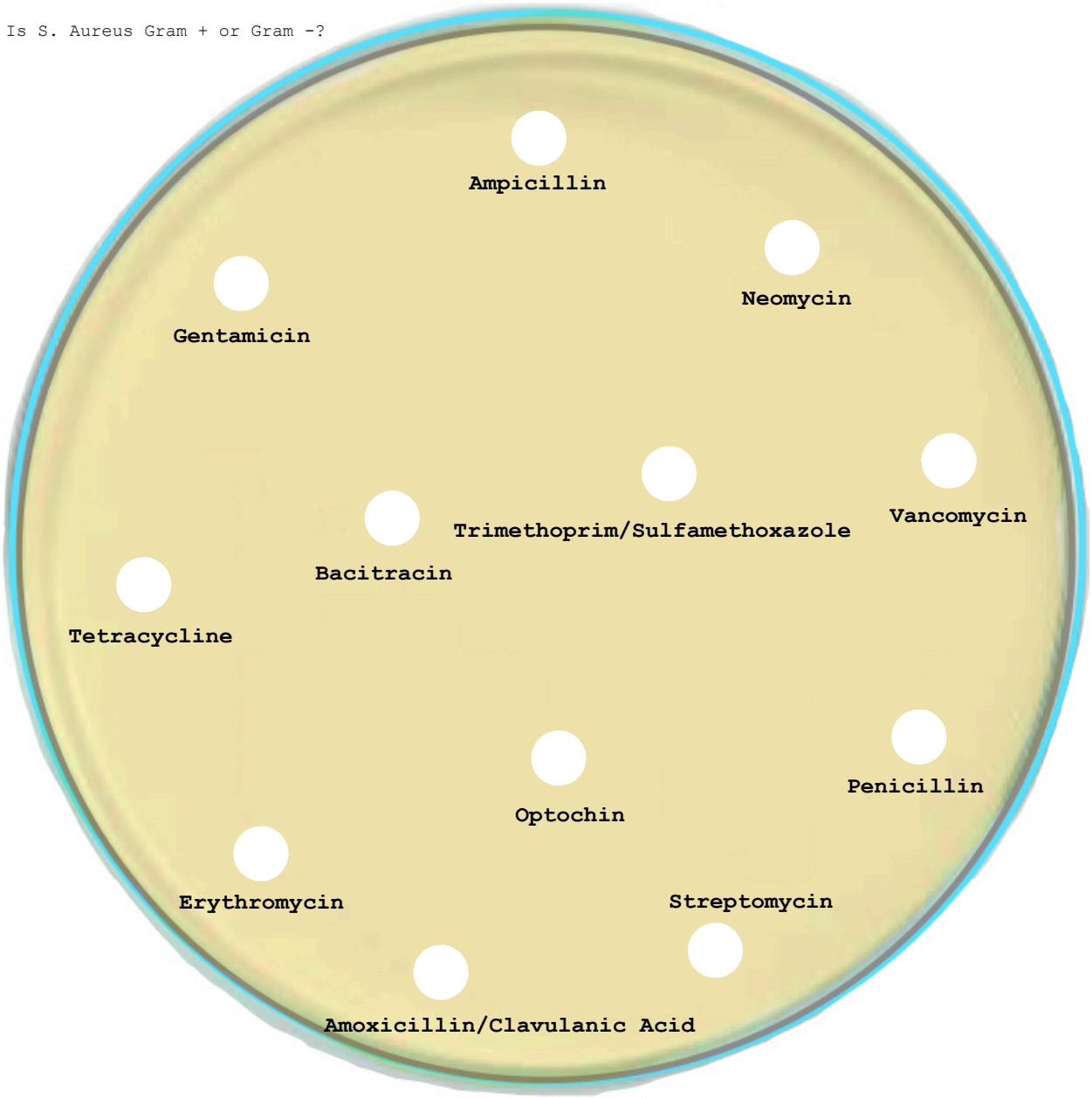
Partially Effective:

Not Effective:

Kirby-Bauer Test Time Lapse with S. Aureus

Your teacher will pause the video, sketch the zone of inhibition for S. Aureus for the following antibiotics.

Is S. Aureus Gram + or Gram -?



S. Aureus

Based on the zone of inhibition, rank the antibiotics into three categories:

Effective:

Partially Effective:

Not Effective:

Analysis Questions

1. Based on the above experiment, if a doctor is unsure of the bacteria responsible for an infection, what antibiotic(s) could she prescribe to increase her patients' likelihood of responding well?

2. How does the zone of inhibition differ between the gram - and gram + bacteria? (Explain general difference and propose a reason for this difference)

Data Collection:

Materials:
Ruler (use millimeters)
Petri Plates (Photos)

Procedure:
1. Get the photo for each group's petri plate (Group A)

Each group (A, B, C, and D) has 1 petri plates, each petri-plate is separated into 3 sections:
1 mg ampicillin, 3 mg ampicillin, and 5 mg ampicillin

2. Use a ruler to measure the distance from the edge of the antibiotic disc to bacterial growth (essentially you are measure the distance where no bacteria is growing, i.e. the zone of inhibition).

3. Add data to your data table below.

5. Repeat for groups B-D petri plates

Bacterial Zone of Inhibition (Susceptibility) in the Presence of Ampicillin			
	1 mg Ampicillin	3 mg Ampicillin	5 mg Ampicillin
Group A	mm	mm	mm
Group B	mm	mm	mm
Group C	mm	mm	mm
Group D	mm	mm	mm
Average Zone of Inhibition (mm)	mm	mm	mm

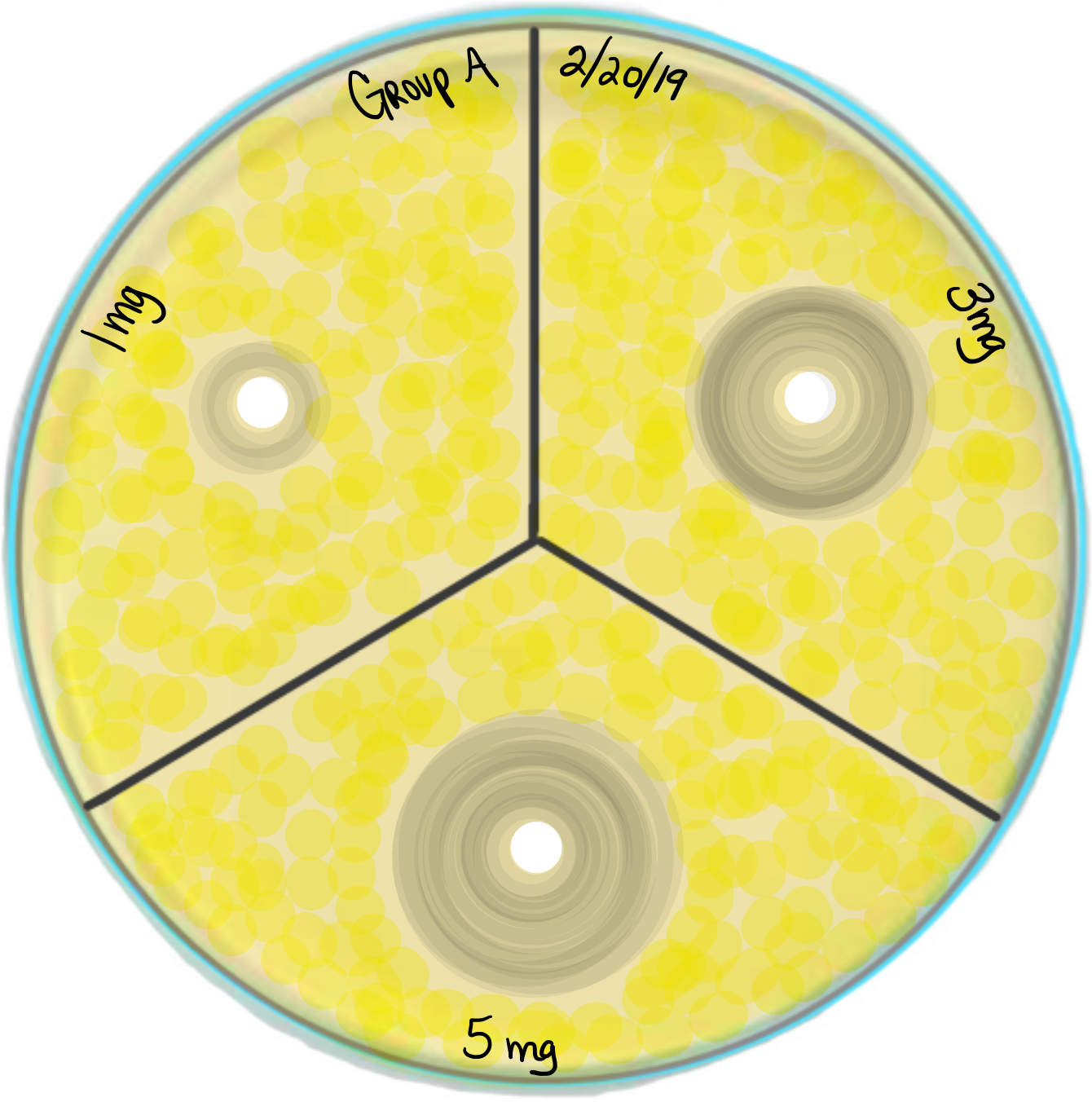
Analysis:

Why is the relationship between the dose (mg) and the zone of inhibition?

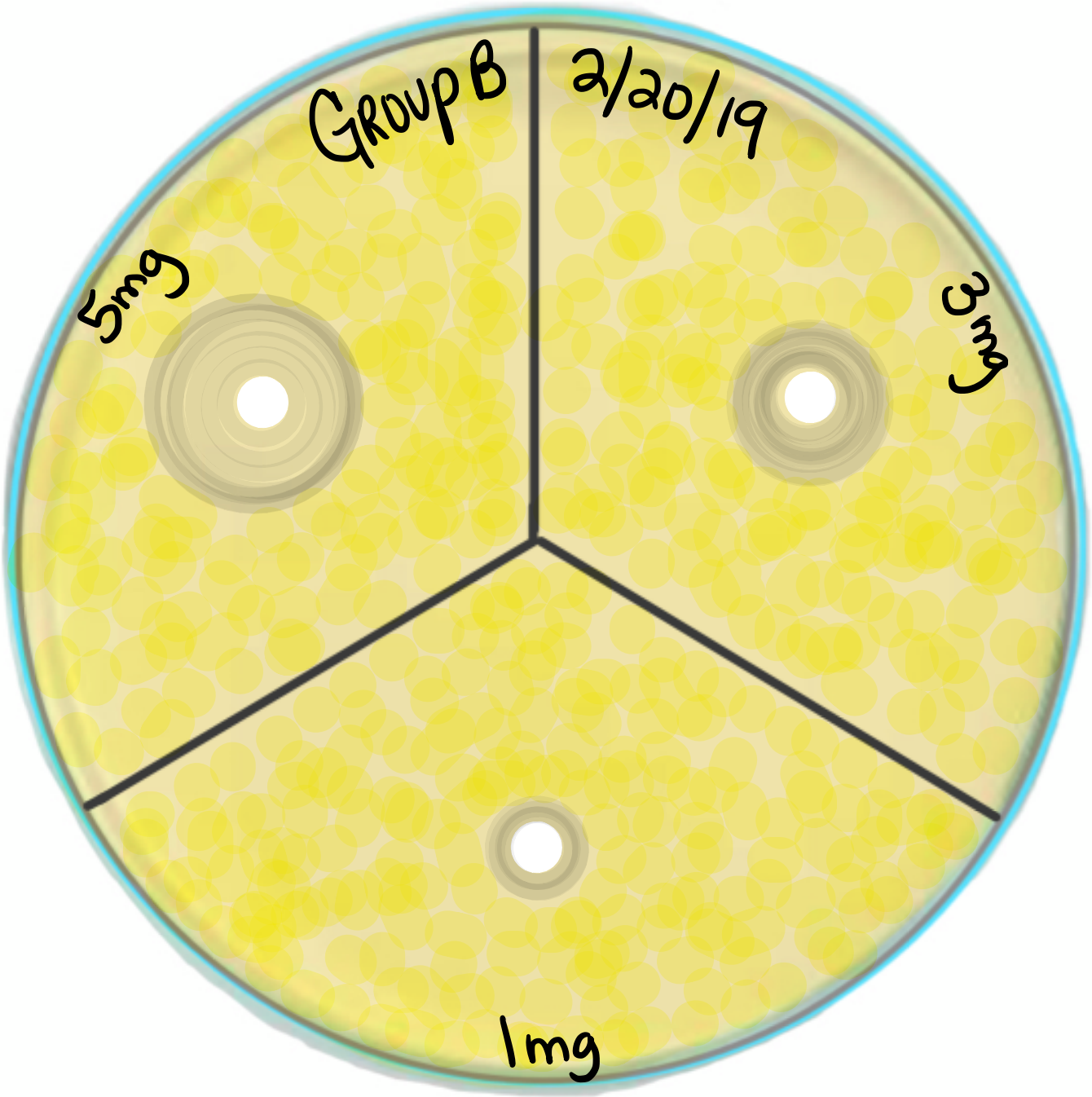
What would it mean if a few colonies of bacteria began to grow in the zone of inhibition?

In terms of Addie's case, how can this lab help us understand why she initially got better?

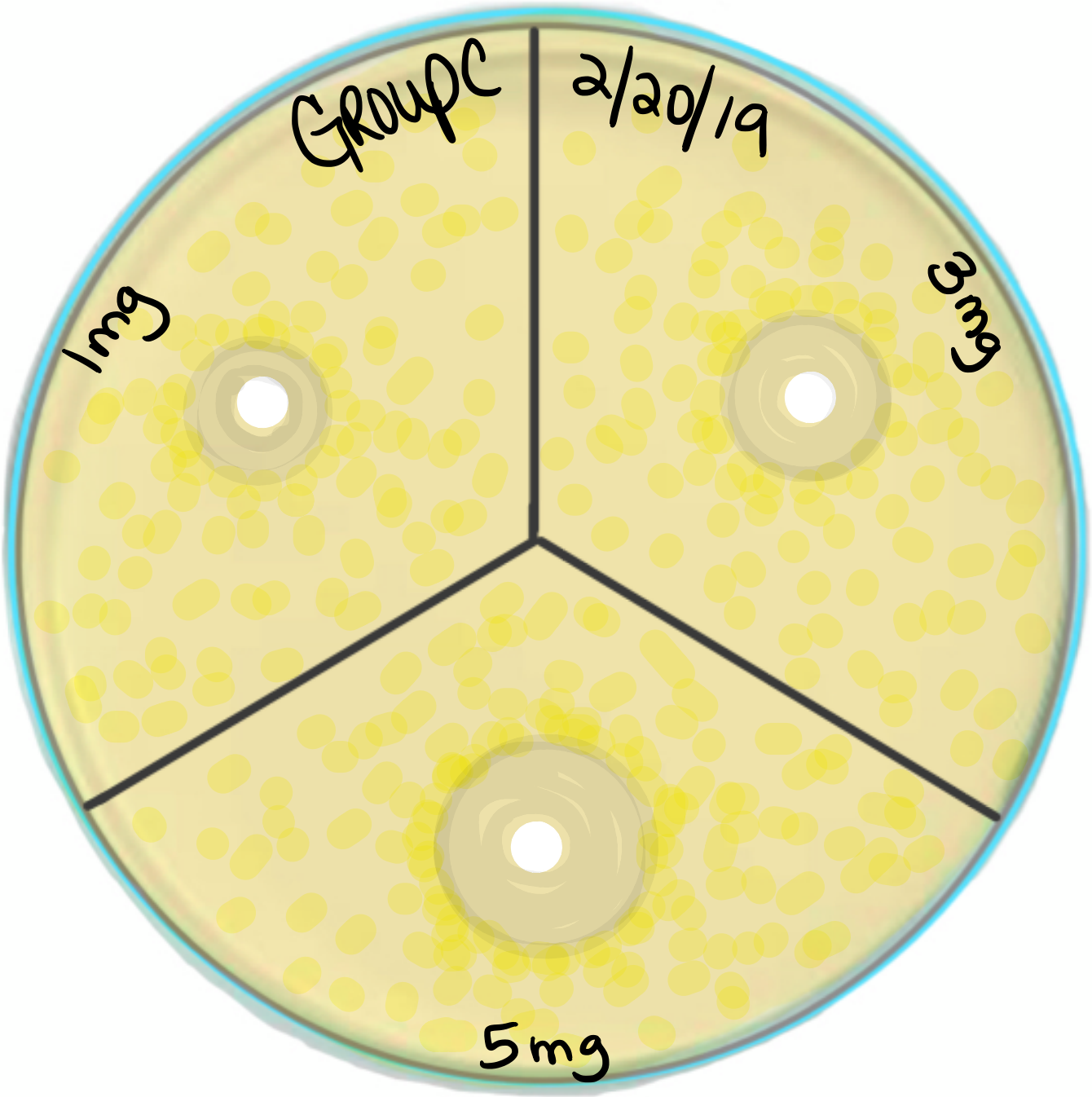
Group A Results



Group B Results



Group C Results



Group D Results