

# Microbiology Laboratory 10

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**Note:** You may want to print out this lab on single sided paper.

## Observations from Last Week's Lab

### Disk Diffusion (Kirby-Bauer) Results

Using a ruler measure the diameter of the **zone of inhibition**. You should measure from one end of the zone, across the disk to the other end of the zone. If the zone of inhibition is too large to measure across, measure the radius. To do so, measure from the center of the disk to the edge of the zone and double the measurement to obtain the diameter.

Report your results on the chalk board.

### Serial Dilutions

On the best plate available count the number of individual colonies. You want a plate with between 30 and 200 colonies. Shoot for around 100 colonies.

Hint: using a Sharpie place a dot on the bottom of the plate for each colony counted.

Then using your dilution calculations from last week, calculate how many CFUs were in 1 ml of milk and report your number on the chalk board.

## **Unknowns**

You will receive two numbered tubes, each contains an unknown bacteria that we have already used in the lab this semester. Your goal over this lab and the next lab is to identify which bacterial species your unknowns are using the methods we have worked on during the semester.

I would suggest first setting up a Gram stain.

While your smear is air drying, I would suggest you make a streak plate (Lab 5) for isolation so next week you can determine culture characteristics.

Based on your Gram stain, determine what type of selective and differential media you want to culture your unknown on. You may (encouraged to) share plates with others. If you report every test you could do (as opposed to the best choice of tests) you will loose points. (We don't want to waste resources.)

Fill out the Unknown Identification Report as you go. Next week at the end of the laboratory you will turn in your Report to the instructor for scoring.

# Unknown Identification Report

Name \_\_\_\_\_

+ = positive      — = negative but test run      n/a = not applicable (test not run)

Unknown ID #		
Observations & Results		
Gram stain		
Colony characteristics - configuration - margin - elevation - pigment		
Cell shape and arrangement		
EMB - growth - lactose fermentation		
MacConkey agar - growth - lactose fermentation		
Mannitol salt agar - growth - mannitol fermentation		
Starch degradation		
Simmons citrate agar - growth		
Lactose broth - growth - acid production - gas production		
Sucrose broth - growth - acid production - gas production		
TSI - slant/butt (K or A) - gas production - H <sub>2</sub> S production		
SIM - sulfur - indole - motility		
Anaerobic growth		
Endospore production		
Catalase		
Miscellaneous		
Organism ID		