

VIRTUAL LAB 4 - DETERMINING THE NUMBER OF BACTERIA IN COMMON PROBIOTIC SUPPLEMENTS

Your digestive tract is colonized with an estimated 100 trillion bacterial cells – in fact there are more microbial cells in you and on you than there are human cells. The bacteria in your gut influence your own health. They help breakdown some of the food you eat, they produce vitamins such as vitamin K that your body needs, and they influence the development of your immune system when you are an infant. In fact, current research suggests that the influence of the microbes in your digestive tract – referred to as your gut microbiome - may influence your health in ways we never imagined, impacting the development of conditions such as diabetes, allergies and even depression.

It is thought that ingesting food or supplements containing live cultures of bacteria may influence the composition of your gut microbiome and provide a health benefit. These live cultures are called probiotics. Many companies market probiotic supplements in pill and liquid form. Furthermore, commercially available yogurt often lists live bacterial culture as an ingredient and is also referred to commonly as a probiotic. In this virtual lab, you will observe the steps taken in order to determine the number of active, live bacteria (the colony forming units, or CFUs) present in several probiotic supplements. You will then analyze the data obtained from these plates, and write a lab report that highlights the data that was obtained.

Materials per supplement:

5 deMan, Rogosa and Sharpe (MRS) agar plates (for selection and growth of *Lactobacillus*, the most common culture in probiotic supplements)
5 tubes containing 9.9 ml of sterile saline
1 sterile test tube
Sterile distilled H₂O
Glass or metal spreader in a beaker of ethanol
Mortar and pestle, if needed
Pipette tips, glass pipettes and pipette aid

Procedure:

1. Dissolve or resuspend 1 pill or capsule of your supplement in 5 ml of sterile distilled water. You might need to use the mortar and pestle to grind it up if it is a pill. If it is a liquid supplement you do not need to dilute it. If you are working with yogurt, weigh out 2 g of yogurt and dilute it in 5 ml of sterile water. Record all the manufacturer's information about the supplement/yogurt, especially the number and species of live bacteria/pill or capsule or ml of solution.

2. Perform a set of serial dilutions of your probiotic or yogurt solution into 9.9 ml saline tubes. If working with diluted yogurt be sure to clean the pipette if any yogurt gets on it. Make sure to label your tubes, vortex your probiotic solution before starting, and vortex any dilution before transferring an aliquot of it to a new tube.

3. Now prepare your spread plates. Label 5 MRS plates as 10^{-5} dilution, 10^{-6} dilution, 10^{-7} dilution, 10^{-8} dilution, 10^{-9} dilution, 10^{-10} dilution, and 10^{-11} dilution. Working 1 plate at a time, plate 100 μ l of the appropriate solution onto each labelled plate. Sterilize the spreader and spread the plate.

4. Incubate your MRS plates at 30°C for 48 hours.

5. Observe your plates and count the colonies after 48 hours of growth. Count plates that have between 25 and 150 colonies only (some employers will request between 20-250).

Questions to Answer:

1. Draw a schematic of the serial dilutions and spread plates that were prepared in the lab (you only need to draw it out ones, as the same dilution set was used for each sample). You may draw by hand and photograph your results.
2. Fill in the data for the logo sample using the information from the lab video. Using the dilution factor of the appropriate plates, calculate the number of CFU/ml in two original probiotic solutions. From this value, calculate the total CFUs in your original capsule or tablet or serving of yogurt. (You will need to either print this table, or copy it into a Word or Excel document to fill it in).

Sample	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Krema	147	3	0	0	0	0	0
logo							
Probiotic capsule A	57	5	0	0	0	0	0
Probiotic capsule B	TNTC	84	15	7	2	3	0
Coconut yogurt	33	2	0	0	0	0	0
Olympic Greek yogurt	TNTC	86	13	10	7	6	0

3. Formal Report: This lab report should be completed as a FORMAL write up. Please hand in a computer typed copy using 12-point font and double-spaced. Your instructor will be primarily marking the scientific content (and how well you follow the instructions attached), but some points will be awarded for the quality of your scientific writing.