December 2021

# FOOD AND BIOTECHNOLOGY
# The Good, the Bad, and the Complex

## Part 1: The Good—Lactic Acid Bacteria

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ABE Master Teacher Fellowship Program

AMGEN® Biotech Experience

Scientific Discovery for the Classroom

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# The Lactic Acid Bacteria: Microbiology 101

## INTRODUCTION

Working with microorganisms in the lab will allow you to develop and use several skills; problem-solving, research skills, organisation and planning, lab techniques, mathematical skills, and lab health and safety.

As you learn to use the tools and techniques of microbiology, you will deepen your knowledge and understanding of cell structure and function, cell division, bacterial growth and nutrition, and other aspects of biology.

The technical skills you are about to learn—aseptic technique, micropipetting, and bacterial culture—will also be useful when you carry out more-advanced molecular biology investigations involving PCR, bacterial transformation, and gene cloning.

## PROBIOTICS

Each year, consumers worldwide spend billions of euro on fermented yoghurt that are marketed as being health-promoting. These live yoghurts contain bacteria that have been claimed to provide health benefits when consumed. The word ‘probiotic’ is used to refer to these kinds of bacteria and the products in which they are contained.

There has been a lot of research on the effectiveness of probiotics on health, and many claims have been made about the health benefits they give. We will look at these health claims in greater detail later.

Many research studies on probiotics suggest that for any observable benefit to occur, a person must ingest a very large number of the probiotic bacteria. For a yoghurt to be labelled as containing “Live and Active Cultures”, the National Yogurt Association in the U.S. requires that a yoghurt contain at least 100 million cells per gram.

# Lesson 1 – Planning the Investigation

## INVESTIGATION: HOW MANY BACTERIA ARE THERE IN A YOGHURT?

One major probiotic manufacturer has stated that each individual yoghurt drink contains 10 billion cells of *Lactobacillus* bacteria.

Your task is to find out if there are really that many cells in a yoghurt drink.


## PLANNING YOUR INVESTIGATION:

*Questions*

- Can you think of any methods you could use to answer the question?
- Are there any lab techniques that you have used that would allow you to estimate the number of bacteria in a sample?
- Do you need to be able to see the bacteria directly to be able to count them?
- What techniques do you know that allow the detection of individual bacterial cells?
- Write an outline of your plan. List the **materials** you might need and write an outline plan of what your **procedure** might be. What do you predict the results will be?

*Materials:*

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.....

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*Procedure:*

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*Diagram:*

*Prediction of results:*

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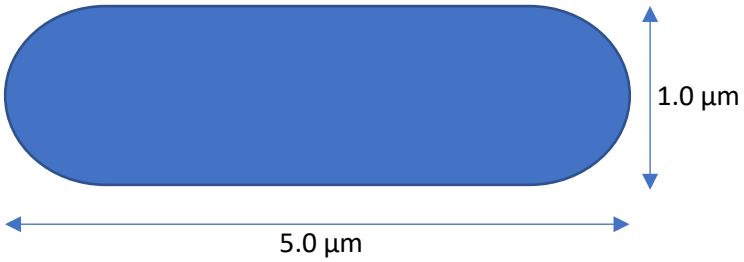
# PROBLEM-SOLVING: CAN YOU FIT 10 BILLION BACTERIA INTO A YOGHURT POT?

*Lactobacillus* is a rod-shaped bacterium of average cell size, roughly cylindrical with hemispherical ends. An average *Lactobacillus* cell is 1.0 µm wide by 5.0 µm.




(Note: a µm, or micrometer, is 1/1000 of a mm, or $10^{-6}$ m)

- Is the volume of 10 billion *Lactobacillus* cells less than the volume of the yoghurt drink?
- Is it even theoretically possible to fit 10 billion bacteria in a small pot of yoghurt?

Carry out some calculations to find out.

# Lesson 2

## ARE THERE MICROORGANISMS IN THE AIR?

You may have already carried out an investigation like this in one of your previous science lessons. You are given two sterile agar plates containing nutrient agar and asked to plan an experiment to investigate if there are microorganisms in the air around us. The nutrient agar contains a mixture of food substances for microorganisms, as well as the semi-solid jelly which provides plenty of water, and a surface to grow it on. The agar plates are **sterile** (which means they contain no living cells) as long as they remain closed.

1. Why do the plates have to be sterile for this experiment?

.....

.....

2. What kinds of nutrients do you think mixed in with the agar?

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3. Microorganisms are too small to see without a microscope. But what happens if microorganisms from the air are allowed to get onto the agar and kept in a warm place for a few days?

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4. Why do you need two plates for this experiment? Will one not do?

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5. What results do you expect to observe after a few days?

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## Exercise:

Draw a labelled diagram showing the process of how a single bacterial cell becomes a colony on an agar plate.

# INQUIRY ACTIVITY: WHAT IS THE GENERATION TIME FOR A BACTERIUM?

If we think of generation time as the difference in age between one generation and the next, we might say that the average generation time for humans is 20-30 years.

1. How long do you think it might be for a bacterium? It can vary quite a lot from species to species but see if you can find out what the time is between successive cell divisions for a fast-growing bacterium.
2. How many cells do you think there are in a bacterial colony big enough to be visible on an agar plate?
3. Using the information you found about the generation time for a typical laboratory bacterium, calculate the number of cells in a colony after 24 hours under optimum conditions.

Calculations:

# Lesson 3

## LAB SKILL: ASEPTIC TECHNIQUE

Aseptic technique underpins all work in microbiology. These are measures that are taken to, as far as is possible, exclude unwanted microorganisms from the materials you are working with. Make sure you are familiar with all these techniques before embarking on the other microbiology protocols.

Only non-pathogenic cultures should be used in schools. It is potentially very dangerous to work with bacteria isolated from environmental (soil, water or air) or human sources (skin, mouth, hair). Sterile equipment and media should be used in the transfer and culture of microorganisms. Aseptic technique should be observed whenever microorganisms are transferred from one container to another.

It is wise to treat all cultures as potentially pathogenic because cultures may have been contaminated. The aseptic techniques described here control the opportunities for contamination of cultures by microorganisms from the environment, or contamination of the environment by the microorganisms being handled.

There are some general rules to follow when using aseptic technique:

- Wear gloves and a lab coat (and a mask would help).
- Close windows and doors to reduce draughts and prevent sudden movements which might disturb the air.
- Disinfect the surface of the bench you will work on. Wiping down with alcohol works best.
- Make sure that everything you need is within easy reach before you start the procedure.
- Vessels such as agar plates or sterile bottles should be open for the minimum amount of time possible.
- While vessels are open, all work must be done close to a Bunsen burner flame where air currents are drawn upwards. This zone of sterility has a diameter of about 20 cm from the centre of the flame.
- On opening a glass test tube or bottle, the neck should be immediately warmed by flaming to sanitize the vessel opening (see procedure below). Do this by holding the vessel as near to horizontal as possible so that any movement of air is outwards from the vessel.
- During manipulations involving an agar plate, limit exposure of the sterile inner surfaces to contamination from the air. Use the clam shell method by only opening the lid 45 degrees and close the agar plate after each manipulation.
- The parts of sterile pipettes or inoculating loops which will be put into cultures or sterile vessels must not be touched or allowed to contact other non-sterile surfaces, such as clothing, the surface of the working area, or the outside of bottles/ test tubes.

More details can be found in this document.

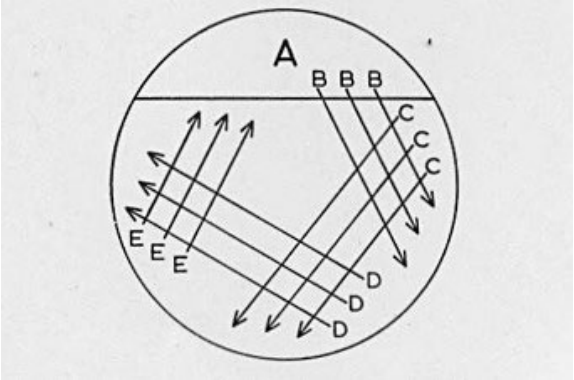
# LAB SKILL: THE STREAK-PLATE TECHNIQUE

**How to isolate a pure culture of *Lactobacillus*.**

In this activity, we will analyse our yoghurt sample for the presence of *Lactobacillus*. In some probiotic yoghurts, there are two or more different strains of bacteria present: one or two which are used to ferment the milk, and a probiotic strain which is added after the fermentation.

MRS agar is *selective* for the growth of *Lactobacillus* species. It contains acetate and has a low pH, both of which inhibit most other bacteria. It is very rich in nutrients for fussy *Lactobacillus* species. MRS growth media contain yeast extract ("Marmite", a by-product of beer brewing) which is rich in the proteins and vitamins required by *Lactobacilli*. Most species of *Lactobacillus* grow optimally at 30°C.

1. Sterilize the inoculating loop by holding it at the top of the small blue cone in the flame of the Bunsen burner until the loop is red-hot. Remove the loop and allow it to cool for 10-15 seconds.
2. While holding the inoculating loop between your thumb and forefinger(s) as you would hold a pencil, slowly insert the sterilized loop into the mouth of the sample container until you reach the liquid. Do not plunge the loop deep into the liquid. Also, be sure not to touch the outside of the sample tube with the loop, as this may introduce contamination to the sample.
3. Once you have some sample on the tip of the inoculating loop, remove the loop.
4. Lift one side of the lid of a plate to form a 45° angle (see the drawing below).
5. Gently wipe the inoculating loop with culture on the edge of an area of the agar to form a reservoir of sample (A) as shown in the diagram. Close the plate.
   *Try to avoid gouging the surface of the agar with the wire loop or digging it up. The weight of the loop on the surface of the agar is enough to allow the sample to be drawn across it successfully. This may take a few attempts to get a feel for it. Don't be offended if the first plate doesn't go so well!*
6. Sterilize the inoculating loop as you did in Step 1. Allow it to cool for 20-30 seconds. (You can set the loop down on the base of the Bunsen burner with the hot end sticking out past the base.)
7. Check to see if the loop is cooled by placing it on an unused part of the agar near the edge of the plate. The sterilized loop should not melt the agar.
8. Open the plate again. Use the loop to gently draw out 3 or 4 lines from the reservoir of sample that you have on your agar, as shown in the diagram (B). Close the plate.
9. Sterilise your loop as before, allow it to cool, and then use it to draw another 3 or 4 lines across and away from the ones you formed in Step 8 (C). Close the plate.
10. Repeat this twice more so that you have 3 or 4 sets of lines. (D and E).



# Lesson 4

## LAB SKILL: MAKING AGAR AND POURING PLATES

At this stage, you have probably used quite a few agar plates, so it is about time that you learned how to make your own. Making agar plates is a relatively straightforward procedure, but here are a few tips that can help to make the perfect agar plate.

1. **Make up the medium according to the recipe or instructions on the jar of powder mix. Add the desired amount of agar powder to the water and stir (or shake).**
   If you autoclave without stirring, with the agar still floating on top of the liquid, you can get an agar cake in the medium.
2. **Autoclave (normally 15-20 mins at 10-15 lbs psi).**
   Before placing the bottles in the autoclave or pressure cooker, make sure that screw-tops are loose and bottles are no more than 3/4 full, which will prevent the bottles from bursting or bubbling over during the autoclave cycle. If using cotton wool stoppers instead of screw-tops, cover the cotton wool with a piece of aluminium foil.

   After autoclaving, you can store the agar in a toughened glass bottle in the fridge, then melt it in a microwave or water bath when needed. Make sure you use toughened glass/Pyrex bottles or flasks. Purpose-made blue-capped media bottles are ideal.
3. **Cool the melted agar to 55°C. For consistent results, cool the bottles of agar in a 55°C water bath.**
   Agar starts to solidify at about 42°C. It's comfortable to handle the bottles at 55°C. If you don't cool them enough, you can cause the plastic of the plates to soften and become misshaped. Using the water bath means you can consistently cool the mixture to just above the setting temperature. If you just cool with air on the lab bench, chances are that you will get lumpy agar. Lumpy plates are no good for spreading!
4. **Add any antibiotics or supplements.**
   Some heat-labile substances may need to be filter-sterilised and added after autoclaving. Add all sterile supplements to cooled agar aseptically.
5. **Pour the plates.**
   Open the plates only as much as you need to and beside a Bunsen flame**.** Flame the neck of the media bottle between plates (unless there is a blue plastic pouring ring on its rim!). Use about 30 ml for each ~100-mm plate, or to about half the depth of the base of the plate.

   The less amount of agar-medium mix in each plate, the faster the agar will dry. Keep in mind, 30 ml is a good amount for long-term storage; 10-20 mL is fine if you are going to use the plates relatively soon. Try to minimise adding bubbles, especially while pouring the last bit of agar at the end of the bottle. You may need to very gently rotate the plate to ensure the agar completely covers the base of the plate, but avoid getting agar on the lid when doing this.
6. **Allow the plates to set.**
   If there are any bubbles in the plates, briefly pass the Bunsen flame over to pop them. A safer option to clear bubbles on the top of agar is to use a sterile 200-uL pipette tip to pop the bubbles before agar solidifies. Once poured, don't try to move the plates before they set. If agar gets onto the inside of the lid, it can form a bridge for outside contamination to enter the plate.

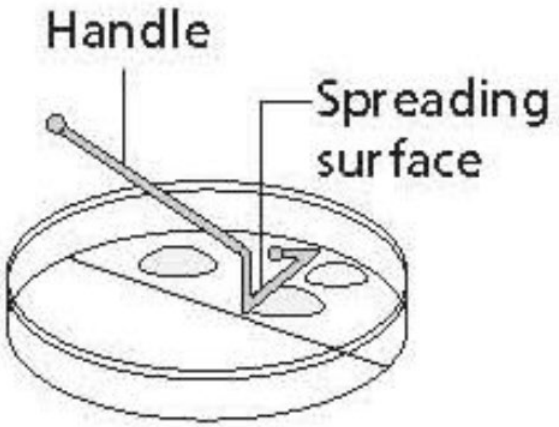
7. **Dry the plates in a 37°C incubator for 1-2 hours, or at room temperature for 24 hours.**
   Allowing the agar to dry a little is important for storing the plates and for growing colonies on them. If the agar plates aren't dried sufficiently, the moisture will evaporate and condense on the lid during storage or during incubation, and will give you horrible wet plates to work with. This condensation may drip down onto a plated experiment and disrupt growing colonies. If the plates are too dry, then they will not have sufficient moisture for the bacteria to grow over several days in the incubator, as the agar will lose moisture during incubation.
8. **Use the plates immediately or seal them before storing.**
   You can use Parafilm to seal each plate or a few grouped together, or you can put them back into the bag that the empty plates came in, sealing the bag with tape. Store the plates at 4°C upside down (agar side up).

   A quick way to label your plates is to have a colour code for each medium type you tend to use (e.g., red for Malt extract, black for nutrient agar, green for LB, blue for MRS). Stack the plates and use the appropriately coloured lab marker to draw a line down the whole stack. Be sure to keep a log of what colour indicates which type of medium and supplements added.

# Lesson 5

## LAB SKILL: THE SPREAD-PLATING TECHNIQUE

Sterile plate-spreaders (or 'hockey-sticks') can be used to evenly spread a sample of liquid across the agar. The volume of liquid should be between 100 µl and 200 µl to get an even spread across the whole agar surface, and so all of the sample is well absorbed by the agar. The agar plates should not be excessively wet before adding samples for spreading.




After incubation, the number of colonies on the agar plate gives you an estimate of how many bacteria were in the sample. This is known as the 'colony count method' for estimating the number of bacteria in a sample.

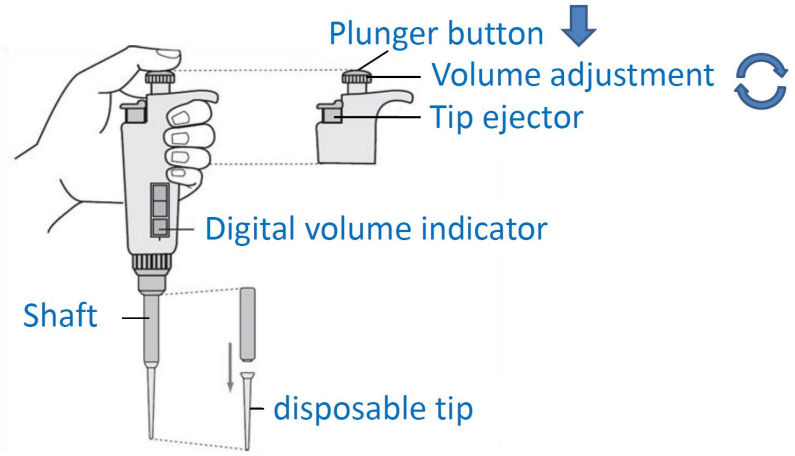
For the colony count method to provide an estimate of the numbers of bacteria in a sample of yoghurt, the volume of sample applied to the plate needs to be accurately measured. This is where the micropipette comes in.

# LAB SKILL: MICROPIPETTING

A micropipette is used to precisely and accurately measure and transfer very small volumes of liquids in either milliliters (ml, thousandths of a liter) or microliters (µl, millionths of a liter), These are the measurements of volume most often used in microbiology, molecular biology and genetic engineering. In the following activity, you will have the chance to learn how to use the micropipette and to see the relative size of different amounts of solution measured by this very precise tool and how precise the amounts that you can measure with it are.

*Materials*

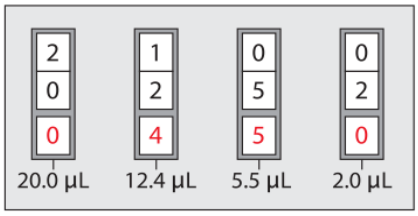
- A plastic microfuge tube rack
- Microfuge tubes
- Red dye solution
- P-20 micropipette (measures 2.0 µl – 20.0 µl)
- P-200 (measures 20 µl – 200 µl)
- Tip box of disposable pipette tips for P-20 and P-200
- Micropipette practice sheet (laminated or placed in a plastic bag)
- Waste container for used tips and microfuge tubes (will be shared among groups)

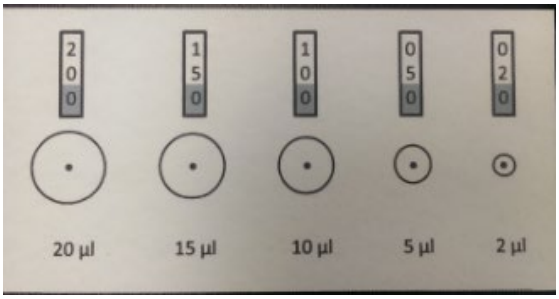



*Method*

1. Review the parts of the micropipette shown in the diagram.
2. Find the display window on the handle of the micropipette.
3. Turn the wheel on the handle of the micropipette clockwise—to the right—to decrease the volume, or counter-clockwise—to the left—to increase the volume.
4. Set the volume to 20.0 µl.
5. Press the plunger button to the first stop position. Then continue pressing the plunger to find the second stop position. Repeat this step to become familiar with the 2 stops on the pipette plunger.
6. Gently but firmly attach a tip to the micropipetter and close the tip box. While holding the plunger at the first stop position, place the tip into the red dye.
7. Slowly release the plunger to draw the liquid into the tip.
8. Withdraw the tip when the plunger is fully back to its original position. The tip should contain 20.0 µl of red dye.

9. To release the liquid from the tip, press the plunger to the first stop, then to the second stop which will remove any remaining liquid from the tip. Do this while holding the pipette very still, while hovering over where the liquid is to be dispensed.
10. Practice pipetting different volumes of red dye onto the micropipette practice sheet.




**Pipetting precautions**

- When loading the micropipette, **only press the plunger to the first stop** or you will draw too much solution into the pipette tip.
- When drawing up the solution**, move your thumb gently upwards**; if you let go too fast, you will end up with the wrong volume and air bubbles in the upper part of the tip.
- **Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward** because fluid could leak back into the pipette and damage the spring mechanism inside it, rendering it inaccurate or inoperable.

# Lesson 6

## PROBLEM-SOLVING: HOW TO COUNT TO 10 BILLION

Now that you have some skills in micropipetting and spread-plating and you have learned about the colony count method, how can you apply these skills to estimating the number of bacterial cells in a yoghurt drink?

If there are 10 billion cells ($10^{10}$) in each yoghurt drink (100 ml), then that is:

100,000,000 cells per ml

*or*

100,000 cells per µl

## CONSIDER THIS

As you plan how to estimate the number of cells in yoghurt, consider the following:

1. The smallest volume that can measured with your P-20 micropipette is 2 µl.
2. If there really are 10 billion cells in each yoghurt, then a 2µl sample would contain 200,000 cells.
3. Two microlitres of liquid is too small a volume to spread evenly across the surface of the agar. The ideal volume to spread on an agar plate is 100-200 µl.
4. If we spread 200,000 cells on an agar plate, the resulting colonies will be so close together that they will merge to form a continuous lawn of bacterial culture, and not individual countable colonies.
5. There needs to be a countable number of colonies on a plate.
    a. If the number of cells is too large, the resulting colonies well merge to form a lawn.
    b. If the number of cells is too small, it may not give an accurate estimate of the number of cells in the sample.
6. It may well be the case that there are far fewer cells in the yoghurt than is being claimed by the manufacturer.

## SERIAL DILUTION, COLONY COUNTING, AND SPREAD-PLATING

You may have concluded that you need to dilute your yoghurt in some way. Since we don't really know how many cells are in the yoghurt, you may have also realised that you need to spread-plate a few different dilutions.

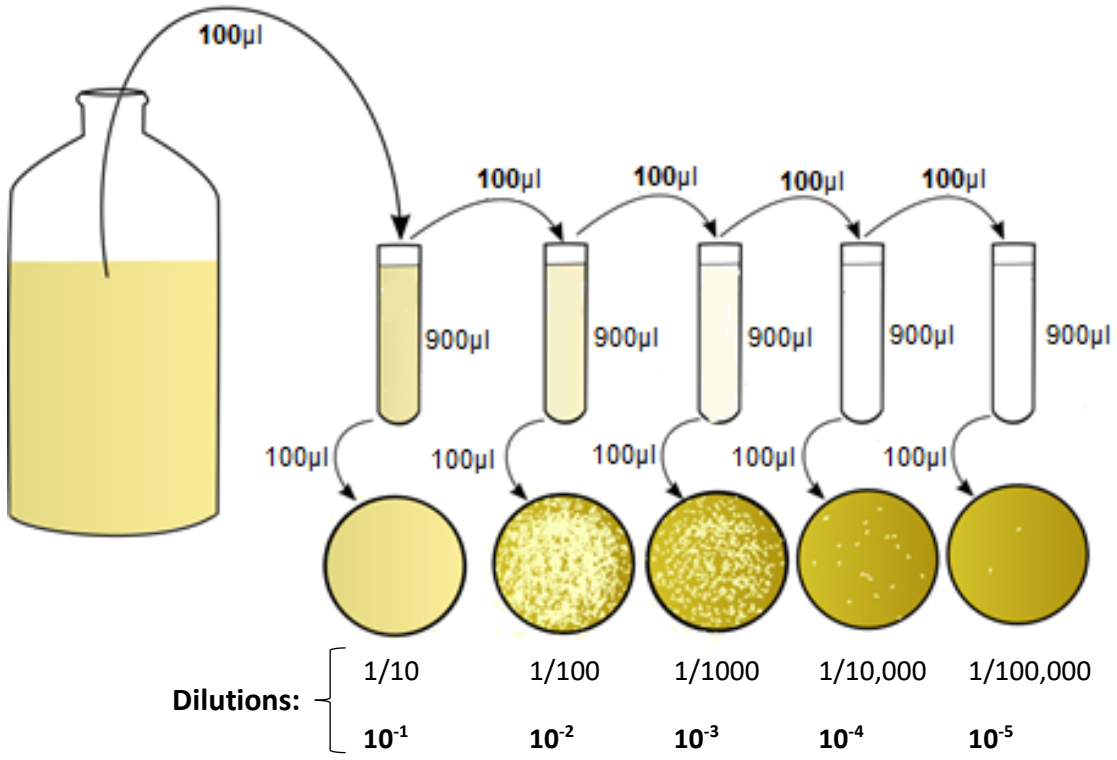
If you need a range of dilutions, it might seem like a good idea to make dilutions such as 1/2, 1/3, 1/5, 1/10, 1/20.

However, it would take a lot of time and effort to make each of these dilutions. You would have to measure different volumes and do different calculations for each dilution, making them up separately.

The range of dilutions in the above case is also uneven (i.e., the differences in concentrations between each dilution is not the same). This could make your results difficult to interpret.

Serial dilution is a better method of making a range of different dilutions of the original sample. It is a useful technique when we are not sure which dilution is the one we require for optimal results. Serial dilutions are made by doing the same dilution over and over, using the previous dilution as the input for the next dilution in each step. This can be done in a series of two-fold dilutions (i.e., 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128).

In this instance, because of the numbers of bacteria that may be present in the original sample, we are going to carry out a **ten-fold serial dilution** as shown in the figure below.




*Ten-fold serial dilution of a sample showing volumes.*

# Practicing serial dilution

## MATERIALS

- Tube of red food colouring
- Tube of water
- Empty tubes (x5)
- P200 Micropipette
- P20 Micropipette
- Box of tips

## TASK

By way of practice, make a range of dilutions in water of the red food colouring similar to those you will need when carrying out your yoghurt investigation.

# Lesson 7

## SERIAL DILUTION, COLONY COUNTING, AND SPREAD-PLATING

### MATERIALS:

- Micropipette (P-200)
- Box of P-200 sterile tips
- 5 x microcentrifuge tubes (1.7 ml)
- Tube rack
- Bottle of sterile saline (0.11% NaCl)
- 5 x MRS agar plates
- Gloves
- Disinfectant or 70% Isopropanol
- Waste tip beaker
- Permanent marker

### METHOD

1. Prepare yourself and your bench for working aseptically and safely (i.e., tie hair back, wear gloves, lab coat, safety glasses, and wipe lab bench with disinfectant).
2. Label five tubes as $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$
3. Into each of the five tubes, pipette 900 µl of sterile saline solution. (Remember to use aseptic technique by flaming the neck of the bottle in the Bunsen burner and ensuring the pipette tip does not touch contaminated surfaces).
4. Pipette 100 µl of yoghurt into the first of your five tubes (the one labelled $10^{-1}$).
5. Close the lid on the tube and mix the contents thoroughly. You can do this by repeatedly inverting the tube or by holding if firmly between finger and thumb of one hand and flicking the tube with the forefinger of the other. If the lab is equipped with a vortex, this can also be used to mix the contents.
   *Note:* It is very important that the contents are evenly mixed before you take a sample of it for the next step.
6. Using a fresh pipette tip, remove 100 µl of the $10^{-1}$ dilution, and add it to the next tube (the one labelled $10^{-2}$).
7. Repeat Steps 5 and 6 to make the $10^{-3}$, $10^{-4}$, $10^{-5}$ dilutions.

8. Label the bottom edge of each of the five MRS agar plates with the corresponding tube labels ($10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$). Also include the date and your initials along the edge of the plate.
9. Pipette 100 µl of the $10^{-5}$ dilution onto the surface of the agar in the plate labelled $10^{-5}$.
10. Repeat this for the $10^{-4}$ plate using the $10^{-4}$ dilution, pipetting 100 µl, and continue with the other tubes by pipetting 100 µl onto their corresponding agar plates. Replace the lid of each plate after adding 100 µl of the diluted culture.
11. Spread each dilution evenly across the surface of the agar using a sterile plate-spreader. *Note:* if you work from the most dilute samples to the most concentrated (i.e., from $10^{-5}$ to $10^{-1}$), you can use the same plate-spreader for all five spreads. This saves on spreaders and saves on waste plastic.
12. Place your five agar plates inverted (agar side up) and stacked in an incubator at 37°C for 3 days. If possible, have students examine the plates each day and report any growth. *Lactobacilli* grow slowly and often appear as small specks before growing into full colonies.

## CALCULATIONS:

## DIAGRAM

# Lesson 8

## INTERPRETING THE RESULTS

1. After incubation, one of the plates will hopefully contain a good countable number of colonies. You don't want too few because the margin of error for your estimate would be too large. Too many will be impractical to count. Somewhere between 40 and 200 colonies would be good.
2. Count colonies by using a fine-tipped permanent marker and touching the marker to each of the colonies as you count them. This helps to avoid double-counting or missing some colonies. If you lose count or make a mistake. Simply use alcohol to wipe off counting dots and dry the plate and start over.
3. Once you have counted the number of colonies for that particular dilution, you can calculate an estimate of the number of cells in the original yoghurt.

### CALCULATING AN ESTIMATE OF THE NUMBER OF CELLS IN THE ORIGINAL SAMPLE

Consider the following example of results

| Agar Plate No. | Dilution | Colony count on agar plate |
|---|---|---|
| **5** | $10^{-5}$ | 9 |
| **4** | $10^{-4}$ | 120 |
| **3** | $10^{-3}$ | Approximately 1000 (too many to count) |
| **2** | $10^{-2}$ | Lawn (no individual colonies) |
| **1** | $10^{-1}$ | Lawn (no individual colonies) |
| **UD** | Undiluted | Lawn (no individual colonies) |

***Precision and Accuracy***

The number of colonies in agar plate 5 is too few to make an accurate estimate of the number of cells in the original yoghurt. **Accuracy** refers to how close the result is to the actual number of cells in the yoghurt. Agar plate 4, however, provides a large enough number of cells to provide us with more confidence regarding *accuracy*.

Note also that each agar plate appears to have approximately 10 times as many cells as the dilution below it. This is expected as the dilutions were all tenfold, and spread-plating techniques were carried out with a high degree of precision. **Precision** refers to the closeness of two or more measurements to each other. In this context, precision comes from good, consistent micropipetting technique (as well as good micropipettes), thorough mixing, spread-plating, and counting. Our results depend on repeatedly measuring 100 µl *precisely* by using the micropipettors.

***Calculation Example***

A colony count of 120 on the $10^{-4}$ plate tells us that there were approximately 120 CFUs (colony forming units, i.e., bacterial cells) in 100 µl of the $10^{-4}$ dilution of the original yoghurt.
This is equivalent to 1,200 CFUs in 100 µl of the $10^{-3}$ dilution.

or

1,200,000 in 100 µl of the original undiluted yoghurt drink.

This gives us a final estimate of 1,200,000,000 cells in 100 ml.

***In Summary***

| CFUs | Volume of sample | Dilution |
|---|---|---|
| **120** | 100 µl | $10^{-4}$ |
| **1,200** | 100 µl | $10^{-3}$ |
| **12,000** | 100 µl | $10^{-2}$ |
| **120,000** | 100 µl | $10^{-1}$ |
| **1,200,000 or 1.2 x $10^{6}$** | 100 µl | undiluted |
| **1,200,000,000 or 1.2 x $10^{9}$** | 100 **ml** | undiluted |

## RESULTS:

| Agar Plate No. | Dilution | Colony count on agar plate |
|---|---|---|
| **5** | $10^{-5}$ | |
| **4** | $10^{-4}$ | |
| **3** | $10^{-3}$ | |
| **2** | $10^{-2}$ | |
| **1** | $10^{-1}$ | |
| **UD** | Undiluted | |

## CALCULATIONS

## CONCLUSION

In the space below, summarize what you have learned about the amount of bacteria in probiotic yoghurt drinks.

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