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MI 100th Learning Journey

13-27 May 2025

Participant's Manual

(v2025-05-07)

This manual was made for: _____

BEFORE READING THE PROTOCOL, sign in to your Google Classroom Account and take the Pre-Event Test to measure your baseline knowledge about microbiology and immunology! Use <https://forms.gle/yEg9Z6Eh83LHRuLY7> or the QR code on the right.



Overview & Learning Objectives

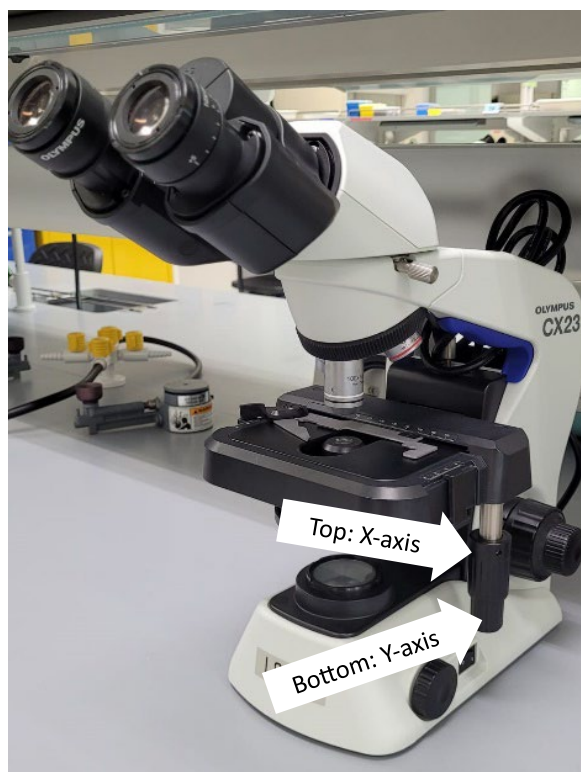
At the end of this Learning Journey, participants should be able to:

1. Deduce that bacteria, fungi, viruses and protozoa are very different types of microbes that are ever-present in nature and may be disease causing (pathogens) or health-promoting (probiotics).
2. Describe our immune system as comprising innate and adaptive responses, using phagocytosis, cytolysis and antibody neutralization as examples of how our body defends against microbial pathogens.
3. Articulate that antimicrobials are important in the fight against infection, but that the rise of antimicrobial resistance (AMR) is compelling us to explore other approaches such as phage therapy to treat multidrug-resistant infections.
4. Examine the levels of probiotics present in consumer products and comment on the benefits of microbes to our healthy development.

Section1: Microbes All Around

Reminders:

1. Wear your gloves and safety glasses.
2. You have the freedom to move between microscopes on your side of the bench at your own pace.
3. Microscopes#1-3 have already been set up and adjusted to focus. Let your Teaching Assistant know if you have difficulty seeing the specimen.
4. Move the stage using the slider bar which controls movement on X and Y axis.
5. Attempt 1.4 if you have completed 1.1-1.3 and have >10min to spare.

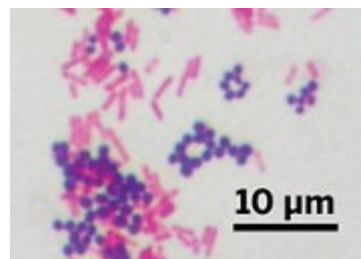
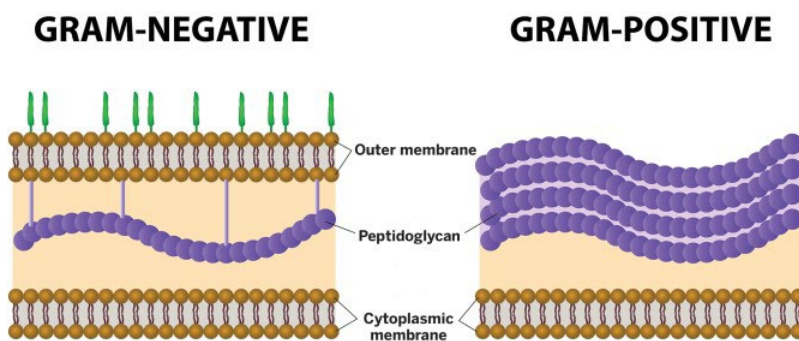


1.1 BACTERIAL Biofilms (Gram Stain)

Background

Microbes are present everywhere, existing predominantly in the form of biofilms, in communities of different types of microbes (mostly bacteria but also fungi, viruses, protozoa, archaea). The slimy biofilm matrix allows microbes to stick to surfaces, avoid dehydration and to resist stressors like antibacterial detergents. For today's setup, we scooped out some biofilms from a Lecturer's washing machine rubber gasket, spread it on a glass slide and performed a Gram stain.

Gram staining is used to differentiate bacterial species into two groups based on the structural differences in their cell walls: Gram-positive and Gram-negative. The process involves several steps: First, bacterial cells are stained with crystal violet dye, which penetrates the cell walls. Then, iodine is applied, forming a complex with the crystal violet that enhances retention within the cell wall. Next, the cells are washed with alcohol, which decolorizes Gram-negative bacteria but not Gram-positive bacteria which have thicker peptidoglycan layer that retains the purple crystal violet-iodine complex. A counterstain, fuchsin, is applied, staining the decolorized Gram-negative bacteria pink, while Gram-positive bacteria remain purple (lighter pink staining is not noticeable).



Gram-positive cocci and Gram-negative bacilli

<https://cen.acs.org/articles/93/web/2015/04/New-Spin-Old-Gram-Stain.html>

Steps to Follow

- Look at the Gram-Stained Biofilm specimen using the 100x objective. Observe the mixture of Gram-positive (purple) and Gram-negative (pink) bacteria, and the different shapes (spherical-shaped cocci, rod-shaped bacilli or corkscrew spirilla). Microbes should have a distinctive shape/size, unlike the debris/dirt which is cloudy looking.
- Record some of your observations and, if you have time, Google/ChatGPT for a possible identity of this washing machine biofilm community member and if it is hazardous to health.

Color (Gram +/-?)	Shape	Possible microbe ID?	Hazardous?

Just Keep Thinking!

- Should the Lecturer be worried about our findings?
- How sure are we about the identity of these microbes?
- Do you think your washing machine will have the same mixture of microbes?

1.2 FUNGAL Colony (Lactophenol Cotton Blue Stain)

Background

Fungi are a special group that can exist in multiple forms: yeasts, moulds and mushrooms. We know that some can be eaten: yeasts to make bread and wine, moulds to make certain cheeses and salami, and delicious mushrooms that we can turn into soup! But we also know fungi can cause infections and spoil our food, our favourite clothes and our Ikea furniture. Unlike plants, fungi lack chlorophyll and therefore do not perform photosynthesis; instead, they obtain nutrients through the absorption of organic matter. Fungi play crucial roles in ecosystems as decomposers, breaking down dead organic material and recycling nutrients.

Steps to Follow

- A. Look at slide with the Lactophenol Cotton Blue-Stained colony of *Aspergillus fumigatus*. Using the “cellotape flag” method, a strip of sticky tape was gently pressed onto the surface of the colony’s edge to retrieve the sample before being affixed onto the glass slide with a droplet of LPCB stain.
- B. Take a photo or draw out a diagram of what you see. The “stalks” are called Stipes, the “petals” are the Conidia and the center holding the “petals” is the Phialides.

My drawing of *Aspergillus fumigatus*:

“Happy Mother’s Day!” 😊

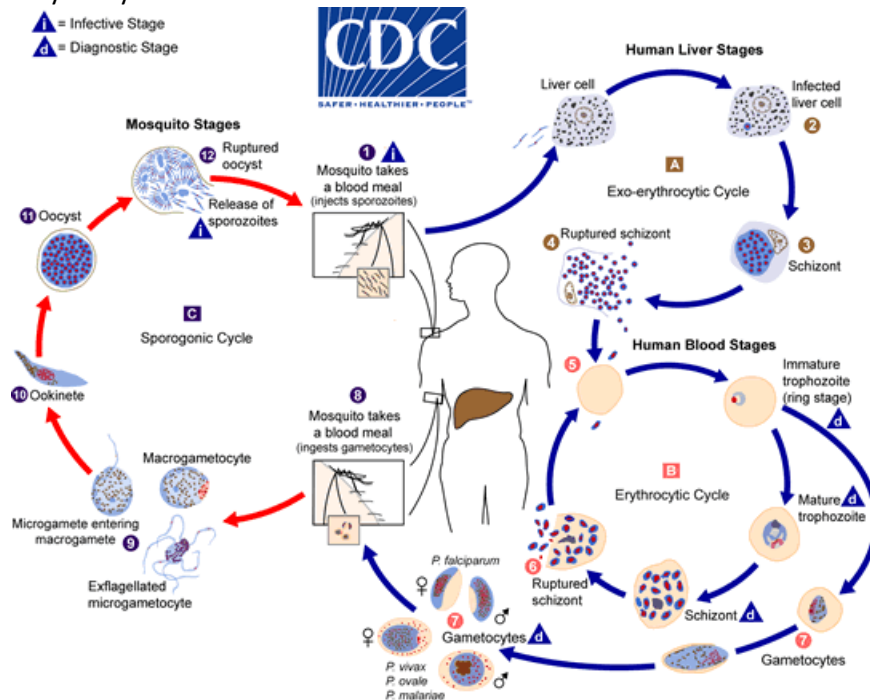
Just Keep Thinking!

- i. How many fungal spores do we breathe in with each breath?
- ii. Who typically suffer from fungal infections?
- iii. Besides getting an infection, how else can fungi harm us?

1.3 PROTOZOAN Parasites Causing Malaria (Giemsa Stain)

Background

The most gorgeous microbes under a microscope, protozoa are single-celled, eukaryotic organisms that are characterized by their ability to move independently, usually through mechanisms like cilia, flagella, or pseudopodia. As a diverse group, protozoa inhabit a variety of environments, including soil, freshwater, and marine habitats. Many protozoa play integral roles in ecological food chains, serving as both predators and prey. While most protozoa are harmless, some are known parasites responsible for significant human diseases, such as malaria caused by *Plasmodium* species. Despite tremendous improvements to detect and cure malaria, and to suppress the *Anopheles* mosquitoes transmitting the parasite, malaria still kills one young child every single minute. The species which accounts for almost all malaria deaths is *Plasmodium falciparum*, which has an amazingly complex life cycle (below). The 48hr red blood cell (erythrocytic) cycle is responsible for falciparum malaria symptoms which can quickly kill if treatment is delayed. Patients can progress from fevers and chills, to convulsions, coma and death in only a day.



Steps to Follow

A. Look at slide with Giemsa-stained thin blood smear with *P. falciparum* malaria parasites of different stages. These intracellular parasites have been grown in a laboratory using human red blood cells as hosts (seen as pink circles). These red blood cells will contain some parasites in different stages: ring stages (resembling a diamond ring), mature trophozoites (filled purple circles within RBCs), and schizonts containing >20 little merozoites. These merozoites will be released from the ruptured red blood cell to infect new red blood cells and perpetuate the deadly erythrocytic cycle.

Just Keep Thinking!

- How can you differentiate between mosquitos that spread malaria and those that spread dengue?
- Singapore was declared malaria-free in 1982 – how did we eliminate malaria?
- Is there anything you can do to help those who are at highest risk of getting malaria?

1.4 WILD microbes (Wet Mount)

Background

Microbes are all around us – they are not only natural but also necessary for life on earth. Let's explore some wild microbes from a freshwater pond. As mentioned, the most beautiful single-cellular microbes are protozoa, but there are also plenty of astonishing microscopic animals that await your discovery!

Steps to Follow

- A.** Go to the Lecturer's bench and help yourself to a clean glass slide.
- B.** Use the scissors, tweezers and dropper to harvest a VERY SMALL SAMPLE of detritus (rotting leaves) or algae that should be placed onto the glass slide. **NB: If the sample is too thick, your cover slip will not stay on and you will not be able to see anything under the microscope!**
- C.** Gently lower a glass cover slip onto the sample – ideally, you should see that water is pulled across the underside of the whole coverslip.
- D.** Head back to your bench, turn on the unused 4th microscope and put your slide with the coverslip facing upwards.
- E.** Turn on the light, select the 4-40x objectives and focus. You may seek assistance from your Teaching Assistant for this.
- F.** Adjust the position of the stage until you find something awesome!

Just Keep Thinking!

- i. What are some of the most striking features of this organism?*
- ii. What do you think these features do for the organism?*
- iii. Can you begin to figure out what organism you are looking at? Here is a video guide from MicrobeHunter: <https://www.youtube.com/watch?v=iHPDW12kG8k>*
- iv. Is it safe for me to swim in a freshwater lake?*

Section2: Immune Defenders

2.1 Detecting Anti-*Streptococcus mutans* Salivary Antibodies

Background

We encounter millions of microbes daily. Yet, for most of us who are healthy, falling ill from these exposures is uncommon, thanks to our body's remarkable defensive immune system which works tirelessly to protect us from severe illness. This sophisticated network of protective mechanisms is designed to identify and eliminate harmful invaders like bacteria, viruses, and other pathogens. This system operates through two primary lines of defence, each featuring various types of immune cells with specialised roles.

Our immune system's first response to threats is via innate immunity. It consists of several immune cell types, including phagocytes, which are among the first to recognise pathogens. These cells not only engulf and destroy invading pathogens but also signal our second line of defence, the adaptive immunity. Although the adaptive immune response takes longer to activate, it is highly specific and has the ability to remember pathogens, allowing for a quicker and stronger response should we encounter the same pathogen again in future. Additionally, B cells within adaptive immunity produce antibodies, often referred to as "magic bullets," because of their precision in targeting and neutralizing pathogens. Together, the innate and adaptive immune systems safeguard our health.

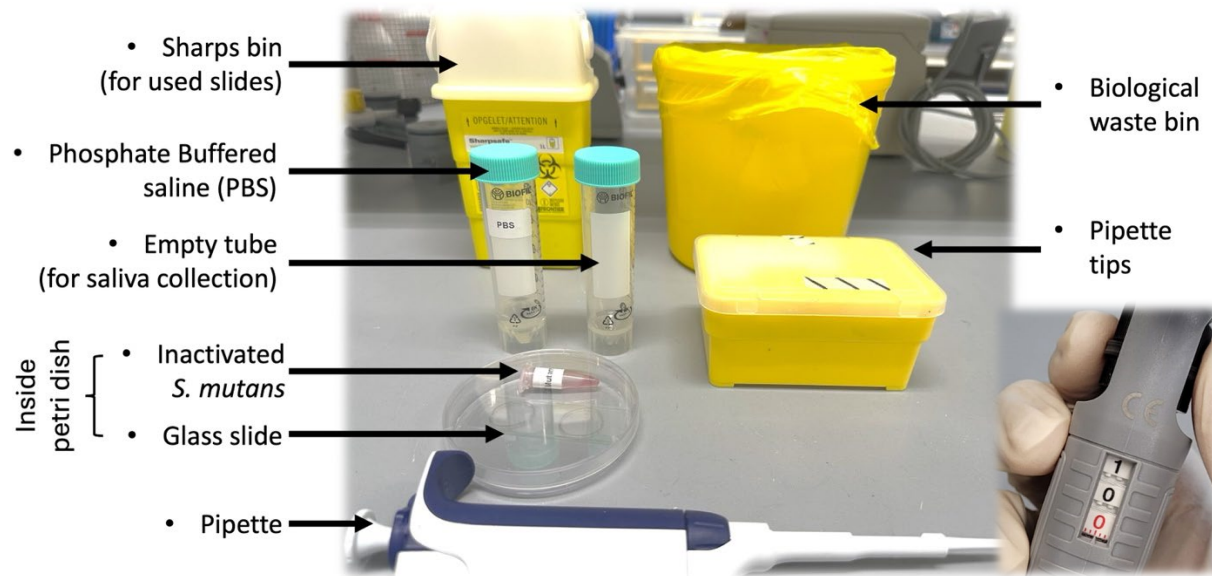
Streptococcus mutans (*S. mutans*), is a Gram-positive bacterium commonly found in our mouths. Ordinarily (and yet extraordinarily!) our adaptive immune system produces specialised antibodies which recognise and bind to glucan-binding protein B on *S. mutans*, limiting its colonisation and growth on our teeth. However, when we take in too much sugar (lollipops, candies and soft drinks), the overgrowth of *S. mutans* is no longer contained by our antibodies, leading to the breakdown of tooth enamel and dental caries.

Today, we investigate the presence of antibodies in your saliva that can bind to inactivated (killed) *S. mutans* by performing an agglutination test. When our antibodies (secretory immunoglobulin A, SIgA) attach to the bacteria, they form aggregates/agglutinates/clumps on the glass slides.

Steps to Follow

- A. Spit out your saliva TWICE into the empty (unlabelled) tube. Do NOT spit phlegm.
- B. Carefully pour some PBS (phosphate-buffered saline) into the tube until it reaches the 5 ml mark.
- C. Cap the Saliva+PBS tube and swirl to mix well.
- D. Place the glass slide from the petri dish in front of you, with the circles (drawn with a wax pencil) facing up.
- E. Pick up the pipette, make sure it is set to 10.0 µl (see photo insert below, the decimal place is indicated by red digits), and insert a yellow pipette tip.
- F. Add 10 µl of PBS to the left circle drawn on the slide and replace the tip before adding 10 µl of Saliva+PBS to the right circle. (10 µl should only fill the bottom 10% of the yellow tip)
- G. Pick up the *S. mutans* tube from the petri dish and flick it 2-3 times to mix the bacteria.
- H. Using a new tip, add 10 µl of the *S. mutans* suspension to the left circle containing the PBS, but without mixing with the PBS droplet.
- I. Using a new tip, add 10 µl of the *S. mutans* suspension to the right circle containing the Saliva+PBS, but without mixing with the Saliva+PBS droplet.
- J. Take a new pipette tip and gently mix the PBS and the *S. mutans* droplets.

- K. Take another new pipette tip and gently mix the Saliva+PBS and the *S. mutans* droplets.
- L. Hold the slide in your hands and gently swirl the solutions in the circles of the slide without spilling the solutions out of the circles.
- M. Observe the formation of agglutinates in the circles after approximately 1 min. You may place the slide over the black card to be better visualize the agglutination.
- N. Dispose the slide into the sharps bin after noting your observations.



Just Keep Thinking!

- i. Do you expect any agglutinates in the circle that contains PBS and *S. mutans*?
- ii. What can you conclude if you see more agglutinates in your sample than in your friend's?
- iii. What could you conclude if no agglutinates form?

Section3: Unexpected Allies

Warning: Plates in this section may contain live, drug-resistant microbes that cause disease. They have been sealed for your safety – do not unseal them.

3.1 Discovery of Antibiotics

Background

The discovery of antibiotics marks one of the most significant breakthroughs in medical history, fundamentally altering the way bacterial infections are treated. The story begins in 1928 when Alexander Fleming, a Scottish bacteriologist, observed an intriguing phenomenon while working at St. Mary's Hospital in London. He noticed that a mould, later identified as *Penicillium notatum* (aka *P. chrysogenum*), had contaminated one of his petri dishes containing *Staphylococcus aureus* bacteria. What caught his attention was the clear zone surrounding the mould colony, where bacterial growth was inhibited. This "zone of inhibition" suggested that the mould was producing a substance capable of killing or inhibiting the bacteria, which Fleming later coined "penicillin."

This serendipitous discovery laid the foundation for the development of antibiotics, revolutionising the treatment of bacterial infections and saving countless lives. Prior to antibiotics, bacterial infections often led to severe illness or death, with few effective treatments available. Penicillin's success prompted further research, leading to the development of a wide array of antibiotics that target different bacterial processes. These drugs have become essential tools in modern medicine, used to treat a vast range of infections and prevent complications during surgeries and other medical procedures.

Steps to Follow

- A.** Look at Plate #1 "Discovery of Antibiotics". Observe the zone of inhibition around the *Penicillium* colony.
- B.** Pass around the plate to your bench mates afterwards.

Just Keep Thinking!

- i. Which fearsome diseases weren't so scary after the discovery of penicillin?
- ii. How do microbes become resistant to antimicrobials?
- iii. Over 70% of all antibiotics are used in farming as growth promoters. How does this impact us?
- iv. Which bacteria are we most concerned about today?
 - The WHO Bacterial Priority Pathogens List 2024
[https://www.thelancet.com/journals/laninf/article/PIIS1473-3099\(25\)00118-5/fulltext](https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(25)00118-5/fulltext)

3.2 Antimicrobial Resistance (AMR) and Countermeasures

Background

AMR is a growing global health concern that occurs when bacteria, viruses, fungi, and parasites evolve to resist the effects of medications that once effectively treated them. The emergence of resistant bacteria is particularly alarming, as it can lead to infections that last for a longer time, are harder to treat and more likely to spread to others. The misuse and overuse of antibiotics in human medicine, agriculture, and animal husbandry are key factors driving the development of resistance. As bacteria are exposed to antibiotics, selective pressure allows only resistant strains to survive and multiply. When used inappropriately, it could lead significant reduction in the effectiveness of these antibiotics.

To combat AMR, understanding the synergy between antibiotics is crucial. Synergy occurs when the combined effect of different antibiotics is greater than the sum of their individual effects. This can be particularly useful in overcoming bacterial resistance. For instance, the “clavulanic effect” (also known as “keyhole effect”) is observed when a combination of antibiotics results in a larger zone of inhibition than either antibiotic alone, indicating enhanced bacterial killing. This phenomenon can be a valuable strategy in treating infections caused by resistant strains, as it can restore the efficacy of antibiotics that have been rendered ineffective due to resistance.

Steps to Follow

- A. Refer to Plates #2 “Antibiotic Sensitive” and #3 “Antibiotic Resistant” and pass them around after recording your observations.
- B. Record the lowest concentration of antibiotics where the growth of *Escherichia coli* stops by reading off the E-strip (the “sharper” tip of the ellipse). This concentration is known as the minimum inhibition concentration (MIC), usually expressed as micrograms per millilitre ($\mu\text{g/ml}$).

Strain	MIC ($\mu\text{g/ml}$ of Ampicillin)
A (Plate#2)	
B (Plate#3)	

- C. Refer to Plate #4 “Antibiotic Synergy” and observe the “keyhole effect” which shows the synergistic action of beta-lactamase inhibitor (clavulanic acid) on the AMC disc (Amoxicillin-Clavulanic Acid) enhancing the antibacterial activity of cefotaxime (CTX).

Just Keep Thinking!

- i. How does MIC guide antibiotic use in a hospital?
- ii. How exactly do resistant bacteria resist antibiotics?
- iii. The *E. coli* from Plate #2 and #3 were from the same patient, have identical genomes, and resistance arose very quickly (within a week), in the context of a polymicrobial wound infection (there were other resistant bacteria also present). Could you speculate how drug resistance emerged?
- iv. What role can you play today to combat antibiotic resistance?

3.3 Phages to the Rescue

Background

Viruses are often perceived as harmful pathogens that cause diseases. However, not all viruses are detrimental; some can play beneficial roles, particularly in the microbial world. A prime example of "good" viruses is bacteriophages, or phages, which are viruses that specifically infect and destroy (lyse) bacteria. Discovered in the early 20th century, bacteriophages have a natural ability to target and destroy bacterial cells, making them a potential tool for combating bacterial infections. They can be found anywhere bacteria inhabit such as rivers, sewage treatment plants or even in our gut!

Lytic bacteriophages attach to bacterial cells, injecting their genetic material, and hijacking the bacterial machinery to replicate themselves. This process ultimately leads to the lysis of the bacterial cell, releasing new phage particles to continue the cycle. When antibiotic therapy has failed, doctors are turning to phages as an alternative. Phage therapy, the therapeutic use of bacteriophages to treat bacterial infections, has been explored for its potential advantages, such as specificity to target pathogenic bacteria without harming the beneficial microbiota.

In this setup, bacteria are grown on 24-well plates and different dilutions (from 10 \times to 1,000,000 \times diluted) of the "wild-caught" phages are added (in triplicates). After incubation, the bacteria are stained to help visualize the zones of clearance (plaques) that form due to the phages activity. The plate map is provided here:

	1	2	3	4	5	6
A	10 ⁶	10 ⁵	10 ⁴	10 ³	10 ²	10 ¹
B						
C						
D	No Lysis Ctrl (-Phage)			Lysis Ctrl (+Phage)		

Steps to Follow

- Refer to Plate#5 "Plaque Assay" (a 24-well plate) and pass it around after observing.
- Record the number of plaques* per well (P) and the corresponding dilution factor (10^x) and calculate the density of bacteriophages originally present in the sample (plaque forming units per millilitre: PFU/ml).

**A plaque refers to a clear zone formed on a lawn of bacteria on an agar plate where bacterial cells have been lysed or killed by bacteriophages.*

Dilution factor	Total # plaques wells of same dilution	Average	PFU/ml
E.g. 10 ⁵	15+10+14=39	39/3=13	13 \times 10 ⁵ = 1.3 \times 10 ⁶

Just Keep Thinking!

- Why is the specificity of phages both an advantage and a limitation in therapy?
- What checks need to be performed on the phages to increase their chances of working?

3.4 When Prevention is Better than Cure - Probiotics

Background

Microbes are often stereotyped as harmful pathogens responsible for causing diseases. However, this perception overlooks the crucial and beneficial roles that many microorganisms play, particularly within the human body. Probiotics are a prime example and defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. These “good” bacteria are typically lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium*, which are naturally found in the human gut. The concept of probiotics originated from the observation that certain populations consuming fermented foods, like yogurt and kefir, exhibited better health and longevity. Probiotics are believed to contribute to gut health by maintaining a balanced microbial environment, restricting the growth of opportunistic pathogens, enhancing the gut barrier, and training the immune system.

Probiotics are commonly consumed as dietary supplements or as components of functional foods and beverages. They are used to promote digestive health, support the immune system, and possibly prevent or treat conditions such as diarrhoea, irritable bowel syndrome and inflammatory bowel diseases. The complex interplay between probiotics and the host's gut microbiota is a growing area of research, with scientists exploring how these microorganisms exert their beneficial effects and their potential role in overall health and disease prevention. Here, we plate out diluted samples of different consumer products purchased from our supermarkets to see how many *Lactobacillus* probiotics are alive within.

Steps to Follow

- A. Look at Plate#6a (all benches) and Plate#6b/c/d/e/f/g/h/i (different depending on your bench).
- B. Count the number of colonies (N) and record the plate's dilution factor (10^x). Calculate the number of colony forming units (CFU) in each ml of the original product by using the formula: $N \times 10^x$.

Product	# colonies	Dil. factor	Initial CFU/ml
<i>E.g. ProductX</i>	29	10^6	2.9×10^7
6a: Yoghurt Drink (Farm Fresh)			
6b: Cultured Milk Drink (Yakult)			
6c. Greek Yogurt (Chobani)			
6d. Drinking Yoghurt (Just Pure)			
6e. Frozen Yogurt (Bulla)			
6f. Kimchi (Top Gourmet)			
6g. Kombucha (Yocha)			
6h. AD Probiotic Cream (Rosken)			
6i. Diarrhea Pills (Lacteol fort)			

Just Keep Thinking!

- i. What factors influence a product's CFU counts?
- ii. Which diseases can be treated successfully with probiotics? How strong is the evidence?
- iii. What evidence can you find about the health benefits of a specific probiotic species or strain?
- iv. How are prebiotics and postbiotics different from probiotics?

6a. Yoghurt Drink (Farm Fresh)



Ingredients:

Cow's Milk, Sugar, Live Culture (*Bifidobacterium*, *Lactobacillus Acidophilus*, *L. Paracasei*, *L. Bulgaricus* & *S. Thermophilus*) & Contains Stabiliser (Pectin) as Permitted Food Conditioner

<https://www.farmfresh.com.my/yogurt-drink-lychee/#focus>

6b. Culture Milk Drink (Yakult)



6c. Greek yogurt (Chobani)



6d. Drinking Yoghurt (Just Pure)



6e. Frozen Yogurt (Bulla)



6f. Kimchi (Top Gourmet)



6h. AD Probiotic Cream (Rosken)



6g. Kombucha (Yocha)



6i. Diarrhea Pills (Lactéol fort)



Product	Comments
6a: Yoghurt Drink (Farm Fresh) https://www.farmfresh.com.my/yogurt-drink-lychee/#focus	Plenty of lactobacilli at high CFU/ml. Ingredients include live culture (<i>Bifidobacterium</i> , <i>Lactobacillus acidophilus</i> , <i>L. paracasei</i> , <i>L. bulgaricus</i> & <i>S. thermophilus</i>). No CFU/ml indicated.
6b: Cultured Milk Drink (Yakult) https://yakult.com.sg/product-faqs/	Plenty of lactobacilli at high CFU/ml. According to website, each 100ml bottle of Yakult contains more than 10 billion of unique probiotics, <i>L. paracasei</i> strain Shirota which have been scientifically proven to withstand gastric juice and bile to reach the intestines alive.
6c. Greek Yogurt (Chobani) https://www.chobani.com/products/yogurt/greek/nonfat-plain-cup	Cultured nonfat milk. No CFU indicated.
6d. Drinking Yoghurt (Just Pure) https://www.pns.hk/en/sipping-yoghurt-original-flavour-chilled-0-4%C2%B0C/p/BP_460717	No lactobacilli detected. A “sterilized yoghurt which has undergone heat treatment and inoculated fermentation with <i>Streptococcus thermophilus</i> and <i>Lactobacillus bulgaricus</i> ” which does not need to be stored cold.
6e. Frozen Yogurt (Bulla) https://www.bulla.com.au/products/bulla-frozen-yoghurt-wildberry/	No lactobacilli detected. Freezing is generally not the best idea as it kills microbes. Indicates Yoghurt culture but no CFU counts.
6f. Kimchi (Top Gourmet) https://www.topgourmet.sg/fermented-vegetables/top-gourmet-kimchi	Microbes are present, but this fermented food has a complex mixture of different types of bacteria and fungi which are usually not clearly defined.
6g. Kombucha (Yocha) https://getyocha.com/pages/frequently-asked-questions-about-kombucha#WhatsKombucha	Kombucha fermentation leads to the development of a thick floating biofilm of bacteria and yeast that are not usually defined. While most of the microbes are located within that biofilm, some will be present in the liquid. To achieve an appealing ‘clear’ look, liquid may be filtered to remove microbes or even pasteurized to prolong shelf-life.
6h. AD Probiotic Cream (Rosken) https://rosken.com.my/product/ad-probiotic-cream/	No lactobacilli detected. Advertised probiotics are yogurt cultures (<i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>). Also has prebiotics (colloidal oatmeal) which enable health-promoting commensals/probiotics to grow.
6i. Diarrhea Pills (Lacteol fort) https://www.watsons.com.sg/forte-capsules-12s/p/BP_12373	No lactobacilli detected. Advertised to contain 10 billion <i>Lactobacillus</i> LB (<u>lyophilized killed</u> microbial bodies) that “stimulates the growth of intestinal flora... inhibits adherence of invasive microorganisms to epithelial cells... increases the synthesis of Immunoglobulin A... to provide instant and effective relief from acute and chronic diarrhea... promotes healthy gut and protects people from unexpected, unwelcome cases of Traveler’s Diarrhea.” Dead probiotics, also known as paraprobiotics, may continue to offer some of the benefits of probiotics such as modulating our immune system.