## WJEC England Biology A Level

## SP C1 04: Investigation into the numbers of bacteria in milk <br> Practical notes

## Introduction

Fermented milks are dairy foods that have been fermented with lactic acid bacteria. They have not been pasteurised so bacteria remain and continue to replicate in them.

This experiment investigates the number of bacteria in fermented milk of varying ages.

## Equipment

- Sample of fermented milk with a distant use-by date
- Sample of fermented milk at its use-by date
- $1 \mathrm{~cm}^{3}$ syringe
- $9 \mathrm{~cm}^{3}$ syringe
- $5 \times 9 \mathrm{~cm}$ Petri dishes
- Graduated pipette
- Molten agar cooled to $50^{\circ} \mathrm{C}$
- $25^{\circ} \mathrm{C}$ incubator
- Distilled water
- 5x screw-cap bottles
- Sellotape
- Permanent marker
- Disinfectant


## Risk assessment

| Hazard | Risk | Precaution | Emergency |
| :---: | :---: | :--- | :--- |
| Pathogenic <br> bacteria | Contamination <br> of culture | Maintain aseptic techniques <br> throughout; culture plates at <br> $25^{\circ} \mathrm{C}$ | N/A |
|  | Infection | Cover any cuts; wear disposable <br> gloves and a lab coat; wash <br> hands after practical; culture <br> plates at $25^{\circ} \mathrm{C}$ (not human body <br> temperature) do not re-open <br> incubated plates; apparatus <br> sterilised in a pressure cooker | Seek medical <br> assistance |
| Disinfectant | Flammable | Make sure that there are no <br> naked flames in the room | Put out small <br> fires with a damp <br> cloth; evacuate <br> the building |

## Method

Sterilise all apparatus in a pressure cooker for 15 to 20 minutes prior to the experiment. During the experiment, place all disposable items into a container labelled 'waste'. Once used, return all apparatus to the pressure cooker to be sterilised.

Carry out the following procedure for both samples of fermented milk:

## Serial dilution

1. Start with the solution of fermented milk. Use a graduated pipette to transfer $0.1 \mathrm{~cm}^{3}$ of the milk into a screw-cap bottle along with $9.9 \mathrm{~cm}^{3}$ of distilled water. Label this $10^{-2}$.
2. Next, use a graduated pipette to transfer $0.1 \mathrm{~cm}^{3}$ of the $10^{-2}$ solution into a screw-cap bottle along with $9.9 \mathrm{~cm}^{3}$ of distilled water. Label this $10^{-4}$.
3. Repeat until a $10^{-10}$ solution is produced:

4. Swirl each screw-cap bottle to gently mix

## Culturing bacteria

1. Take five sterile Petri dishes and label with $10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}$ and $10^{-10}$
2. Using a graduated pipette, add $1 \mathrm{~cm}^{3}$ of the $10^{-2}$ milk dilution and $12 \mathrm{~cm}^{3}$ of molten MRS agar into the centre of the corresponding Petri dish. Swirl to mix and to evenly cover the bottom of the dish. Ensure that the contents of the beaker are not transferred to the sides of the dish.
3. Add 2 to 4 pieces of sticky tape to tape the base of the Petri dish to the lid
4. Repeat steps 2 to 4 for the four other milk dilutions
5. Leave the Petri dishes at room temperature until the agar has solidified
6. Incubate at $25^{\circ} \mathrm{C}$ for five days. A temperature of $25^{\circ} \mathrm{C}$ discourages the growth of bacteria pathogenic to humans. Store upside down to prevent condensation disrupting the culture.

## Bacterial count

1. Remove the Petri dishes from the incubator. Observe the plates and select the dilution that produces the most distinct colonies.
2. Count the number of bacterial colonies present on the selected plate. Use a marker to highlight each colony counted on the Petri dish to prevent re-counting.
3. Estimate the bacterial count of the initial fermented milk sample. Each bacterial colony arises from a single cell, enabling the estimation of the number of cells in the initial culture.
e.g. 56 colonies counted on the $10^{-6}$ dilution Petri dish
$\therefore 56 \times 10^{6}=5.6 \times 10^{7}$ bacteria per $\mathrm{cm}^{3}$ fermented milk

To increase the reliability of the results, the experiment can be repeated a further two times for the dilution that produced the most distinct colonies. This gives three bacterial colony counts, enabling the calculation of a mean.

## Conclusion

The expected result is that milk at its use-by date will have greater numbers of bacteria per $\mathrm{cm}^{3}$ than milk with a distant use-by date. The increased length of time from production allows a greater period of time for reproduction.

