Bacterial Enumeration

There are various ways of counting or monitoring microbial growth in a culture.

Serial dilution involves taking a sample and diluting it through a series of standard volumes of sterile diluent, e.g. distilled water or 0.9 % saline. Then a small measured volume of each dilution is used to make a series of pour or spread plates.

By diluting the sample in this controlled way it is possible to obtain an incubated plate with an easily countable number of colonies (30–100) and calculate the number of microbes present in the sample.

Materials:

- Culture of bacteria or yeast or sample of natural material
- 6 sterile test tubes containing 9 ml
- sterile diluent, fitted with a cap or cotton wool plug, labelled 1→6 and with the dilution factor as shown in the diagram
- 12 sterile, plugged Pasteur pipettes
- 1 ml syringe barrel fitted with rubber tubing
- Pot of Virkon disinfectant

Procedure

1. Take a sterile pipette.

2. Place the syringe onto the plugged end of the pipette.

3. Draw up 1 ml of a well mixed sample/culture into the pipette.

4. Add this sample to the first tube. The volume of this tube is now 10 ml. This provides an initial dilution of 10^{-1}.

5. Mix the dilution thoroughly, by emptying and filling the pipette several times.

6. Discard this pipette into the pot of disinfectant, but keep the syringe for making the next dilution.

7. Take a new pipette, fit it to the syringe and draw up a 1 ml sample of the 10^{-1} dilution and place it in the second tube.

8. Mix well as before. This gives a 10^{-2} dilution.

9. Discard the pipette in disinfectant.

10. Repeat this for the remaining tubes, removing 1ml from the previous dilution and adding it to the next 9 ml of diluent.

If 6 tubes are used, the final dilution for the bacteria will be 10^{-6} (1 in 1,000,000).

Plating and counting procedure

Use a known volume of each dilution to make either pour plates or spread plates . By starting with the highest dilution, the same pipette may be used throughout. For statistical purposes, replicate plates should be prepared.

After incubation the plates will show a range of numbers of colonies. Choose the plate that has an easily countable number (about 30–100) and carefully count every colony. Using a marker pen helps to avoid counting the same colony twice.

Then calculate the number of micro-organisms in the sample:

Number of microbes/ml = number of colonies × dilution of sample