

PROTOCOL FOR BACTERIAL SAMPLING FROM THE MOUSE INTESTINE

1. Switch on the steriliser and sterilise the instruments that you will need to dissect the mouse.
2. Weigh as many empty capped Eppendorf tubes as there are mice to dissect.
3. If sampling mesenteric lymph nodes, prepare as many yellow top (15ml) tubes with 3mls of sterile BSS inside as there are mice to sample.
4. Kill the mouse with an overdose of ether and spray with alcohol.
5. Open the skin and the peritoneum as usual, sample the mesenteric lymph nodes in a sterile way if appropriate (into 3ml of sterile BSS)
6. Lay the caecum on a paper towel away from any contaminating alcohol and cut off the end with a clean sterile scalpel blade.
7. Squeeze out a blob of faeces and use the blade to transfer it into an Eppendorf tube.
8. Reweigh the Eppendorf tubes after all the mice have been dissected (the difference is the weight of faeces in the sample).

Faeces (process this without delay, some anaerobes are killed by oxygen).

9. Add 1ml of sterile BSS to each Eppendorf tube.
10. Mix on the vortex vigorously until the suspension is homogenous.
11. Quickspin in the Eppendorf microfuge (press the 'short spin' until the machine reads over 5krpm i.e. about 3-4 seconds).
12. Prepare 0.9 mls of sterile BSS in each well of a 24 plate. Each row is for the dilutions from one faecal sample. Dilute a single sample then plate it immediately, followed by diluting the next sample (i.e. do not do all the dilutions then the plating).
13. Add 0.1ml of bacterial suspension to the left hand well with an old 200 μ l pipette, work up and down to mix. Change tips then transfer 0.1ml to next well and mix, and thus do 10fold dilutions across the plate. CHANGE TIPS EVERY TIME BETWEEN DILUTIONS.
14. Plate out 0.1ml of "1 in 1" and "1 in 10" concentrations onto LB agar for aerobic growth, and 0.1ml of "1 in 10" and "1 in 1000" onto Wilkins-Chalgren agar for anaerobic growth.
15. Dry 5 μ l of "1 in 1", "1 in 10", "1 in 100", and "1 in 1000" onto a slide for each animal. Perform Gram stain (Fluka 77730 Gram Staining Kit) and count.
16. Read plates and assess colony numbers after 24 and 72 hours.