McCoy and Macpherson Lab
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Protocol 907_Gavaging for high affinity IgA induction

Reagents

- Bacterial culture
- Sterile-filtered, autoclaved PBS
- Sterile gavage needles and 1ml syringes
- Sterile PBS containing 0.5% Turgitol. You cannot autoclave Turgitol so sterile filter!
- Sterile 2ml tubes containing baked (and therefore also sterile) steel ballbearing. Weighed! 3 tubes per mouse.
- Appropriate agar plates
- Sterile tubes and PBS for doing dilutions
- Sterile instruments

Method

1. Grow up large overnight culture of bacteria
2. Centrifuge either in many sterile 50ml falcon tubes
3. Wash 3x with sterile PBS to remove LB and soluble contaminants (you can pool everything into 1 falcon tube after the first spin)

4. Resuspend at 10^10-10^11 bacterial per ml in sterile PBS. For E. coli overnight culture expect a starting density of around 10^9/ml
    - NB: less than 10^9 bacteria does not give reliable induction of specific IgA when gavaged

5. Carefully gavage 500µl per mouse
6. Wait 18hrs
7. Sacrifice mice. Open skin with forceps and scissors set 1. Spray peritoneal membrane with ethanol and proceed with forceps and scissors set 2 to open the peritoneum taking great care not to puncture the intestine. If you do, you have to discard the mouse from the experiment.
8. Remove the spleen to the first 2ml tubes.
9. Remove the mesenteric lymph nodes – you can use a sterile petri-dish to remove the fat but NOT tissue as this will contaminate the prep.
10. open the cecum and take an aliquot of content into the third tube
11. Place all tubes on ice
12. Wash instruments in water then ethanol, then in 4% formaldehyde, then again in ethanol to sterilise.
13. Repeat for next animal (NB always use forceps/scissors 1 to open the skin and set 2 for everything else).
14. Weight all of the tubes to calculate tissue weight
15. Add 500µl PBS turgitol to spleen and content and 100µl to mesenteric lymph nodes
16. Beat in the outermost position of the tissuelyser for 2mins at 25
17. Plate 50µl of neat spleen suspension; mesenteric lymph nodes neat and 1:200; cecal content 1:40000, 1:8000000.
18. Incubate overnight at 37ºC and count colonies