

Human PMN Phagocytosis Assay

1. Start overnight culture of bacteria in 5ml lysogeny broth (LB) for 16-18 hours at 37°C
2. Sub-culture (1:30) for 2-3 hours at 37°C in LB
3. While the sub-culture is growing, isolate healthy human neutrophils as previously described in "Isolating PolyMorphoNuclear Cells (PMNs) Protocol"
4. Resuspend neutrophils in Roswell Park Memorial Institute (RPMI) 1640 medium and add 250,000 cells (in 250ul RPMI) to round-bottom polystyrene tubes
5. Add 1ml of the sub-cultured bacteria to a 1.5ml Eppendorf tube
6. Centrifuge at 13,000rpm for 2min and wash with 1ml PBS
7. Resuspend bacteria in 1ml RPMI
8. Measure OD₆₀₀ of a 1:10 dilution of the bacteria
9. Calculate the volume of bacteria needed to treat the neutrophil at multiplicity of infection (MOI) 10 or 25 (in 250ul RPMI)
10. Add 250ul bacteria to the neutrophils in the round-bottom polystyrene tubes, gently mix by flicking the tube
11. Incubate the neutrophils and bacteria at 37°C for 45min
12. Add 200ul of gentamicin in RPMI (final concentration of 67ug/ml) for 20min at 37°C
13. Pellet the neutrophils by centrifugation at 1000rpm for 5min
14. Store supernatant for further analysis at -80°C
15. Wash neutrophils 2x with 500ul RPMI
16. Resuspend neutrophils in 500ul sterile-filtered 0.1% TritonX-100 in PBS
17. Pipette up and down to lyse cells
18. Plate 10ul of lysed cells on a 1.5% LB agar plate and incubate overnight at 37°C
19. Count colony forming units (CFU)