Culturing macrophages from bone marrow, blood and bronchoalveolar lavage

Cells were isolated as per the cell isolation protocol (ROSLIN_SOP_Harvest_of_Large_Animal_Tissues_20160516).

**Materials**

- Bone marrow cells
- PBMCs
- Bronchoalveolar lavage
- RPMI
- Sheep or Fetal Calf Serum
- Glutamax
- Penicillin Streptomycin
- Recombinant human CSF1
- Bacteriological plastic culture dishes
- 6 well tissue culture plates
- Lipopolysaccharide (*Salmonella enterica* serotype minnesota Re 595)
- Trizol

**Method**

**To differentiate bone marrow and monocyte derived macrophages**

Bone marrow cells and PBMCs were cultured for 7 or 11 days, for sheep and buffalo respectively, on bacteriological plastic in RPMI + 20% sheep or fetal calf serum, 1% glutamax, and 25U/ml Penicillin Streptomycin, in the presence of $10^4$U/ml recombinant human CSF1 (rhCSF1).
Cells were fed after 3-5 days in culture and harvested from plates at 7 or 11 days (depending on species) and replated in 6 well plates at $10^6$ cells/ml. Cells were cultured overnight in 6 well plates then media was removed and cells lysed in 1ml Trizol.

**Alveolar macrophages**

Cells isolated from bronchoalveolar lavage were cultured in 6 well plates at $10^6$ cells/ml overnight in the above complete RPMI medium in the presence of $10^4$U/ml rhCSF1. After overnight incubation media was removed and cells were lysed in 1ml Trizol.

**For stimulation of macrophage cultures with LPS**

Bone marrow derived macrophages stimulated with LPS were incubated with fresh medium containing 100ng/ml LPS (Salmonella enterica serotype minnesota Re 595) for 2, 4, 7 and 24 hrs for sheep or 7hrs only for buffalo. The media was removed and the cells lysed in 1ml Trizol at each time point.