"ALEXANDER FLEMING"
Biomedical Sciences Research Center

RESEARCH SAMPLE COLLECTION AND PREPARATION EXAMPLES FROM MICE

FLOW CYTOMETRY UNIT
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SAMPLE PREPARATION OUTLINE

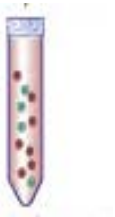
Directly conjugated Ab
Biotinylated Ab
Streptavidin conjugated
1ry Ab non conjugated
2ry Ab non conjugated

Fluid

Tissue

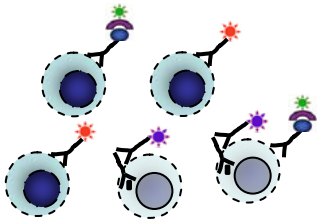
Dissociate
Lyse RBCs

Filter
Count

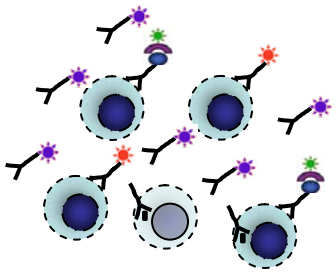


Single cell
suspension

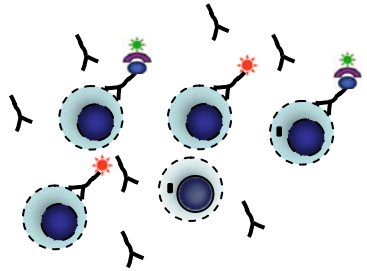
1. CELL HARVESTING



Stained cells

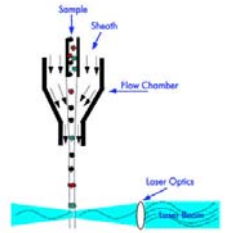


2ry Ab incubation

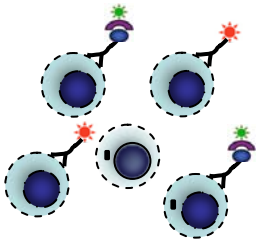


1ry Ab incubation

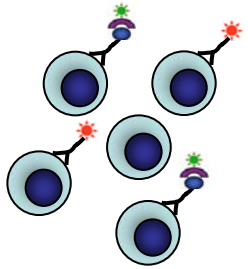
3. INTRACELLULAR STAINING



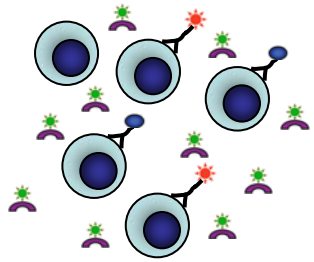
4. ANALYSIS



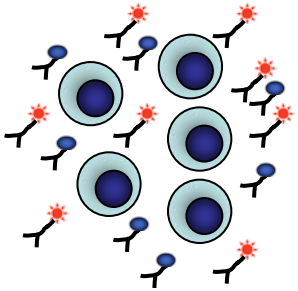
Fix/Perm



Stained cells



Ab incubation



Ab incubation

2. SURFACE STAINING

CELL HARVESTING I: Cells from mouse body fluids

Blood:

•Contains single cell suspension of leukocytes ideal for flow cytometry
•Can be obtained from veins in live animal and cardiac puncture in sacrificed animals
•Need to use anticoagulation agents to prevent clotting (e.g. heparin)
•Need to lyse RBCs

Peritoneal lavage:

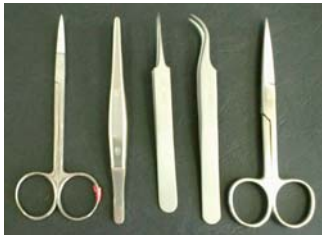
•Good source of macrophages upon elicitation through the use of inflammatory stimulus before collection (e.g. thioglucollate medium).
•Involves rinsing the peritoneal cavity with saline.

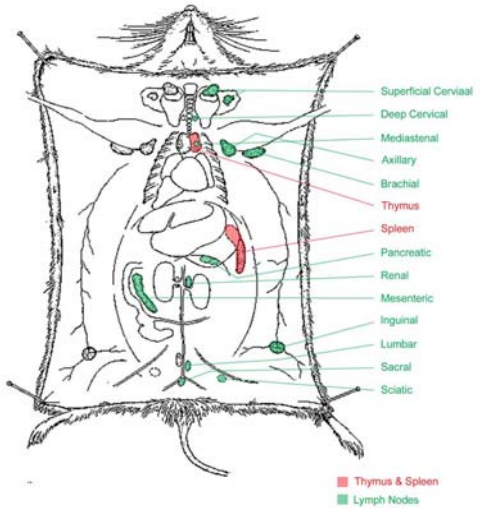
Bronchial lavage:

•Source of alveolar macrophages.
•Involves airway flushing (typical yield $5x10^5$ cells/mice)

CELL HARVESTING II: Cells from mouse solid organs

METHODS
- Tissue Grinding
- Tissue Flushing
- Complex Protocols: enzymatic digestion, filtrations, centrifugations



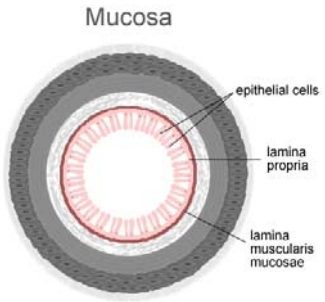


<u>Tissue Grinding:</u> suitable for tissues in which cells are not tightly bound to each other by tight junctions or by substantial amount of connective tissue
e.g. **Thymus, lymph nodes, spleen**

<u>Tissue Flushing</u>: suitable from **bone marrow** cell extraction
Marrow plug is expelled by forcing liquid through the bone with the use of a needle.

Complex protocols: generally, non lymphoid tissues in the mouse (e.g. kidney, gut, lung) have to be disaggregated by enzymatic digestion. In addition, their cell type heterogeneity has generated a diversity of preparation techniques, many of which involve a combination of enzymatic digestion as well as centrifugation and filtration steps.

Isolation of Lamina Propria (LP) Mononuclear Cells (LPMCs)



Small intestine

↓ **Mechanical dissection of intestinal layer**

Mucosa

↓ **Epithelial cell depletion by EDTA/DTT treatment and filtrations**

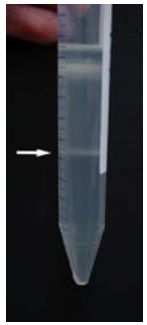
LP, muscle layer

↓ **Digestion with Collagenase, DNase, Dispase**

Lymphoid cell suspension

↓ **Purification with Percoll gradient**

LPMCs
Purified concentrated cell suspension



CELL HARVESTING III: cell culture expansion

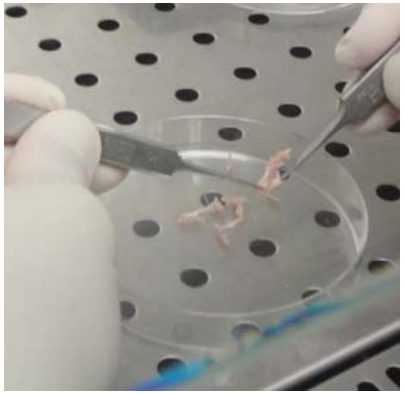
Cell culture expansion is utilized to obtain large numbers of homogeneous populations of **primary** cells e.g:

BMDMs: Bone Marrow Derived Macrophages

- Macrophages derived from bone marrow precursors
- Involves culturing of bone marrow cell suspension with growth factors to continue hematopoiesis *in vitro*

SFs: Synovial Fibroblasts

- CD45-negative cells of mesenchymal origin involved in supporting and lubricating the joint by providing nutrients and proteoglycans. Involved in RA.
- Involves collagenase digestion of dissected joints and *ex vivo* culturing of the synovial cell suspension for up to 20days (minimum 3 passages)



A standardized protocol for the isolation and culture of normal and arthritogenic murine synovial fibroblasts Maria Armaka, Vassiliki Gkretsi, Dimitris Kontoyiannis, and George Kollias. Protocol Exchange 01/05/2009 doi:10.1038/nprot.2009.102