

How we prepare material other than blood and bone marrow for reliable flow cytometry analysis?

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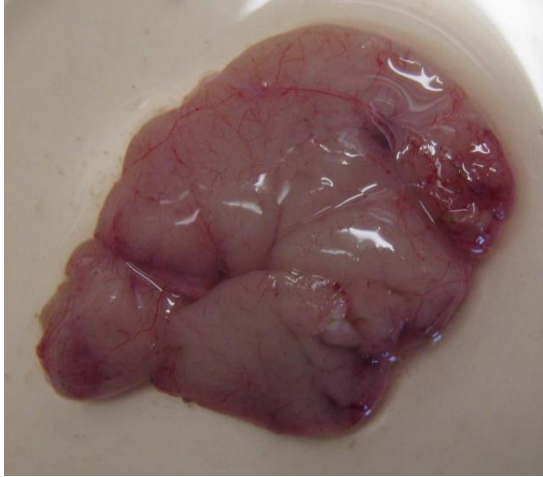
1. Tissue

Tissue is a collection of cells that have the same origin, shape, and perform the same main function.

- We have approx. 50 tissue samples examined in our lab each year
- Mostly, these are tissues suspected of lymphoma
- The size of the tissue is usually 10 x 5 x 3 mm (*the priority is given to histology*)

1.Tissue

Photos of different tissues



Brain



Liver



Node



Node

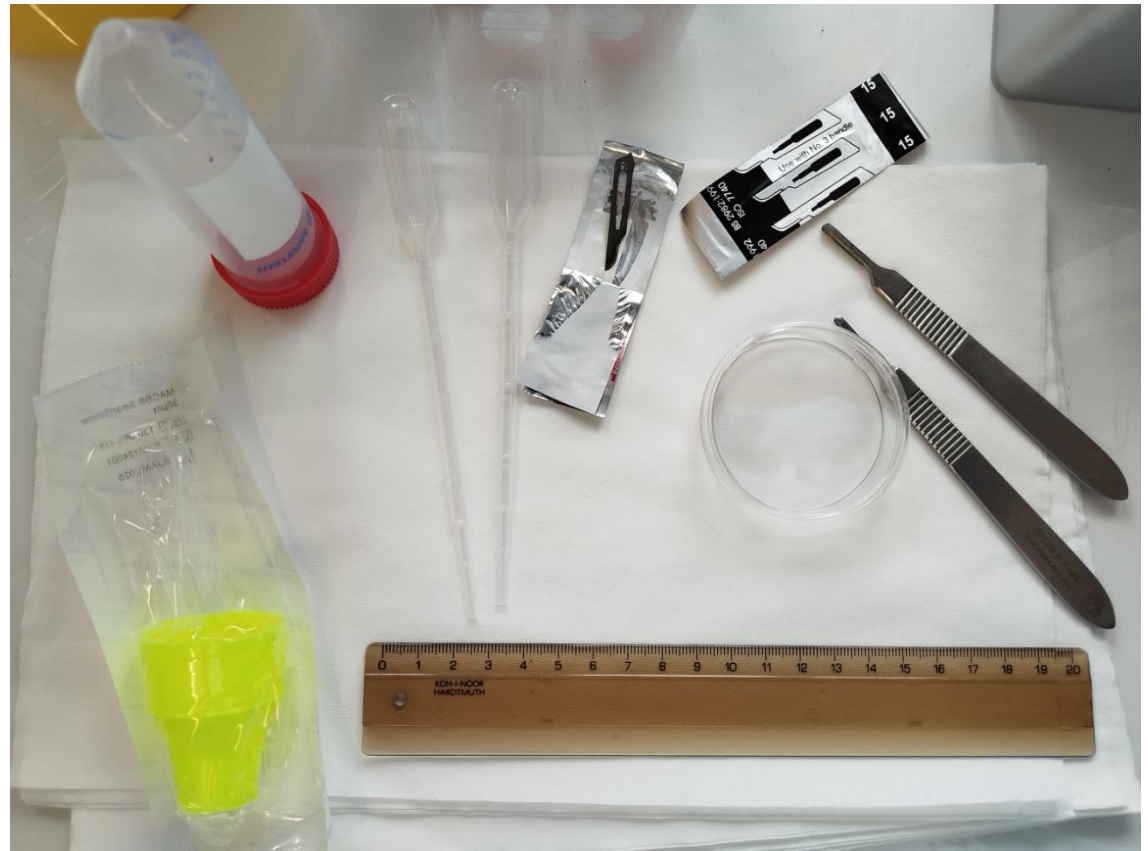


Spleen

1.Tissue

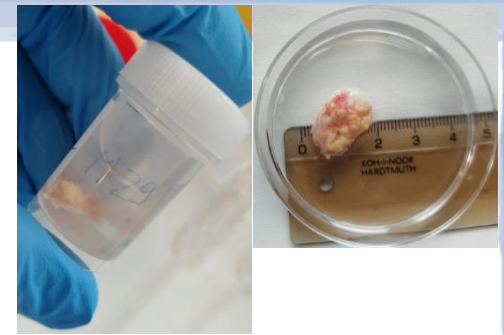
What tools do you need?

- PBS with 2 mmol/l EDTA
- Test tube
- Filter (30 μm)
- Scalpel
- Petri dish
- Pasteur
- Shaker
- Centrifuge

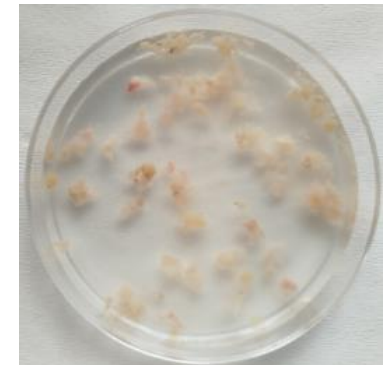


1.Tissue

Work procedure



- Filter the fluid in which the tissue is taken
- Chop the tissue into small pieces in PBS/2 mM EDTA
- Let the cells isolate from tissue for 15 minutes on shaker
- Filter and add the filtrate to the already filtered liquid
- Centrifuge
- Gentle remove the supernatant
- Resuspend **the pellet** in PBS/2 mM EDTA
- The suspension is labeled with fluorescently labeled antibodies in the same way as blood.

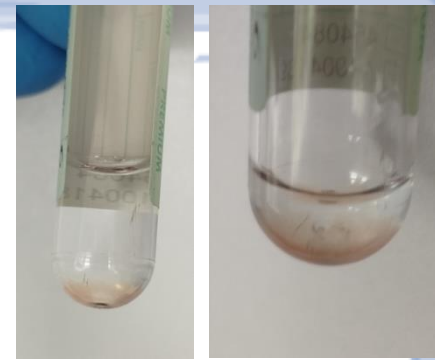


2. Cerebrospinal fluid

Cerebrospinal fluid (CSF) is a clear, colorless fluid that surrounds and protects the brain and spinal cord. It is almost cell-free.

- we have approx. 270 CSF samples per year in our laboratory
- 60% is leukemia patient, the rest of the patients came with a neurological problem, ear problem or other malignant neoplasms
- 5% of CSF are fixed in Transfix (external hospital)

Work procedure

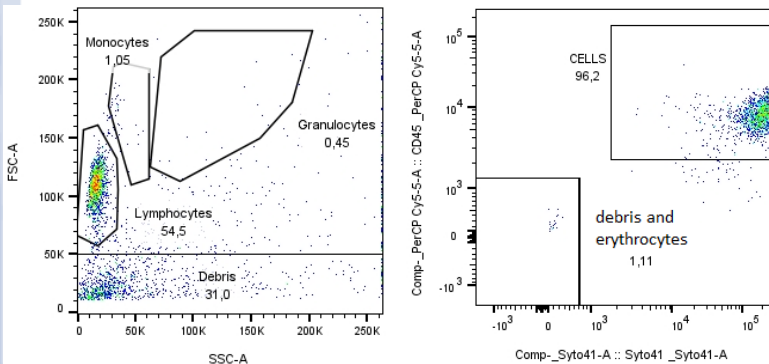


- Centrifuge the CSF
- Check if the pellet is red (= contamination with peripheral blood)
- Gentle remove the supernatant, leave 100 μ l per test + reserve
- Resuspend and mark the suspension with antibodies
- After 15 minutes in dark, add the lysing agent (NH_4Cl)
- After 15 minutes in dark, measure (= no wash – no cells are lost)
- Results are given in cell counts per microliter

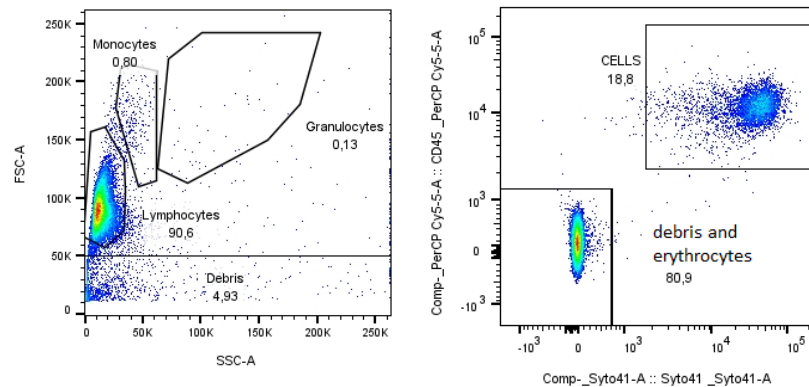
2. Cerebrospinal fluid

Cytometric data of cerebrospinal fluid

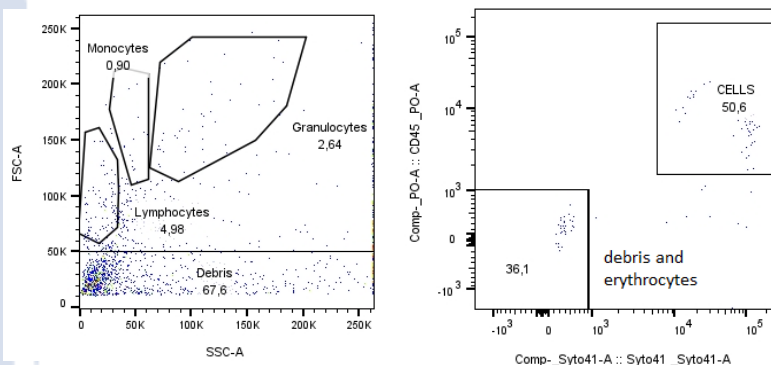
Native w/o peripheral blood



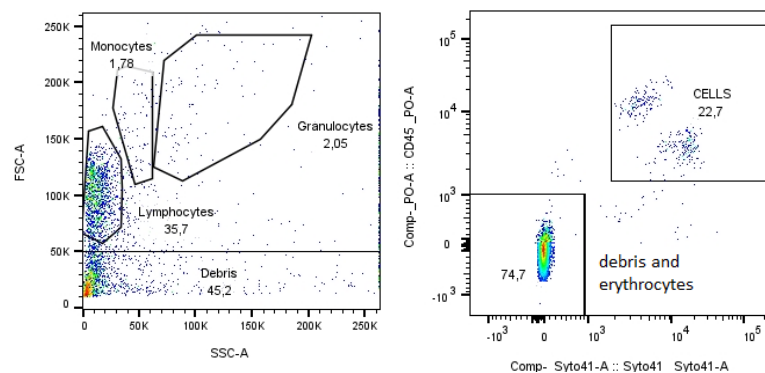
Native with peripheral blood



Transfix w/o peripheral blood



Transfix with peripheral blood



2. Cerebrospinal fluid

Native cerebrospinal fluid vs TransFix fixed cerebrospinal fluid

Native

- must be processed within 2 hours of collection

TransFix

- possibility to measure the sample up to 72 hours after fixation
- clinic staff must be familiar with how to properly collect cerebrospinal fluid into a fix tube
- more expensive because a special fixative must be used

3. Bronchoalveolar lavage fluid (BAL) or effusion

Bronchoalveolar lavage (BAL): a bronchoscopic examination in which saline solution is poured into the segmental bronchus in the lungs and then sucked back out.

Effusion is fluid that has accumulated in a body cavity.

- we have approx. 4 samples of BAL per year in our laboratory
- we have approx. 35 samples of effusion per year in our laboratory (*fluidothorax, pericardial effusion and others*)

3. Bronchoalveolar lavage fluid (BAL) or effusion

Work procedure

- Filter BAL or effusion (30 μm) to new tube
- Centrifuge the filtrate
- Gentle remove the supernatant (*a large admixture of erythrocytes is common in effusions*)
- Resuspend **the pellet** in PBS/2 mM EDTA
- Mark the suspension with fluorescently labeled antibodies in the same way as blood or CFS (*depends on cellularity and erythrocyte infiltration*)

Tip and tricks

- If you cannot process the tissue immediately, it is better to leave it intact in the refrigerator for next day examination
- For most tissues, you do not need to incubate in lysis reagent for 15 minutes to remove erythrocytes, just wash with lysis reagent
- Larger amounts of erythrocytes in cerebrospinal fluid are better removed by spinning and removing the supernatant after lysis (*unfortunately the information about the amount of target cells per μl is lost*)

THANK YOU FOR YOUR ATTENTION

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