

Practical Aspects of the Mouse Immune System

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Overview

1. Overview of the immunological organs and tissues in the mouse
2. Overview of the immune cells in the mouse
3. Isolation/separation of immune cells
4. Cell culture of immune cells
5. Analysis of immune cells

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1. Overview of the immunological organs and tissues in the mouse



BALB/c mouse



C57BL/6 mouse

1. Overview of the immunological organs and tissues in the mouse

GENERAL BIOLOGY AND PHYSIOLOGICAL DATA:

- Most active at night (nocturnal)
- Curious and investigative behaviour
- Poor vision, acute sense of hearing and smell
- Social animals, adult males may require separation if aggressive
- Average body temperature: 37°C
- Respiratory rate: 95-165 breaths/minute
- Heart rate: 325-800 beats/minute
- Daily water consumption: 5 ml
- Daily food consumption: 5 g

http://www.medicine.mcgill.ca/arc/forms/ed_train/Handout%20Mouse%20Module%201-Sept%2009.pdf

1. Overview of the immunological organs and tissues in the mouse

- Average litter size: 6-12
- Gestation period: 19-21 days
- Average birth weight: 0.5-1.5 g
- Weaning age: 21-28 days
- Sexual maturity: 6-7 weeks in males; 7-8 weeks in females
- Reproductive span: 7-9 months
- Male adult weight: 25-40 g
- Female adult weight: 20- 40 g
- Life span: 1.5-3.0 years

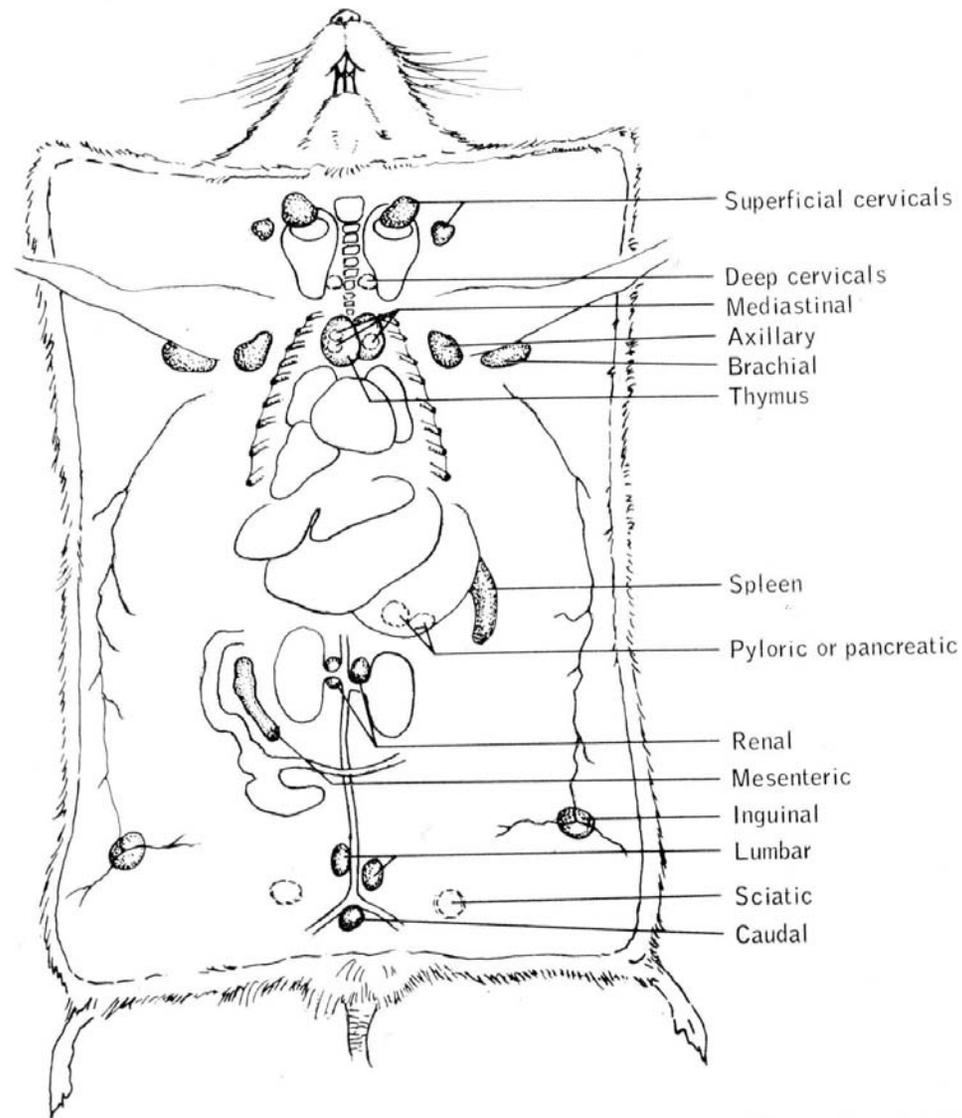
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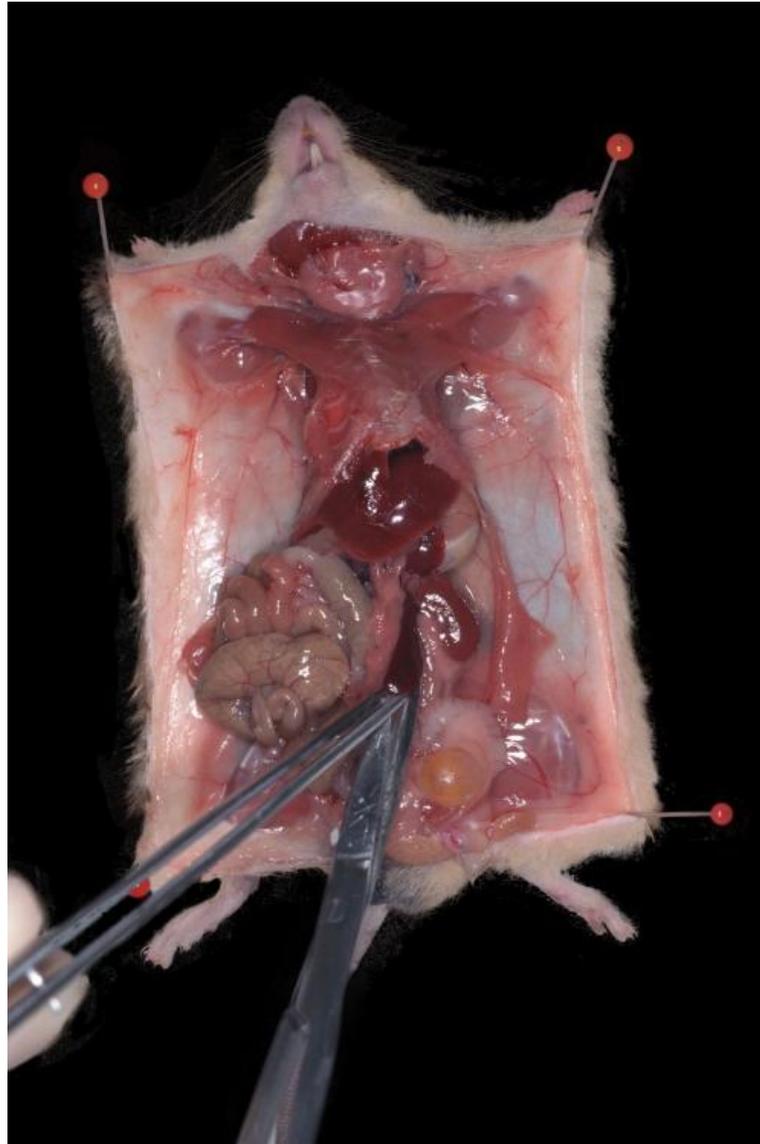
Euthanasia

- CO₂ asphyxiation
- Cervical dislocation

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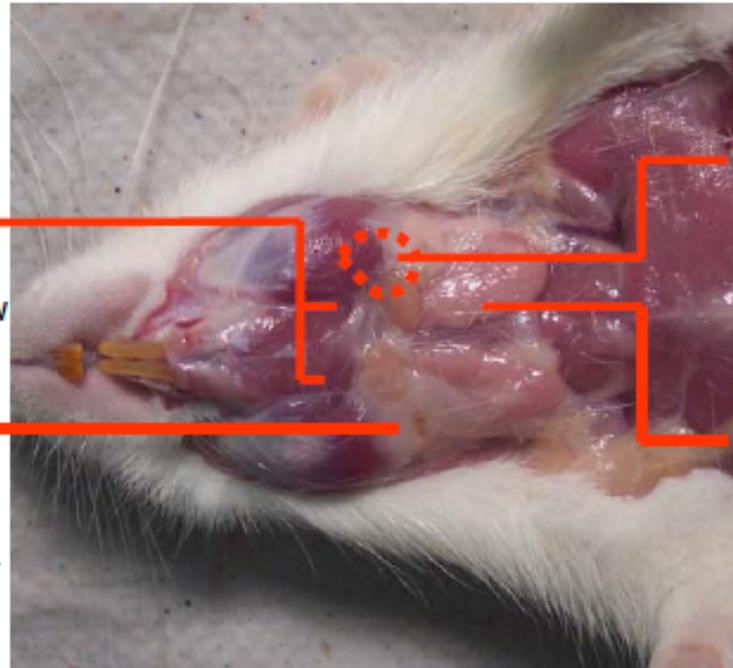
SALIVARY GLANDS

Lymph Nodes

Brown solid nodes found near the jaw line.

Parotid

This is the white, half-moon shaped tissue lying on top.



Sublingual Gland

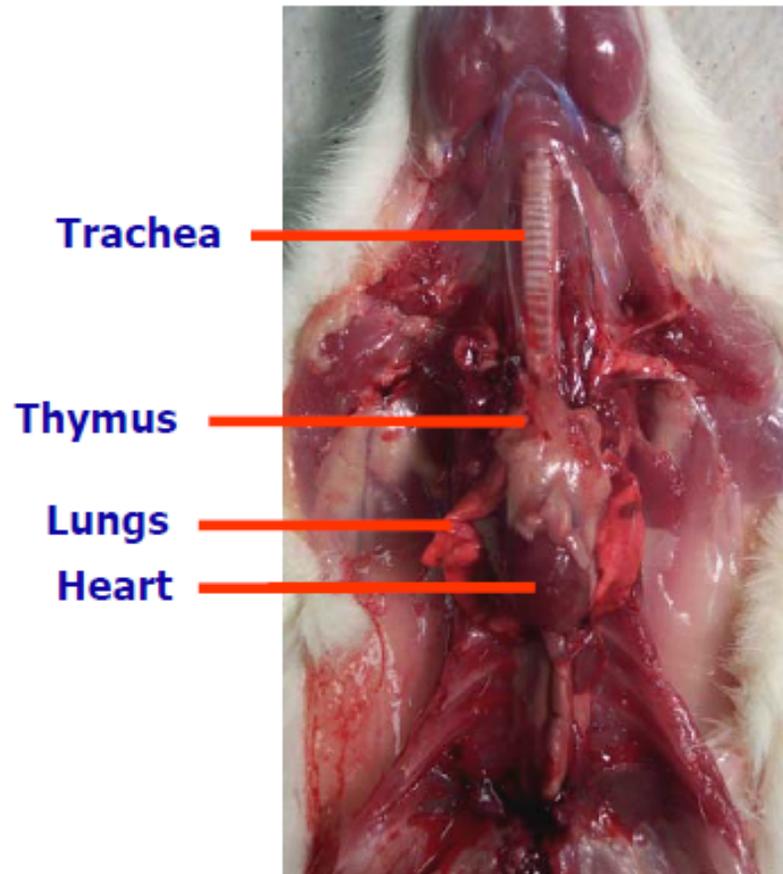
Also known as Submaxillary gland is found attached to the corner of the submandibular gland.

Submandibular Gland

The largest salivary gland, responsible for most of the secretions.

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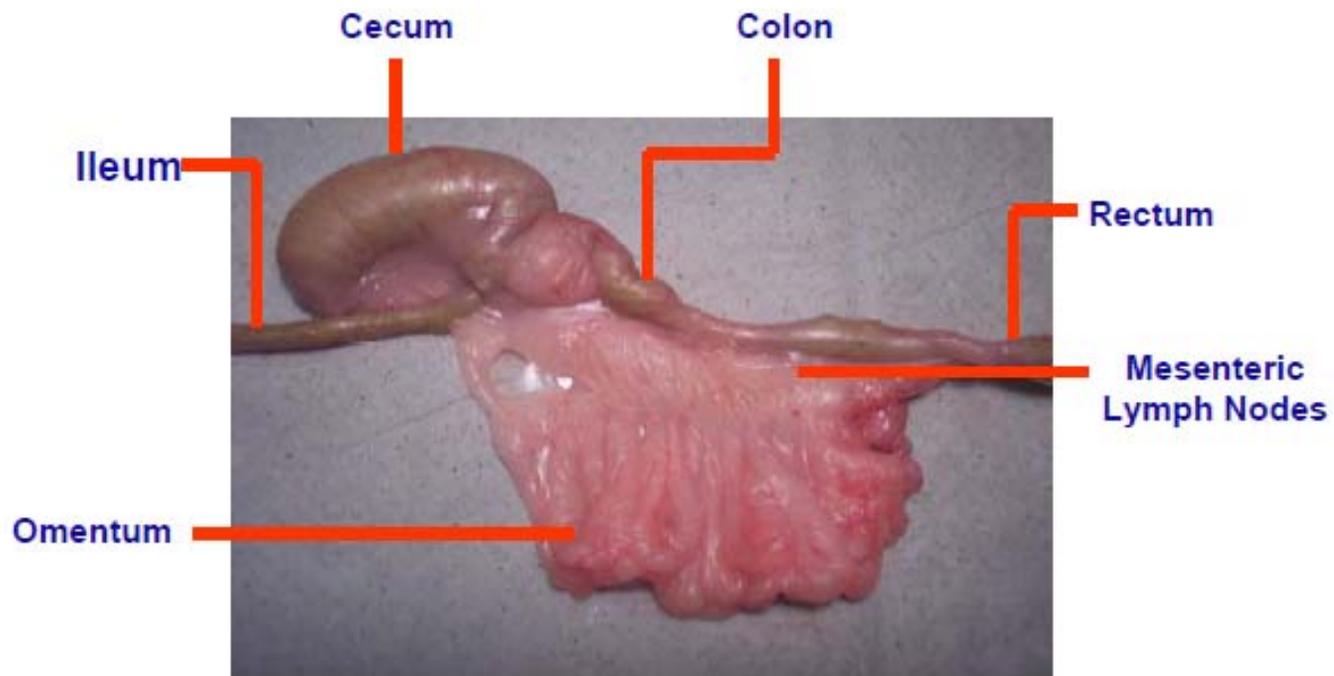
TRACHEA, THYMUS, LUNGS AND HEART



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1. Overview of the immunological organs and tissues in the mouse

CECUM, MESENTERIC LYMPH NODES, COLON AND RECTUM.

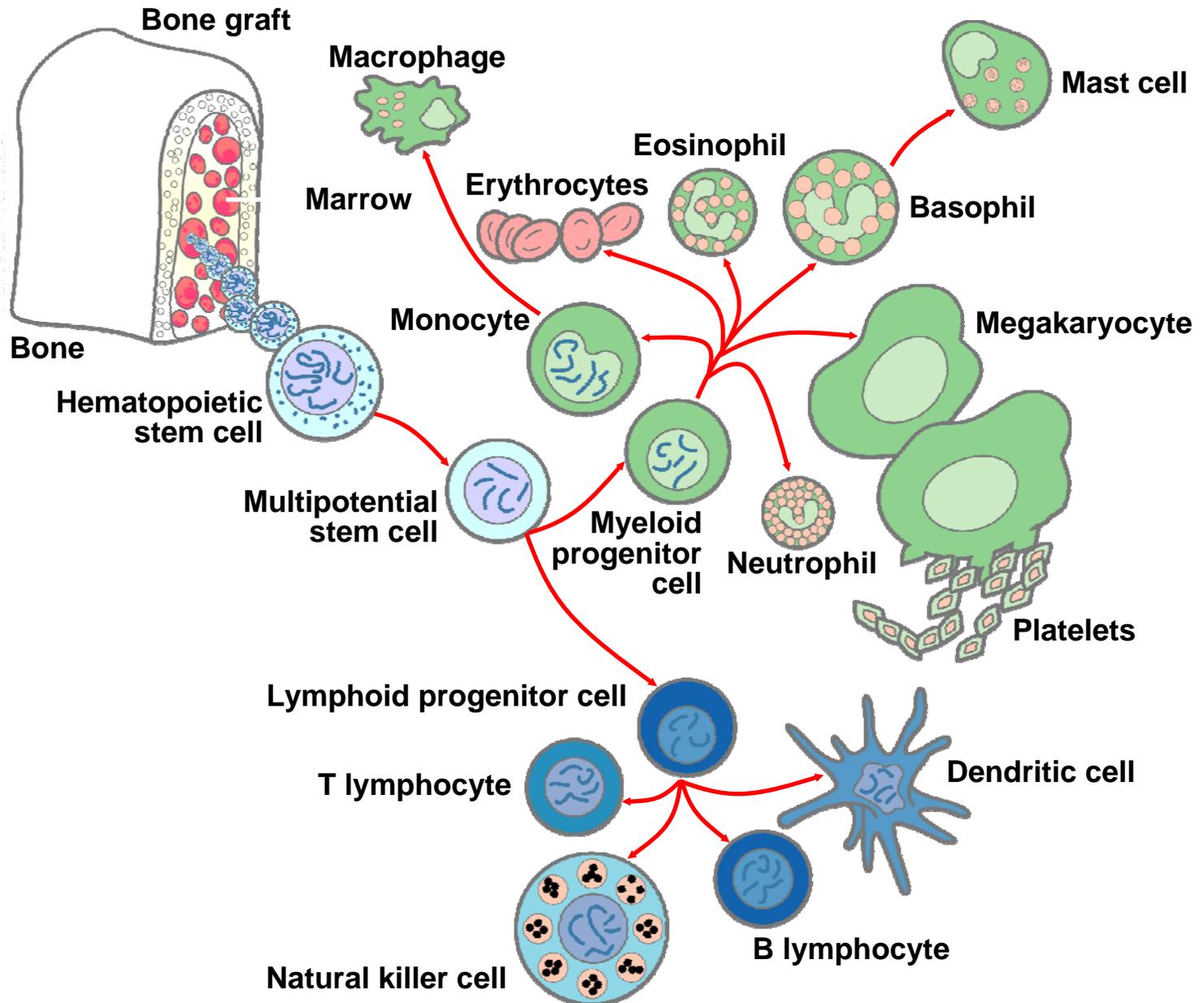


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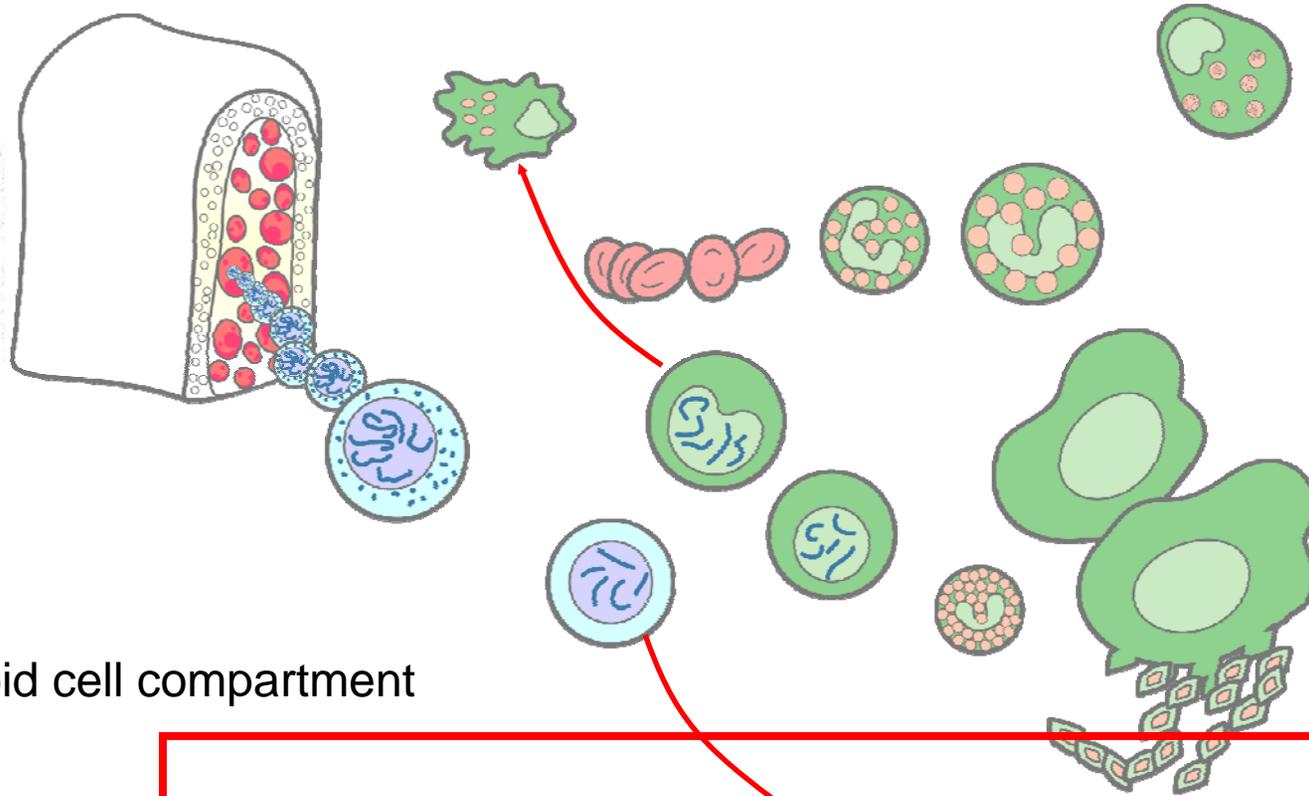
2. Overview of the immune cells in the mouse

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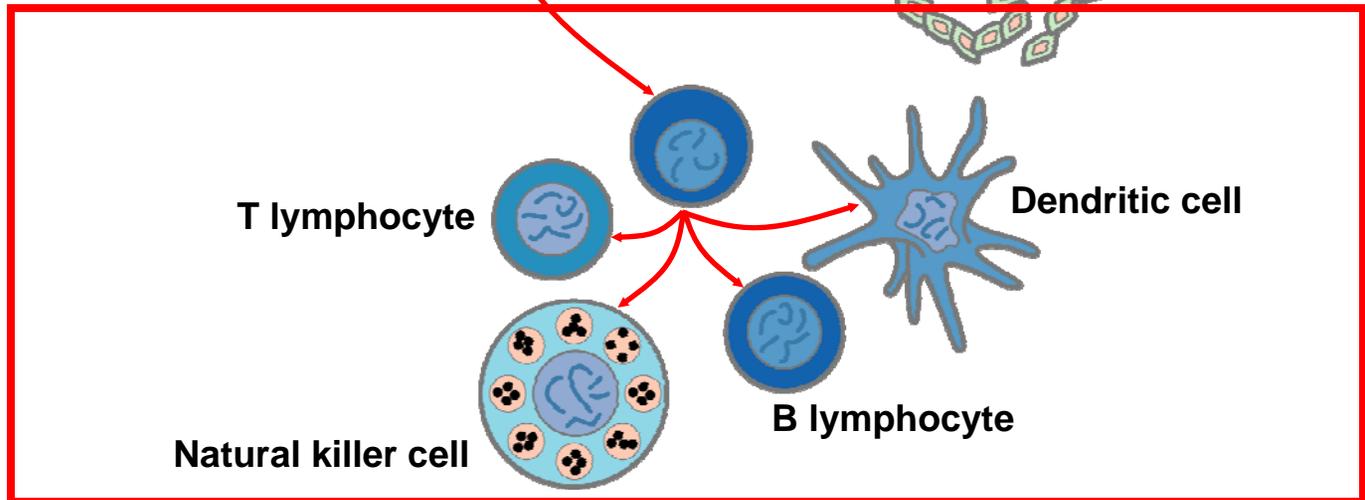
Cells of the Immune System



Cells of the Immune System



The lymphoid cell compartment



2. Overview of the immune cells in the mouse

Where are the cells?

T cells: LN, spleen and thymus

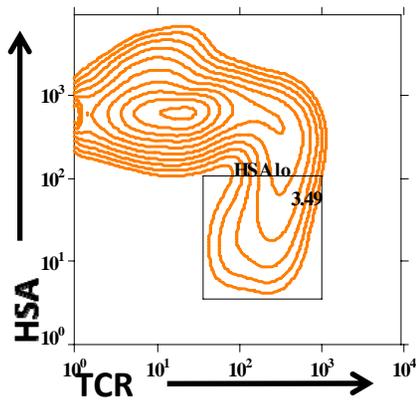
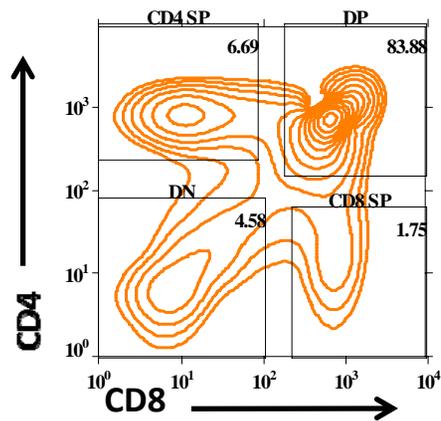
B cells: LN, and spleen

NK cells: LN, and spleen

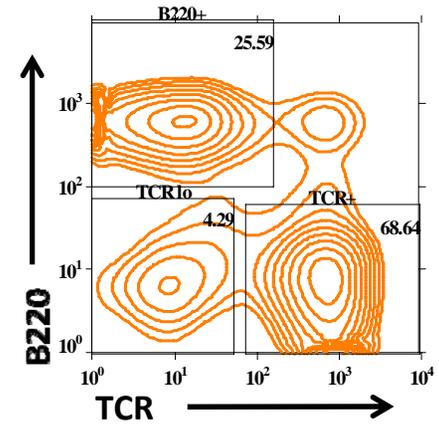
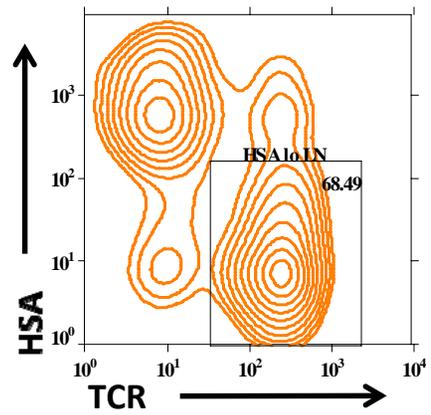
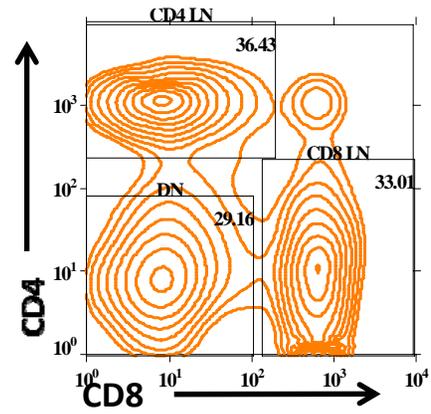
NKT cells: Thymus, spleen and liver

2. Overview of the immune cells in the mouse

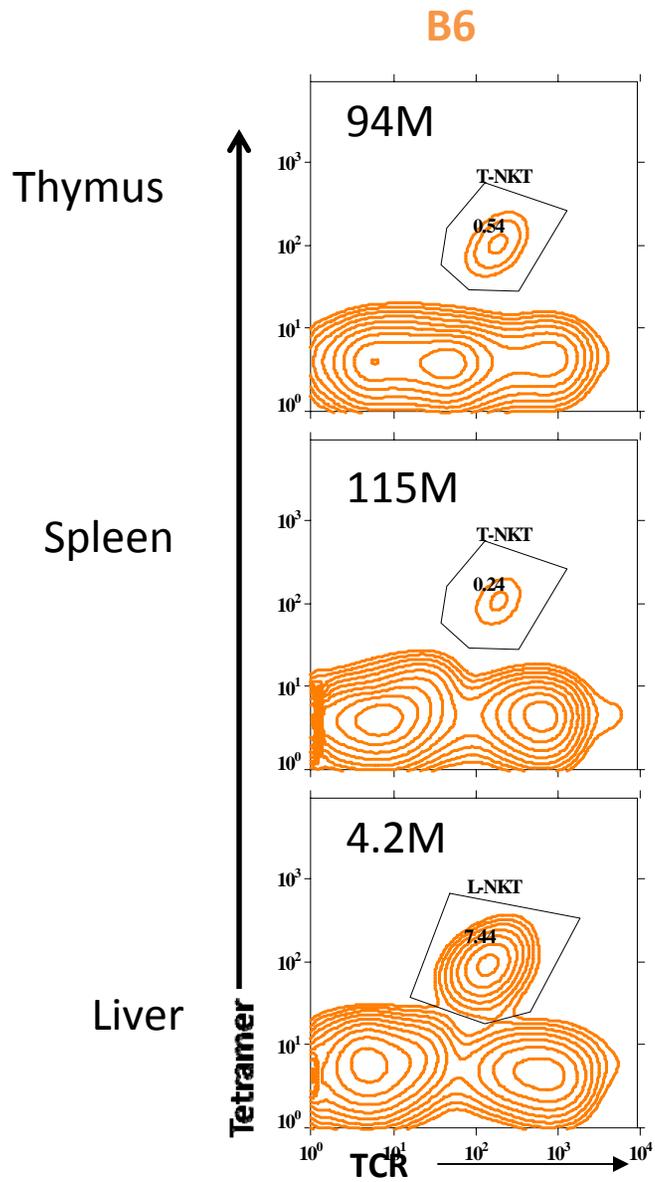
Thymus



Lymph node



2. Overview of the immune cells in the mouse



NKT cells are detected using tetramers

3. Isolation/separation of immune cells

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Dissecting the mouse

1. Use approved CO₂ chamber to euthanize mice
2. Once death is confirmed, the mouse is wet with 75% EtOH
3. Large scissors can then be used to make large incision on mouse's back
4. Skin can then be pulled up over head or toward by rear legs, this exposes the majority of the mouse's lymph nodes.
5. Using forceps, lymph nodes are next removed in the following order: inguinal, axillary, submandibular and mesentery

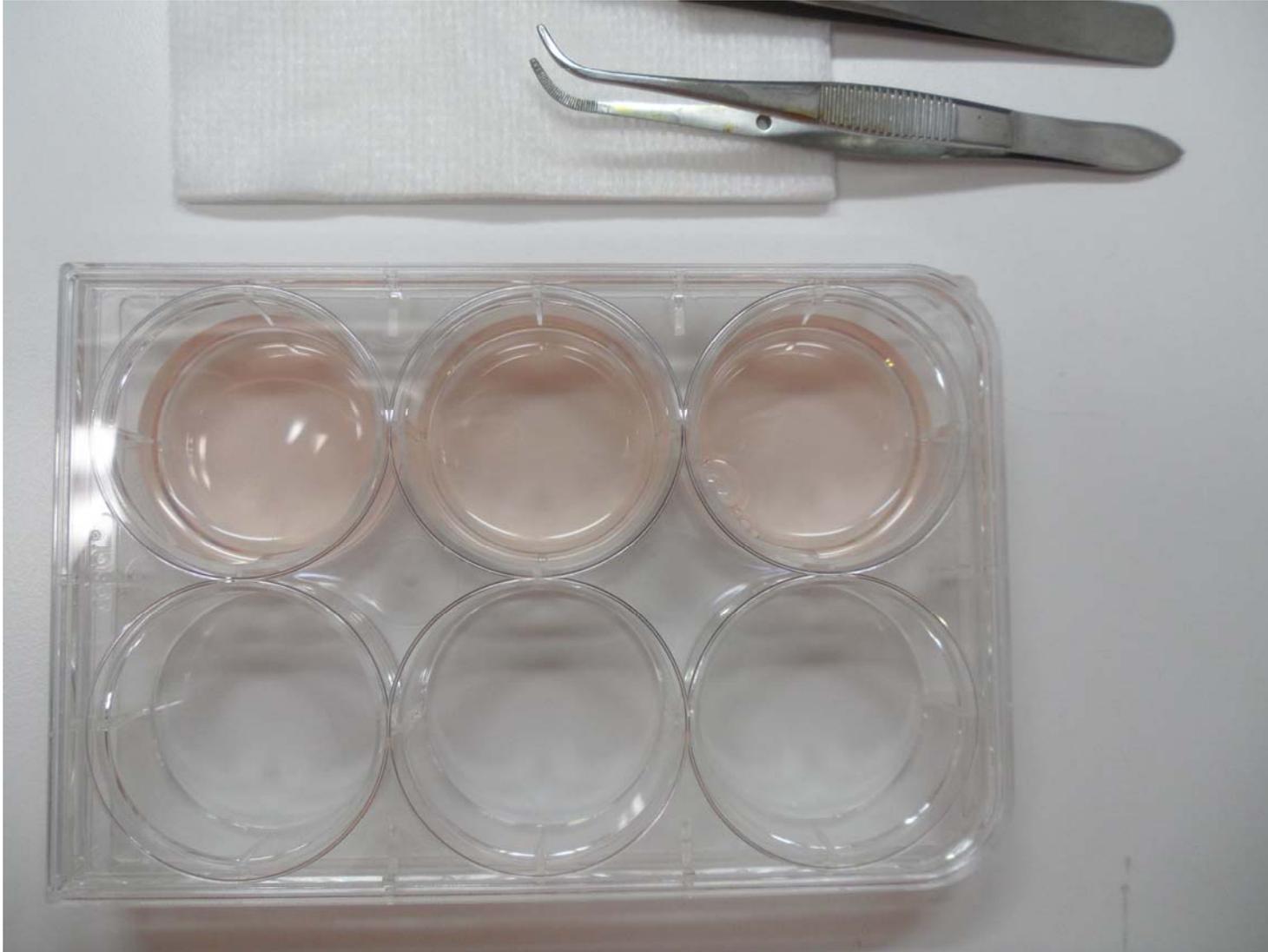
Dissecting the mouse

6. Once removed from the mouse, organs are immediately placed into a plate containing lymphocyte specific media on ice
7. Next the spleen is removed with forceps
8. Finally, using small scissors incisions are made under the rib cage and cuts are made upwards toward the heart, this exposes the thymus. Very carefully, using forceps the thymus is tweezed out of the chest cavity
9. Organs are processed using slides and forceps
10. Cells are placed into single cell suspension and cells are counted using a hemacytometer

3. Isolation/separation of immune cells



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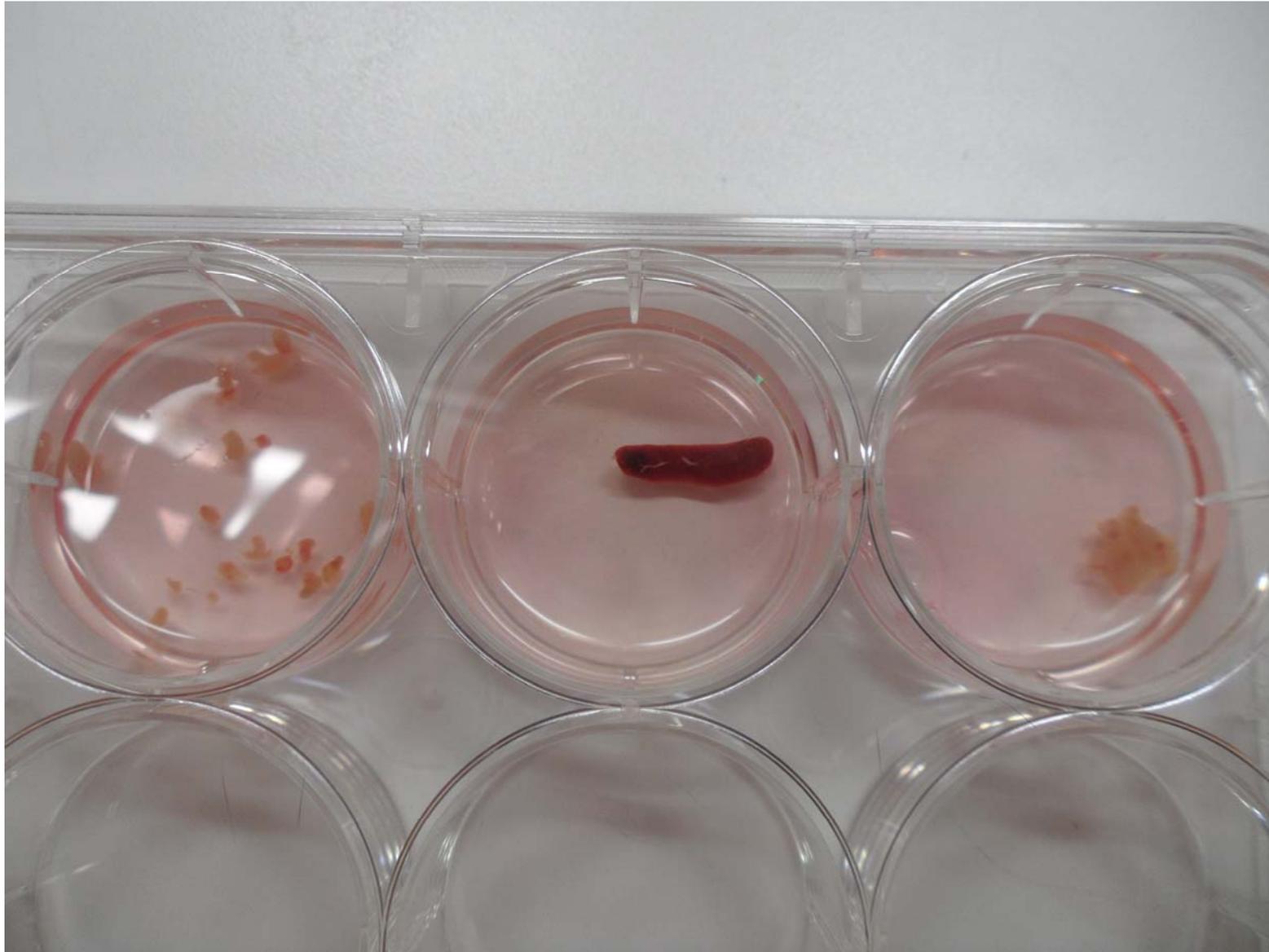
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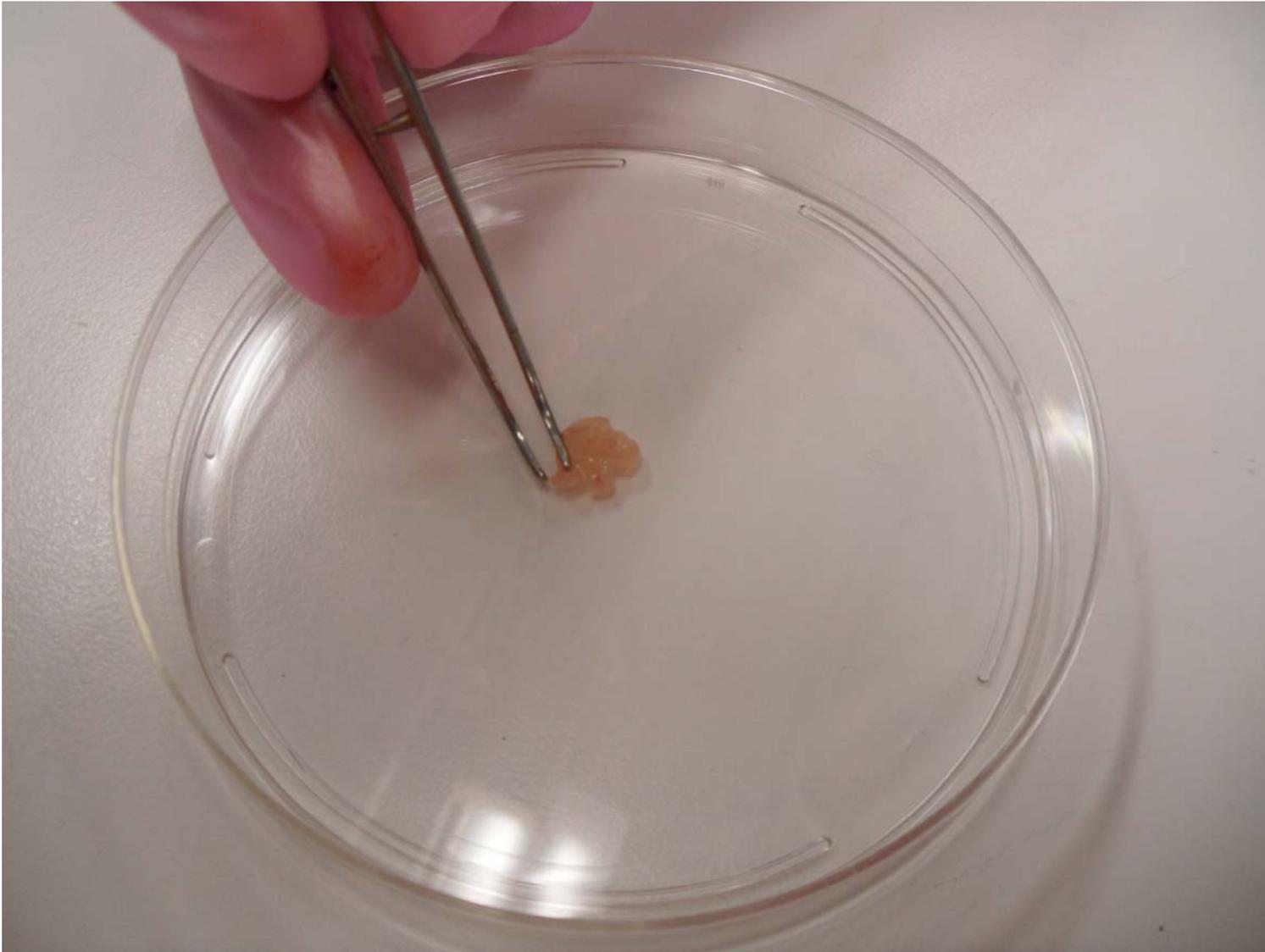
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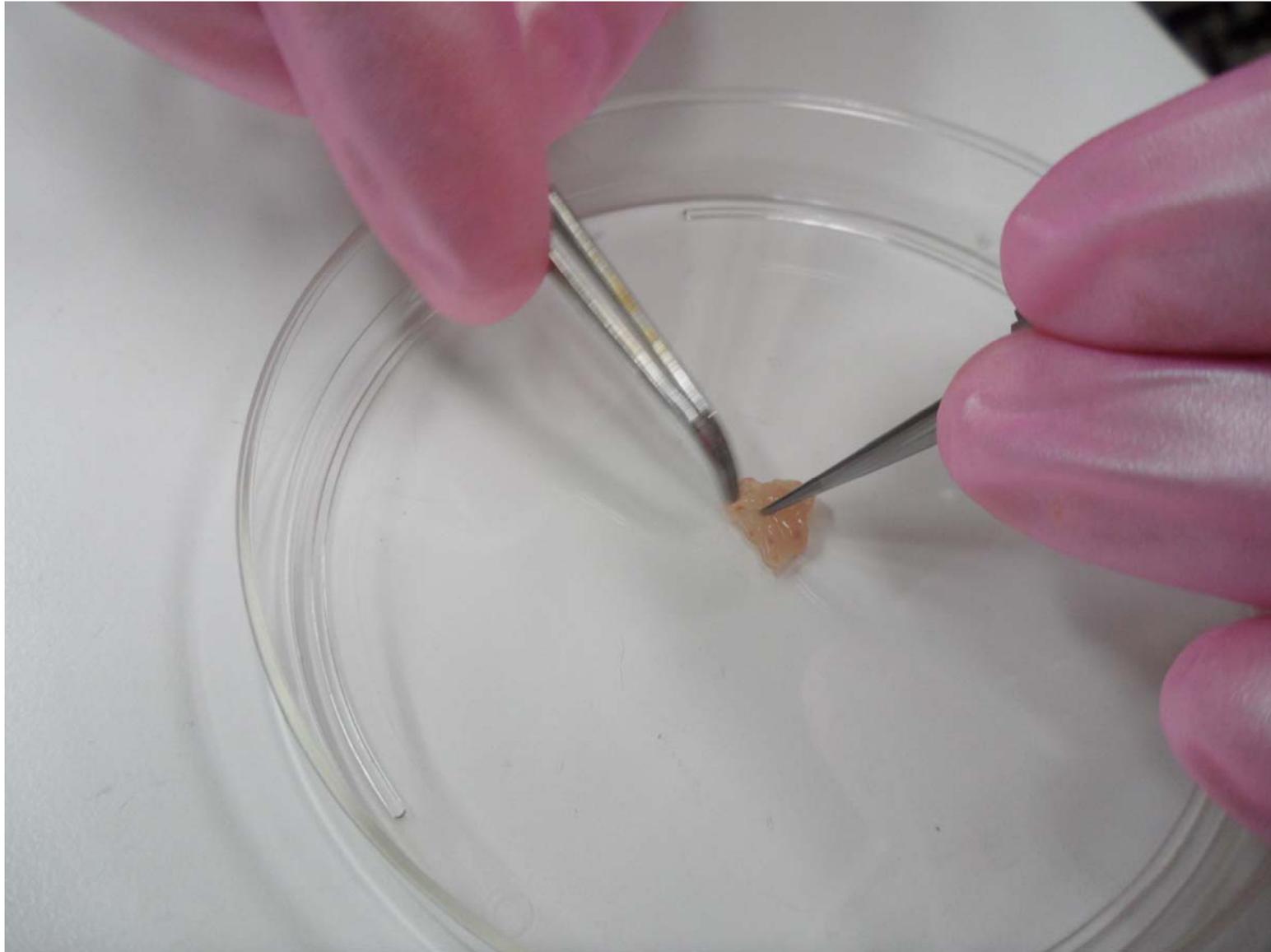
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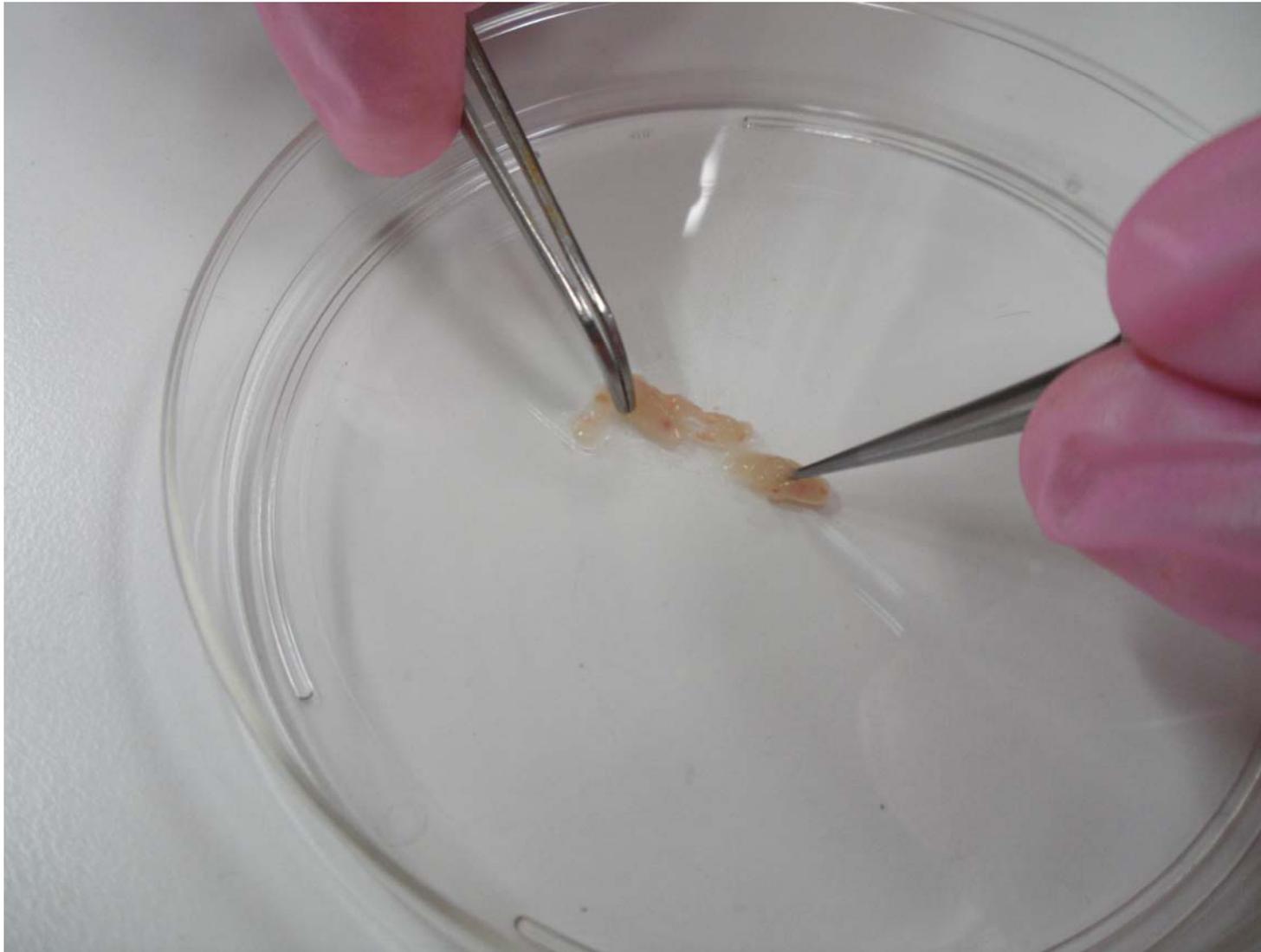
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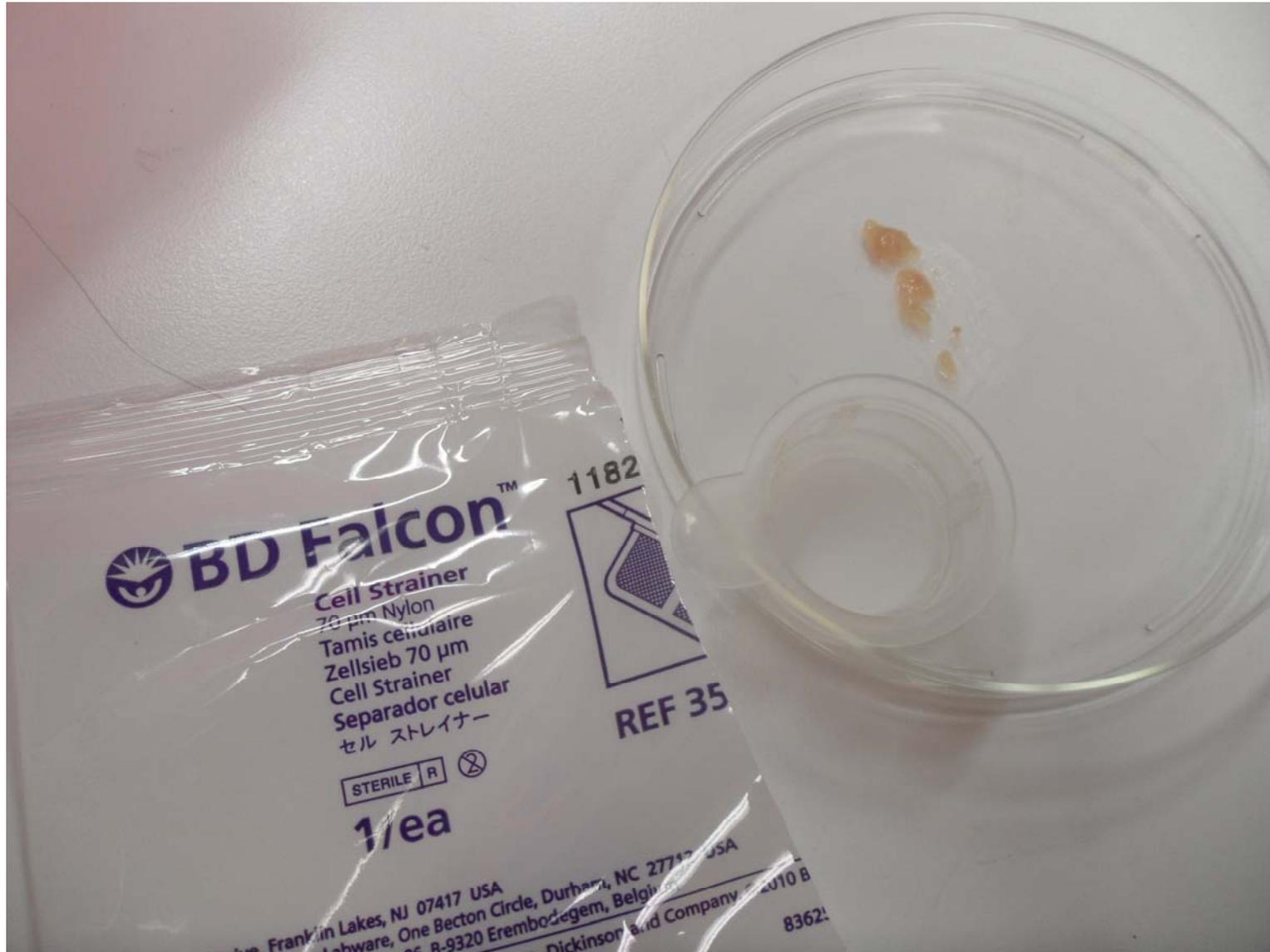
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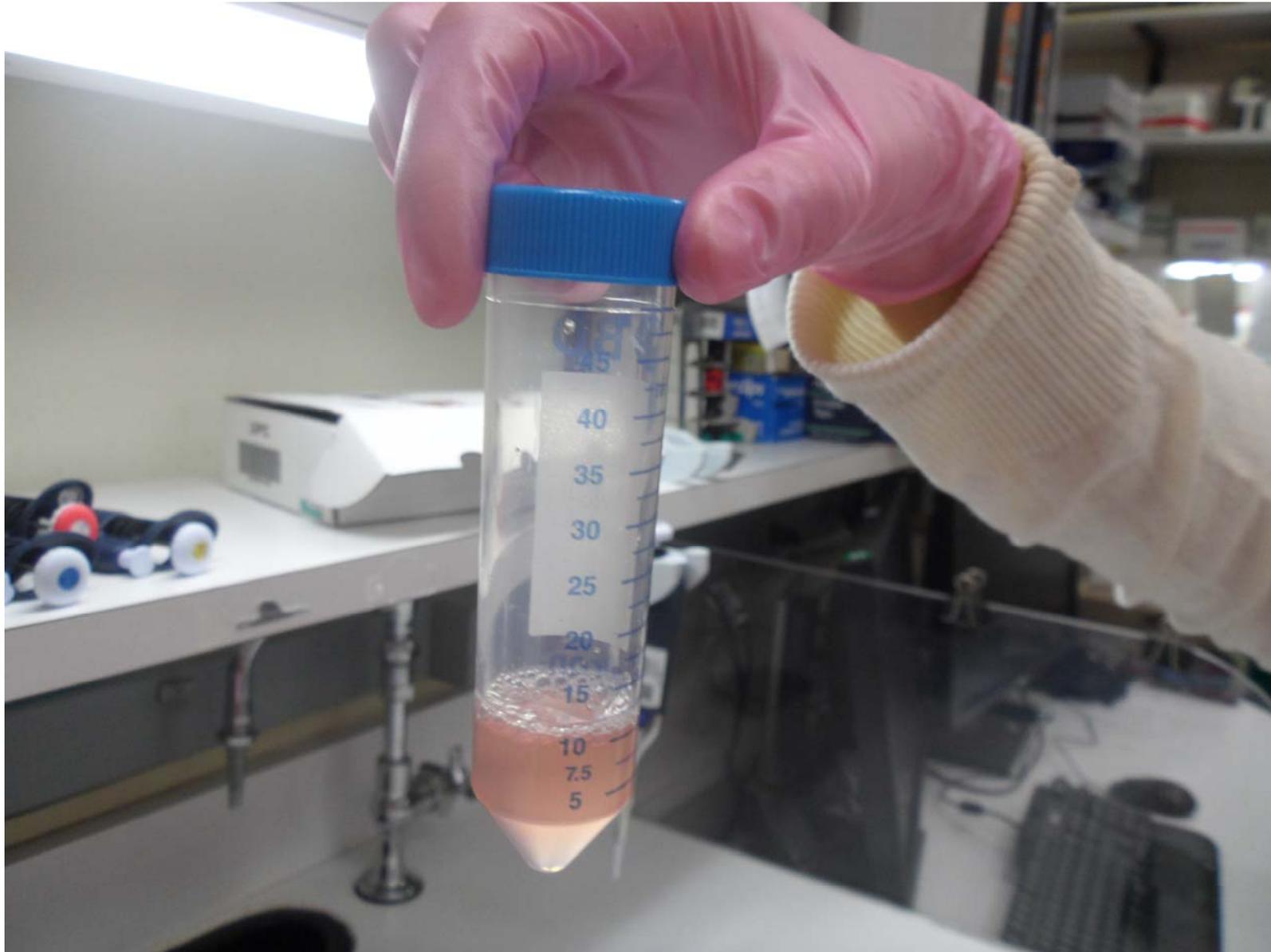
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Intra Epithelial Lymphocyte (IEL) preparation

1. Use small forceps to cut open skin exposing intestines
2. Very carefully tweeze small intestine from mouse, disconnecting it from the stomach and removing as much fat as possible
3. Place the intestine into a petri dish containing FBS and HBSS and wash several times
4. After washing, use small scissors to open up the intestine, and then cut intestine into small 1 cm pieces
5. Shake intestine pieces in FBS + DTT to allow lymphocytes to detach from epithelial cells

Intra Epithelial Lymphocyte (IEL) preparation

6. Dilute 100% Percoll in PBS to create 20%, 40% and 80% gradient
7. Filter cells into 50mL falcon tube, spin down to pellet and resuspend cells in 20% Percoll
8. Set up Percoll gradient in tube: With 80% on the bottom, 40% layered on top, followed by 20% containing your cells
9. Spin down for 25 minutes, the pipette out lymphocytes which can be found between the 40% and 80% gradient
10. Count cells using a hemacytometer

Lymphocyte isolation from liver

1. Use forceps to remove liver from mouse
2. Place liver onto 70um filter located in a petri dish on ice
3. Using the back end up a 6mL syringe smash the liver, creating a single cell suspension
4. Using PBS filter the liver cells through the 70um filter into a 50mL falcon tube
5. Wash the filter and plate several times
6. Spin down cells to remove fat and erythrocytes

Lymphocyte isolation from liver

7. Dilute 100% Percoll in PBS to create 40% and 70% gradient
8. Resuspend cells in 40% Percoll
9. Set up Percoll gradient with 70% on the bottom followed by 40% Percoll containing the cells
10. Spin down for 30 minutes, the pipette out lymphocytes which can be found between the 40% and 70% gradient
11. Count cells using a hemacytometer

4. Cell culture of immune cells

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Cell culture media

CML media: RPMI-1640-based, and supplemented with 10% FCS (pre-heat-inactivated, and charcoal-absorbed).

Absorption with charcoal ensures you to get rid of all the serum hormones that might interfere in the cell culture.

Charcoal Stripping

For 2000 ml

20 ml 1M Tris, pH 8.0

10 g Activated Charcoal (Sigma Catalog #: C-3345)

.05 g Dextran (Sigma Catalog #: D-1537)

2 L dH₂O

Spin 50 ml slurry in conical tubes (3000 rpm, 5 min)

Pour off supernatant

Add 25 ml Fetal Calf Serum/charcoal pellet

Incubate for 30 min at 56°C

Spin (3000 rpm, 5 min)

Decant supernatant into new 50 ml conical tubes and freeze at -20°C

Cell culture media

CML media: RPMI-1640-based, and supplemented with 10% FCS (pre-heat-inactivated, and charcoal-absorbed).

Absorption with charcoal ensures you to get rid of all the serum hormones that might interfere in the cell culture.

To make CML media add to a 500 ml RPMI-1640 bottle w/o glutamine (Life Science, in the white 4C refrigerator), one 50 ml tube of charcoal-stripped FCS (pre-aliquoted and stored in the -20 C) plus 25 ml of media mix (pre-aliquoted and in the -20 C: contains Penicillin, Streptomycin, Sodium Pyruvate, and non-essential amino acids) and finally 2-ME (also pre-aliquoted into snap-cap tubes and in the -20 C).

Thaw everything in a 37 C water bath, then mix and filtrate. Label with date and name.

In vitro cell

Cells are cultured in 5×10^6 millions/ml concentration in flat culture dishes or plates

CO₂ incubators are necessary for pH and temp

Lymphocytes are normally very small and will undergo cell death unless supplemented with survival factors or activated

5. Analysis of immune cells

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Flow cytometric analysis: single cell analysis

Western blot analysis: Different than when using cell lines (such as EL4 *etc*)

Proliferation assays: radioactive/non-radioactive

***In vitro* differentiation assays:** CD4 helper cell differentiation

THANKS