

# Module of Immunology Practical Course

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# OVERVIEW

## **01 Basic of mice handling**


Basic methodology for handling the mice before further analysis or treatment

## **02 Isolation of lymphoid organs**

Isolation of various organs related to immune system

## **03 Analysis of infection effect towards lymphoid organs**

Observation of infection effect toward various lymphoid organs





# 01

## **Basic of mice handling**

Basic methodology for handling the mice before further analysis or treatment

# Basic of mice handling

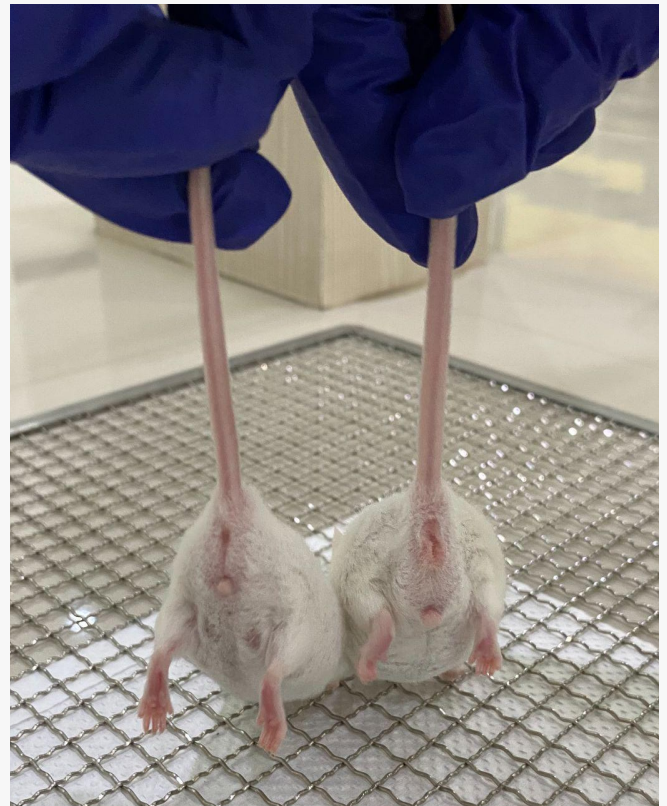


**Figure 1.** Mice balb/c in cage

Mice are small rodents with a pointed nose, furry round body, large ears, and a long, often hairless, tail. Mice are nocturnal or active at night. In brief, mice typically grow from 1-7 inch (2.54 to 18 centimeters) in length and weight between 0.5-1 ounce (Bradford, 2014).

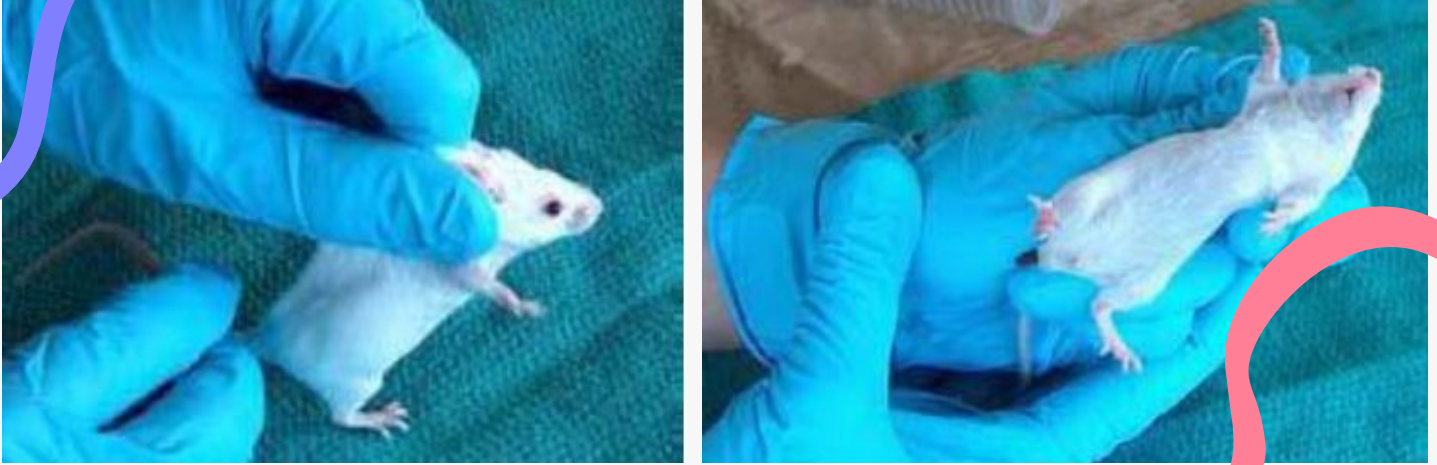
The process of sexing in mice often depends on variations in the genitalia. Male mice had testes and penis, while female mice had mammary glands (occasionally with nipples visible) and vagina. Therefore, the observation of mice sexing could be differentiated by tail lifting with forearm.

In behavioral observation, male and female mice exhibit different primal behavioral which essential for survival and reproduction. Female mice tend to be receptive while male mice are more aggressive and active.



**Figure 2.** Female mice (left) and male mice (right)

# Basic of mice handling



**Figure 3.** Basic of mice handling  
(Queen's University)

The basic of mice handling could be referred as follows:

- Mice should be carefully removed from the cage. Grab the tail to transport mice as needed.
- Using thumb and forefinger, grasp the upper third of the tail, then set the animal down on the wire top of a cage.
- In order to reduce the possibility of the mice escaping, grab the tail until restraint is secured. Furthermore, restrainer or tube could be used to transport mice so that it less stressful than grab the mice tail.
- Preparation for treatment could be started by lifting the mice from the wire cage, or any other surface.
- The thumb and index finger are used to grab the loose skin over the neck, shoulders and back. The head of mice should be immobilized.
- It essential to not hold the skin too tightly since it could potentially restrict the breathing and suffocate the mice.
- Moreover, secure the tail between the small finger and the palm of hand. The position of hand could be adjusted depend on the specific procedure or treatment.

# Practice




## Materials:

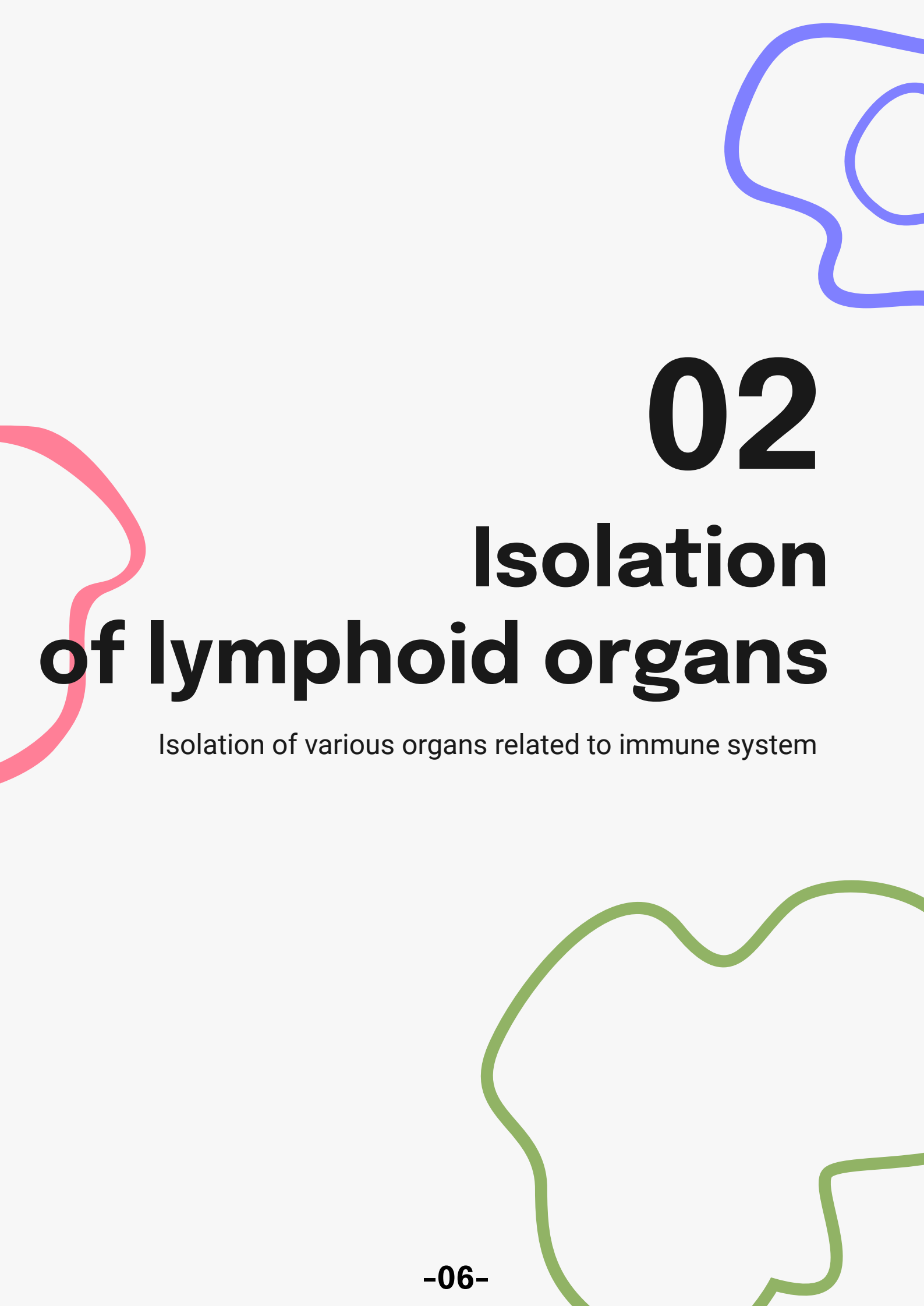
- Mice balb/c
- Lab coat
- Gloves
- Underpad
- Hair-nett

## Methodology

1. Prepare yourself by using lab coat, hair-nett, and gloves.
2. Remove the mice balb/c from the cage carefully by grasp the tail.
3. Placed the mice in table or wire cage. Let mice grab the table or wire cage.
4. Use the thumb and index finger to grab the scruff or neck of mice. Pinch the loose skin over the neck carefully.
5. Take the mice and expose the abdomen region.
6. Evaluate the position of your finger and hand. Adjust the position of your finger or hand for further treatment

## Questions:

1. How is the obstacles while experiencing the mice handling ?
  2. Is there any different for handling between male mice and female mice ?
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# 02

# Isolation of lymphoid organs

Isolation of various organs related to immune system

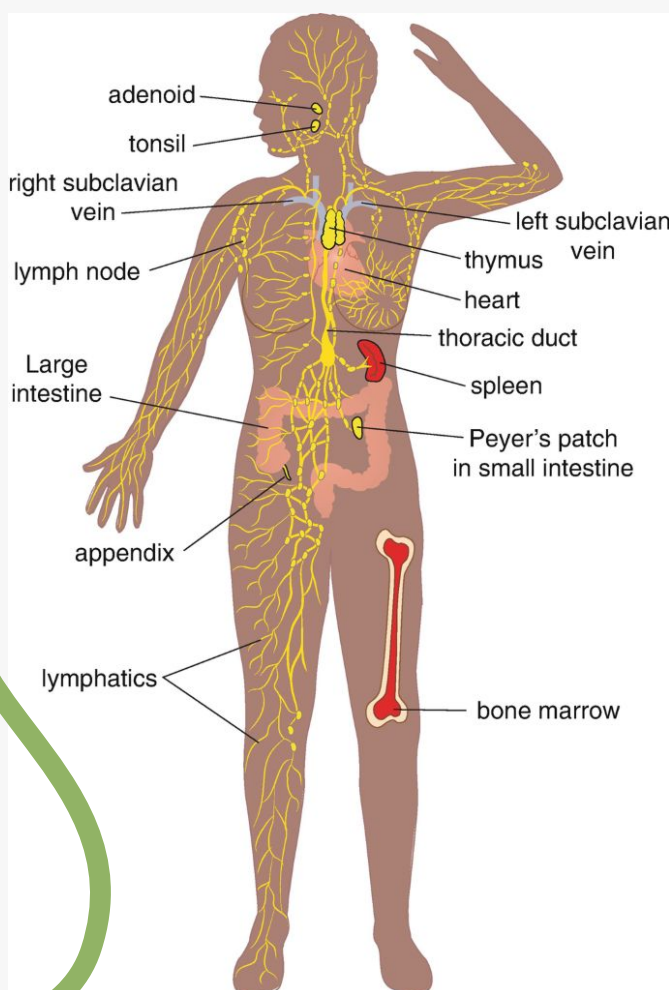


# Isolation of lymphoid organs

## Immune system

Immune system is a complex of biological systems that protects an organism from diseases. It responds to a wide variety of pathogens, ranged from viruses to parasitic worms, as well as cancer cells.

In brief, the immune system includes various components such as white blood cells, proteins, and chemicals that work together to protect the body. Furthermore, it also supported by lymphoid organs (Marshall et al., 2018)



**Figure 4.** Lymphoid organs in human (Kavathas et al., 2019)

## Lymphoid organs

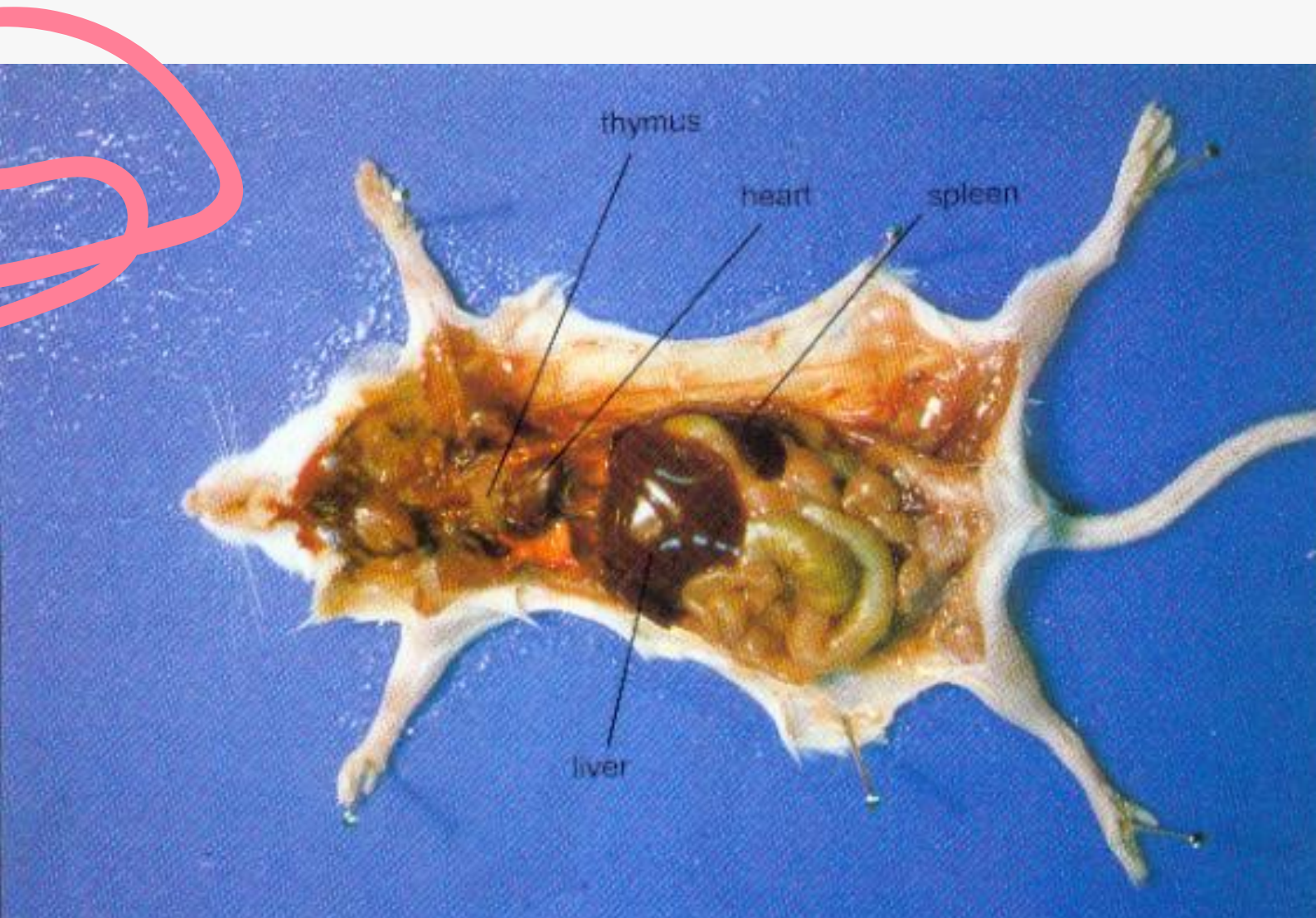
The immune system is composed of **primary and secondary lymphoid organ**.

Primary lymphoid organs are responsible for the production and maturation of lymphocytes. It include the thymus and the bone marrow. The thymus is where T cells mature, and the bone marrow is where both B and T cells are produced and mature.

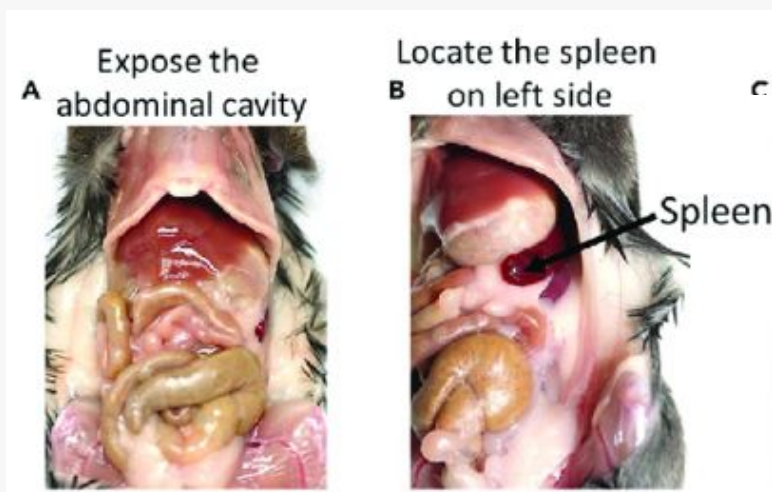
While, secondary lymphoid organ are responsible for the activation of immune cells. These organs include the spleen, lymph nodes, tonsils, Peyer's patches, and mucosa-associated lymphoid tissue (MALT) (Janeway et al., 2001)



# Isolation of lymphoid organs



**Figure 5.** Lymphoid organs in mice (University of Maryland)



**Figure 6.** Spleen organ in mice (Bafor et al., 2023)

Both humans and mice have lymphoid organs that play crucial roles in the immune system. In mice, primary lymphoid organs also include the thymus and bone marrow, but the thymus is more prominent in mice, and it plays a more significant role in the development of T cells.

# Practice

## Materials:

- Mice balb/c
- Alcohol swab
- Surgery kit
- Underpad
- Straight pin
- Dissection tray
- Lab coat
- Gloves
- Hair-nett
- Tube with PBS

## Methodology

1. Prepare yourself by using lab coat, hair-nett, and gloves.
2. Placed the underpad and dissection tray on the table.
3. Mice balb/c were anesthetized then euthanized.
4. Euthanized mice were positioned in dissection tray and secured the limbs using straight pin
5. Swab the abdomen region of mice by using alcohol swab.
6. Dissect the abdomen skin by using surgery kit, collect several lymphoid organs.
7. Collected lymphoid organs were placed into tube contained of PBS for further observation.

## Questions:

1. How many lymphoid organs that collected ?
2. How the morphology of each collected lymphoid organs ?



# 03

## **Analysis of infection effect towards lymphoid organs**

Observation of infection effect toward various lymphoid organs

# Analysis of infection effect towards lymphoid organs

## Infection

Infection refers to the invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body. Those can lead to various symptoms and diseases, ranging from mild to severe, depending on the type of the infection (Drexler, 2010).

The infection of bacteria could be transmitted through poor hygiene, raw eating, or direct contact. Therefore, it essential to avoid the transmission for reducing the risk of infection.

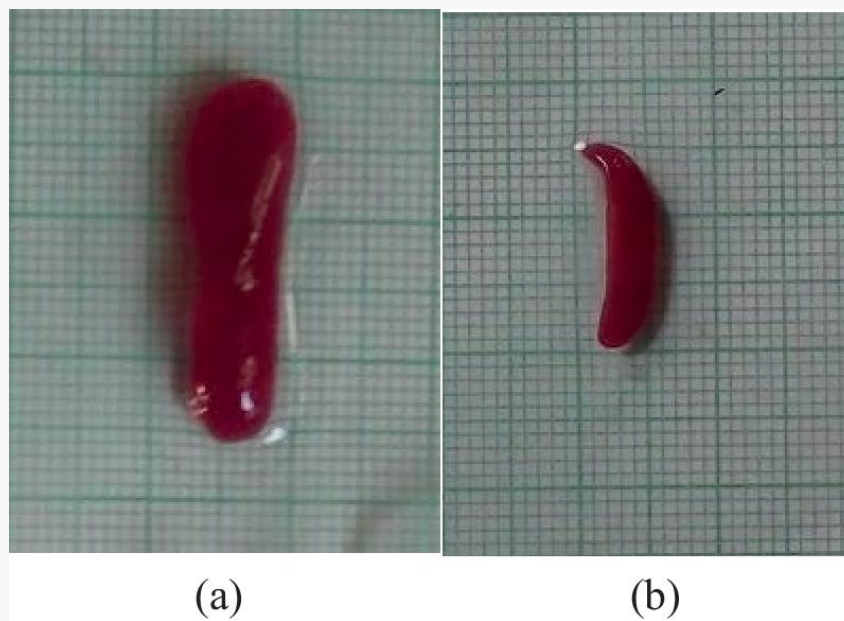
## Research of immune response toward infection

*Escherichia coli* (*E.coli*) is a type of negative gram bacteria that can cause a number of illnesses, including digestive system, urinary tract, and meningitis infections. The component of cell wall's lipopolysaccharide (LPS) triggers immunological responses in a variety of ways. The study identify the effect of *E. Coli* infection the amount of lymphocytes and weight of the spleen in mice (Mitasari et al., 2017).



# Analysis of infection effect towards lymphoid organs

Result showed that *E. coli* infection significantly increase both weight of the spleen and number lymphocyte. In conclusion, mice responded to *E. coli* infection by increasing the number of lymphocyte (Mitasari et al., 2017).



**Figure 7.** The different size of spleen in mice which infected with *E.coli* (a) and uninfected with *E.coli* (b) (Mitasari et al., 2017)

The study used a randomized design with treatment as follows:

- Control (-): mice without treatment
- Control (+): mice were injected with 1 mL of PBS
- Treatment 1: mice infected with *E. coli*  $1.5 \times 10^3$  cfu in 1 mL PBS
- Treatment 2: mice infected with *E. coli*  $1.5 \times 10^5$  cfu in 1 mL PBS
- Treatment 3: mice infected with *E. coli*  $1.5 \times 10^7$  cfu in 1 mL PBS

# Practice

## Materials:

- Mice balb/c
- Alcohol swab
- Surgery kit
- Underpad
- Straight pin
- *E.coli* culture
- Dissection tray
- Lab coat
- Gloves
- Hair-nett
- Tube with PBS
- Syringe

## Methodology (Mitasari et al., 2017)

1. Prepare yourself by using lab coat, hair-nett, and gloves.
2. Mice balb/c were grouped into treated group and untreated group.
3. Treated group was injected with *E.coli* suspension (150.000 CFU in 1 ml of PBS) (Stoddart, 2011; Vembadi et al., 2019) through intraperitoneal injection. While, mice in untreated group were not injected.
4. After 5 days, mice were anesthetized then euthanized.
5. Euthanized mice were positioned in dissection tray and secured the limbs by using straight pin.
6. Swab the abdomen region with alcohol swab.
7. Dissect the abdomen skin by using surgery kit, collect the spleen and other lymphoid organs.
8. Collected spleen and other lymphoid organs were placed in tube for further observation.

## Questions:

1. How the morphology of spleen between untreated mice and treated mice ?
2. Is there any difference among others lymphoid organs in untreated mice and treated mice ?

# References

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# Task submission

Every section of practice is completed by comprehensive assignment or task. It aimed to measure the capability of students within practical course activity. Each of task or submission is need to be submitted individually after the practical course through link or QR code below.

## Basic of mice handling

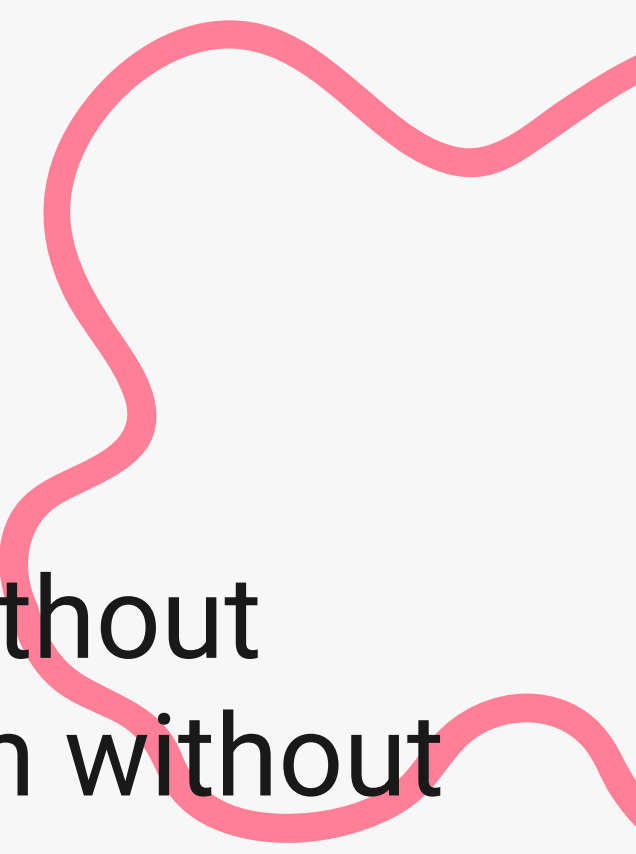



## Isolation of lymphoid organs



## Analysis of infection effect towards lymphoid organs





**“No research without  
action, no action without  
research.”**

**–Kurt Lewin**

# Thank you!

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