

GEroNIMO

**Protocol: sampling of tissues from laying hens
(aged between 70 and 90 weeks)**

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Here we describe the procedures used to sample tissues from laying hens aged between 70 and 90 weeks.

Tissues analyzed in GEroNIMO are:

- Adipose tissue
- Brain
- Hypophysis (Pituitary gland)
- Hypothalamus
- Liver
- Spleen
- Muscle
- Intestine (jejunum)

1. Protocol description

1.1 Required reagents and instruments

Reagents:

- Liquid nitrogen (in a storage bank)

Consumables:

- Cryogloves
- Dry ice
- Disposable scalpels
- Scissors
- Knives
- Pruning shears
- Sterile disposable Petri dishes
- Thick aluminium foil (sterile) 14.5x9.5 cm per 120
- Pre-labelled 2 mL cryotubes, use cold-resistant labels, which will have been checked before, the label shows animal ID, tissue
- A permanent marker to label the aluminium foil
- Water bottle
- Ethanol spray bottles
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Equipment:

- Cryoprotection gloves
- Protective gloves for handling sharp objects (Size M and L)
- Storage tank (30L)
- Small styroform box for temporary storage of liquid nitrogen
- Precision scales

1.2 Preparatory step

Prepare the workplace by putting aluminum foil and paper towel on the working bench. Place on each workplace scalpels, gloves, Petri Dishes...

1.3 Animal dissection

Laying hens are stunned by electronarcosis before being slaughtered by bleeding. Each organ is rapidly

extracted from the carcass: A) on one side, the brain tissues with the hypophysis & the hypothalamus and then the remaining brain B) on the other, different tissues in a pre-determined order: liver, adipose tissue, spleen, magnum, uterus, muscle, intestine (jejunum), caecum.).

1.4 Tissue processing

Each dissected organ is put in Petri Dishes (100 mm), pre-labelled with the tissue sample ID.

Some tissues as abdominal adipose tissue, liver, spleen and muscle were weighed on a precision scale just before being stored.

For adipose tissue, liver, spleen, magnum and muscle, little pieces of the tissue sample (roughly 1g) are cut and stored in thick aluminum foil..

For brain samples and intestine, the samples are stored in a 2 ml cryotube.

For the intestine sampling, about 6 cm long of the jejunum was cut and placed in a Petri dish on a cold ice pack and then cut lengthways. The content of the segment was cleaned with sterile water. Then, the intestinal mucosa is scraped stored in a 2 ml cryotube.

Tubes and aluminum foils are immediately snap frozen by immersion into liquid nitrogen and then, stored in dry ice and transported back to the laboratory. Samples are finally stored at -80°C.