Necropsy Procedure for Old Mice. Updated 11/03/2015

Note: Any right or left designations are from perspective of mouse.

1. After CO2 asphyxiation, the mouse is **WEIGHED**. Check teeth for **BROKEN INCISORS**.

2. **SERUM**: Blood is drawn using the heart stick method after mouse termination. Keep tube at room temp, spin down for 5 minutes (14000g x 5 min) at 4C in the cold room, and keep on ice. Once spun down, transfer serum to labeled Eppendorf tubes and store at -20C.

3. Pin down, face up, and spray with ethanol. Make a ventral midline incision from groin to chin and reflect skin back. Gross photos can be taken now or throughout the procedure as indicated.

4. **HEART**: The trachea with attached lungs and heart are removed together. Heart is separated from the lungs and after dabbing out blood, the heart is weighed. It is then cut into 4 pieces with a razor in the Heart Block (see diagram).

5. Place sections 1, which contains the valves, and 3, which contains the papillary muscles, in tissue cassette A in 10% NBF. Place sections 2 and 4, which includes the bottom (apex) in foil and freeze in liquid nitrogen to be stored at -80C.

6. **LUNGS**: Left lung is placed in a labeled foil, frozen in liquid nitrogen, and stored at -80C. The right lung is gently infused/inflated with formalin from a syringe threaded through the trachea, and placed in cassette B into the formalin jar.

7. **LIVER**: The liver, spleen and pancreas are removed. Major lobe of liver is placed in foil, frozen in liquid nitrogen, and stored at -80C. Place the rest of the liver in one or two cassettes C and into the formalin jar. If there are any tumors in the major lobe or any lobes, consider saving a section in a cassette as well for histopathology.

8. **SPLEEN & PANCREAS**: Place the pancreas and spleen in cassette D to be stored in the formalin jar.

9. **KIDNEYS**: Kidneys are removed with the adrenals. Left Kidney with adrenal is placed in a foil and frozen in liquid nitrogen to be stored at -80C. Right kidney with adrenal is placed in cassette D and into the formalin jar.

10. **GUT**: The colon will be jelly rolled and placed in cassette I to be stored in the formalin jar.
11. **REPRODUCTIVE TRACT**: Note if abnormalities are present, making any additional notes, and leave with the carcass to be placed in formalin.

12. **MUSCLE & BONE**: Measure the length of the left tibia. The left thigh, or quadriceps, is separated from the bone by severing the tendons. Place it in foil to freeze in liquid nitrogen and stored at -80°C. The right thigh muscle, or quadriceps, along with the femur bone is to be collected and placed in cassette F into formalin.

13. **LYMPH NODES & ENDOCRINE GLANDS**: Note if abnormalities are present by checking the corresponding box, make any additional notes, and leave with the carcass to be placed in formalin.

14. **EYES**: Note if abnormalities are present, making any additional notes, and leave with the carcass to be placed in formalin.

15. **HEAD AND BRAIN**: Remove the head from the rest of the body at the level of the neck. The brain will remain within the head. At the level of the foramen (opening) that goes into the brain from the spinal cord, snip slightly (~0.5 cm) with scissors on either side of the spinal cord/brainstem through the bone, soft tissue and skin to allow for formalin penetration. Replace formalin with 70% ethanol after 72 hours of fixation.

16. **PAW AND CARPAL**: Remove the right paw and carpal from the forelimb to be placed in cassette H and into formalin jar.

17. **TUMORS & ABDOMINAL TISSUES**: Any tumors should be removed and placed in a cassette in formalin. If tumor is large, cut it in half with razor blade. Also, try to leave it attached to whatever organ it has grown from or is attached to. If a tissue designated for freezing has tumors, freeze a piece or the half that does not have tumors and make note of it.

18. **FIXATION**: The entire remaining carcass is placed in formalin. The carcass and tissues are fixed for 24-48 hours and then replaced with 70% ethanol.
LABELING:

**Index Cards**
- Mouse ID
- Mouse Strain
- Sex
- DOB
- DOD

**Formalin Cups**
- Study Title
- Mouse ID
- Mouse Strain
- DOBW
- Date of Death
- Age Group
- Sex

**FREEZING – aluminum foil**
- Study Title
- Strain
- Mouse ID
- Age Group
- Organ
- Date of death

**Materials:**
- Plasma separator tubes
- Aluminum foil - labeled
- 1.5ml tubes
- 1ml or 3ml syringe
- 21G or 23G needles
- 70% Ethanol
- Razor blades
- Heart block (for slicing)
- Scissors and forceps
- Spatula (helps for removing brain)
- Styrofoam base with needles for pinning
- Tissue cassettes
- Cups and lids
- 10% Neutral Buffered Formalin
- Paper Towels
- Liquid nitrogen
- CO2
- Dry Ice
- CAMERA
- Necropsy form

**Cassette Labels**
- A: Heart
- B: Lungs
- C: Liver
- D: Spleen and Pancreas
- D: Kidney and adrenals
- E: Fat (retro, mesen, inguin, abdom)
- F: Muscle and bone
- G: Brain
- H: Paw and carpel
- I: Colon or GI Tract