If brain can be collected within 5 hours of death
Follow PMI < 5 hour procedure on page 2

Ape Death

If brain cannot be collected within 5 hours of death
Follow PMI > 5 hour Procedure on page 3
Step 1: Photograph whole brain and weigh it

Step 2: Cut brain in half along the midsagittal plane

Step 2: Cut left hemisphere into 5 mm slabs in the coronal plane (see adjacent Figure)

Step 3: Label each slab of the left hemisphere and wrap in foil

Step 4: Flash freeze the left hemisphere slabs and store in a -80 degree freezer (see ADJACENT PAGE for instruction)

Step 5: Place right hemisphere in 4% or 10% formalin

Step 6: After two weeks, ship the formalin fixed right hemisphere to William Hopkins: Ship frozen slabs to Chet Sherwood (contact information on next page). Please contact Chet Sherwood prior to shipping to make sure staff is available for pick up of the materials.

Step 7: MRI scan at 7T will be conducted on right hemisphere sent to William Hopkins. A brief summary of any clinical abnormalities in gross morphology will be provided by WDH.

Step 8: After scanning, right hemisphere will be shipped to Dr. Sherwood for histological analyses by he and Dr. Hof. A report on any histological abnormalities will be provided to the ApeTag committee or participating zoos. Of course, tissues from any brain region can be provided to zoo authorities or research institutions upon request in order to conduct their own pathology analyses.

Step 9: Frozen sections will be sent to Dr. Sherwood. Tissue should be placed in a box with dry ice and shipped priority with FEDEX or related company. These tissues will be banked and tissue samples will be provided to participating zoos or any research institution upon request.
Freezing methods for tissue slabs or brains

We realize that not all facilities will have the same capacity for freezing brain tissue, there we have outlined below, three options (in preferred order) for pathologists.

1. **Isopentane/dry ice.** The preferred method of freezing of tissue slabs is a slurry of isopentane/dry ice at -30 to -40 C.
2. **Liquid nitrogen.** The second method of choice is freezing in liquid nitrogen by immersion.
3. **-80C freezer.** The third method is freezing the samples on a tray in a -80 degree freezer.

Place hemisphere on a horizontal surface with the medial surface down and the lateral side facing up. If possible, take images with a digital camera before and during sectioning. Cut serial/coronal slices of the hemisphere from the frontal pole and proceeding caudally at about 1 cm intervals. The number of sections/slices per brain varies depending on the age and size of the brain. Snap freeze all slices using one of the three freezing method described above and number sequentially with a letter designating the left (L) or right (R) hemisphere. Store each slice in its own individual Ziploc or vacuum plastic bag at -80C.
Shipping Instruction

For Formalin Fixed Tissue:

1) Place in sealed container
2) Write “Biological samples – nonhazardous” on the label of the container (formalin will kill any pathogenic agent)
3) Ship to addresses on next page following instructions from previous pages

For Frozen Tissue:

1) Place in sealed container
2) Write “Biological samples – nonhazardous” on the label of the container if the individual has no known pathogenic agent is present
3) Write “Biohazard materials” if there is a known pathogenic label.
4) Ship to addresses on next page following instructions from previous pages
PMI > 5 Hour Procedure

Step 1: Photograph whole brain and weigh it

Step 2: Place entire brain in 4% or 10% formalin, which is ever is available

Step 3: After two weeks, ship the formalin fixed whole brain to William Hopkins (contact information on next page)

Step 4: Samples can be shipped in a labeled box with a minimum amount of formalin. Shipping can take place any day of week except Friday (i.e., no weekend delivery). Please contact William Hopkins (contact information provided on Page 4) before shipping to coordinate pick up and tracking of the brain.

Step 5: MRI scan at 7T will be conducted on whole brain sent to William Hopkins. A brief summary of any clinical abnormalities in gross morphology will be provided by WDH.

Step 6: Copies will be made available of all MRI scans upon request to any interested parties

Step 7: After scanning, whole brain will be shipped to Dr. Sherwood for histological analyses by he and Dr. Hof. A report on any histological abnormalities will be provided to the ApeTag committee or participating zoos.

Step 8: Tissues from any brain region can be provided to zoo authorities or research institutions upon request in order to conduct their own pathology analyses.
Contact Information

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FEDEX account: 1692-6745-6
Internal Billing: 39471 (you will need this in section 7 of the FEDEX Airbill form for us to pay)

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