

# ***ELEPHANT TAG/SSP RESEARCH AND NECROPSY PROTOCOL***

*(Elephas maximus and Loxodonta africana)*



*The American Zoo and Aquarium Association  
Elephant Taxon Advisory Group  
Species Survival Plan*

*February 2025*

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## ABSTRACT / SUMMARY

Due to the length of this protocol, a brief summary is provided here as a reminder for those who have previously performed an elephant necropsy. Those persons or institutions who have not previously performed an elephant necropsy should read the protocol in its entirety to ensure completion of a **safe**, efficient, and accurate necropsy procedure.

This necropsy protocol should be used in conjunction with the optional SSP research and tissue requests (included at the end of this document) to facilitate collection of a complete tissue, sample, and data set. Several pathologists, clinical veterinarians, and scientists are potentially available to assist institutions with elephant necropsies. Assistance may be in the form of telephone consultation or, if given sufficient notice and time to travel, on-site guidance (contact information available at the end of this document). Two of the more important disease processes in elephants include **endotheliotropic herpes virus (EEHV) infection** and **tuberculosis** (caused by the human pathogen, *Mycobacterium tuberculosis*). Additional information regarding these diseases is available from other sources and would be beneficial to review prior to initiating a necropsy procedure in these species (EEHV: [eehvinform.org](http://eehvinform.org); TB: USDA website – [http://www.aphis.usda.gov/animal\\_welfare/index.shtml](http://www.aphis.usda.gov/animal_welfare/index.shtml)), “Guidelines for the Control of Tuberculosis in Elephants protocol” and IEF website - <https://elephantconservation.org/stay-informed/recommendations-for-tb-in-elephants-in-human-care/>, “Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care”). If the TB test status of the elephant is unknown, suspect, or positive, close attention should be paid to the tuberculosis alert in this protocol to ensure the safety of staff participating in the necropsy and to prevent contamination of the surrounding areas or animals. Most equipment listed in the protocol is similar to that used in smaller animal necropsies with the addition of heavy equipment (tractor), chain saw or reciprocating saw, an axe, numerous knives, chains, straps, and the very important TB protective equipment. To complete a detailed necropsy, a team of at least 6-8 people should be assembled for 8-10 hours of work. Team members should be organized and assigned specific roles (supervising pathologist or clinician, prosectors, dedicated knife sharpener, and various assistants to collect samples, take notes, and take photos). Heavy equipment or chain hoists should be used to remove and move large body parts (limbs, head, etc.) for safety and efficiency reasons. The gastrointestinal tract of the elephant is massive but relatively simple, and the remaining organs are similar to those in other mammals (with some exceptions listed in the protocol). The thoracic cavity should be examined last and in those cases with unknown, suspect, or positive TB-results, special precautions are required (**see TB alert**). Removal of the brain is difficult and requires use of a chain or reciprocating saw. The protocol provides some guidance to facilitate this task. Disposal of an elephant carcass is a job in and of itself. Ideally, the necropsy should be performed within or adjacent to a burial site large enough to accommodate the carcass. Special burial permissions may be required depending on city, county, and state regulations, and those agencies should be contacted as soon as possible.

Post-mortem examination of an elephant can be a daunting task, but with proper personnel, planning, and experience, the procedure can be done safely and efficiently. If at all possible, institutions should make preparations or contingency plans for the movement, necropsy, and disposal of an elephant ahead of time to avoid the stress of planning at the time of death. The information gained from an elephant necropsy is hugely valuable to institutions, the AZA, and to elephants in captivity and in the wild.

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February 2025

## INTRODUCTION

This protocol is an effort of the Elephant Species Survival Plan (SSP) Propagation Group of the American Zoo and Aquarium Association (AZA). The purpose is to provide a format for the systematic collection of information and samples that will facilitate diagnostic investigation and add to our knowledge of elephants. All North American institutions holding elephants will receive a copy.

An elephant necropsy may be a daunting procedure; however, it should be viewed as an important learning opportunity. Although it may not be feasible to collect all the information and samples requested in each case, collection of as much as possible is encouraged. With the increased availability of digital cameras, photographic documentation of both normal and pathologic structures for future reference is also strongly recommended.

All current research requests for postmortem elephant samples are presented at the end of this document. Collection of the requested data sets/samples is optional; however, voluntary compliance is essential for continued advances in the understanding of elephant physiology in both health and disease. Please consider making necessary efforts to fulfill research requests.

Familiarity with the protocols in these documents and having the necessary equipment ready will facilitate sample collection. It is highly suggested that holding facilities have regular preparedness meetings and institution-specific elephant necropsy protocols and SOPs established as part of the complete health plan rather than dealing with the pressure of assembling at the time of an unexpected death. It is suggested that a necropsy team be designated in advance; the ability to mobilize skilled individuals quickly will save valuable time. Veterinarians, anatomists, and pathologists from nearby universities and zoos may be enlisted to assist the institution's staff. In addition, a list of researchers interested in participating in elephant necropsies is included in this protocol.

The Elephant TAG/SSP Research and Necropsy Protocol will be revised/updated annually or as required. Contact Jaime Landolfi with any changes. **A copy of the completed final necropsy and any other lab reports should be forwarded, with digital images if applicable, to Dr. Landolfi for inclusion in the TAG/SSP pathology archive.**

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## **ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS (EEHV) ALERT**

**All elephants that die or are euthanized for any reason should have a systematic collection of information and samples that will contribute to our knowledge of Elephant Endotheliotropic Herpesvirus (EEHV). Research sample requests are included at the end of this document.** Contact the listed researchers for specific questions. The EEHV Advisory Group website ([www.eehvinfo.org](http://www.eehvinfo.org)) is an excellent source of additional information.

Whole blood and serum samples from sick or dead elephants should be obtained for diagnostic testing in any suspected case of herpesvirus infection.

Postmortem examination should include thorough investigation and documentation of any gross lesions suggestive of EEHV-hemorrhagic disease (HD). The oral, thoracic and peritoneal cavities should be evaluated for mucosal and/or serosal abnormalities such as hemorrhage, edema, cyanosis/congestion and ulceration. Distribution and size of lesions should be noted; written documentation should be augmented by digital imaging if available.

Lungs should be visually examined and thoroughly palpated for the presence of EEHV-associated pulmonary lymphoid nodules on surface and “bread-loafed” cut sections. These lesions have been found in a high proportion of African elephants culled in the wild. These lung nodules have also been reported in Asian elephants. Affected lungs may have rare to many, 3-30 mm diameter, white to gray, soft spongy nodules. Nodules may occur in the absence of other pulmonary disease in otherwise healthy elephants. In the absence of visible or palpable lesions, collect 4 representative sections of lung for histopathology (2 sections from each side including one cranioventral and one caudodorsal section).

Skin, particularly from the trunk and face, should be examined for raised nodules with darker fibrous centers. Such EEHV-associated skin lesions have been found occasionally in otherwise healthy juvenile African elephants.

Mucosa of the distal female reproductive tract (vulva and vagina) should be evaluated for reddened, raised nodular or depressed ulcerative lesions. Such lesions have been associated with EEHV in the genital tract of female African elephants.

Multiple, representative sections of any and all suspect EEHV-associated lesions (i.e. hemorrhage/edematous tissues, pulmonary lymphoid nodules, raised cutaneous lesions, vulvar/vaginal mucosal nodules) should be collected for histopathology and frozen archive.

## **ELEPHANT TUBERCULOSIS ALERT**

An intense search for lesions of tuberculosis (TB) is encouraged in all elephant necropsies. **This should include all elephants that die or are euthanized for other reasons (even if TB is not suspected).** Be advised that elephant TB is likely to be caused by *Mycobacterium tuberculosis* which is contagious to humans. Therefore, participants must be prepared with proper protective apparel, and any suspicious organs or lesions should be contained as soon as possible.

Ideally, elephants should be bled for currently available TB diagnostic test development and validation, and trunk wash(es) collected just prior to euthanasia. Elephants that die naturally should have a postmortem trunk wash performed, and serum should be harvested from postmortem blood for serological assays. Available online documents (**2017 Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care** [[http://www.nasphv.org/Documents/ElephantTB\\_NASPHV.pdf](http://www.nasphv.org/Documents/ElephantTB_NASPHV.pdf)] and **Guidelines for the Control of**

**Tuberculosis in Elephants** [<https://www.federalregister.gov/documents/2013/01/04/2012-31644/guidelines-for-the-control-of-tuberculosis-in-elephants>] may be consulted for further information.

#### Protective equipment for tuberculosis cases

Respiratory protective equipment should be utilized for all elephant necropsy procedures regardless of the historical TB testing status of the animal. In animals with an unknown, suspect, or positive TB test history, respiratory protection should be considered **mandatory**. OSHA standards (29CFR1910.134) require that “workers present during the performance of high hazard procedures on individuals (humans) with suspicious or confirmed TB” be given access to protective respirators (at least N-95 level masks). Similar precautions should be taken during an elephant necropsy. According to the draft CDC guidelines for the prevention of transmission of tuberculosis in health care settings, respiratory protective devices used for protection against *M. tuberculosis* should meet the following criteria:

1. Particulate filter respirators approved include (N-,R-, or P-95,99,or 100) disposable respirators or positive air pressure respirators (PAPRs) with high efficiency filters
2. Ability to adequately fit wearers who are included in a formal respiratory protection program with well-fitting respirators such as those with a fit factor of greater than or equal to 100 for disposable or other half-mask respirators
3. Ability to fit the different face sizes and characteristics of wearers. This can usually be met by supplying respirators in at least 3 sizes. PAPRs may work better than half-masks for those persons with facial hair.

See website links below for OSHA and CDC guidelines.

#### **Necropsy procedures**

All elephants require careful examination of the tonsillar regions and submandibular lymph nodes for possible tuberculous lesions. These lymph nodes may be more easily visualized following removal of the tongue and laryngeal structures during the dissection. All lymph nodes (including extra-thoracic) should be carefully evaluated for lesions since other sites may also be infected (e. g. reproductive or gastrointestinal tract). Any nodes that appear caseous or granulomatous should be collected for both culture (freeze or ultrafreeze) and fixation (in buffered 10% formalin).

Thoracic organs should be carefully evaluated for early stages of TB as follows:

- After removal of the lungs and trachea, locate the bronchial nodes at the tracheal bifurcation. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node to be submitted for mycobacterial culture (NVSL or another experienced laboratory is recommended). Culture should be done **even if no gross lesions are evident**. Submit sections in formalin for histopathology.
- Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm B-B shot to larger, nodular lesions. Take **NUMEROUS (5 or more)** sections of any suspicious lesions. In the absence of visible or palpable lesions, collect 4 representative sections of lung for histopathology (2 sections from each side including one cranioventral and one caudodorsal section).
- Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly.
- Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas.
- Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.

Samples for TB diagnostics should be submitted to the National Veterinary Services Laboratory (NVSL).

## INTERNET SITES

These guidelines and other elephant protocols are available on the internet at the following sites:

1. [http://www.aphis.usda.gov/animal\\_welfare/index.shtml](http://www.aphis.usda.gov/animal_welfare/index.shtml)
2. [www.aazv.org](http://www.aazv.org)
3. [www.elephantcare.org](http://www.elephantcare.org)
4. [http://www.aphis.usda.gov/animal\\_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf](http://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf) – Elephant Postmortem Examination
5. <http://www.osha.gov/SLTC/tuberculosis/standards.html> - OSHA TB standards and rules
6. <http://www.eehvinfo.org> – Information on Elephant Endotheliotropic Herpesvirus
7. [http://www.nasphv.org/Documents/ElephantTB\\_NASPHV.pdf](http://www.nasphv.org/Documents/ElephantTB_NASPHV.pdf) - Information on Elephant TB; updated 2017
8. [https://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/downloads/NecropsyGuideline.pdf](https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/NecropsyGuideline.pdf)

## EQUIPMENT CHECKLIST

1. At least 10 quality large necropsy knives, knife sharpener, diamond, steel, and/or stone
2. Standard large animal necropsy instruments, multiple scalpel handles, duplicates or triplicates of other instruments, extra box of scalpel blades
3. Sterile instruments for culture collection
4. 10% neutral buffered formalin (at least 5 gallons)
5. Field acid-fast staining kit (to determine the presence or absence of *Mycobacteria* sp.)
6. Gluteraldehyde, 2.5-4% (at least 100mls); *optional*
7. Large containers for sample collection
8. Culture swabs, conical vials, sterile urine cups, glass slides
9. Serum tubes for blood and urine collection
10. Plastic bags for freezing tissues (whirl-paks of various sizes); aluminum foil if toxicosis is suspected
11. Labels and waterproof marking pens
12. Scale for obtaining organ weights
13. Tape measure (metric), at least 2 meters long
14. Chain saw, axe, or reciprocating saw to cut through the cranium and ribs
15. Hammers, chisels and handsaws
16. Small hand meat hooks x 6
17. Hoist/crane/small tractor
18. Heavy straps, chains (and chain locks/anchors), ropes
19. Carts on rollers to move heavy parts
20. Coveralls, boots, gloves, masks, protective eye and head gear, face shields (waterproof disposable suits are ideal)
21. Accessible water supply with hose
22. Camera and size reference (ruler)
23. First aid kit
24. Surgical masks approved for TB exposure
  - OSHA/CDC guidelines require N,R, or P-type particulate filter respirators with at least 95% efficiency (ie. N95,N99,N100; R95,R99,R100; P95,P99,P100) (example: 3M model N95)
  - Positive air pressure respirators (PAPRs)
25. Biohazard bags (red bags)
26. Leak-proof boxes
27. Disinfectant solution (tuberculocidal)
  - Approved tuberculocidal disinfectants should list *Mycobacteria* sp. as susceptible on the label and are classified as “intermediate-level” disinfectants. Numerous products are commercially available.

## LOGISTICS AND NECROPSY TIPS

The necropsy of an elephant should proceed in the same manner as the necropsy of any smaller mammalian species. Although the size and scope of an elephant necropsy may seem intimidating, the procedure can be accomplished in 8-10 hours (or less) by a team of dedicated prosectors and assistants. The necropsy should be performed with the elephant in left lateral recumbency. An external examination is performed to evaluate body condition and lesions. The oral cavity should be closely examined for evidence of lesions consistent with **elephant endotheliotropic herpes virus (EEHV) infection**. The trunk should be examined according to above guidelines in the **tuberculosis** section.

Heavy equipment is necessary to move a dead elephant. For an on-site necropsy, chains and a tow truck may be sufficient to reposition the animal or to move it a short distance. If the animal must be transported to a remote site, a truck with a hoist will be needed. It may be easier to manipulate the animal onto a flatbed trailer. Vehicles must be able to handle these approximate weights: female Asian: 2,300 - 3,700 kg; male Asian: 3,700 - 4,500 kg; female African: 2,300 - 4,000 kg; male African: 4,100 - 5,000 kg. Trucks can generally be rented. If a flatbed carrier is used, the animal will need to be strapped to the bed and covered with a tarp. If transportation will be delayed, the carcass can be covered with ice (800-1000lbs of ice can be laid on top of and next to the carcass and will preserve the carcass quite well even in summer heat).

Assigning specific tasks to team members will help the necropsy proceed in an orderly manner. For example, a team may be assigned to each of these areas: head, fore limbs, hind limbs, abdominal region. One person should oversee the collection, labeling, and processing of research materials and any communication concerning research requests. It may be helpful to designate a media spokesperson. One of the most important tasks to be assigned is the task of knife sharpener. One person with knife sharpening experience should be assigned to be continually sharpening knives and cycling sharpened knives to prosectors. Removal of the legs, head, skin, and rib cage is made easier through the use of chain hoists or a small tractor or backhoe. This equipment should be used to lift the very heavy body parts in the interests of safety and efficiency and to preserve the strength of primary prosectors.

Dissection of the head is best completed after separating it from the body. A good portion of the cranium must be damaged to remove the brain intact; a chain saw, large axe, and chisels are needed to penetrate the thick cranium. A battery-operated reciprocating saw with a replaceable metal cutting blade may be safer and easier to handle. Alternatively, a posterior approach to brain removal can be made by 3 connecting deep cuts with a chain saw in the margins of the flattened triangle formed at the base of the elephant skull. The bony plate is then removed in chunks with a curved crowbar. The brain itself is removed in portions (i.e. the cerebellum and brainstem, then one cerebral hemisphere followed by the other). Use of a chain saw on bone can be hazardous and cause shrapnel-like fragments to be launched. Protective eye, head and face gear should be worn by the chain saw operator and personnel in the immediate area.

During examination of an elephant with unknown, suspicious, or positive **TB test history**, dissection of the thoracic cavity should always be performed last, and should be done by two people with proper (at least N-95) face masks and other protection against *Mycobacterium* sp.. All other personnel should be dismissed from the area before the thoracic cavity is entered. After the abdominal viscera have been removed, the diaphragm can be cut from its costosternal attachments. The lungs are then palpated from a caudal approach for tuberculous nodules as the lobes are being separated from the closely adhered visceral and parietal pleura. The heart, lungs, and associated structures may then be removed “en bloc”.

## CARCASS DISPOSAL AND DISINFECTION

Options for disposal include incineration, tissue digestion, rendering, and burial (the most common option). Few institutions possess an on-site incinerator, but a bio-hazardous waste company may be of assistance in locating incineration services. Incineration often requires that the carcass be broken down into manageable pieces (50-100lbs) for transportation and is a very difficult and time-consuming process. Tissue digesters, more and more popular for human biohazard waste disposal, are uncommon except in a few veterinary schools around the country. Some veterinary schools may be willing to dispose of carcasses for a fee (especially smaller carcasses). Rendering may be available in some states once it has been determined that no infectious disease agents are present. **Burial is the option most commonly used and is the easiest option logistically.** Ideally, the necropsy should be performed adjacent to a hole large enough to contain the carcass and deep enough to prevent odors and excavation by scavenging animals. In the event of a **TB suspect necropsy**, it is ideal for the hole to be large enough that the entire procedure can occur in the hole to eliminate the chances of contamination of the surrounding area.

Please be aware that special permissions or permits may be required from city, county, or state government for burial of a carcass and may be especially important in the event of burial of a TB suspect animal.

# ELEPHANT NECROPSY PROTOCOL GROSS EXAMINATION WORKSHEET

Institution/Owner \_\_\_\_\_

Address \_\_\_\_\_

Species \_\_\_\_\_ Accession/ISIS# \_\_\_\_\_ Studbook# \_\_\_\_\_

Name \_\_\_\_\_

Birth date/Age \_\_\_\_\_ Sex \_\_\_\_\_ Weight (Kg) \_\_\_\_\_

Actual || Estimate ||

Death date \_\_\_\_\_ Death location \_\_\_\_\_

Necropsy date \_\_\_\_\_ Necropsy location \_\_\_\_\_

Post mortem interval \_\_\_\_\_

Captive Born || Wild Caught ||

History (clinical signs, circumstances of death, clinical lab work, diet & housing)

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

## GROSS EXAMINATION

*(If no abnormalities are noted, mark as normal or not examined (NE); use additional sheets if needed)*

General Exam (physical and nutritional condition, skin, body orifices, superficial lymph nodes).

**Examine skin of head and trunk especially for nodular lesions; these have been associated with EEHV in African elephants (samples for fresh/frozen/formalin should be saved).**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Musculoskeletal System (bones, marrow, joints, muscles). **Chronic osteoarthritis/degenerative joint disease and a variety of foot lesions are common. Thorough examination of feet and all joints is required for documentation of disease.**

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Body Cavities (fat stores, pleura, thymus, lymph nodes). **Examine all lymph nodes for caseous lesions suggestive of TB.**

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Spleen. **Capsular fibrous tags and siderotic plaques are common, age-related incidental lesions.**

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Respiratory System (trunk passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes; submit lung lesions for TB culture; **bronchial lymph nodes should be cultured for TB even if normal in appearance**). **In addition to evaluation for pulmonary TB lesions, lungs are examined for EEHV-associated lymphoid nodules (samples for fresh/frozen/formalin should be saved).**

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Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers). **Epi-/endocardial hemorrhage is highly suggestive of EEHV-HD. Closely examine abdominal aorta for subtle or obvious aneurysms.**

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Digestive System (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes). **Glossal cyanosis is a common lesion of EEHV-HD. Gastric ulcers are seen commonly. Note GI contents and especially cecal contents; cecal impaction and sequela are common causes of colic.**

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Urinary System (kidneys, ureters, bladder, urethra)

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Reproductive System (testes/ovaries, uterus & cervix, penis/vagina, urogenital canal, prostate, seminal vesicles, bulbo-urethral gland, mammary gland, placenta). **Examine endometrium for changes consistent with polyploid and/or cystic endometrial hyperplasia. Uterine masses/tumors are extremely common in Asian cows and multiple tumor types may be present – please record number and size of masses as well as gross description. Ovarian and uterine serosal fibropapillomas also occur frequently in Asian and African cows. Mucosal nodular lesions in the distal urogenital tract have been associated with EEHV infections (samples for fresh/frozen/formalin should be saved).**

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Endocrine System (thyroids, parathyroids, adrenals, pituitary)

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Central Nervous System (brain, meninges, spinal cord)

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Sensory Organs (eyes, ears)

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Additional Comments or Observations:

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Prosector: \_\_\_\_\_ Date: \_\_\_\_\_

Summarize Preliminary Diagnoses:

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**Laboratory Studies: Please attach results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, x-ray, photographs, or other data collected.**

## TISSUE CHECK LIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (brain, heart, lung, liver, kidney, spleen) in small plastic bags. Freezing at -70 degrees Celsius in an ultra-low freezer is preferred. If this is unavailable, freezing at conventional temperatures is acceptable (use a freezer without an automatic defrost cycle if possible).

**Any lesions noted in the lungs should be submitted to NVSL or other qualified mycobacterial laboratory for mycobacterial culture. Bronchial lymph nodes should be cultured for TB even if normal in appearance.** Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. **NOTE: There is no need to fix and label each tissue separately with the exception of lymph nodes.** Ideally, tissues should be processed for histopathology as soon as possible following 24-48 hours of formalin fixation. Send tissues required for diagnosis to a primary pathologist. Diagnostic histopathology is available through the University of Illinois Zoological Pathology Program (ZPP) for a fee. (slide costs = approximately \$15/block); contact the SSP pathologist, Dr. Jaime Landolfi for submission instructions and information. Also, freeze postmortem serum (from heart), urine and any abnormal fluid accumulations. Consult the **Research Tissues/Samples Requests** (at the end of this document) for specific project sample requests.

- |   |   |  |   |
|---|---|--|---|
| <input type="checkbox"/> Adrenal              | <input type="checkbox"/> Kidney   | <input type="checkbox"/> Penis                     | <input type="checkbox"/> Thymus                   |
| <input type="checkbox"/> Blood *              | <input type="checkbox"/> Large intestine  | <input type="checkbox"/> Pituitary                 | <input type="checkbox"/> Tongue                   |
| <input type="checkbox"/> Bone with marrow     | <input type="checkbox"/> Liver (x2)   | <input type="checkbox"/> Prostate                  | <input type="checkbox"/> Trachea                  |
| <input type="checkbox"/> Bulbo-urethral gland | <input type="checkbox"/> Lung (x 4 at least)  | <input type="checkbox"/> Salivary gland            | <input type="checkbox"/> Trunk cross section      |
| <input type="checkbox"/> Brain (entire)       | <input type="checkbox"/> Parathyroid  | <input type="checkbox"/> Temporal gland            | <input type="checkbox"/> Seminal vesicles         |
| <input type="checkbox"/> Cecum                | <input type="checkbox"/> Mammary gland  | <input type="checkbox"/> Skin                      | <input type="checkbox"/> Ureter                   |
| <input type="checkbox"/> Diaphragm            | <input type="checkbox"/> Muscle   | <input type="checkbox"/> Small intestine           | <input type="checkbox"/> Urinary bladder          |
| <input type="checkbox"/> Esophagus            | <input type="checkbox"/> Nerve (sciatic)  | <input type="checkbox"/> Spinal cord               | <input type="checkbox"/> Vaginal/urogenital canal |
| <input type="checkbox"/> Eye                  | <input type="checkbox"/> Ovary/testis   | <input type="checkbox"/> Spleen                    | <input type="checkbox"/> Uterus/cervix            |
| <input type="checkbox"/> Hepatic bile duct    | <input type="checkbox"/> Epididymis   | <input type="checkbox"/> Tonsillar lymphoid tissue |   |
| <input type="checkbox"/> Heart/aorta          | <input type="checkbox"/> Pancreas   | <input type="checkbox"/> Stomach                   | <input type="checkbox"/> Thyroid gland            |
| <input type="checkbox"/> Hemal node           | <input type="checkbox"/> Lymph nodes (tracheobronchial, submandibular, tonsillar, mesenteric) |  |   |

\* Collect postmortem blood, separate serum and freeze for retrospective studies.

Primary Pathologist (Name): \_\_\_\_\_

Lab \_\_\_\_\_

Address \_\_\_\_\_

Phone \_\_\_\_\_

**A combined diagnostic and research sample collection checklist follows for use at time of necropsy. Consult the specific research requests listed later in this document for detailed collection instructions.**

**Please send a copy of the final report including significant gross as well as histopathologic findings and pertinent digital or color slides to:**

Jaime Landolfi, DVM, PhD, Diplomate ACVP; SSP Pathology Advisor, Elephants

Zoological Pathology Program, University of Illinois

c/o Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513

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Email: [jaimeland@gmail.com](mailto:jaimeland@gmail.com); [landolfi@illinois.edu](mailto:landolfi@illinois.edu)

## Elephant TAG-SSP diagnostic and research sample collection checklist

Tissue	Histopath (formalin)	SSP (frozen -80C)	Baylor (culture media)	Fox Chase (fresh or frozen)	Utah (formalin and frozen - 80C)	Utah (culture media)	Colossal (saline)	Harvard (frozen)	NVSL, TB diagnostics (frozen - 80C)	Indiana Univ (formalin and frozen - 80C)	Smithsonian (frozen - 80C)
Temporal gland	X										
Eye	X										
Trunk (skin and mucosa)	X										
Tongue	X				X						X
Salivary gland	X		X	X							
Submandibular lymph node	X										
Trachea	X										
Esophagus	X										
Tonsillar lymphoid tissue	X										
Thyroid	X										
Parathyroid	X										
Liver	X (2 pieces)	X			X						X
Hepatic bile duct	X										
Spleen	X	X		X	X						X
Stomach	X										
Duodenum	X										
Jejunum	X		X		X						X
Ileum	X										
Pancreas	X										
Mesenteric lymph node	X										
Colon	X		X		X						



Brain	X	X			X			X		X	
Trunk tip								X			
Fore foot subsolar tissue								X			
ANY TUMORS	X				X	X					
MATCHED NORMAL FOR ANY TUMORS					X	X					
WHOLE BLOOD				X		X				X	
SERUM											
PLASMA					X						

## INDIVIDUALS INTERESTED IN PARTICIPATING IN NECROPSY PROCEDURES

The following people may be available to participate in necropsies. If you are interested, please contact them as soon as possible after an animal dies or before euthanasia.

Name/Location	Phone Numbers	Email	Availability
Jaime Landolfi, DVM, PhD, DACVP Chicago, IL	W: 312-585-9050 Cell: 708-305-0611	jaimeland@gmail.com; landolfi@illinois.edu	On site participation and phone consultation
Rita McManamon, DVM Athens, Georgia	W: 678-429-3134	ritamcmdvm@gmail.com	Phone consultation
Susan Mikota DVM Hohenwald, TN	W: 931-628-5962 Cell: 931-628-5963	smikota@elephantcare.org	On site participation and phone consultation
Scott Terrell, DVM, DACVP Orlando, Florida	W: 407-938-2746 Cell: 321-229-9363	scott.p.terrell@disney.com	Phone consultation
D MacAloose, DVM, DACVP Bronx, NY	W: 718-220-7105 Cell: 646-852-4962	dmcaloose@wcs.org	Phone consultation and local/regional on-site participation
Brittany McHale, DVM, MPH, DACVP Athens, GA	W: 706-542-5812 Cell: 815-575-2954	brittany.mchale25@uga.edu	On-site participation and phone consultation

## RESEARCH REQUESTS

**Institutional reminder** - all requests made are conditional, not automatic, and may require the researcher's presence if they want detailed measurement info and/or complicated samples that are difficult to obtain and ship. Please contact the researchers in advance if you would like help in the collection of more complicated/labor intensive samples.

These requests are not a requirement for completion of a detailed diagnostic necropsy.

The following table summarizes all current research requests. Specific details regarding protocols/procedures, shipping information, and/or other special instructions are addressed in the following text section.

### Research tissues summary:

<b>Samples</b>	<b>Requesting facility</b>	<b>Purpose</b>	<b>Preservation</b>	<b>Transport</b>	<b>Special instructions</b>
- Serum - Brain - Heart - Lung - Liver - Spleen - Kidney	TAG-SSP	Frozen archive	Frozen (-80C)	NA – maintain at holding facility	
- Placenta - Swab of herpetic lesions - Heart - Liver - Tongue - Spleen - Intestine - Kidney - Tissues with hemorrhagic lesions	Smithsonian's National Zoo National Elephant Herpes Lab	EEHV screening	Frozen (-80C)	Priority overnight	
- Whole blood (EDTA) - Salivary gland - Spleen	Fox Chase Cancer Center	EEHV research	Fresh or frozen (tissues)	Priority overnight on ice packs; ship within 24 hrs of collection	
- Temporal bone - Trunk tip - Fore foot subsolar tissue - Brain	Harvard University School of Medicine	Hearing loss research	Frozen (-20C or lower)	Priority overnight	Researcher available to assist sample collection and/or offset costs
- Tumors	University of	Cancer	Fresh, unfrozen	Priority	Wash tissues

- Non-neoplastic tissue adjacent to tumors - Whole blood (EDTA)	Utah/Huntsman Cancer Institute	research		overnight on ice packs	with PBS, then transport media required (researcher can provide)
- Colon - Heart - Lung - Spleen - Tongue - Thymus - Uterus - Liver - Kidney - Brain - Any tumors - Small intestine - Skin - Muscle - Adrenal gland - Plasma	University of Utah/Huntsman Cancer Institute	EEHV research	Formalin (tissues)  Frozen (-80C; plasma and tissues)	Priority overnight (tissues)  Priority overnight on dry ice (plasma and frozen tissues)	Researcher can provide required supplies
- Small intestine - Large intestine - Cecum - Rectum - Salivary gland	Baylor College of Medicine	EEHV research	Fresh, unfrozen	Priority overnight on ice packs	Wash tissues with PBS, then transport media required (researcher can provide)
- Brain - Whole blood	Indiana University	Cognitive function	Frozen (-80C) and formalin-fixed	Priority overnight on dry ice (frozen) Ambient temp (formalin-fixed and blood)	Contact researcher to facilitate shipment
- Ovary - Testes - Skin - Blood - Adipose	Colossal Biosciences	Immortalized and primary cell lines and stem cells	Fresh at 4C (on ice packs)	Priority overnight	Contact researcher for supplies

## CURRENT TISSUE/SAMPLE RESEARCH REQUESTS

### 1. **Jaime Landolfi, DVM, PhD, Diplomate ACVP**

SSP Pathology Advisor, Elephants

Zoological Pathology Program, University of Illinois

c/o Chicago Zoological Society, 3300 Golf Road, Brookfield, IL 60513

W (312) 585-9043; Cell (708) 305-0611

Email: [jaimeland@gmail.com](mailto:jaimeland@gmail.com); [landolfi@illinois.edu](mailto:landolfi@illinois.edu)

Dr. Landolfi requests an electronic copy of the **final necropsy and histopathology report** including all pertinent culture results for inclusion in the TAG/SSP Pathology Archive.

Tissues for histopathology may be sent to the institution's primary pathology service. Diagnostic histopathology is also available through the University of Illinois Zoological Pathology Program (ZPP) for a fee ("at cost"; approximately \$15/block); contact Dr. Landolfi for submission instructions and information. Please check with your primary pathology service to determine their policy for retention and disposal of paraffin blocks. If your primary pathology service retains paraffin blocks in perpetuity, then no further action is needed. **If your primary pathology service disposes of paraffin blocks after a defined time period, please request that those blocks be sent the zoo or to the SSP pathologist once the case is finalized.** Paraffin blocks retain valuable diagnostic, molecular and retrospective information and are easier to ship and store than formalin fixed tissues. Formalin fixed tissues present significant long term shipping, storage and archival challenges and have little or no ancillary diagnostic/research value. **After finalization of the case, formalin-fixed tissues may be disposed of at the discretion of the holding facility.**

## 2. Elephant TAG/SSP

The elephant TAG/SSP recommends that serum be collected and stored from all elephants on a regular basis for the benefit of retrospective analyses by the holding institution or requests for future research projects. A **minimum of 4 mls serum/elephant** should be collected on an annual basis, frozen and stored by the current facility, ideally at -80°C.

In addition to tissues collected in formalin for histopathology, it is recommended that at least 50 g of the following tissues be **collected and stored frozen at -80°C by the current facility** from any elephant that dies or is euthanized: brain, heart, lung, liver, spleen, kidney (50 g each)

## 3. National Elephant Herpes Lab

Erin Latimer

Smithsonian's National Zoo and Conservation Biology Institute

Department of Pathology

3001 Connecticut Ave. NW

Washington, D.C. 20008

Work: 202-633-4252

Cell: 703-855-9611

Email: [latimere@si.edu](mailto:latimere@si.edu)

The following samples are requested for EEHV screening: **1. Placenta** – freeze 1 inch<sup>3</sup> piece in liquid nitrogen or dry ice, then store at -80C freezer until shipped. Also, serum and whole blood from dam and baby. **2. Suspected herpetic lesions** – wet a cotton swab with small amount of sterile saline, swab lesion and place in sterile 15 ml plastic test tube; store at -80C until shipped. **3. Necropsy tissues (heart, liver, tongue, spleen, intestine, kidney, any tissue with hemorrhages, any kind of nodules – skin, lung, vestibular)** – aseptically place 3x3 cm piece of tissue in small Ziploc or WhirlPak bag. Label with type of tissue, elephant ID, date. Use separate bag for each tissue; store at -80C until shipped. **Shipping** – FedEx overnight; email tracking number to [latimere@si.edu](mailto:latimere@si.edu). EEHV Lab will pay for shipping of samples; contact lab for shipping labels.

## 4. Fox Chase Cancer Center

Virginia Riddle Pearson, Visiting Scientist  
Campbell Laboratory  
Fox Chase Cancer Center  
333 Cottman Ave,  
Philadelphia, PA 19111  
mobile 215-816-5734  
[virginiarpearson@gmail.com](mailto:virginiarpearson@gmail.com)

Dr. Pearson is working on EEHV pathogenesis. For this work, the following are requested: .05 ml whole blood in EDTA and one 0.5 cm each of spleen and salivary gland from African and Asian elephant necropsies, including EEHV suspect cases. Blood and tissues may be fresh or frozen. Please advise in advance of any planned euthanasia.

Please ship on wet ice for next day delivery to:

Virginia Riddle Pearson  
701 West Gravers Lane,  
Philadelphia, PA 19118

**6. Harvard University School of Medicine, Caitlin O’Connell-Rodwell, PhD**

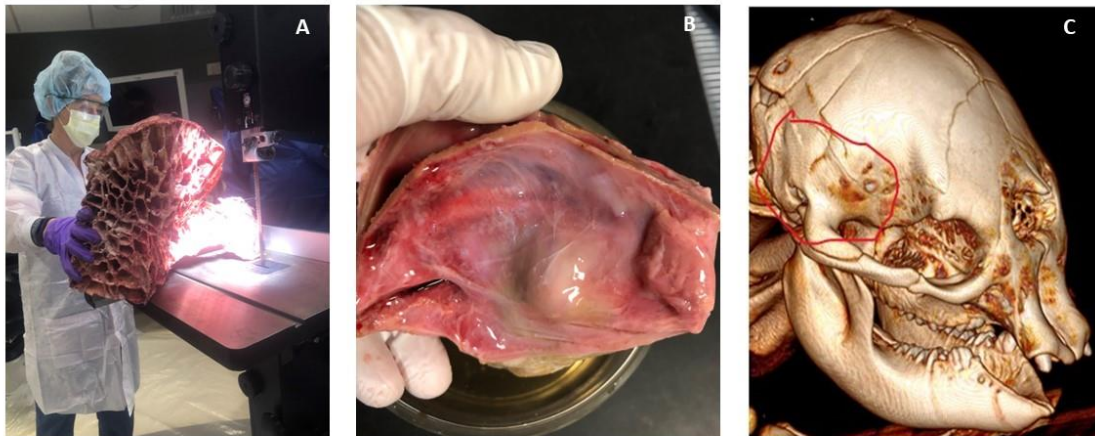
Eaton Peabody Lab  
Massachusetts Eye & Ear Infirmary  
243 Charles Street  
Boston, MA 02114  
[ceoconnell@stanford.edu](mailto:ceoconnell@stanford.edu)  
(650) 868-2251

**Samples requested: 1) temporal bone (no fixative); 2) trunk tip (Zamboni fixative) 3) fore foot subsolar connective tissue (Zamboni fixative); 4) whole brain (10% neutral buffered formalin). See below for more details**

Dr. O’Connell-Rodwell is requesting samples as part of a study to understand the mechanics of low frequency hearing and sound processing in elephants.

1) Temporal bone: It is requested that a whole block of tissue that would include the external auditory meatus through to the dura side of the brain case to ensure that the temporal bone is included in the sample (Figure 1.). Collection of this tissue block can be done with either a reciprocating or ban saw for smaller specimens, or a chain saw for larger specimens. The lower jaw is not needed. Depending on what the necropsy facility has on hand, the larger the block will ensure that the entire temporal bone specimen is included in the sample. Sample should be stored frozen and shipped priority overnight. It is very important that **NO FIXATIVE** be used in the sample preparation as this will alter the behavior of the middle ear.

Figure 1: (A) One quarter of an adult Asian elephant skull (half of the upper jaw portion of the skull), including opening to the ear canal through to the dura side of the brain case (B) In calf specimens, the dura side, (opposite to the ear canal opening) has a prominent protrusion containing the middle ear and cochlea. This protrusion is less pronounced in adult specimens. (C) CT of elephant calf with the temporal bone circled in red. To reiterate, in order to harvest the temporal bone, the specimen needs to include the ear canal opening, through to the brain case. Please call if you have questions! 650-868-2251



2) Trunk tip: A 3-4 cm section from the dorsal aspect of the trunk tip is requested. Do not freeze. Collect into Zamboni's fixative (2% paraformaldehyde, <1% picric acid, <1% sodium hydroxide).. Please contact Dr. O'Connell-Rodwell if these reagents are not available.

3) Fore foot subsolar tissue: Please collect a an approximately 5 cm section of tissue from the subsolar region behind the middle nail, towards the center of the foot and just deep to the solar fat pad. Do not freeze. Collect into Zamboni's fixative (2% paraformaldehyde, <1% picric acid, <1% sodium hydroxide). Please contact Dr. O'Connell-Rodwell if these reagents are not available.

4) Brain\*: Brain needs to be extracted intact without any damage to parenchyma. Remove dura to facilitate fixation. Immerse in cold 10% neutral buffered formalin immediately after extraction and refrigerate during fixation. Fixation occurs on site prior to shipment to the lab. Formalin needs to be changed during the fixation process: daily for the first week, every other day for the next week, and every 3 days for the following 2 weeks.

**\* NOTE – Fulfillment of this research request precludes evaluation of the brain for diagnostic purposes.**

Please contact Dr. O'Connell-Rodwell for further collection details and arrangement of shipping.

**NOTE – Dr. O'Connell-Rodwell may be available to come and perform/assist with on-site sample collection and/or support the cost of challenging sample preparation:**

#### **7. University of Utah/Huntsman Cancer Institute, Lisa Abegglen and Joshua Shiffman**

Lisa Abegglen/Schiffman Lab  
University of Utah/Huntsman Cancer Institute  
2000 Circle of Hope  
Salt Lake City, UT 84112  
[Lisa.Abegglen@hci.utah.edu](mailto:Lisa.Abegglen@hci.utah.edu); 801-718-8629

Drs. Abegglen and Shiffman are requesting samples of elephant tumors and matched non-neoplastic tissue from the affected organ as well as samples of whole blood from affected animals to aid in on-going investigations into carcinogenesis, specifically the role of tumor suppressor gene expression, in elephants.

For this research, a 2 x 2 x 2 cm sample of tumor (including tumor only and no adjacent normal tissue) along with an additional 2 x 2 x 2 cm sample of normal tissue, collected separately, from the same organ are requested. Also requested is whole blood in a single EDTA blood tube.

1. If a skin sample is being obtained, then clean area with ethanol prior to harvest. If the sample is coming from the inside of the animal and the area has become dirty, then clean with ethanol or rinse with PBS prior to harvest.
2. Take the tissue sample (try to avoid including normal tissue with tumor)
3. Drop the sample or samples into a labeled conical with cell growth media (which we will ship overnight to harvest location prior to procedure). Please label the tube with the type of tissue added to the tube and whether the sample contains tumor or normal tissue.
4. Ship the samples to our lab to arrive within a day or two of retrieval, with an ice pack (not dry ice) overnight. Please fill the conical with media prior to shipment (also will be provided). Be careful when opening the media, because it will support the growth of any microorganisms that are accidentally introduced into the tubes or bottle. Store the media at 4C.
5. EDTA blood tube can be shipped on ice packs along with tissue samples

Please contact Dr. Abegglen with any questions and/or to arrange acquisition of tissue media and sample shipping. Dr. Abegglen can cover shipping costs.

**8. University of Utah/Huntsman Cancer Institute, Lisa Abegglen and Gareth Mitchell**

Gareth Mitchell/Schiffman Lab  
University of Utah/Huntsman Cancer Institute  
2000 Circle of Hope  
Salt Lake City, UT 84112  
801-587-4774  
[Lisa.abegglen@hci.utah.edu](mailto:Lisa.abegglen@hci.utah.edu); 801-718-8629

FedEx Account # is 111960402 (no spaces) Add this Internal Billing number: 01322-6000-22276-64300

In the middle of the FedEx shipping label/form, there is a space for "Your Internal Billing Reference."

Drs. Abegglen is requesting samples from elephants to facilitate their research into the immunopathogenesis of EEHV infections. For this work, a variety of normal tissue samples (see below) collected from non-EEHV-HD affected animals as well as plasma samples from both EEHV-HD and non-EEHV-HD affected animals are needed.

**Tissues requested (non-EEHV-HD affected animals):** in order of priority.

1. Colon
2. Heart
3. Lung
4. Spleen
5. Tongue
6. Thymus
7. Uterus
8. Liver
9. Kidney
10. Brain
11. Any tumors, including benign like uterine leiomyomas
12. Matched normal for any tumors

13. Intestine (small and large)
14. Skin
15. Muscle
16. adrenal gland

Two small sections of each tissue should be collected, allocated into tissue cassettes/cryosettes (labeled with tissue type and animal ID); the cassette is placed in 10 percent neutral buffered formalin, and the cryosette is frozen (-80C). Investigators will provide cassettes/cryosettes upon request. Formalin samples can be stored at room temperature.

**Plasma samples (both EEHV-HD and EEHV-HD affected animals)**

Collect a whole blood sample in an EDTA purple top tube (investigators can provide). Fill the tube to the top. If the tube is partially full, please indicate approximately how full it is (1/4, 1/2, 3/4).

- **The blood must be processed within 2 hours of the draw.** Please indicate the amount of time between draw and centrifugation.
- Spin the tube at 423 g for 5 minutes
- Transfer supernatant to a new tube
- Spin the supernatant at 3800 g for 5 minutes
- Collect plasma (Supernatant) and make aliquots of 500 µL/each
- Store the aliquots in labeled cryovials at -80 °C

**Please ship frozen plasma samples on dry ice to keep it frozen in transit.**

For all samples, the following information is requested: Sample collection date, Species common name, animal house name, Institution # for animal if available, AZA # if has one, sex, date of birth, age at sample collection (or estimated age), location of birth (zoo vs wild, place if possible), sample site (location on body), diagnosis, date and time of sample collection, EEHV strain (info can be written in a sample report letter and emailed to investigators).

Ship all samples **FedEx priority overnight on Mon, Tue, or Wed to the Schiffman lab address above.** If via **shipping on a Thurs or Fri via FedEx overnight**, then please contact the lab to get a home address. If necropsy occurs on a Sun, then please ship on Monday. **Frozen tissue samples and plasma should be packaged on dry ice.** Formalin-fixed samples can be shipped at room temperature. FedEx account information for shipping samples is provided above under shipping address.

**9. Baylor College of Medicine**

Dr. Paul D. Ling  
 Baylor College of Medicine  
 One Baylor Plaza  
 Department of Molecular Virology and Microbiology  
 Houston, Texas 77030  
 Ph: 713-798-8474  
 Email: [pling@bcm.edu](mailto:pling@bcm.edu)

Dr. Ling is requesting tissue samples from the gastrointestinal (GI) tract to grow what is commonly referred to as organoid cultures. These cultures capture some of the key attributes of the tissues they were derived from and have been instrumental in allowing the culture of some viruses for the very first time. Once established, the organoid cultures will be extremely useful for two purposes: 1) they can be developed into a model system for investigating cancer in elephants, and most importantly, 2) serve as a useful system for growing EEHV. Dr. Ling's lab is working towards development of an effective vaccine against elephant endotheliotropic herpesvirus (EEHV). Successful propagation of the virus in the laboratory will greatly accelerate efforts to understand EEHV and develop an effective vaccine against it.

**To comply with this research request, viable, living tissue is required. Thus, collection from humane euthanasia cases will be most feasible. It is optimal to contact Dr. Ling in advance to allow for shipment of necessary supplies.**

Protocol for sample collection:

1. Collect 3 independent 30mm<sup>2</sup> tissue specimens each from the: a) small intestine; b) large intestine; c) rectum; d) cecum; e) salivary glands
2. Rinse/wash sample by pouring sterile saline or PBS (researcher will provide) to clean off debris and/or feces material. If available, sterile saline or PBS can also be poured into a disposable cup or glass and the tissue “dipped” and agitated in the solution to clean it off. Place each specimen in a 50ml tube prefilled with 35ml media (Advanced DMEM/F12, Glutamine, Hepes, Pen/Strep/Fungizone) provided by the researcher; contact researcher ahead of time to obtain (store at 4<sup>0</sup>C). Seal outside of screw cap with a strip of parafilm.
3. Fill shipping container with ice packs to maintain 4<sup>0</sup>C and pad with paper or other packing material to prevent tubes from moving around.

In the absence of media tubes provided ahead of time by the researcher, facilities may employ the following alternate protocol:

1. Collect 8mm<sup>2</sup> specimens as described for the standard protocol and then cut them into 2- to 3-mm<sup>2</sup> fragments and transfer into cryopreservation vials containing 1ml freezing medium (Dulbecco’s modified Eagle medium/F12; 10% fetal bovine serum; 10% dimethyl sulfoxide)
2. Place tubes into a Mr. Frosty cell-freezing container and freeze overnight at -80C.
3. Place vials into a shipping container with plenty of **dry ice** to keep the samples frozen during shipping and ship as described above.

Ship samples overnight to the following address using the Fed Ex reference number: 342808905

#### 10. **Indiana University**

Daniella Chusyd, PhD  
 1025 E 7<sup>th</sup> St. SPH 394  
 Bloomington IN 47405  
 W: (812) 855-0240  
 C: (954) 552-8994  
[dchusyd@iu.edu](mailto:dchusyd@iu.edu)

Dr. Chusyd is evaluating how cognitive function changes with age in African and Asian elephants. Investigation aims to determine and characterize cognitive pathologies that may develop associated with aging. Dr. Chusyd’s lab is also establishing a national elephant brain bank to facilitate research and enhance the use of elephants as a reference species for comparative aging studies (“National Elephant Brain Resource”). Dr. Chusyd is requesting **both fresh and formalin-fixed samples of brain and an associated whole blood sample (1 acid citrate dextrose (ACD) tube (yellow top), 6-8ml)**. Whole blood will ultimately be used for epigenetic analyses to help identify blood biomarkers for brain health. Please see below for brain sampling procedures.

#### **Blood sampling procedure:**

Collect 1 acid citrate dextrose (ACD) tube of whole blood (6-8mL). Tube should be labeled with elephant name, date and time of collection, and stored at 4C until shipment at ambient temperature overnight with the formalin-fixed brain tissue. If an ACD tube is not available, please use an EDTA tube.

**Brain sampling procedure for specimens from animals deceased for less than 12 hours:**

- This procedure was developed to prioritize brain tissue for histopathology, MRI, and frozen archive collection.
- This brain sampling procedure is intended as a reference. If it is not possible to collect as outlined, please do what you can.
- Diagnostic sampling is prioritized over research requests. Samples of brain for diagnostic histopathology should be collected as necessary prior to satisfying requests below, especially in cases where clinical neurologic disease is suspected.

**Step 1: Weigh and photograph** brain sections (cerebellum and brainstem, right cerebral hemisphere, left cerebral hemisphere) from dorsal view, ventral view, and lateral views.

**Step 2: Select one intact hemisphere and fix in formalin.** Place the hemisphere in a bucket large enough to accommodate it without touching the sides. This should ensure preservation of the anatomical structures with little distortion. For optimal fixation, add enough 10% buffered formalin to cover the entirety of the hemisphere (minimum 1 gallon/brain hemisphere) and let stand for 14 days, stirring the bucket gently every few days. Once the brain is fully fixed it can be removed from the formalin, gently bagged, boxed or bottled, and shipped with a few teaspoons of formalin to keep it moist in transit.

**Step 3:** In conjunction with standard TAG/SSP diagnostic procedures, after one hemisphere and cerebellum and brainstem are fixed in formalin, **collect approximately 2 cm thick slabs for snap freezing from the other fresh cerebral hemisphere and brainstem/cerebellum, with particular attention to the frontal and temporal lobes.** If there was any clinical indication of CNS disease, and the clinical history indicates a one-sided lesion of the brain, select this area for sample collection. Photograph the slabs with anterior face upright.

**Step 4: Place every fresh section in a plastic (Ziplock™) or plastic tissue bags, and label them to indicate the anatomical region. These bagged sections should be frozen in isopentane/dry ice, liquid nitrogen, or in -80 freezer, maintaining the tissue flat to preserve architecture. The frozen sections should be stored in -80 degree freezer until shipped (see shipping directions, Step 5).**

**Step 5: Frozen tissue packed on dry ice in styrofoam containers and/or formalin fixed sections/sectioned in sealed plastic bags -please ship to address above. Dr. Chusyd will cover shipping costs. Please contact prior to shipment to coordinate.**

**Brain sampling procedure for specimens from animals deceased for more than 12 hours:**

If the animal died 12 hours or more prior to tissue harvesting, brain imaging and histopathology are prioritized. ***Submitting institutions are encouraged to aid the quality of the MRI by fixing one intact hemisphere, and then shipping.*** Place the hemisphere in a bucket large enough to accommodate it without touching the sides. This should ensure preservation of the anatomical structures with little distortion. Add enough 10% buffered formalin to cover the entirety of the hemisphere (minimum 1 gallon/brain hemisphere) and fix for 14 days, stirring the bucket gently every few days. After MRI and on request, NEBR can dissect regions requested by the donating institution and return them for their own histopathology evaluation. Alternatively on request, NEBR will section the brain and slides will be examined by the NEBR consulting pathologist, and report issued to the submitting institution.

**11. Colossal Biosciences**

Steve Metzler: [steve@colossal.com](mailto:steve@colossal.com); 863-206-1605

Joshua Heinrich: [jheinrich@colossal.com](mailto:jheinrich@colossal.com); 214-293-3722

Darya Tourzani: [darya@colossal.com](mailto:darya@colossal.com); 201-316-4823

SHIPPING INFORMATION:  
 COLOSSAL BIOSCIENCES  
 ATTN: Darya Tourzani/Josh Heinrich  
 3033 Irving Blvd  
 Dallas, TX 75247

The goal of this research is to develop and optimize assisted reproductive technologies (ART) in elephants, with the specific aim of establishing a tissue repository, immortalized and primary cell lines, stem cells, and developing IVF techniques in both Asian and African elephants.

**TISSUE REQUESTS: (in order of priority)**

<b>TISSUE</b>
Ovaries (whole; both pairs highly preferred)
Testes
Skin – skin patch with dense hair
Blood
Adipose tissue

**OVARIES (whole, both pairs highly preferred)**

1. Materials required (if planned necropsy, a collection kit can be provided by Colossal Biosciences)
  - a. WASH MEDIA: BoviPlus – OPU Recovery Media containing 2% antibiotic/antimycotic  
OR Saline with containing 2% antibiotic/antimycotic
  - b. SAMPLE CONTAINER: BoviPlus – OPU Recovery Media containing 2% antibiotic/antimycotic OR Saline with containing 2% antibiotic/antimycotic
  - c. Biohazard Specimen container and transport bag
  - d. Styrofoam shipping box
  - e. Strips of Parafilm to seal the container
2. Prepare the sample container:
  - a. Name/Local ID
  - b. EN/microchip number (if applicable)
  - c. Age (if known, approximate)
  - d. Gender
  - e. Date of collection
3. Isolate the ovaries and wash with provided media or saline equivalent prior to placing into the sample container containing fresh media
4. Seal the container with the provided paraffin strips to prevent leaking and place securely into the shipping container. Add in ice packs to maintain 4C or cool temperature during shipment.
5. In order to achieve the best results, please immediately ship the tissue sample to Colossal Biosciences, overnight priority.
  - a. If advance notice is given, we can provide a shipping label (within 24 hours)

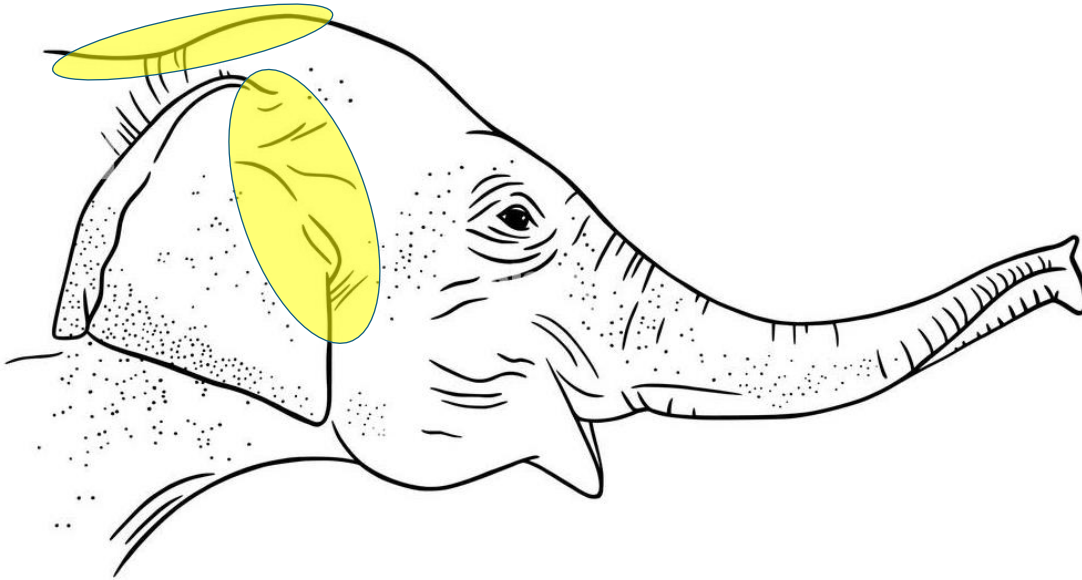
**TESTES with epididymis**

1. Materials required (if planned necropsy, a collection kit can be provided by Colossal Biosciences)
  - a. WASH MEDIA: BoviPlus – OPU Recovery Media containing 2% antibiotic/antimycotic  
OR Saline with containing 2% antibiotic/antimycotic
  - b. SAMPLE CONTAINER: BoviPlus – OPU Recovery Media containing 2% antibiotic/antimycotic OR Saline with containing 2% antibiotic/antimycotic
  - c. Biohazard Specimen container and transport bag
  - d. Styrofoam shipping box
  - e. Strips of Parafilm to seal the container
2. Prepare the sample container:
  - a. Name/Local ID

- b. EN/microchip number (if applicable)
  - c. Age (if known, approximate)
  - d. Gender
  - e. Date of collection
3. Isolate the testes and associated epididymis (if possible) and wash with provided media or saline equivalent prior to placing into the sample container containing fresh media
  4. Seal the container with the provided paraffin strips to prevent leaking and place securely into the shipping container. Add in ice packs to maintain 4C or cool temperature during shipment.
  5. In order to achieve the best results, please immediately ship the tissue sample to Colossal Biosciences, overnight priority.
    - a. If advance notice is given, we can provide a shipping label (within 24 hours)

### **SKIN PATCH with dense hair follicles**

1. Materials required (if planned necropsy, a collection kit can be provided by Colossal Biosciences)
  - a. WASH MEDIA: Saline with containing 2% antibiotic/antimycotic
  - b. SAMPLE CONTAINER: Saline with containing 2% antibiotic/antimycotic
  - c. Biohazard Specimen container and transport bag
  - d. Styrofoam shipping box
  - e. Strips of Parafilm to seal the container
  - f. Chlorohexidine scrub
2. Prepare the sample container:
  - a. Name/Local ID
  - b. EN/microchip number (if applicable)
  - c. Age (if known, approximate)
  - d. Gender
  - e. Date of collection
3. Identify a region of the skin with dense hair follicles (please see diagram below with yellow areas indicating preferred areas) and use the chlorohexidine scrub brush to clean off the outer skin layer, and remove any large pieces of debris. Use the provided media or saline equivalent to wash away the scrub
4. Isolate skin patches with hair and wash with provided media or saline equivalent prior to placing into the sample container containing fresh media
  - a. Minimum size per patch: 3 inches x 4 inches
5. Seal the container with the provided paraffin strips to prevent leaking and place securely into the shipping container. Add in ice packs to maintain 4C or cool temperature during shipment.
6. In order to achieve the best results, please immediately ship the tissue sample to Colossal Biosciences, overnight priority.
  - a. If advance notice is given, we can provide a shipping label (within 24 hours)



### **Blood**

1. Materials required (if planned necropsy, a collection kit can be provided by Colossal Biosciences)
  - a. Blood Collection Vials: Yellow-top ACD tubes
  - b. Sample Bag (bubble wrap for extra protection)
  - c. Styrofoam shipping box
2. Prepare the sample bag:
  - a. Name/Local ID – add to blood tubes if possible
  - b. EN/microchip number (if applicable)
  - c. Age (if known, approximate)
  - d. Gender
  - e. Date of collection
3. Draw blood from animal prior to euthanasia if possible from whatever source is easiest, otherwise after is also ok
4. Fill the blood tubes with a minimum of 4 mL of blood.
  - a. Minimum amount needed: 28 mL (4 yellow top tubes)
5. Invert the tubes at least 10X and place into sample bag
  - a. Avoid extreme conditions if possible
    - i. Avoid direct sunlight if possible to reduce over-heating the samples
    - ii. Avoid cool temperatures to prevent freezing/rapid cooling
6. In order to achieve the best results, please immediately ship the blood sample to Colossal Biosciences, overnight priority.
  - a. If advance notice is given, we can provide a shipping label (within 24 hours)

### **Adipose tissue**

1. Materials required (if planned necropsy, a collection kit can be provided by Colossal Biosciences)
  - a. WASH MEDIA: Saline with containing 2% antibiotic/antimycotic
  - b. SAMPLE CONTAINER: Saline with containing 2% antibiotic/antimycotic
  - c. Biohazard Specimen container and transport bag
  - d. Sterile, disposable scalpel
  - e. Sterile, disposable tweezer
  - f. Styrofoam shipping box
  - g. Strips of Parafilm to seal the container
  - h. Chlorohexidine scrub
2. Prepare the sample container:
  - a. Name/Local ID

- b. EN/microchip number (if applicable)
  - c. Age (if known, approximate)
  - d. Gender
  - e. Date of collection
3. Identify the location to which you plan on isolate adipose tissue – there is no preference for the particular area where you extract the tissue from
  4. Using the chlorohexidine scrub, clean off the skin and rinse off with wash media.
  5. Remove the skin layer, and rinse off with wash media
  6. Using a sterile scalpel and sterile tweezer/forceps, isolate a piece of adipose tissue and rinse with wash media
    - a. Minimum size: 1 x 1 x 1 inch
  7. Place into the sample container
  8. Seal the container with the provided paraffin strips to prevent leaking and place securely into the shipping container. Add in ice packs to maintain 4C or cool temperature during shipment.
  9. In order to achieve the best results, please immediately ship the tissue sample to Colossal Biosciences, overnight priority.
    - a. If advance notice is given, we can provide a shipping label (within 24 hours)

# FORM FOR REQUESTING ELEPHANT TISSUE/BLOOD SAMPLES

Name \_\_\_\_\_ Date of request \_\_\_\_\_

Affiliation \_\_\_\_\_

Address \_\_\_\_\_

Work phone (\_\_\_\_) \_\_\_\_\_ Home phone (\_\_\_\_) \_\_\_\_\_

Fax (\_\_\_\_) \_\_\_\_\_ Email \_\_\_\_\_

Sample(s) requested \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Purpose of study**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Duration of study** \_\_\_\_\_

**Instructions for sample preparation** – attach protocol

**Shipping instructions (dry ice? Overnight? Will you pay for shipping?)**

\_\_\_\_\_

**Special instructions**

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Attach any additional information. Send to by email to Dr. Jaime Landolfi ([jaimeland@gmail.com](mailto:jaimeland@gmail.com); [landolfi@illinois.edu](mailto:landolfi@illinois.edu)).**

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