

ELEPHANT TAG/SSP RESEARCH AND NECROPSY PROTOCOL

(Elephas maximus and Loxodonta africana)



*The American Zoo and Aquarium Association
Elephant Species Survival Plan*

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TABLE OF CONTENTS

| | |
|--|-------|
| Abstract/summary | 3 |
| Introduction | 4 |
| Elephant Herpesvirus Alert | 5 |
| Elephant Tuberculosis Alert..... | 5-6 |
| Internet Sites..... | 6 |
| Equipment Checklist | 7 |
| Logistics / Necropsy Tips | 8 |
| Carcass disposal | 9 |
| Gross Examination Worksheet..... | 10-12 |
| Tissue Check List..... | 13 |
| Research requests..... | 14 |
| Form for requesting elephant tissue/blood samples for research projects..... | 15 |
| Literature cited..... | 16 |

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 TAG/SSP research and tissue request protocol to facilitate collection of a complete tissue, sample, and data set. Two of the more important disease processes in elephants include elephant endotheliotropic herpes virus infection and tuberculosis (caused by the human pathogen, *Mycobacterium tuberculosis*). Specific sample collection protocols are available for these diseases (a separate Elephant Endotheliotropic Herpesvirus Research and Tissue Protocol is available at www.aazv.org under the SSP/TAG protocols; and Guidelines for the Control of Tuberculosis in Elephants protocol is available through the USDA website – http://www.aphis.usda.gov/animal_welfare/index.shtml) and Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care through the IEF website - <https://elephantconservation.org/stay-informed/recommendations-for-tb-in-elephants-in-human-care/>. These protocols should be reviewed in detail if either disease is suspected. 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. This is especially important to ensure the safety of staff participating in the necropsy and to prevent contamination of the surrounding areas or animals. A variety of types of equipment are listed in the protocol and most are similar to what would be used in smaller animal necropsies with the exception of the need for heavy equipment (tractor), an axe, numerous large knives, chains, straps, and TB personal protective equipment. A team of at least 6-8 people should be assembled for 8-10 hours of work to complete a detailed necropsy. Various roles should be assigned to team members including a supervising pathologist or clinician, prosectors to do the actual cutting, a specific 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 chest 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. Disposal of an elephant carcass is a job in and of itself. Ideally, the necropsy should be performed within or adjacent to hole large enough to bury 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, it 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 following the death of the animal. The information gained from an elephant necropsy is potentially hugely valuable to institutions, the AZA, and to elephants in both captivity and in the wild.

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INTRODUCTION

This protocol is an effort of the Elephant Species Survival Plan (SSP) Propagation Group of the American Zoo and Aquarium Association (AZA). Its purpose is to provide a format for the systematic collection of information and samples that will add to our knowledge of elephants. All North American institutions holding elephants will have access to this document.

We hope that most institutions will not have to face the immense task of performing an elephant necropsy, but should a death occur, it should be viewed as an important learning opportunity. Although it may not be feasible to collect all the information and samples requested, we encourage the collection of as much as possible. With the increased availability of digital cameras, it is strongly recommended that photographs of both normal and pathologic structures be recorded for future reference.

Sample and data collection information for research is contained at the end of this document. The requested data sets are optional. Some of these requests may apply to living animals. Therefore, this protocol, the **Elephant Endotheliotropic Herpesvirus Research and Tissue Request Protocol, Guidelines for the Control of Tuberculosis in Elephants**, and **Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care** should be referred to when planning a procedure that might facilitate data collection.

Acquainting one's self with the protocols in these documents and having the necessary equipment ready will facilitate sample collection. It is suggested that a necropsy team be designated in advance; the ability to mobilize skilled individuals quickly will save valuable time particularly in the event of a sudden death. 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 Research and Tissue Request Protocol will be revised as required. Contact Michele Miller or Scott Terrell, with any changes. A copy of the completed gross pathology protocol with preliminary findings should be sent right after the necropsy and followed by the histopathology and any other lab reports when completed to the veterinary advisors.

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ELEPHANT HERPESVIRUS DISEASE 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). Please refer to the Elephant Endotheliotropic Herpesvirus Research and Tissue Request Protocol (available at www.aazv.org). Contact the Elephant SSP Veterinary Advisors or listed researchers for specific questions.

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 though TB is not suspected.** Be advised that elephant TB is likely to be caused by *Mycobacterium tuberculosis* which is a zoonotic disease. Therefore be prepared with proper protective apparel, and contain any suspicious organs or lesions 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 post mortem trunk wash performed and serum should be harvested from post mortem blood for serological assays. Consult the current version of the **Guidelines for the Control of Tuberculosis in Elephants** (http://www.aphis.usda.gov/animal_welfare/index.shtml) and **Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care** (<https://elephantconservation.org/stay-informed/recommendations-for-tb-in-elephants-in-human-care/>) for further information.

Protective equipment for tuberculosis cases

Respiratory protective equipment should be available during any elephant necropsy procedure 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 undergoing necropsies should have a careful examination of the head lymph nodes for tuberculous appearing lesions. These lymph nodes may be more easily visualized following removal of the tongue and laryngeal structures during the dissection. All lymph nodes should be carefully evaluated for lesions since other sites may also be infected (ex. reproductive or gastrointestinal tract). Take any nodes that appear caseous or granulomatous for culture (freeze or ultrafreeze), and fixation (in buffered 10% formalin). In addition, search thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node and submit for TB culture to NVSL or a laboratory experienced in mycobacterial culture and identification (**even if no 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 nodular size lesions. Take **NUMEROUS (5 or more)** sections of any suspicious lesions. 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 for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.

For further information on laboratories performing diagnostic tests for TB, consult the current version of the **Guidelines for the Control of Tuberculosis in Elephants and Recommendations for the Diagnosis, Treatment and Management of Tuberculosis (*Mycobacterium tuberculosis*) in Elephants in Human Care**.

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
2. www.aazv.org
3. www.elephantcare.org
4. 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. <https://elephantconservation.org/stay-informed/recommendations-for-tb-in-elephants-in-human-care/> - Information on Elephant TB

EQUIPMENT CHECKLIST

1. At least 6 quality large necropsy knives, knife sharpener, steel, and/or stone
2. Standard large animal necropsy instruments. Multiple scalpel handles, duplicates or triplicates of other instruments. Extra box of scalpel blades, knife sharpener, and a continual supply of sharp knives.
3. Sterile instruments for culture collection.
4. 10% neutral buffered formalin (at least 2 gallons).
5. Field acid-fast staining kit (to determine the presence or absence of Mycobacteria sp.)
6. Gluteraldehyde, 2.5-4% (at least 100mls)
7. Containers for sample collection. Cylindrical plastic tubes.
8. Culture swabs, sterile urine cups, glass slides.
9. Serum tubes for blood and urine collection.
10. Aluminum foil and plastic bags for freezing tissues. Whirl-paks of various sizes work well.
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.
15. Hammers, chisels and handsaws.
16. Small hand meat hooks x 6
17. Hoist/crane/small tractor
18. Heavy straps, chains, ropes
19. Carts on rollers to move heavy parts.
20. Coveralls, boots, gloves, caps, 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 bag (red bags)
26. Leak proof styrofoam boxes or other 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. A review of elephant necropsy techniques and tips is available at http://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf. Although the size and scope of an elephant necropsy may seem intimidating, the procedure can be accomplished in 8-10 hours (sometimes 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 **endotheliotropic herpes virus infection**. The trunk should be examined according to above guidelines in the **tuberculosis** section.

Heavy equipment may be 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, forelegs, hind legs, 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 for purposes of safety and efficiency 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. 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. Then remove the bony plate in chunks with a curved crow-bar. 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 and the lungs 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

The task of disposing of an elephant carcass can be immense. 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 cut into manageable pieces (50-100lbs) for transportation. This can be very difficult and time consuming. 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 be performed in the hole to eliminate the chances of contamination of the surrounding area. In at least one TB-positive case, all personnel, equipment, and materials remained within a large hole for the entire necropsy procedure. At the completion of the procedure, all biohazardous materials deemed appropriate were buried with the remains of the carcass. This greatly reduced the chances of contamination.

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 _____ 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). **Skin nodules have been associated with EEHV in African elephants* (samples for fresh/frozen/formalin should be saved).**

Musculoskeletal System (bones, marrow, joints, muscles)

Body Cavities (fat stores, pleura, thymus, lymph nodes)

Spleen

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). **Lymphoid nodules in lungs may be associated with EEHV infections* (samples for fresh/frozen/formalin should be saved).**

Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers, **be sure to closely examine abdominal aorta for subtle or obvious aneurysms**)

Digestive System (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes)

Urinary System (kidneys, ureters, bladder, urethra)

TISSUE CHECK LIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) in small plastic bags. Freezing at -80 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 (ie. National Jewish Diagnostic Lab, Colorado). 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. Fix diced (1x1 mm) pieces of kidney, liver, spleen and lung in a suitable EM fixative if possible - glutaraldehyde base e.g., Trump-McDowell fixative. NOTE: There is generally no need to fix and label each tissue separately. Take 2 sets of fixed tissue. Bank one set. Send tissues required for diagnosis to primary pathologist; the SSP pathologist, Dr. Scott Terrell, should be contacted with any questions. Also, freeze post mortem serum (from heart), urine and any abnormal fluid accumulations. Consult **Elephant Research and Tissue Request Protocol** 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 | <input type="checkbox"/> Prostate | <input type="checkbox"/> Trachea |
| <input type="checkbox"/> Bulbo-urethral gland | <input type="checkbox"/> Lung | <input type="checkbox"/> Salivary gland | <input type="checkbox"/> Trunk cross section |
| <input type="checkbox"/> Brain | <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"/> Epididymus | <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 post mortem blood, separate serum and freeze for retrospective studies.

Primary Pathologist (Name): _____

Lab _____

Address _____

Phone _____

(Please send a copy of this protocol with gross descriptions and preliminary diagnoses to SSP Pathology and SSP Veterinary Advisors). Send final report with histopathologic findings and any pertinent digital or color slides to):

Scott P. Terrell, DVM, Diplomate ACVP

SSP Pathology Advisor, Elephants

Disney's Animal Kingdom, 1200 N Savannah Circle, Bay Lake, FL 32830

Email: Scott.P.Terrell@disney.com

RESEARCH REQUESTS

Institutional reminder - all requests made are conditional and 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.

CURRENT TISSUE/SAMPLE RESEARCH REQUESTS

1. Scott Terrell
Disney's Animal Kingdom
1200 N Savannah Circle
Bay Lake, FL 32830
Work: (407) 938-2746 Fax: (407) 938-1909
Email: Scott.P.Terrell@disney.com

The SSP pathologist cannot provide primary histopathology services except in a very few special cases.

Dr. Terrell requests an electronic copy of the **final necropsy and histopathology report** including all pertinent culture results.

Tissues for histopathology should be sent to the institution's primary pathology service. 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 block 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 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 molecular diagnostic value.

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.

- Heart, liver, spleen, kidney (50 g each)

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. Michele Miller (michelemiller128@gmail.com).

LITERATURE CITED and SELECTED BIBLIOGRAPHY

- Laws, R.M., Parker, I.S.C. and Archer, A.L. (1967). Estimating live weights of elephants from hindleg weights. *East Afr Wildl J* 5:106-111.
- Mikota, S.K., Sargent, E.L., and Ranglack, G.S. (1994). *Medical management of the elephant*. Indira Publishing House, West Bloomfield, Michigan, 298 pp.
- Mikota, S.K., Larsen, R.S., and Montali, R.J. (2000) Tuberculosis in elephants in North America. *Zoo Biology* 19: 393-403.
- Richman, L.K., Montali, R.J., Garber, R.L., Kennedy, M.A., Lehnhardt, J., Hildebrandt, T., Schmitt, D., Hardy, Alcendor, D.J., and Hayward, G.S. (1999) Novel endotheliotropic herpesvirus fatal for Asian and African elephants. *Science*, 283(5405): 1171-1176.
- Robertson-Bullock, W. (1962). The weight of the African elephant *Loxodonta africana*. *Proc Zool Soc Lond* 138:133-135.
- Roth, V.L. and Shoshani, J. (1988). Dental identification and age determination in *Elephas maximus*. *J Zool (Lond)* 214(4):567-588.
- Shoshani, J., Alder, R., Andrews, K., Baccala, M.J., Barbish, A., Barry, S., Battiata, R., Bedore, M.P., *et al* (1982). On the dissection of a female Asian elephant (*Elephas maximus maximus* Linnaeus, 1758) and data from other elephants. *Elephant* 2 1):3-93.
- Sreekumar, K.P. and Nirmalan, G. (1989). Estimation of body weight in Indian elephants (*Elephas maximus indicus*). *Vet Res Commun* 13:3-9.

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