

ELEPHANT NECROPSY PROTOCOL

(Elephas maximus and Loxodonta africana)

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

February, 2010

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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 request protocol 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 if given sufficient notice and time to travel (contact information available at the end of this document). Two of the more important disease processes in elephants include **endotheliotropic herpes virus infection** and **tuberculosis** (caused by the human pathogen, *Mycobacterium tuberculosis*). Specific sample collection protocols are listed in the following pages and should be followed 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), chain saw or reciprocating saw, an axe, numerous large knives, chains, straps, and the very important TB 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 and requires use of a chain or reciprocating saw. Hints and tips are given. 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.

Scott P. Terrell, DVM, Dipl. ACVP
SSP Pathology Advisor, Elephants

Michele Miller, DVM, PhD
SSP Veterinary Advisor, Elephants

February 2010

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 receive a copy.

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 is contained in a separate document, **Elephant Research and Tissue Request Protocol**. The *Search List* describes those parts of the anatomy for which data is lacking or about which previous observations need to be confirmed or refuted. The requested data sets are optional and included in an accompanying document, Elephant Research and Tissue Request Protocol. Some of these observations may be applied to live animals. Therefore, this protocol should be referred to when planning a procedure that might facilitate data collection.

Acquainting one's self with the protocols in both documents (Elephant Necropsy Protocol and Elephant Research and Tissue Request Protocol) 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.

A revised Elephant Research and Tissue Request Protocol will be forwarded periodically as new requests are received and projects end. Contact Michele Miller or Scott Terrell for current requests. 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, with digital or color slides to Drs. Scott Terrell and Michele Miller.

Scott Terrell, DVM, Dipl. ACVP
 Head, Department of Pathology
 Veterinary Services, Disney's Animal Kingdom
 1200 N Savannah Circle
 Bay Lake, FL 32830
 Work: (407) 938-2746 Fax: (407) 938-1909
 Home: (407) 251-0545; Cell: (321) 229-9363
 Email: Scott.P.Terrell@disney.com

Michele Miller, DVM, PhD
 Chief Veterinary Officer
 Palm Beach Zoo
 1301 Summit Blvd.
 West Palm Beach, FL 33405
 Work: 561-833-7130 ext 224 Fax: 561-833-7135
 Cell: 561-727-9630
 Email: mmiller@palmbeachzoo.org

ELEPHANT HERPESVIRUS DISEASE ALERT

Elephant herpesvirus infection is a highly fatal disease of elephants in captivity and the wild, and is associated with a group of unique herpesviruses (11 species of which 5 have caused fatal disease). These herpesviruses affect mainly young elephants (<10 years of age) and can have a fatal outcome within hours to a week after onset of signs. Clinical signs are often vague and include lethargy, lameness, colic, anemia, thrombocytopenia, edematous swellings of the head and thoracic limbs, oral ulceration and cyanosis of the tongue. Necropsy findings are consistent with vasculitis and include extensive cardiac and serosal hemorrhages and edema, hydropericardium, cyanosis of the tongue and oral and intestinal ulcers. Histological features are microhemorrhages with very mild inflammation in the heart, liver and tongue accompanied by intranuclear inclusion bodies in the capillary endothelium. Transmission electron microscopy of the inclusion bodies shows 80-90 nm diameter viral capsids consistent with herpesvirus morphology.

There have been 34 known acute cases in North America since 1977 with 27 deaths (25 in Asian elephants). EEHV1A is the most common type (18 cases) with EEHV1B (4 cases), EEHV2 (2 cases), and 1 case each of EEHV3, EEHV4, EEHV5 and EEHV6. Diagnosis in ill elephants is made by detecting herpesvirus in EDTA whole blood using polymerase chain reaction (PCR). Of eight elephants that were treated with famciclovir, four survived. Ganciclovir has also been more recently used. The onset of the disease may be very rapid with few prodromal signs and peracute death within 24 to 36 hours. Recent evidence suggests that there may be asymptomatic carriers among North American elephants. A pilot trunk wash study showed shedding in adult Asian elephants. Of the 20 cases to date, there are significant differences even among the 18 EEHV1As.

Serological tests have been developed in Asian elephants to detect antibodies to some of the EEHVs. However, diagnostic tests are confounded by the inability to cultivate the virus *in vitro*. The 8% of captive Asians known to be serologically positive are all wild-born animals over the age of 30 years. Therefore, it is likely that captive elephants in North America brought EEHV1 strains with them from parts of Asia or Africa.

If you suspect an elephant in your care may have died from this disease or shows clinical signs, please contact one of the principals listed below. Consult the Tissue Checklist section of this necropsy protocol for instructions on sending diagnostic samples from any elephants suspected of having this disease.

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

Small numbers of white to gray nodules with a spongy texture (3-30 mm in cross sectional diameter) in lungs have been found in a high fraction of African elephants culled in the wild and these contain high levels of EEHV2 and EEHV3 at least (subclinical or latent infection). These lung nodules have also been reported in Asian elephants and thorough search for lung nodules by slicing through the lung at regular intervals (“breadloafing”) at necropsy should facilitate collection of such nodules in both Asian and African elephants. The nodules may be very small and rare within the lung, or could be obvious and more numerous and are found in otherwise healthy elephants. Similarly, raised skin nodules with darker fibrous centers have been found occasionally in otherwise healthy juvenile African elephants and in one outbreak in Florida; these contained EEHV1. A third type of lesion has been associated with EEHV1: variably sized, red ulcers or vesicles in the distal vestibulum of the genital tract of African elephants. More samples of all of these types of lesions (lung and skin nodules, vestibular ulcers/vesicles) are required from both captive and wild Asian and African elephants to evaluate the natural history of the EEHVs. **Please search carefully for and collect “benign”herpes” lung nodules especially in all elephant necropsies.**

Contacts: Laura K. Richman
National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008

W: (301) 398-4741
e-fax (301) 398-9741
Email: richmanl@comcast.net

Michele Miller
Palm Beach Zoo
1301 Summit Blvd
West Palm Beach, FL 33405
W: (561) 833-7310 ext 224
Cell: (561) 727-9630
Email: mmiller@palmbeachzoo.org

Scott Terrell
Disney's Animal Kingdom
1200 N Savannah Circle
Bay Lake, FL 32830
Work: (407) 938-2746
Cell: (321) 229-9363
Email: Scott.P.Terrell@disney.com

Erin Latimer
National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
W: (202) 633-4252
Email: latimere@si.edu

Dennis Schmitt
217 Karls Hall-SMSU
901 S. National Ave.
Springfield, MO 65804
W: (417) 836-5091
Cell: (417) 861-9572
Email: dschmitt@feldinc.com

Martha Weber
St. Louis Zoo
Forest Park, 1 Government Dr.
St. Louis, MO 63110-1396
Work: (314) 781-0900 ext 4565
Email: Weber@stlzoo.org

Richard Montali
Pathologist
Cell: (530) 304-1482
Email: montalirj@yahoo.com

Ramiro Isaza
University of FL-Gainesville
2015 SW 16th Ave.
Gainesville, FL 32610
W: (352) 392-2226 ext 5700

Email: Isazar@vetmed.ufl.edu

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 contagious to humans. Therefore be prepared with proper protective apparel, and contain any suspicious organs or lesions as soon as possible.

Ideally, elephants should be bled for serology (Elephant TB STAT-PAK, MAPIA), 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 Guidelines for the Control of Tuberculosis in Elephants 2008 (http://www.aphis.usda.gov/animal_welfare/publications_and_reports.shtml).

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 tonsillar regions and submandibular 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 **Guidelines for the Control of Tuberculosis in Elephants 2008**. In the event of an elephant necropsy (elective or otherwise), please notify Dr. Terrell (see contact list) for further instructions and possible participation.

Contacts: Scott P. Terrell, DVM, Diplomate ACVP, SSP Pathology Advisor, Disney's Animal Kingdom, 1200 N Savannah Circle, Bay Lake, FL 32830, W (407) 938-2746; H (407) 251-0545; Cell (321)229-9363; email Scott.P.Terrell@disney.com

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/publications_and_reports.shtml (available to the public)
2. www.aazv.org (available to AAZV members by password)
3. www.elephantcare.org (available to the public)
4. <http://www.osha.gov/SLTC/tuberculosis/standards.html> - OSHA TB standards and rules
5. http://www.cdc.gov/nchstp/tb/Federal_Register/New_Guidelines/TBICGuidelines.pdf
Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005

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. 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 -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 (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 and request a duplicate set of slides for the SSP pathologist, Dr. Scott Terrell who should be contacted for further instructions. 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 pathologist. 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

W (407) 938-2746; H (407)251-0545; Cell (321)229-9363

Email: Scott.P.Terrell@disney.com

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 | Work Number | Home Number | Fax Number |
|---|--------------------------------------|----------------|----------------|
| Scott Terrell, DVM, DACVP Orlando, Florida Email: scott.p.terrell@disney.com | (407) 938-2746 Cell: 321-229-9363 | (407) 238-0693 | (407) 938-1909 |
| Richard Montali, DVM, DACVP Email: montalirj@yahoo.com | Cell: 530- 304-1482 | | |
| Dee MacAloose, DVM, DACVP Bronx, NY Email: dmcaloose@wcs.org | (718) 220-7105 Cell: 646-852-4962 | na | (718) 220-7126 |
| Genevieve Dumonceaux, DVM Florida Aquarium, Tampa, Florida Email: gdumonceaux@flaquarium.org | (831) 367-4055 Cell: 831-465-9234 | (831) 907-5795 | |
| Susan Mikota DVM smikota@elephantcare.org | (931) 628-5962 Cell: 931-628-5963 | (931) 796-7102 | |

Feb 2010 mm