

***ELEPHANT RESEARCH AND TISSUE  
REQUEST PROTOCOL***

*(Elephas maximus and Loxodonta africana)*

*The American Zoo and Aquarium Association  
Elephant Species Survival Plan  
And  
The Elephant Research Foundation*

*August, 2006*

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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) and the Elephant Research Foundation. 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 immobilizing or performing an elephant necropsy, but this 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 in this document. (Specific necropsy information is contained in a separate document, **Elephant Necropsy 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 *Measurements Checklist* may seem tedious, but only this type of attention to detail will allow us to expand our knowledge of elephant anatomy. Both of these requested data sets are optional and included in this document. 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. Please send the completed measurements checklist to Dr. J. Shoshani (contact information on page 11) and a copy to Dr. Michele Miller.

Acquainting oneself 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. A team should be designated in advance for data and sample collection to save valuable time. A list of researchers interested in participating in elephant necropsies is included in the Elephant Necropsy Protocol.

A revised Elephant Research and Tissue Protocol will be forwarded periodically as new requests are received and projects end. Contact Dr. Michele Miller or Scott Terrell for current requests. A copy of the completed data should be sent to the appropriate researcher. A copy of the necropsy report should be completed and sent to Drs. Scott Terrell and Genny Dumonceaux (see Elephant Necropsy Protocol for details).

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](mailto:Scott.P.Terrell@disney.com)

## ELEPHANT HERPESVIRUS DISEASE ALERT

The cause of a highly fatal disease of elephants in North American and European Zoos has been identified recently as a new type of herpesvirus. The herpesvirus affects mainly young elephants and usually has a fatal outcome within an hour to a week of onset. Clinical signs are variable and include lethargy, edematous swellings of the head and thoracic limbs, oral ulceration and cyanosis of the tongue. Necropsy findings 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.

Serological tests have been recently developed (2002) using molecular techniques to express antigens because it has not been possible to cultivate the virus *in vitro*. Some of the epidemiological aspects of the disease are not yet clear and are still under study. Although African elephants are known to carry the virus that is fatal for Asian elephants, there have been a number of cases in Asian elephants in which no direct contact occurred with African elephants. Asian calves (less than two years of age) from different facilities in the U.S. became ill with the clinical signs noted above, and were found to have the herpesvirus by a blood test using polymerase chain reaction (PCR). Of seven elephants that were treated with famciclovir, three recovered. The onset of the disease may be very rapid with few prodromal signs and peracute death within 24 to 36 hours. This occurred in 1999-2000 in a six and eight year old Asian elephant that both died even though famciclovir was administered several hours after herpes infection was suspected.

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

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Contacts: Laura K. Richman  
Pathologist/Scientist II  
MedImmune, Inc.  
One MedImmune Way  
Gaithersburg, MD 20878  
W: (301) 398-4741; e-fax (301) 398-9741; [RichmanL@MedImmune.com](mailto:RichmanL@MedImmune.com)

Scott P. Terrell, Disney's Animal Kingdom, Orlando, FL. W: 407-938-2746, H: 407-251-0545, Cell: 321-229-9363; Email: [Scott.P.Terrell@disney.com](mailto:Scott.P.Terrell@disney.com)

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Michele Miller  
Disney's Animal Kingdom  
Department of Veterinary Services  
P.O. Box 10,000 Lake Buena Vista, FL 32830-1000  
Work: (407) 939-7316 Fax: (407) 938-1909  
Email: [Michele.Miller@disney.com](mailto:Michele.Miller@disney.com)  
**FOR HELP WITH TREATMENT ADVICE**

## 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 (RAPID TEST, 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 2003 ( [www.aphis.usda.gov/ac/TBGuidelines2003.html](http://www.aphis.usda.gov/ac/TBGuidelines2003.html) ).

### 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 is **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 2003**. 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](mailto:Scott.P.Terrell@disney.com)

## INTERNET SITES

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

1. [www.aphis.usda.gov/ac/TBGuidelines2003.html](http://www.aphis.usda.gov/ac/TBGuidelines2003.html) (available to the public)
2. [www.aazv.org](http://www.aazv.org) (available to AAZV members by password)
3. [www.elephantcare.org](http://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](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

## SEARCH LIST (OPTIONAL)

The following are anatomical features that need to be confirmed or refuted, or for which few data exist. They are not arranged in order of importance, but rather as one studies the elephant by regions from the tip of the trunk to the tip of the tail. Please be aware of these anatomical questions and attempt to obtain the needed additional data as you proceed in your dissection.

1. Record the number of toenails.
2. Weigh skin after dissection from limbs and carcass.
3. Search for sesamoids especially under tendons. There may be one at the proximal end of the humerus, but check other sites as well.
4. Obtain total skeletal weight. Remove as much soft tissue as possible.
5. Note any pathological conditions in the joints. Slight erosions on articular surfaces can be viewed best in fresh tissues and should be examined soon after death. Grooves and fractures on articular surfaces cannot be mistaken and should be sought. Look also for "joint mice," calcium deposits, and any other abnormal signs.
6. Measure the volume of the nasal passages by instilling water soon after death or by measuring the diameter of the passages at intervals (record total length of trunk and diameter of passages at intervals of 10 cm).
7. Look for the intercommunicating canal between the two nasal passages of the trunk and the associated fibrous arches by sectioning the trunk every 10-20 cm. These structures were described as being located 13 cm from the tip of the trunk in a young female Asian elephant. Other searches in adult Asian females have revealed neither the arches nor the canals (Shoshani *et al.*, 1982).
8. Harvest the lenses from the eyes and weigh them (or keep intact eyes frozen).
9. Search for the trachea-esophageal muscle. This muscle is small and may be overlooked or cut during dissection so we suggest that a section about 20 cm posterior and 50 cm or more anterior to the bifurcation be removed and examined carefully outside the carcass. This muscle was found in only three of twelve elephants examined (Shoshani *et al.*, 1982).
10. Examine the dividing arrangement of the arteries from the aortic arch. There are two possibilities three branches or two branches. In the three-branch arrangement the sequence is right subclavian, a trunk common to the two carotids and the left subclavian. In the two-branch arrangement, the right subclavian and the common carotids merge into one vessel and the left subclavian remains separate.

## **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.

## 1. Dr. Bets Rasmussen

Professor of Biochemistry  
Oregon Graduate Institute  
20,000 NW Walker Rd  
Beaverton, Oregon 97006 USA  
Cell: 971-645-9485

Work: 503-748-1263  
Home: 503-621-1435  
Fax: 503-748-1464  
Email: [betsr@ebs.ogi.edu](mailto:betsr@ebs.ogi.edu)

***For any MALE ELEPHANT please call Bets Rasmussen immediately at 503-748-1263 or 503-621-1435. She will come on the next airplane as certain tissues are of extreme and immediate value in her studies.***

**Frozen at liquid nitrogen temperatures and maintained at dry ice temperature for shipping. Small pieces ( 1cm by0.5cm) in cryo tubes**

**1. temporal gland 2. Palatal pits 3. Incisive duct openings 4. Olfactory tissue 5. Liver 6. ovary**

**Fixatives.** Fixatives are available from Dr. Rasmussen. If there is an immediate need, contact your local medical school. Preferred fixative is 4% glutaraldehyde (EM grade) in a pH 7.2 buffer. Cut pieces for EM 0.5cm<sup>3</sup>. Save (in 10% buffered formalin) the larger section from which the EM piece was taken. If glutaraldehyde is not available submit tissues in 10% buffered formalin.

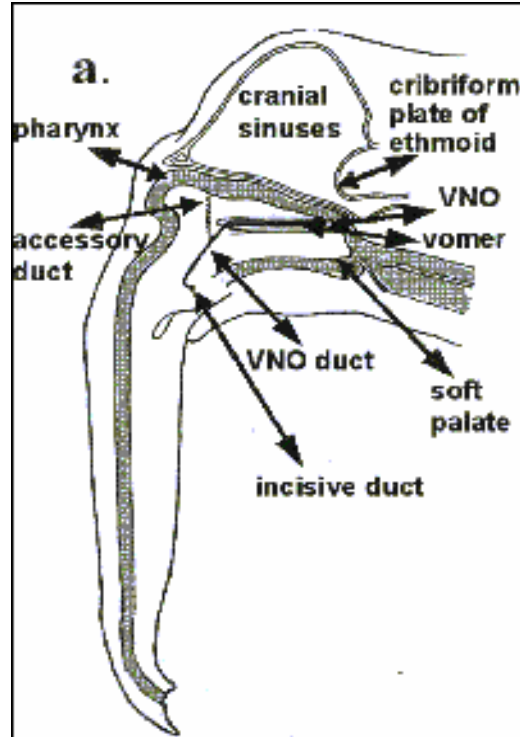
**Vomerinal organ** (RNA & DNA studies; electron microscopy). See diagram a. **Adults:** Only the incisive ducts are requested. These paired openings on the roof of the mouth are 1-2 cm in diameter, several cms posterior to the juncture of the mucosa of the mouth with the lip region.

**Fetal, neonates & young elephants.** The whole vomeronasal organ is requested. It is found in the vomer bone, dorsal and posterior to the incisive ducts. It is paired, pear-shaped and surrounded by cartilage. In immediately post-natal elephants it does not connect with the ducts. In these young elephants to obtain the organ, work dorsal/posteriorly from the ducts, looking for shiny white cartilage surrounding receptive tissue, which is hollow in the center. **FIRST PRIORITY** is (1cm<sup>3</sup>) pieces frozen in cryotubes in liquid nitrogen. Second priority is fixed tissue.

**Palatal pits.** (Histological and cytological studies.)

The palatal pits are dual series of small (smaller than VNO duct opening) openings (0-13), asymmetrically and bilaterally located along the approximate demarcation line in the upper head between the hard palate and the trunk. Push aside the upper lip to locate. Dissect out a pit (0.5-1.0cm), making sure at least 2 cm of underlying tissue are included (4% buffered glutaraldehyde).

**Brain.** The anterior, olfactory bulb region is requested if the brain is being removed. Especially the anterior-ventral region where connections to olfactory turbinates and vomeronasal nerve occur. This is a special request and Dr. Rasmussen will be present for such procurement.



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2. Dr. C. Earle Pope  
Audubon Center for Research of Endangered Species (ACRES)  
14001 River Road  
New Orleans, Louisiana, 70131 USA  
Work: (504) 398-3161 Home: (504) 734-5381 Fax: (504) 391-7707 Email: [epope@acres.org](mailto:epope@acres.org)

**Intact ovaries** (To recover oocytes for *in vitro* maturation and culture). Remove ASAP; rinse with saline to remove blood and adhering tissue. Wrap in sterile gauze pre-soaked in saline and place in plastic bag or specimen container. Keep at room temperature if sample can be shipped the same day; if longer, pack in crushed ice. Ship overnight; will pay shipping.

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3. Dr. Linda Munson  
Department of Veterinary Pathology, Microbiology Immunology  
University of California at Davis  
1126 Haring Hall / One Shields Ave.  
Davis, CA 95616 USA  
Work: 530-754-7567 Fax: 530-752-3349 Email: [lmunson@ucdavis.edu](mailto:lmunson@ucdavis.edu)

**Sections of uterine endometrium** (Characterization of endometrial lesions.) Endometrial samples including any polyps, cysts, tumors or other lesions. Samples should include lesion and adjacent normal tissue. Fix in 10% formalin. Ship by U.S. mail. Will pay shipping.

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4. Dr. Scott Terrell  
Disney's Animal Kingdom  
1200 N Savannah Circle  
Bay Lake, FL 32830  
Work: (407) 938-2746 Home (407) 251-0545 Fax: (407) 938-1909  
Email: [Scott.P.Terrell@disney.com](mailto:Scott.P.Terrell@disney.com)

**One complete set of histopathology H&E slide recuts for review.**

**Complete set of formalin fixed tissues (as per SSP Necropsy Protocol).** For formalin fixed tissues 0.5-1.0cm thick sections. Call before shipping. Will pay shipping. Dr. Terrell will review histopathology slides if pathology services are not available.

**A complete set of formalin fixed tissues is required only if your institution does not retain possession of those tissues in perpetuity. If the tissues will be or may be disposed of at any time in the future, please send them to Dr. Terrell. The tissues will be kept archived forever.**

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5. Laura K. Richman, DVM, PhD, Diplomate, ACVP  
Pathologist/Scientist II  
MedImmune, Inc.  
One MedImmune Way  
Gaithersburg, MD 20878  
W: (301) 398-4741; e-fax (301) 398-9741; [RichmanL@MedImmune.com](mailto:RichmanL@MedImmune.com)

**1. Ultra frozen (-70°C) heart, liver, tongue, spleen, oral ulcers and lymphoid patch sections from distal vestibulum. 2. Whole blood (5-10 ml); serum (5-10 ml).** (For elephant herpes virus study). Take samples to be frozen as soon as possible place in sterile container and freeze. Send frozen tissue overnight on dry ice. Send blood and serum samples overnight on wet or dry ice. Call before shipping. Will pay shipping.

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6. Anthony T. Boldurian, Ph.D.  
Professor of Anthropology  
University of Pittsburgh at Greensburg  
Smith Science Building  
1150 Mt. Pleasant Road  
Greensburg, Pennsylvania, 15601 USA  
Work: (724) 836-9989 Fax: (724) 836-7129 E-mail: [folsom@pitt.edu](mailto:folsom@pitt.edu)

**Femur or humerus from either species; adult or sub-adult.** (For experimental archeological study to replicate mammoth shaft wrench artifact.) Minimum width 60 mm; minimum thickness 20 mm. Samples must be in a “green” or unweathered state, preferably from a recently deceased individual. Pack in ice or cool packs if tissue still adhering. Call before shipping. Will pay shipping.

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7. Mark Stetter, DVM, DACZM  
Director of Veterinary Services  
Disney’s Animal Programs  
Department of Veterinary Services  
P.O. Box 10,000  
Lake Buena Vista, Florida 32830-1000 USA  
Work: (407) 939-7352 Fax: (407) 938-1909 E-mail: [Mark.Stetter@disney.com](mailto:Mark.Stetter@disney.com)

**Please call if an elephant euthanasia is being planned. Project involves abdominal laparoscopy for developing reproductive intervention methods (vasectomy/ovariectomy).**

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8. Michele Miller, DVM, PhD  
Disney’s Animal Kingdom  
Department of Veterinary Services  
1200 N. Savannah Circle East  
Bay Lake, Florida 32830  
Work: (407) 939-7316 Fax: (407) 938-1909 E-mail: [Michele.Miller@disney.com](mailto:Michele.Miller@disney.com)

**Minimum 5-10 ml frozen serum for SSP serum bank.** Instructions for sample preparation: Serum samples should be separated within 1 hr of collection and frozen in 1-2 ml aliquots in cryovials. Freeze at –70C until shipment. Ship on dry ice or ice packs overnight to address above. Please complete serum bank submission form (see below) and send with shipment.

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9. Dr. Fahad Sultan  
Department of Cognitive Neurology, University Tubingen  
Auf der Morgenstelle 15  
72076 Tubingen, Germany  
Work: +49-7071-2980464 Fax: +49-7071-295724 E-mail: [fahad.sultan@uni-tuebingen.de](mailto:fahad.sultan@uni-tuebingen.de)  
Cell: 0160 98730310

**Intact elephant brain** immersed in 3.5% paraformaldehyde. (For neurologic study) Studying quantitative comparative neuroanatomical aspects of mammalian cerebella. Part of the research has been dealing with the size and form of the unfolded cerebellar cortex. Duration of study: 2-5 years. Special instructions: Brains should be as intact as possible (after careful removal from skull), immerxe in 3.5% paraformaldehyde fixative. Researcher will pay for shipping. CITES permit for shipping samples required.

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10. **Name:** Robin Dunkin and Dr. Terrie Williams  
**Date of Request:** 2-20-2005  
**Affiliation:** University of California at Santa Cruz  
**Address:** Center for Ocean Health  
100 Shaffer Road  
Santa Cruz, CA 95060  
**Work phone:** (831) 334-0640 or (831) 459-5428 **Fax:** (831) 459 – 3383  
**Email:** [dunkin@biology.ucsc.edu](mailto:dunkin@biology.ucsc.edu) or [williams@biology.ucsc.edu](mailto:williams@biology.ucsc.edu)

#### **REQUEST FOR ELEPHANT TISSUE/BLOOD SAMPLES**

##### **Sample(s) Requested**

We are requesting samples (~30cm<sup>2</sup>) of full depth, dorsal integument from both Asian and African elephants.

##### **Purpose of Study**

We are conducting a study of the thermal properties of elephant integument as part of a larger project on elephant thermoregulation and water conservation. These samples will be used to measure thermal conductivity and conductance of the integument both in its dry state as well with water or mud coating the surface of the skin.

##### **Duration of the Study**

Approximately 4-5 years

##### **Instructions for Sample Preparation**

The sample should be collected from the dorsal surface at the level of the umbilicus. Sample dimensions should be roughly 30cm<sup>2</sup> and should include the full depth of the integument. Alternatively, other areas on the dorsal surface are acceptable, however, the location where the sample was taken should be noted. The sample should be collected and then notched at the dorso-cranial margin to maintain orientation. The sample should then be wrapped in Saran-wrap, double bagged in a zip-lock bag, and frozen until shipped.

##### **Shipping Instructions**

Samples should be shipped with either ice packs or dry ice. Samples should be shipped overnight and we are happy to pay for shipping and packaging supplies.

##### **Special Instructions**

Along with the sample, we would like to request some basic information on the animal including age, sex, mass, whether the animal was in robust or emaciated body condition, and whether any skin lesions or other skin problems were present

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11. Dr. Jeheskel (Hezy) Shoshani or Dr. William Kupsky  
Elephant Research Foundation Phone: (313) 745-2542; email: [wkupsky@dmc.org](mailto:wkupsky@dmc.org)  
106 East Hickory Grove Road or Gary Marchant  
Bloomfield Hills, Michigan 48304 USA Phone: (248) 559-2278; email: [merchant@ic.net](mailto:merchant@ic.net)

Work: (248) 540-3947  
Email: [jshosh@sun.science.wayne.edu](mailto:jshosh@sun.science.wayne.edu)

or Mahmood Mokhayesh  
Phone: (313) 557-2872;  
email: [carnassial@hotmail.com](mailto:carnassial@hotmail.com)

**Intact brain** from any age, sex or species of elephant. Preserve in 10% formalin.

Copy of completed measurements checklist (Anatomical studies).  
Please send to Dr. Shoshani.

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12. Tim Griffin and Chelsea He  
Orthopedic Bioengineering Laboratory  
Duke University Medical Center  
375 MSRB, DUMC Box 3093  
Duke University Medical Center  
Durham, NC 27710

Email: [tmgriff@duke.edu](mailto:tmgriff@duke.edu)      [chelsea.he@duke.edu](mailto:chelsea.he@duke.edu)

Work: 919-684-3583    Home: 919-931-4260    Fax: 919-681-8490

We are interested in studying how cartilage biomechanical properties vary in animals that span a wide range of body sizes in order to characterize the relationship between body mass, cell density, and tissue mechanical properties.

Sample requested: **Intact knee joint**

Duration of study: Open

Instructions / sample: Refrigerate joint within 24 hours of animal's death. Freeze within 72 hours. Femoral shaft can be cut proximal to the knee joint and tibial shaft distal to the knee. Skin and muscle can be trimmed or left intact. **Please do not open knee capsule.**

Shipping: Recipient will pay, ship in sealed bag on dry ice.

# REQUEST FOR 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 \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

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

\_\_\_\_\_

Special instructions \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Attach any additional information. Send to: Michele Miller, Disney's Animal Kingdom, Department of Veterinary Services, P.O. Box 10,000, Lake Buena Vista, Florida 32830-1000. Work: (407) 939-7316; Fax: (407) 938-1909; Email: Michele.Miller@disney.com

**Elephant Serum Bank Submission Form**

Institution/owner: \_\_\_\_\_  
 Submitter: \_\_\_\_\_  
 Address: \_\_\_\_\_  
 Tel: \_\_\_\_\_ Fax: \_\_\_\_\_ Email: \_\_\_\_\_

***Animal Information***

Asian  African  ISIS# \_\_\_\_\_ Studbook # \_\_\_\_\_  
 Name \_\_\_\_\_ Age: \_\_\_\_\_  actual  estimate  
 Sex:  male  female

***SAMPLE COLLECTION INFORMATION***

Date of sample collection: \_\_\_\_\_ Time of collection : \_\_\_\_\_  
 Site of sample collection:  ear vein  leg vein  other: \_\_\_\_\_  
 Health status of animal:  normal  abnormal  
 Fasted:  no  yes – how long \_\_\_\_\_  
 Weight \_\_\_\_\_  actual  estimated  
 Type of restraint:  manual  anesthetized/sedated  behavioral control  
 Temperament of animal:  calm  active  excited

Type of blood collection tube:

- no anticoagulant (red-top)  
 EDTA (purple)  
 heparin (green)  
 other: \_\_\_\_\_

Sample handling:  separation of plasma/serum by centrifugation  
 (check all that apply)  stored as whole blood  
 frozen plasma/serum  
 other – describe \_\_\_\_\_

**TB EXPOSURE STATUS**

- Known infected animal  
 Known exposure to culture positive source within the past 12 months  
 Known exposure to a culture positive source within the past 1-5 years  
 No know exposure to a culture positive source in the last 5 years

**TREATMENT INFORMATION**

**Is elephant currently receiving any medication or under treatment?**  yes  no  
**If yes, please list drugs and doses:** \_\_\_\_\_

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

Time between blood collection and last treatment: \_\_\_\_\_

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Ship samples overnight frozen with shipping box marked "PLACE IN FREEZER UPON ARRIVAL"

**Send completed form with samples to:****Dr. Michele Miller**

Disney's Animal Kingdom-Dept. of Vet. Services  
 1200 N. Savannah Circle West  
 Bay Lake, FL 32830  
 (407) 939-7316; email: Michele.Miller@disney.com  
 2/20/03

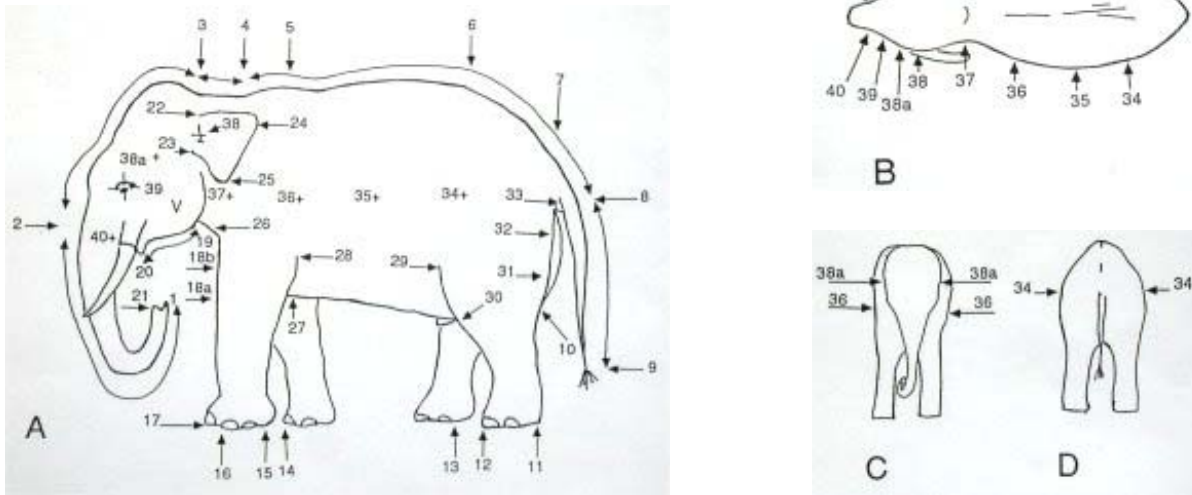
**MEASUREMENTS (OPTIONAL)**

This data sheet is a general guideline to the pre-euthanasia or post mortem measuring of an elephant. Refer to the anatomical diagrams, Figures 1 and 2. The numbering system begins at the trunk and continues in a clockwise direction. All measurements should be taken in a straight line, except when indicated otherwise. Measurements to be taken between corresponding points on opposite sides of the body are marked with a plus symbol (+). These should be taken in a straight line, essentially through, not around, the elephant. Calipers can be improvised from two long straight poles or straight edges. Place the end of each pole on one of the two points, keeping the poles parallel to one another. Measure the straight line distance between the free ends of the two parallel poles.

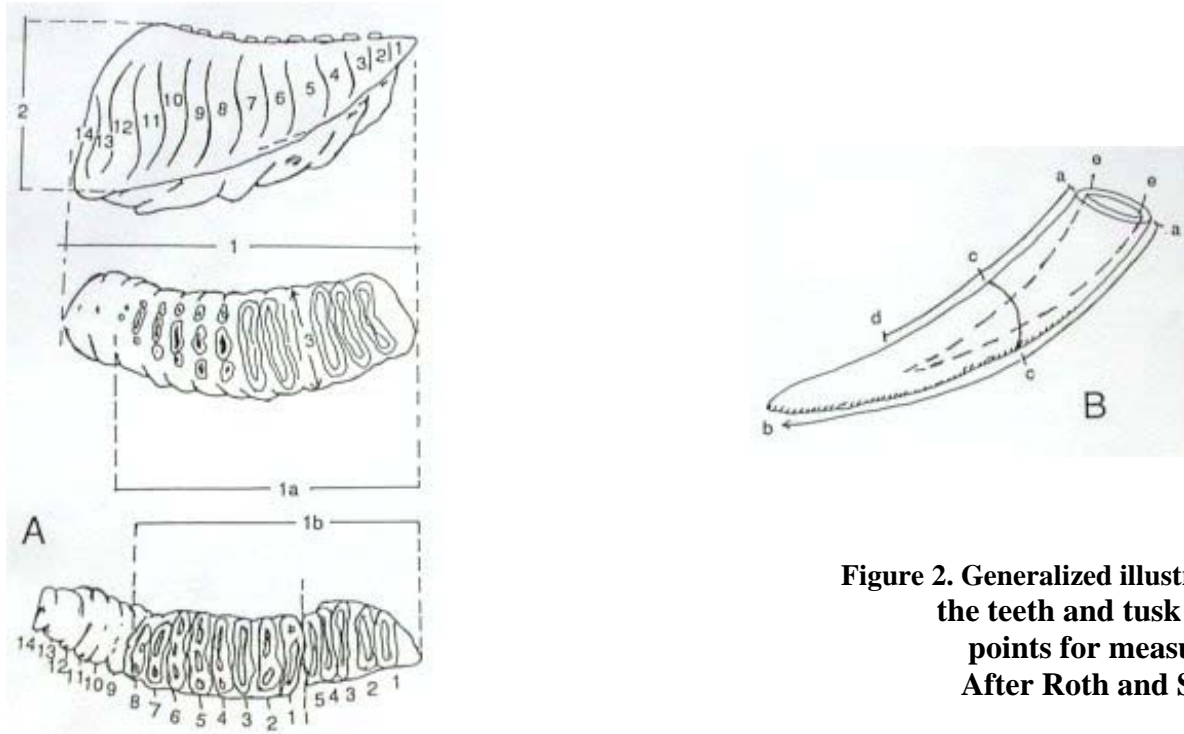
GENERAL		
Subject	Reference numbers on figures	Measurement between these points (cm)
Tip of trunk to tip of tail (along the curve)	Fig.1: 1-9	
Length of trunk (along the curve)	Fig.1: 1-2	
Length of tail	Fig.1: 8-9	
Shoulder height	Fig.1: 5-14	
Dorsum height (the highest point of back or "hump")	Fig.1: 6-13	

DETAILED			
TRUNK	Tip to base	Fig.1: 1-2	
	Tip width	Fig.1: 1-21	
	Base width	Fig.1: 40+	
HEAD	Dorsal length (along the curve)	Fig.1: 2-3	
	Ventral length (along the curve)	Fig.1: 19-20	
	Neck height	Fig.1: 3-19	
	Width between ears	Fig.1: 38+	
	Width between temporal glands	Fig.1: 38a+	
	Width between eyes	Fig.1: 39+	
	Width of mouth	Fig.1: 40+	
EAR	Anterior width	Fig.1: 22-23	
	Posterior width	Fig.1: 24-25	
	Dorsal length	Fig.1: 22-24	
	Ventral length	Fig.1: 23-25	
NECK	Length	Fig.1: 3-4	
	Width	Fig.1: 37+	
	Height	Fig.1: 3-19	
BODY	Dorsal length (along the curve: number 7 is in a straight line with number 11)	Fig.1: 4-7	
	Middle length (make sure this and the next measurement are taken parallel to each other)	Fig.1: 32-26	
	Bottom length (make sure this and the previous measurement are taken parallel to each other)	Fig.1: 10-27-18a	
	Width at front	Fig.1: 36+	
	Width at middle	Fig.1: 35+	
	Width at back	Fig.1: 34+	
	Height at front of forelimb	Fig.1: 5-27	
	Height at front of hindlimb	Fig.1: 6-30	
Height at back of hindlimb	Fig.1: 7-10		
TAIL	Length (excluding hair)	Fig. 1: 8-9	
	Width at base	Fig. 1: 8-33	

DETAILED			
FORELIMB	Length (height)	Fig. 1: 16-26	
	Width at top	Fig. 1: 18b-28	
	Width at bottom ( include side width, if different)	Fig. 1: 15-17	
HIND LIMB	Length (height)	Fig. 1: 11-31	
	Width at top	Fig. 1: 29-31	
	Width at bottom (include side width since it is narrower)	Fig. 1: 11-12	
FEET	Count number of “toenails”		Left front
			Right front
			Left hind
			Right hind
TEETH (can be measured soon after death or at a later date)	Total number of plates (including very small ones)	Fig. 2A: (1b)	
	Total length	Fig. 2A: (1)	
	Maximum width	Fig. 2A: 3 (1a)	
	Maximum grinding length of individual teeth	Fig. 2A: (1a)	
	Maximum grinding length of entire grinding surface	Fig. 2A: 1b	
	Maximum height	Fig. 2A: 2	
	Weight (in grams)		
TUSKS	Present_____Absent_____	Fig. 2B	
	Length from tip to gum line	Fig. 2B: b-c	
	Length from gum line to base	Fig. 2B: a-c	
	Length of pulp cavity	Fig. 2B: a-d	
	Width of pulp cavity	Fig. 2B: e-e	
	Total length	Fig. 2B: a-b	
PENIS	Circumference at base		
	Circumference at head		
	Length		
CLITORIS	Circumference at base		
	Circumference at head		



**Figure 1. Generalized illustrations of an elephant showing points for measurement: A) after Deraniyagala (1955); all others by Shoshani. Letter “V” on the head indicates the approximate location of the vent gland.**



**Figure 2. Generalized illustrations of the teeth and tusk showing points for measurement. After Roth and Shoshani (1988).**

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