

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

*(Elephas maximus and Loxodonta africana)*

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

*February, 2010*

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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 International Elephant 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 if this occurs, 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 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 forms 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 Michele Miller (see Elephant Necropsy Protocol for details).

Scott P. Terrell, DVM, Diplomate ACVP  
SSP Pathology Advisor                      SSP

Michele Miller, DVM, PhD  
Veterinary Advisor

## 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  
W: (301) 398-4741  
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Erin Latimer Richard  
National Zoo  
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Washington, DC 20008  
: (202) 633-4252  
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Montali  
Pathologist  
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Email: [montalirj@yahoo.com](mailto:montalirj@yahoo.com)

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W: (561) 833-7310 ext 224  
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Schmitt Ram  
217 Karls Hall-SMSU University  
901 S. National Ave.  
Springfield, MO 65804  
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Cell: (417) 861-9572  
Email: [dschmitt@feldinc.com](mailto:dschmitt@feldinc.com) Em

iro Isaza  
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Gainesville, FL 32610  
W: (352) 392-2226 ext 5700  
ail: [Isazar@vetmed.ufl.edu](mailto:Isazar@vetmed.ufl.edu)

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

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

Em ail: [Weber@stlzoo.org](mailto:Weber@stlzoo.org)

## 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](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](mailto: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](http://www.aphis.usda.gov/animal_welfare/publications_and_reports.shtml) (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.

## CURRENT TISSUE/SAMPLE RESEARCH REQUESTS

1. 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)

1. **One complete set of histopathology H&E slide recuts for review. 2 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 payshipping. Dr. Terrell will review histopathology slides if pathology services are not available. 3. 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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2. Michele Miller  
 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  
 E-mail: [mmiller@palmbeachzoo.org](mailto:mmiller@palmbeachzoo.org)

**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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3. Jeff Stanton, DVM or Paul Ling, PhD  
 Department of Molecular Virology & Microbiology  
 Baylor College of Medicine  
 Mail Stop BCM-385  
 One Baylor Plaza  
 Houston, TX 77030  
 Work: (713) 253-9282 (Jeff Stanton)  
 Email: [jstanton@bcm.edu](mailto:jstanton@bcm.edu); [pling@bcm.edu](mailto:pling@bcm.edu)

**Trunk Wash Collection Protocol for EEHV Screens:** Generally follow standard trunk wash collection protocol for *M. tuberculosis* culture: Instill 50mL sterile saline solution into the nares. Elevate the trunk 2-3ft for 30-60 seconds. Have elephant blow into a clean container – use new ziplock/plastic bags for each sample to reduce the chance of cross contamination. Transfer sample to a 50mL conical tube – the more mucous and exfoliated respiratory epithelial cells the better (SNOT IS GOOD). Chill the sample on ice until it can be centrifuged. Centrifuge at 1500Xg for 10min @ 4°C or room temperature. Gently pour off and discard the supernatant. Ideally the cell pellet is stored @ -80°C and shipped on dry ice, however it is acceptable to store cell pellets @ -20°C and ship on ice.

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4. Erin Latimer/Laura Richman/Gary Hayward (John Hopkins)  
 Smithsonian, National Zoological Park  
 Department of Pathology  
 3001 Connecticut Ave. NW  
 Washington, D.C. 20008  
 Work: (202) 633-4252  
 Email: [latimere@si.edu](mailto:latimere@si.edu)

1. **Serum** – 2 mls collected weekly; transfer to plastic screw-top tube and store at -80C or non-defrosting freezer until shipped (for EEHV titers). \*Contact Debbie Olsen ([dolson@elephantconservation.org](mailto:dolson@elephantconservation.org) for study number to label tubes (use study number and date collected ONLY). Ship samples quarterly when requested by email, overnight with ice packs or dry ice. 2. **Whole blood** – 1-2 mls in EDTA tube, then transfer to plastic screw-cap for storage at -80C freezer until shipped (for EEHV detection). 3. **Placenta** – freeze 1 inch<sup>3</sup> piece in liquid nitrogen or dry ice, then store at -80C freezer until shipped Also, serum and whole blood from dam and baby. 4. **Herpes lesions** – wet a cotton swab with small amount of sterile saline, swab lesion and place in sterile 15 ml plastic test tube; store at -80C until shipped. 5. **Saliva** – scoop up saliva into 15 ml sterile plastic tube; store at -80C until shipped. 6. **Necropsy tissues (heart, liver, tongue, spleen, intestine, any tissue with hemorrhages, any kind of nodules – skin, lung, vestibular)** – aseptically place 3x3 cm piece of tissue in small Ziploc or WhirlPak bag. Label with type of tissue, elephant ID, date. Use separate bag for

each tissue; store at -80C until shipped. **7. Trunk wash, urine, semen** – place up to 5 ml in a sterile plastic tube; store at -80C until shipped. **Shipping** – FedEx overnight; email tracking number to [latimere@si.edu](mailto:latimere@si.edu). EEHV Lab will pay for shipping of sample from healthy elephants; contact lab for account information.

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5. Mary Beth Matychak/Noha Abou-Madi/Julia Flaminio  
Cornell University, College of Veterinary Medicine  
Department of Clinical Sciences  
Room C3521  
Ithaca, NY 14853  
Work: (607) 253-3493  
Email: [mbm10@cornell.edu](mailto:mbm10@cornell.edu)

**1. Whole blood from EEHV infected or surviving elephants**-10-30 mls whole blood in EDTA, ideally collected prior to starting treatment, and every 6-8 hours if possible (if bled again). Samples should be sent overnight on ice for morning delivery (for EEHV culture). **2. Umbilical cord vessels and blood** – notify investigators of birth 1-2 days ahead for shipping supplies (email – [mbm10@cornell.edu](mailto:mbm10@cornell.edu), [na24@cornell.edu](mailto:na24@cornell.edu)). Once placenta passed, mix 10 mls antibiotic solution in each of liters of saline provided. Identify end of umbilical cord closest to calf, cut and discard first 2 inches of cord, then harvest vessels in segments of 1-1.5 feet. Do not tie segments into knots. Use saline bottles to rinse outside of cord; don't rinse inside of cord. Transfer 300 ml of saline into empty one and place section in saline bottles. Wrap tape around neck of bottles; wrap bottle in 1-2 diapers and place in baggie. Place in Styrofoam box, add 2 ice packs. Collect blood from any remaining cord into EDTA tubes. Ship overnight for AM delivery (culture of endothelial cells). **3. Necropsy tissues (heart, lung, liver, spleen, tongue, skeletal muscle, brain)** – collect 2-3 cm<sup>2</sup> samples and place in individual containers of antiviral transport media (supplied by investigators). Collect pericardial and peritoneal fluid in EDTA tubes. Ideally, collect 10-30 mls EDTA whole blood pre- or immediately post-mortem. Ship samples on ice packs overnight for AM delivery (EEHV culture). **Shipping** – use FedEx account number. Contact Dr. Mary Beth Matychak or Noha Abou-Madi (607-253-3278).

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6. Joseph Flanagan/Lauren Howard  
Houston Zoo  
Veterinary Hospital  
1513 North MacGregor  
Houston, TX 77030  
Clinic: (713) 533-6632  
Email: [jflanagan@houstonzoo.org](mailto:jflanagan@houstonzoo.org)/[lhoward@houstonzoo.org](mailto:lhoward@houstonzoo.org)

**Serum from confirmed EEHV cases** – 10-100 mls serum for EEHV sequencing. Place serum in screw top plastic tubes and store at -80C until shipped on dry ice. Call ahead or email prior to shipping.

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7. Jeff Talcott/Noha Abou-Madi  
Animal Health Diagnostic Center  
College of Veterinary Medicine  
Cornell University  
Upper Tower Road  
Ithaca, NY 14853  
Work: (607) 253-3900  
Email: [na24@cornell.edu](mailto:na24@cornell.edu)

**Necropsy tissues (lung nodules)** - 0.5 cm by 0.5 cm should be collected from lung nodules (two samples each) and placed in a container with antiviral transport media (supplied by investigator). The sample should be shipped back on ice packs overnight for AM delivery. FED EX account number: 1834-4049-7 (EEHV isolation).

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8. Steven Arnold  
University of Pennsylvania, Center for Neurobiology and Behavior  
2205 TRL, 125 S. 31<sup>st</sup> St.  
Philadelphia, PA 19104  
Work: (215) 573-2840  
Email: [sarnold@exchange.upenn.edu](mailto:sarnold@exchange.upenn.edu)

**Brain tissue from old elephants: 1) hippocampus; 2) inferior temporal cortex; 3) lateral prefrontal cortex.** Comparative neuroanatomical study of aging in elephants and humans. Can work with fresh, frozen, fixed, or fixed paraffin blocks. **Shipping** – investigator will provide UPS or FedEx account number and pay all costs.

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9. Gary Hayward  
 Johns Hopkins School of Medicine, Viral Oncology Program  
 3M09, Bunting-Blaustein Cancer Research Building,  
 1650 Orleans St,  
 Baltimore, MD 21287 (Fedex)  
 PH 410-955-8684 Fax 410-955-8685 (work)  
 PH 410 821-8187 (Home/weekend)  
 Email: ghayward@jhmi.edu

Fresh whole blood, serum and tissue transported on ice/refrigerated packs (Please do not freeze) for EEHV latency detection and cell culture work from acute disease cases and from selected known seropositive elephants.

1. Live or Euthanised: 20 to 200 ml of fresh whole blood collected pre-or immediate post-mortem in purple top blood tubes or EDTA-coated butterfly catheter.
2. Necropsy: Multiple pieces of fresh lung necropsy tissue from bronchiolar area with or without “herpes” lung nodules at necropsy. Transported in sealed wide-mouth 500ml plastic bottles or in multiple 40ml plastic tubes provided containing sterile PBS plus antibiotics. Please collect multiple lung nodules if present.
3. Necropsy: Fresh lung necropsy tissue (several pieces) from upper airway for epithelial cell culture. Transport in 40 ml plastic tubes provided containing epithelial cell culture media plus antibiotics or if larger in the second 500ml wide-mouth bottle with PBS.

Birth of Asian elephant. For primary endothelial cell and lymphocyte cell cultures.

1. Fresh unfrozen umbilical cord (several 6 to 8 in. segments) in 1x or 2x 500ml wide-mouth bottles provided with sterile PBS plus antibiotics (wash thoroughly with contents of second bottle). Transport on ice/refrigerator packs ASAP with return collect Fedex package provided.
2. Fresh unfrozen whole cord blood (4-10 ml if possible) in EDTA tubes for PBMC fractionation. Transport on ice/refrigeration packs provided.

Checklist for EEHV Sample Research ( written by Debbie Olson)

<b>SAMPLE CATEGORY</b>	<b>SAMPLES NEEDED BY NOHA AT CORNELL</b>	<b>SAMPLES NEEDED BY NEHL AND JOHNS HOPKINS</b>	<b>SAMPLES NEEDED BY HOUSTON ZOO/BAYLOR GROUP</b>
Healthy adult Asian elephant	EDTA WB from known survivors or known herds with a high incidence of EEHV	EDTA WB, serum quarterly *	
Healthy Asian elephant calf	EDTA WB from known survivors or calves in high risk situation - herds with a high incidence of EEHV	EDTA WB, serum monthly*	
Healthy adult African elephant	EDTA WB from known survivors or known herds with a high incidence of EEHV	EDTA WB, serum quarterly*	
Healthy African elephant calf	EDTA WB from known survivors or calves in high risk situation - herds with a high incidence of EEHV	EDTA WB, serum monthly*	
Birth of Asian calf	Umbilical cord and blood	Placenta, EDTA WB and serum from mom & baby	
Birth of African calf	Umbilical cord and blood	Placenta, EDTA WB and serum from mom & baby	
Unconfirmed illness**	EDTA WB	EDTA WB, serum, plus swabs/biopsies if lesions present, bank EDTA WB and serum from patient and every member of herd ***	
Confirmed illness prior to treatment	EDTA WB	EDTA WB, serum, saliva, urine**** on patient and every member of herd, banked EDTA WB and serum from patient and every member of herd up	10 – 100 ml of serum frozen and sent on dry ice to Houston Zoo, for viral sequencing

Confirmed illness after treatment	EDTA WB	to 3 weeks prior to diagnosis. EDTA WB, serum, saliva**** on patient and every member of herd	10 – 100 ml of serum frozen and sent on dry ice to Houston Zoo, for viral sequencing
Necropsy tissue from EEHV death in Asian or African elephant	EDTA WB, heart, liver, lung, spleen, tongue, skeletal muscle, brain, pericardial and peritoneal fluid.	Tongue, heart, liver, spleen, intestine, WB, serum, any tissue with a lot of hemorrhages. Any kind of nodules – skin, vestibular etc.	
Necropsy tissue from non-EEHV death in Asian or African elephant	2 sets of tissues for “normal” reference	Tongue, heart, liver, spleen, WB, serum. Any kind of nodules – skin, vestibular etc. Hilar, mandibular, thoracic and mesenteric lymph nodes. <b>Look hard for hidden lung lymphoid nodules (likely few, white to gray, spongy texture, small 3-30mm in diameter- will likely need to breadloaf the lung to locate and collect samples).</b>	
Seropositives		Periodic EDTA WB, serum, saliva, trunk washing, urine.	
Other samples		Semen aliquot each time collected	

**KEY**

NEHL – National Elephant Herpesvirus Laboratory

**HIGH PRIORITY**

\* after participation in the 2-year EEHV serology study and if alternate collection protocol is not provided.

\*\* anytime calf exhibits any abnormal behavior of lameness, stiffness, lethargy, in appetite to the more common symptoms of EEHV

\*\*\* samples will be important should EEHV be confirmed.

\*\*\*\* urine and saliva may or may not be useful prior to treatment – yet to be determined.

**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 \_\_\_\_\_

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

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

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

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

Attach any additional information. Send to: Michele Miller, Palm Beach Zoo, 1301 Summit Blvd., West Palm Beach, Florida 33405. Work: 561-833-7130 ext 224; cell: 561-727-9630; Fax: 561-833-7135; Email:mmiller@palmbeachzoo.org

## Elephant SSP Serum/Tissue 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: \_\_\_\_\_

---

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

Send completed form with samples to:

Dr. 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](mailto:mmiller@palmbeachzoo.org)

# Consent Form for Use of Sample by Elephant SSP

I give consent for the sample submitted to the Elephant Species Survival Plan (SSP) serum/tissue bank to be used for research on any elephant related issues based on recommendations by the veterinary advisor and/or steering committee.

The results could be reviewed and used by the SSP veterinary advisor in providing health-related recommendations and publications.

I understand that all results and recommendations regarding the individual elephant will be kept confidential.

\_\_\_\_\_ Yes, I agree to allow the SSP to use our sample for designated research and testing results.

\_\_\_\_\_ No, I do not consent to the use of our sample and test results unless specified.

\_\_\_\_\_  
Signature, title      Date

\_\_\_\_\_

\_\_\_\_\_  
Printed name      Phone

\_\_\_\_\_  
number

\_\_\_\_\_  
Institution      Em

\_\_\_\_\_  
ail address

\_\_\_\_\_  
Address

\_\_\_\_\_

\_\_\_\_\_

Comments: \_\_\_\_\_

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Feb 2010 mm

*Appendices*

***Instructions for Elephant Endotheliotropic Herpesvirus (EEHV)  
Sample Submission***

Please submit all samples on plenty of dry ice in a sealed styrofoam container. Please contact us for our FedEx number.

\*\*Please send at least 1-2 ml of whole blood (EDTA, lavender top tube). It is best to transfer the blood to a freezer-safe tube (not glass) after thoroughly mixing with EDTA. We have received broken glass tubes in the past.

\*\*Please send 3 serum tubes. Freeze ASAP.

\*\*Please collect the following tissues and place in labeled 50 ml tubes/Whirlpak bags/Ziploc bags. Freeze ASAP.

hilar and mandibular lymph nodes

heart

liver

spleen

hidden lung lymphoid nodules

skin or vestibular nodules

**FedEx to:**

**Erin Latimer/Laura Richman  
Smithsonian, National Zoological Park  
Department of Pathology  
3001 Connecticut Ave. NW  
Washington, DC 20008  
202-633-4252  
703-855-9611**

# Request for EEHV testing

Department of Pathology  
Smithsonian National Zoo  
3001 Connecticut Ave. NW  
Washington, DC 20008  
(202) 633-4252

Date \_\_\_\_\_

Requestor's name \_\_\_\_\_

Institution \_\_\_\_\_

Address \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Phone/email \_\_\_\_\_

Animal Name/ISIS # \_\_\_\_\_

Age \_\_\_\_\_

Species \_\_\_\_\_

Reason for request (i.e. current symptoms of possible EEHV, or possible exposure to  
EEHV) \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Type of samples being sent \_\_\_\_\_

Before sending samples, please call Erin Latimer 202-633-4252 (W)  
703-8 55-9611 (H)

Please send samples **overnight on dry ice in a sealed styrofoam container**. The best day to send samples is Wednesday, for Thursday delivery.

## **Baylor College of Medicine Trunk Wash Collection Protocol for EEHV Screens**

Generally follow standard trunk wash collection protocol for *M. tuberculosis* culture

- 1) Instill 50mL sterile saline solution into the nares.
- 2) Elevate the trunk 2-3ft for 30-60 seconds.
- 3) Have elephant blow into a clean container – use new ziplock/plastic bags for each sample to reduce the chance of cross contamination.
- 4) Transfer sample to a 50mL conical tube – the more mucous and exfoliated respiratory epithelial cells the better (SNOT IS GOOD).
- 5) Chill the sample on ice until it can be centrifuged.
- 6) Centrifuge at 1500Xg for 10min @ 4°C or room temperature.
- 7) Gently pour off and discard the supernatant.
- 8) Ideally the cell pellet is stored @ -80°C and shipped on dry ice, however it is acceptable to store cell pellets @ -20°C and ship on ice.

Questions: Call Jeff Stanton (713) 253-9282

Shipping Address: Jeff Stanton, DVM or Paul Ling, PhD

Department of Molecular Virology & Microbiology  
Baylor College of Medicine  
Mail Stop BCM-385  
One Baylor Plaza  
Houston, Texas 77030

## **EDTA WB, serum Sample collection for the EEHV Lab at the National Zoo Sample Handling for Prospective EEHV Study**

This is a blind study and in order to maintain confidentiality, please Contact Debbie Olson at [dolson@elephantconservation.org](mailto:dolson@elephantconservation.org) with your elephant studbook number and name. Debbie will provide a study number for each elephant

### **Serum**

Please collect serum weekly. Transfer to a plastic screw-cap tube after centrifuging for storage and shipping (the glass tubes break). We would like 2 ml, if possible. Store at -80 or in a NON-DEFROSTING freezer if an ultralow is not available until shipped.

### ***Whole blood***

Please collect whole blood weekly. Collect at least 1-2 ml whole blood in a purple top (EDTA) tube. Fill at least half full (to dilute out the EDTA), mix well and transfer to a plastic screw-cap tube for storage and shipping (the glass tubes break); store at -80 or in a NON-DEFROSTING freezer until shipped if an ultralow is not available.

With a Sharpie, please mark on the tube directly the animal's study number and the date collected. Please do not put the name of your institution on the tube.

Ship quarterly when requested by email to ship samples. Additional directions will be given in the email. Ship overnight with ice packs or dry ice.

### **FedEx to:**

**Erin Latimer/Laura Richman  
Smithsonian, National Zoological Park  
Department of Pathology  
3001 Connecticut Ave. NW  
Washington, DC 20008  
(202) 633-4252  
latimere@si.edu**

## **Protocol for collecting samples for Herpesvirus culture**

**Erin Latimer, Laura Richman, Gary Hayward**

**Placenta**-- freeze a 1 inch<sup>3</sup> piece (preferably in liquid nitrogen or dry ice, or store in a -70 C freezer); store at -80 or in a NON-DEFROSTING freezer until shipped.

**Swabs**—Wet a cotton swab with a small amount of sterile saline, swab lesion and place swab in a sterile 15 ml plastic test tube (or tube that swab came with); store at -80 or in a NON-DEFROSTING freezer until shipped.

**Saliva\***—scoop up saliva into a 15 ml sterile plastic tube; store at -80 or in a NON-DEFROSTING freezer until shipped.

**Necropsy tissues**—aseptically place 3 x 3 cm piece of tissue in a small Ziploc or WhirlPak bag. Label with type of tissue, elephant name, date. Please do not put more than one tissue type in a bag—each tissue type should go in a separate bag. Store at -80 or in a NON-DEFROSTING freezer until shipped.

**Trunk wash, urine\*, semen**—Place up to 5 ml in a sterile plastic tube; store at -80 or in a NON-DEFROSTING freezer until shipped.

\*EDTA WB, serum, saliva, urine from a confirmed case before treatment—please collect as much of each of these as possible for possible sequencing/viral isolation

**Shipping**—Please ship FedEx overnight; email the tracking number to [latimere@si.edu](mailto:latimere@si.edu). Please pack in a styrofoam cooler, with dry ice and plenty of ice packs. Do not send in a cardboard box without a cooler. The EEHV Lab will pay for shipping of samples from healthy elephants; please contact the lab for FedEx account information

### **Address for sample shipment:**

Erin Latimer  
Dept of Pathology  
National Zoo  
3001 Connecticut Ave, NW  
Washington, DC 20008  
202-633-4252

# **Elephant Endothelial Cells: a System to Isolate Elephant Endotheliotropic Herpesviruses**

## REQUEST FOR BLOOD SAMPLES FROM SICK ANIMALS

Noha Abou-Madi, Mary Beth Matychak, Julia Flaminio

Cornell University, College of Veterinary Medicine

February 2009 (updated)

### **Contact information:**

Mary Beth Matychak

Email: [mbm10@cornell.edu](mailto:mbm10@cornell.edu)

Cornell University, College of Veterinary Medicine

Ithaca, NY 14853

607 253-3493 (lab)

Noha Abou-Madi, DVM, MSc

Email: [na24@cornell.edu](mailto:na24@cornell.edu)

Cornell University, Department of Clinical Sciences

College of Veterinary Medicine

Ithaca, NY, 14853

607 253-3278 (off.) 607- 227-1238 (cell)

Julia Flaminio, DVM, PhD

Email: [mbf6@cornell.edu](mailto:mbf6@cornell.edu)

Cornell University, Department of Clinical Sciences

College of Veterinary Medicine

Ithaca, NY, 14853

607 253-3119 (off.) 607 253-3493 (lab)

**Methods:**

Briefly, elephant umbilical cord-derived endothelial cell monolayer cultures will be used to isolate EEHV from peripheral blood and post mortem tissues from clinical cases. Tissue and blood samples from infected or surviving animals will be tested for the presence of the virus using PCR and real time PCR (qPCR). African and Asian endothelial cells will be co-cultured with these samples, passaged and monitored for evidence of infection. At each passage, infected endothelial cells will be checked for presence of virus and viral replication using PCR and qPCR. Positive cell cultures will be passaged, and aliquots will be saved in liquid nitrogen and at -80°C. When viral titer in culture is high, immunohistochemistry and electron microscopy will be used. This project is approved by Cornell University IACUC.

**Samples requested:**

We understand the challenges associated with collecting blood in elephants especially when they are sick. We will take any volume of blood that is available, but would prefer a minimum of 10 ml (maximum of 30 ml) of whole blood collected in EDTA (purple top). Any size of EDTA tubes can be used; large tubes (10ml) may facilitate collection.

Ideally, the first blood sample should be collected prior to the start of the treatment with Fanciclovir (however, we will take any sample available). Then if the animal is bled again, samples collected every 6-8 hours will improve the recovery of the virus.

The samples should be sent for overnight delivery, on ice, to be delivered in the morning. Our FED EX account number is 1834-4049-7. The address and contact person are indicated below. Do not delay shipping samples if additional samples are collected and please send additional samples the following day if possible.

Mary Beth Matychak

Cornell University

College of Veterinary Medicine

Ithaca, NY 14853

607 253-3493

## **Request for permission to communicate results of EEHV testing:**

It is important for our laboratory to know if the blood samples collected are positive for EEHV using the tests developed by Laura Richman and Erin Latimer.

With this document, we (name of authorized person) \_\_\_\_\_

from (name of institution) \_\_\_\_\_

authorize Laura Richman and Erin Latimer to send us results of EEHV testing on (name of animal)

\_\_\_\_\_ .

Signature:

Name and Date:

# **Necropsy protocol for collecting necropsy samples for Herpesvirus culture**

Cornell University, College of Veterinary Medicine, Ithaca, NY

## **Contact information:**

### **Mary Beth Matychak**

Email: [mbm10@cornell.edu](mailto:mbm10@cornell.edu) Em  
Cornell University, College of Veterinary Medicine  
Ithaca, NY 14853 College  
607 253-3493 (lab) Ithaca,

### **Noha Abou-Madi, DVM, MSc**

ail: [na24@cornell.edu](mailto:na24@cornell.edu)  
Cornell University, Dept. of Clinical Sciences  
of Veterinary Medicine  
NY 14853  
607 253-3278 (off.) **607- 227-1238 (cell) best**

### **Julia Flaminio, DVM, PhD**

Email: [mbf6@cornell.edu](mailto:mbf6@cornell.edu)  
Cornell University, Department of Clinical Sciences  
College of Veterinary Medicine  
Ithaca, NY, 14853  
607 253-3119 (off.) 607 253-3493 (lab)

## **SAMPLE COLLECTION:**

We have shipped to your institution sterile vials (50ml) containing viral transport media (labeled and 3 extra), plastic bags, EDTA tubes and the return FED EX label. You can use the same box to ship the samples back to us. The diapers are used to absorb fluids in case of a spill in transit.

Our goal with this protocol is to optimize the recovery of LIVE viruses; if possible, a blood sample should be collected immediately post-mortem in EDTA tubes (purple top). We would prefer a volume of at least 10 ml and no more than 30ml, though we will take any volume that you may be able to collect. The sample should be shipped as soon as possible on ice packs for early morning delivery. See shipping address below.

During necropsy, samples measuring approximately 2-3 cm by 2-3 cm should be collected from the heart, lung, liver, spleen, tongue, skeletal muscle, and brain as cleanly (sterile?) as possible. Each sample should be placed individually in a tight container with antiviral transport media. Make sure the appropriate tube is used or correct the label with their origin. If available, pericardial and peritoneal fluid can be collected in the EDTA tubes. To prevent accidental spills, all the containers should be sealed and placed in 2 plastic bags. The sample should be shipped as soon as possible on ice packs for early morning delivery.

Our FED EX account number is: 1834-4049-7

Mary Beth Matychak  
Cornell University  
College of Veterinary Medicine  
Department of Clinical Sciences  
Room C3521  
Ithaca, NY 14853  
607 253-3493

IF THE SAMPLES ARE SHIPPED ON A FRIDAY (for Saturday delivery, please use my home address:

Noha Abou-Madi  
123 E. King Road Apt C1  
Ithaca, NY 14850  
607- 227-1238

Please do not hesitate to contact us if you have any question. Thank you for your collaboration in such difficult times.

Noha Abou-Madi, DVM, MSc ([na24@cornell.edu](mailto:na24@cornell.edu))

## **Viral Sequencing Of Frozen Serum From Confirmed EEHV Elephant At The Houston Zoo**

Houston Zoo  
Joseph P. Flanagan, DVM  
Houston Zoo Veterinary Hospital  
1513 North MacGregor  
Houston TX 77030  
Office: (713) 533 6628  
Main clinic number: (713) 533 6632  
Fax: (713) 533 6764  
[jflanagan@houstonzoo.org](mailto:jflanagan@houstonzoo.org)

Houston Zoo  
Lauren Howard, DVM, Dipl. ACZM  
Houston Zoo Veterinary Hospital  
1513 North MacGregor  
Houston TX 77030  
Office: (713) 533 6630  
Main clinic number: (713) 533 6632  
Fax: (713) 533 6764  
[lhoward@houstonzoo.org](mailto:lhoward@houstonzoo.org)

The Houston Zoo requests 10 -100 ml of serum from confirmed EEHV cases. This serum will be used for viral sequencing to better understand the epidemiology and spread of this deadly virus. Whole blood should be collected into serum separator tubes and centrifuged. Ten to 100 ml of serum should be placed in freezer-safe, screw top, plastic containers for storage, and labeled with date, time and elephant's identification information. Samples should be stored frozen in a -80 °C freezer if possible, if not, then short term (< 1 week) storage in a standard freezer is adequate. Samples should be shipped frozen on dry ice to Joe Flanagan, DVM, Houston Zoo Veterinary Hospital, 1513 North MacGregor, Houston, TX 77030, phone number for shipping is (713) 533 6632. Please call ahead and/or email ([jflanagan@houstonzoo.org](mailto:jflanagan@houstonzoo.org), [lhoward@houstonzoo.org](mailto:lhoward@houstonzoo.org), [lavendano@houstonzoo.org](mailto:lavendano@houstonzoo.org)) so we know to look for the sample. The sample will be stored at the Houston Zoo until it can undergo viral sequencing at the Baylor College of Medicine.

If there are questions or any clarification is required, please contact Joe Flanagan or Lauren Howard at the above numbers. If they are unavailable, secondary contact information is: Lisa Marie Avendano, Hospital Manager, (713) 533 6636, [lavendano@houstonzoo.org](mailto:lavendano@houstonzoo.org), or Stephanie Durkee, Medical Clerk, (713) 533 6626, [sdurkee@houstonzoo.org](mailto:sdurkee@houstonzoo.org).