

GUIDELINES FOR COMPREHENSIVE ELEPHANT HEALTH MONITORING PROGRAM
Elephant Species Survival Plan, American Zoo and Aquarium Association
May 2005

Routine health monitoring should be performed on all elephants on an ongoing basis. Animals should be trained to permit sampling and examination. The following protocol advises that specific baseline laboratory tests be performed for the purpose of evaluating current health status. Additional tests are recommended to increase baseline information on other diseases to determine their significance to elephant health. The final decision for specific procedures should be made by the institutional animal care and veterinary staff based on individual circumstances. For additional information, refer to the Elephant Husbandry Manual, AZA Standards for Elephant Management and Care, and the AAZV Preventive Medicine Recommendations. Additionally, it is recommended that the veterinarian review the behavioral profile of the individual animals with elephant management staff on a regular basis.

Minimum Database:

1. Signalment - age, sex, origin, studbook#, ISIS#, picture of individual (as viewed from the front and sides) should be included in the permanent record.
2. Anamnesis - summary of information regarding health screens, medical problems, diagnostic test results, and treatment over the previous year (complete "Individual Annual Elephant Medical Survey" form and send to SSP Veterinary Advisor – will be available in 2006).
3. Complete physical exam by a veterinarian familiar with elephant health problems. This should include a review of all systems.
4. Body weight – actual weight should be recorded at least annually.
5. Blood collection
 - a. Complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis
 - b. Serologic (ELISA) test for elephant herpesvirus – contact Erin Latimer to submit samples, or Dr. Laura Richman (1).
 - c. Bank a minimum of 10-20 ml serum (duplicate sample for SSP serum bank) – all banked samples should be labeled with species, studbook #, age, sex, and date collected. Use submission form for serum samples sent to the SSP serum bank (maintained at Disney's Animal Kingdom).
6. Serum progesterone analysis in females – Serial samples should be collected weekly on an ongoing basis to evaluate estrous cycles (2). Assays can be performed at the National Zoo's Conservation & Research Center or Southwest Missouri State University. Contact specific institutions (Dr. Janine Brown - CRC (National Zoo), Dr. Dennis Schmitt - S.M.S.U.) for submission instructions (see protocol below).
7. Fecal analyses
 - a. Parasite screen - Fecal samples should be collected at least semiannually; direct, flotation, and sedimentation should be performed on every sample to detect intestinal parasitism.

- b. Enteric pathogen screen - Aerobic culture of feces for enteric pathogens should include special media for the detection of *Salmonella spp.* Because *Salmonella* organisms may be shed intermittently, at least 3-5 fecal cultures should be performed (may be done on consecutive days) on an annual basis.
8. TB culture - refer to the current USDA Guidelines for the Control of Tuberculosis in Elephants (3). Protocol can be accessed on the USDA website: www.aphis.usda.gov/ac/ElephTBGuidelines2003.html. At this time, annual trunk wash cultures are the only required test; however, collection of other samples for research is strongly encouraged (minimum 2 ml serum collected at the time of trunk washes should be sent to Dr. Michele Miller for use in validating other serologic tests for TB diagnosis).
- a. Samples for cultures must be collected under the direct supervision of a licensed veterinarian.
 - b. Three trunk wash samples should be collected on separate days, ideally within a 7- day period. Trunk swabs are no longer acceptable.
 - c. All samples should be frozen immediately after collection and shipped frozen.
 - d. Ship by overnight express to NVSL (or other laboratory facility offering comparable procedures). Request mycobacterial culture with speciation (use VS Form 10-4 submission form for NVSL).
9. Vaccinations
- a. Tetanus toxoid *— annual vaccination with a commercial equine product is recommended. Follow label instructions for product use (usually 1 ml IM). Data are insufficient at this time to determine adequate protective vaccine doses and titers.
 - b. Rabies vaccine* - vaccination with a commercial killed rabies product approved for horses should be considered if the animal resides or will be traveling to an endemic area. Follow label instructions for product use (usually 2 ml IM). Vaccination with Imrab 3 has induced detectable titers to rabies virus in African elephants (M. Miller, pers. comm.)¹. Biannual vaccination is recommended. Data are insufficient at this time to determine adequate protective vaccine doses and titers.

* Both tetanus and rabies have been reported to occur in elephants (4, 5).

¹ This is based on the persistence of titers (>1:50) in one herd of African elephants in response to Imrab 3 for more than 2 years.

Additional Preventive Health Recommendations:

1. Serological screening for EMC (encephalomyocarditis virus), leptospirosis (multiple serovars), and WNV (West Nile Virus). Although these tests are not species-specific and have not been validated for elephants, they may detect cross-reactive antibodies in exposed animals. The presence of antibodies does not necessarily denote infection/disease. Encephalomyocarditis virus may cause clinical disease and death in elephants (6). Antibodies to leptospirosis have been detected in both Asian and African elephants (7, 8). At the time of this writing, EMC serologic testing was not available. Insufficient data is available at this time to determine the significance of WNV antibodies in elephants; it is important to include the history of exposure and vaccination to WNV when interpreting results.
2. Serum vitamin E levels – submit heparinized plasma to Michigan State University.
3. Reproductive tract examination – whenever feasible, a complete reproductive examination should be conducted which includes transrectal ultrasound, semen collection and analysis, serum collection (weekly best, or bi-weekly to monthly) for testosterone analysis, cytology and microbial cultures of the lower urogenital tract (to be screened for bacteria, Chlamydia, protozoa, and Herpesvirus). Herpesvirus has been identified in biopsies of vaginal lymphoid patches in an African elephant (9). A high prevalence of uterine leiomyomas has been observed in captive Asian elephants and could be detected via transrectal ultrasound (10). Since both of these conditions have potentially significant effects on reproduction, a careful evaluation is warranted if the animal is being considered for breeding. All elephants (male and female) over the age of 5 years should have both ultrasound and hormonal (testosterone in males; progesterone/LH in females) assessments performed.
4. Urinalysis – fluid and sediment evaluation of clean voided sample; +/- microbial culture.
5. Foot radiographs – baseline radiographs of all feet are strongly recommended; see Gage for description of technique (11). It may be appropriate to annually monitor selected elephants (i.e., those that have a history of chronic foot problems).
6. Ancillary diagnostic tests for tuberculosis – ELISA, etc. recommended for data gathering; see Guidelines for the Control of Tuberculosis in Elephants for current recommendations (3).
7. Other vaccination regimens will depend on regional requirements and exposure risks (consider vaccination for equine encephalitis viruses, Clostridial diseases, Leptospirosis). Insufficient information is available at this time to provide a recommendation for West Nile Virus vaccination of elephants. Contact the SSP veterinary advisor for current information.

Elephant Serum Bank Submission Form

Institution/owner: _____

Submitter: _____

Address: _____

Tel: _____ Fax: _____ Email: _____

Animal InformationAsian African ISIS# _____ Studbook # _____Name _____ Age: _____ actual estimateSex: 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 abnormalFasted: no yes – how long _____Weight _____ actual estimatedType of restraint: manual anesthetized/sedated behavioral controlTemperament 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

Disney's Animal Kingdom-Dept. of Vet. Services

1200 N. Savannah Circle East

Bay Lake, FL 32830-1000

(407) 939-7316; email: Michele.Miller@disney.com

TB ELISA SAMPLE SUBMISSION FORM FOR ELEPHANT SAMPLES (LAB 1)												PAGE ___ OF ___	
Please send samples to: Mycobacterium bovis Testing Laboratory Animal Population Health Institute Environmental Health Bldg. RM 107 Colorado State University Fort Collins, CO 80523-1676 Attn: Joni Phone: (970) 491-2379; Fax: (970) 491-2940						For Questions Regarding Sample Submission, Test Results, or Interpretation Please Contact: Dr. Scott Larsen School of Veterinary Medicine University of California Davis, CA 95616 Phone: (530) 752-6059; Fax (530) 752-0414 slarsen@ucdavis.edu						LAB USE ONLY JTPID: Date Rec'd: Location:	
SUBMITTED BY:				PHONE:								NUMBER OF SAMPLES:	
CLINIC/INSTITUTION:				FAX:									
ADDRESS:				E-MAIL:									
CITY: STATE: ZIP:				OWNER OF ANIMALS:									
Date Serum Taken	Elephant Name	Stud-book or ISIS #	Species	Sex	Age	Birth Date	Time at Current Facility	Trunk Wash Date	Trunk Wash Results	Skin Test Date	Skin Test Results	PCR Date	PCR Results
SAMPLE SUBMISSION 1. Samples should be submitted in 12 mm X 75 mm serology tubes or 1.7 ml microcentrifuge tubes. 2. Collect blood into red top clotting tube. Allow to clot, centrifuge, and transfer serum into another vial. 3. If possible, ship at least 3 ml of serum for each sample submitted. 4. Shipping boxes must say "REFRIGERATE UPON ARRIVAL." 5. Send via overnight shipment at 4C or frozen. Please take care that samples will not arrive on a weekend or holiday.													

Instructions for Elephant Endotheliotropic Herpesvirus (EEHV) Sample Submission

Please submit all samples on plenty of dry ice in a sealed styrofoam container.

- For a sick elephant with suspected active EEHV infection, 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.
- We can test any tissue for EEHV from deceased elephants, but it must be frozen (preferably in liquid nitrogen or dry ice, or store in a -70 C freezer). Heart, liver and spleen are the best organs to test for EEHV.
- We would like to test placenta from all newborn/stillborn/aborted elephants. Please freeze a 1 inch³ piece (preferably in liquid nitrogen or dry ice, or store in a -70 C freezer).
- For EEHV titers, please send at least 2 mls of serum (preferably more). Freeze immediately and send on dry ice.
- Please include all pertinent elephant information (including history) on the attached form.
- The best day to ship (FedEx) would be Wednesday for a Thursday arrival. Please call Erin or Laura before shipping samples:

Erin Latimer: 202-633-4252 (W)
703-471-2168 (H)
LatimerE@nzp.si.edu

OR

Laura Richman 301-398-4741 (W)
301-253-8723 (H)
RichmanL@MedImmune.com

FedEx to:

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

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)
703-471-2168 (H)

or Laura Richman 301-398-4741 (W)
301-253-8723 (H)

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

SERUM COLLECTION PROTOCOL FOR MONITORING REPRODUCTIVE ACTIVITY IN ELEPHANTS

Janine L. Brown, PhD

Elephant SSP Reproductive Advisor
Endocrine Research Laboratory

Females

- Blood samples should be collected once weekly to establish if females exhibit normal ovarian cycles (e.g., estrous cyclicity). **Note:** the Elephant SSP requires that females between 8 and 35 years of age be hormonally assessed, and recommends that all elephants be monitored throughout their lifespan.

Males

- Blood samples should be collected weekly (bi-weekly or monthly if weekly is not possible) to assess testicular steroidogenic activity. **Note:** the Elephant SSP recommends that elephant bulls be monitored throughout their adult lifespan.

Sample collection and shipment

- For best results, allow blood to clot for ~1 hour at room temperature, or for ~2 hours at refrigerator temperature. Avoid exposing blood to ambient temperatures for longer than 3 hours (blood cells can metabolize progesterone and affect results). Plasma (collected in either EDTA or heparinized tubes) can also be analyzed, although serum is preferred.
- Centrifuge blood (~1000 x g for 10-15 min) and decant serum into a polypropylene vial (best sizes, 12 x 75 mm or 12 x 55 mm) with a tight-fitting cap that pushes or screws on and is flush with the tube (i.e., cap doesn't hang over the side of the tube). We recommend tubes with a frosted writing space and caps from the Sarstedt company (1-800-257-5101). The catalogue number for the tubes is 62.526.003 PP, and for the push caps is 65.809. Smaller tubes are okay, but do not use ones that exceed a 5-ml capacity. Information on the tube should include: animal name, date (mo/day/year), and facility name or abbreviation. Please provide a minimum 1 ml of serum for each sample. Store frozen (-20°C or colder).
- Ship samples in a styrofoam container with dry ice or cold packs. We will return box and any shipping materials. Use an overnight express courier (e.g., Airborne Express or FedEx) and ship only on Mon, Tues or Wed. Never ship on Fri or before a government holiday.
- Include in the shipment a written request as to what hormone analyses are required. If you need results immediately (emergencies only, please), let us know in the paperwork you provide. If you will be requesting Prolactin or LH (or any other protein hormone), call a week in advance so that we can schedule an iodination.
- Address samples to: Nicole Abbondanza, Conservation and Research Center, 1500 Remount Rd., Front Royal, VA 22630. Please notify us when samples are shipped (540-635-6521, ext. 225; abbondanzan@crc.si.edu).
- We will issue an invoice at the time data are sent. Please make checks payable to: Conservation & Research Center Foundation, c/o Janine Brown.
- For our records, please provide the studbook number, name and age of your elephant(s). If you have any questions, please contact Dr. Janine Brown: phone (540) 635-6586, fax (540) 635-6506, email jbrown@crc.si.edu. Lab hours are 8:00 am-5:00 pm est.

REFERENCES

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Available on the Internet: www.aphis.usda.gov/ac/ElephTBGuidelines2003.html
For regulatory questions, contact:
Dr. Denise Sofranko
USDA, APHIS, Animal Care
1629 Blue Spruce Drive, Suite 204
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Voice Mail (703) 812-6682; FAX: (505) 293-7466
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4. Burke, T.J. 1975. Probable Tetanus in an Asian Elephant. *J. Zoo Anim. Med.* 6:22-24.
5. Wimalaratne, O. and D.S. Kodikara. 1999. First Reported Case of Elephant Rabies in Sri Lanka. *Vet. Rec.* 144:98.
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APPENDIX

*Elephant SSP Veterinary Advisor

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Dr. Laura Richman
(301) 398-4741
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National Veterinary Services Laboratories (NVSL)
Dr. Janet Payeur
1800 Dayton Rd.
Ames, IA 50011
(515) 663-7676 or 663-7548 FAX: (515) 663-7315
Email: Janet.B.Payeur@usda.gov

TB ELISA – Dr. Scott Larsen
School of Veterinary Medicine
University of California
Davis, CA 95616
(530) 752-6059
Email: slarsen@ucdavis.edu
See attached submission form for samples

For Leptospiral and WNV titers, contact:
Cornell University, College of Vet. Med.
Diagnostic Lab
Upper Tower Rd.
Ithaca, NY 14853
(607) 253-3900

For vit E analysis, contact:
Michigan State University
Animal Health Diagnostic Lab
4125 Beaumont Rd.
East Lansing, MI 48910
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Other contacts for general elephant medical information:

Dr. Susan Mikota
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