

GUIDELINES FOR COMPREHENSIVE ELEPHANT HEALTH MONITORING PROGRAM

March 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 body weights should be recorded at least annually.
5. Blood collection
 - a. Complete blood count (CBC), serum chemistry panel, fibrinogen, serum protein electrophoresis, evaluated at least annually
 - 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 for your institution and collect a duplicate sample for the 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). Samples should be taken at least annually; ideally during the time trunk wash samples are collected.
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). Other labs are available to run progesterone

assays – please contact SSP advisors for current list. For those institutions considering breeding cows, LH assays should also be included to predict specific breeding dates. These can be run at the same labs.

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 (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). **Verify that your overnight carrier that not irradiate packages.**

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 (Merial) has induced detectable titers to rabies virus in African elephants (M. Miller, pers. comm.). Biannual vaccination is recommended.¹ 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 (Merial) 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 (see appendix for submission information).
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 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 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

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 ___

Mycobacterium bovis Testing Laboratory
Center of Veterinary Epidemiology and
Animal Disease Surveillance Systems
Colorado State University
Environmental Health Bldg. RM 107
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. R Scott Larsen
School of Veterinary Medicine
University of California
Davis, CA 95616
Phone: (530) 752-1393; 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.

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.

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@si.edu

OR

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

FedEx to:

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

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

I give consent for the results of Elephant Endothelial Herpes Virus (EEHV) testing to be used by the Species Survival Plan (SSP) and the National EEHV Laboratory to make recommendations on any elephant related issues (including, but not limited to, recommendations on relocations and breeding).

The results that could be reviewed and used by the SSP and the National EEHV Lab include Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA), and DNA sequencing of the EEHV virus.

I understand that all results and recommendations will be kept confidential.

_____ Yes, I agree to allow the SSP and the National EEHV Lab to use our testing results.

_____ No, I do not consent to the use of our testing results.

Signature, title

Date

Printed name

Phone number

Institution

Email address

Address

REFERENCES

1. Richman, L.K., R.J. Montali, R.C. Cambre, D. Schmitt, D. Hardy, T. Hildebrandt, R.G. Bengis, F. M. Hamzeh, A. Shahkolahi, and G.S. Hayward. 2000. Clinical and Pathological Findings of a Newly Recognized Disease of Elephants Caused by Endotheliotropic Herpesviruses. *J. Wildl. Dis.* 36:1-12.
2. Brown, J.L. 2000. Reproductive Endocrine Monitoring of Elephants: An Essential Tool for Assisting Captive Management. Special Issue on Elephant Biology. *Zoo Biol.* 19:347-368.
3. Guidelines for the Control of Tuberculosis in Elephants.
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
Ft. Collins, CO 80524-2013
Voice Mail (703) 812-6682; FAX: (505) 293-7466
Email: Denise.M.Sofranko@aphis.usda.gov
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

Dr. Michele Miller
Disney's Animal Kingdom
Dept. of Veterinary Services
P.O. Box 10,000
Lake Buena Vista, FL 32830-1000
(407) 939-7316 FAX: (407) 938-1909
Email: Michele.Miller@disney.com

*Elephant SSP Pathology Advisor
Dr. Scott Terrell
Disney's Animal Kingdom
Dept. of Veterinary Services
P.O. Box 10,000
Lake Buena Vista, FL 32830-1000
(407) 938-2746 FAX: (407) 938-1909
Email: Scott.Terrell@disney.com

*Elephant SSP Reproductive Advisor
Dr. Janine Brown
Conservation and Research Center
1500 Remount Rd.
Front Royal, VA 22630
(540) 635-6586 FAX: (540) 635-6506
Email: jbrown@crc.si.edu

*Elephant SSP Nutritional Advisor
Dr. Ellen Dierenfeld
St. Louis Zoological Park
Forest Park
1 Government Dr.
St. Louis, MO 63110-1396
Email: dierenfeld@stlzoo.org

Dr. Laura Richman
(301) 398-4741
RichmanL@MedImmune.com

Erin Latimer (EEHV tech)
Dept. of Pathology
Smithsonian National Zoo
3001 Connecticut Ave., NW
Washington, D.C. 20008
(202) 633-4252 FAX: (202) 673-4660

Dr. Dennis Schmitt

SMSU – Agriculture Dept.
217 Karl's Hall
901 South National Ave.
Springfield, MO 65804
(417) 836-5091 FAX: (417) 836-6979
Email: dennisschmitt@smsu.edu

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

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

For vitamin E samples, contact:
Michigan State University
Animal Health Diagnostic Lab
4125 Beaumont Rd
East Lansing, MI 48910-8104
(517) 353-1683
www.AHDL.MSU.edu

TB ELISA – Dr. Scott Larsen
(916) 264-8808
Email: slarsen@ucdavis.edu
See attached submission form for samples

Other contacts for general elephant medical information:

Dr. Susan Mikota
438 N. Central Ave.
Waveland, MS 39576
(228) 467-9622
Email: smikota@yahoo.com

Dr. Genny Dumonceaux
Busch Gardens Tampa Bay
P.O. Box 9158
Tampa, FL 33674-9158
(813) 987-5561
Email: Genevieve.dumonceaux@anheuser-busch.com