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**VETERINARY RESEARCH AND NECROPSY PROTOCOL FOR  
RHINOCEROS  
March 2016**

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## INTRODUCTION HOW TO USE THIS MANUAL

Thank you for your efforts to collect samples for the numerous research projects regarding the health of rhinoceroses. To date, your cooperative efforts have supplied a variety of research projects with material for their studies. Numerous scientific publications and presentations have resulted. Most importantly, these findings are building a firmer base for our understanding of rhinoceros health.

To submit samples, just go through the following steps:

1. Collect the appropriate sample as outlined in the “Summary of Tests and Samples”.
2. Make copies of the “Submission Form” for each animal and collection date. Once it is completed, it can be copied and mailed to each researcher.
3. Store or ship the samples as outlined in the “Summary of Tests and Samples”. Addresses for express shipments are listed in the Directory of Researchers and Veterinary Advisors.
4. If collecting samples for a suspected specific disease or study, check the list of recommended diagnostic and research samples and Summary of Research Project Information.
5. If possible, contact each researcher prior to shipment of samples to check availability for receipt or additional instructions. Names and phone numbers, email and FAX numbers are located in the Directory of Researchers and Scientific Advisors. **NOTE: some of the tests listed require a lab fee; please check the listed information carefully. An asterik (\*) indicates a fee-based test. Call the contact individual with any specific questions.**
6. If you have specific questions, consult with the individual included in the description of each project. If you have additional questions, please contact:

Michele Miller

Veterinary advisor, Rhinoceros TAG and Black rhinoceros SSP

Phone: +27 (0)73 812 9141

Email: michelemiller128@gmail.com

OR

R. Eric Miller

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Phone: 314- 646-4557

FAX: 314- 647-7969

Email: remiller@stlzoo.org

**RHINOCEROS SAMPLE SUBMISSION FORM**  
(MAKE EXTRA COPIES AS NEEDED)

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Please fill out one form for sample collection date. Please make copies and send one to each researcher that receives samples.

SUBMITTER: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

PHONE/FAX: \_\_\_\_\_

EMAIL: \_\_\_\_\_

SPECIES: \_\_\_\_\_  
(If possible, please indicate subspecies; e.g., eastern or southern black rhinoceros)

SEX: \_\_\_\_\_ ANIMAL NAME: \_\_\_\_\_

STUDBOOK NUMBER: \_\_\_\_\_ (Other ID only if Studbook number not available)

DATE OF BIRTH (MON/DAY/YR): \_\_\_\_\_  
(If unknown, give approx. age)

DATE SAMPLE COLLECTED (MON/DAY/YR): \_\_\_\_\_

SAMPLE COLLECTION INFORMATION: (check all that apply)  
 serum  hep. plasma  EDTA plasma  
 EDTA whole blood  hep whole blood  other: specify \_\_\_\_\_

Volume \_\_\_\_\_ Tissue(s) \_\_\_\_\_

Processed within:  <2 hrs  2-12 hrs  >12 hrs  
Sample storage:  frozen immediately  refrigerated  room temp.  
 other: specify \_\_\_\_\_

Sample collected:  live animal  post-mortem

REASON SAMPLE COLLECTED (ROUTINE EXAM, ILL, ETC):

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OTHER RELEVANT INFORMATION:

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Please submit this form along with any samples to the appropriate recipient/researcher.

## SUMMARY OF TESTS AND SAMPLES

Codes:

ON = overnight express

DI = dry ice

WI = wet ice

### BLOOD COLLECTION SAMPLES

<u>Researcher/Purpose</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
Rhino TAG/future research	Serum and hep plasma (2-4 ml aliquots; at least 2-4 aliquots)	*Please store at current facility	N/A Freeze (-80°C)
Dr. Oliver Ryder/ Genetics	10-20 ACD or hep whole blood	Ship same or next day	ON, room temp

### GENETICS/SKIN BIOPSY SAMPLES

<u>Researcher/Purpose</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
Dr. Oliver Ryder/ Genetics	Skin biopsy in bx media (vials provided)	Ship same or next day	ON, room temp

### REPRODUCTIVE/SPERM BANK SAMPLES

<u>Researcher/Purpose</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
Dr. Terri Roth/ Sperm Rescue	Both testicles with epididymus and vas deferens	Ship day of collection	ON, WI

### NECROPSY TISSUES

<u>Researcher/Test</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
Dr. Mary Duncan/ Pathology *Black rhino only	Full tissue set in formalin plus sections of main organs in glutaraldehyde	Ship at zoo's convenience	Regular US mail
Dr. Mike Kinsel/ Pathology *White rhino only	Full tissue set in formalin 50-100 g of liver, spleen, kidney, heart, brain	Ship at zoo's convenience Freeze (-80°C) Ship at zoo's convenience	Regular US mail ON, DI

<u>Researcher/Test</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
Dr. Trupkiewicz/ Pathology *Indian rhino only	Full tissue set in formalin	Ship at zoo's convenience	Regular US mail
Rhino TAG Tissue Bank	200 grams each: liver, kidney, spleen	Freeze (-80°C)	N/A
Dr. Eric Miller/ Encephalomalacia	½ brain in formalin ½ brain frozen	Ship at zoo's convenience (-80°C)	Regular US mail ON, DI
Dr. Oliver Ryder Genetics	2 g heart	Freeze (-80°C) Ship at zoo's convenience	ON, DI

**INDIVIDUALS INTERESTED IN PARTICIPATING IN RHINO NECROPSIES**

Dr. Kirk Suedmeyer                      Kansas City Zoo  
 Would appreciate opportunity to participate in necropsy of any male black rhinoceros.

**CHECKLIST OF SAMPLES TO COLLECT  
ROUTINE BLOOD SAMPLING (INCLUDING SICK RHINOS)**

<b>Sample</b>	<b>Volume</b>	<b>Investigator</b>	<b>Shipping</b>	<b>Project</b>
Serum	4-10 ml (2-4 aliquots of 2 mls serum – freeze at -80°C and store at current facility)	Rhino TAG	N/A	Resource bank
Heparinized whole blood	20 ml (2 x 10 ml ACD or hep blood tubes – leave as whole blood)	Dr. Oliver Ryder	ON, room temp	Genetics

**NECROPSY TISSUE CHECKLIST**

**All Rhino Species**

<b>Tissue</b>	<b>Amount</b>	<b>Investigator</b>	<b>Shipping</b>	<b>Project</b>
Brain-frozen	200 grams	Dr. Eric Miller	ON, DI	Tissue bank
Heart – frozen	2 grams	Dr. Oliver Ryder	ON, DI	Genetics
Skin biopsy – fresh/room temp Punch biopsy In bx media (vials provided)		Dr. Oliver Ryder	ON, room temp	Genetics
Testicles-fresh/cooled	Both testes	Dr. Terri Roth	ON, WI	Sperm Bank

**Black Rhinoceros Only**

<b>Tissue</b>	<b>Investigator</b>	<b>Shipping</b>	<b>Project</b>
Complete set of formalinized tissues or slide set (see below for tissue list)	Dr. Duncan	US Mail	Pathology
1 mm squares of major tissues in glutaraldehyde	Dr. Duncan	US Mail	Pathology
Liver, kidney, spleen, heart, duodenum- 200 grams of each, frozen at -80°C	Rhino TAG	N/A	Store at own facility



**Greater Asian One-Horned Rhinoceros Only**

<b>Tissue</b>	<b>Investigator</b>	<b>Shipping</b>	<b>Project</b>
Complete set of formalinized tissues	Dr. Trupkiewicz	US Mail	Pathology

**White Rhinoceros Only**

<b>Tissue</b>	<b>Investigator</b>	<b>Shipping</b>	<b>Project</b>
Complete set of formalinized tissues	Dr. Kinsel	US Mail	Pathology
Liver, kidney, spleen, heart, brain- 50-100 grams of each, frozen at -80°C	Dr. Kinsel	ON, DI	Pathology

**Basic Checklist of Tissues to Collect in Formalin (Priority Samples in Bold)**

Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. Freeze 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) in small plastic bags. Freezing at -80 degrees Celsius in an ultra-low freezer is preferred.

- |  |  |   |                                  |
|--|--|---|----------------------------------|
| <input type="checkbox"/> <b>Adrenal</b>  | <input type="checkbox"/> <b>Kidney</b>   | <input type="checkbox"/> Penis            | <input type="checkbox"/> Thymus  |
| <input type="checkbox"/> Blood   | <input type="checkbox"/> Large intestine | <input type="checkbox"/> Pituitary        | <input type="checkbox"/> Tongue  |
| <input type="checkbox"/> Bone with marrow  | <input type="checkbox"/> <b>Liver</b>    | <input type="checkbox"/> Prostate         | <input type="checkbox"/> Trachea |
| <input type="checkbox"/> Bulbo-urethral gland  | <input type="checkbox"/> <b>Lung</b>     | <input type="checkbox"/> Salivary gland   |                                  |
| <input type="checkbox"/> Brain   | <input type="checkbox"/> Parathyroid     | <input type="checkbox"/> Seminal vesicles |                                  |
| <input type="checkbox"/> Cecum   | <input type="checkbox"/> Mammary gland   | <input type="checkbox"/> Skin             | <input type="checkbox"/> Ureter  |
| <input type="checkbox"/> Diaphragm<br>bladder  | <input type="checkbox"/> <b>Muscle</b>   | <input type="checkbox"/> Small intestine  | <input type="checkbox"/> Urinary |
| <input type="checkbox"/> Esophagus   | <input type="checkbox"/> Nerve (sciatic) | <input type="checkbox"/> Spinal cord      | <input type="checkbox"/> Vagina  |
| <input type="checkbox"/> Eye   | <input type="checkbox"/> Ovary/testis    | <input type="checkbox"/> <b>Spleen</b>    | <input type="checkbox"/> Uterus  |
| <input type="checkbox"/> Epididymus  |  |   |                                  |
| <input type="checkbox"/> <b>Heart/aorta</b>  | <input type="checkbox"/> <b>Pancreas</b> | <input type="checkbox"/> Stomach          | <input type="checkbox"/> Thyroid |
| <input type="checkbox"/> <b>Lymph nodes</b> (sampling of head/neck, thoracic, abdominal, and peripheral) |  |   |                                  |
| <input type="checkbox"/> Any abnormal fluids   |  |   |                                  |

**Recommended Diagnostic/Necropsy Testing for Suspected Specific Diseases or Studies in All Rhino Species (\* indicates fee-based test – paid by submitting institution)**

<b>Tissue</b>	<b>Investigator</b>	<b>Shipping</b>
<b>Encephalomalacia</b>		
Brain – ½ in formalin 100-200 grams frozen	Dr. Eric Miller	US Mail ON, DI
Feed samples – frozen at –80°C	Dr. Eric Miller	ON, DI
Liver – 100 grams frozen at –80°C	Dr. Eric Miller	ON, DI

**Iron overload disorder (iron storage disease) of browsing rhinos**

Iron profile testing may be performed in commercial diagnostic and research laboratories. The KSU laboratory is the commercial laboratory with a ferritin assay for rhinoceros.

\*Tests available: serum iron, TIBC, ferritin, transferrin saturation, haptoglobin. In addition, research laboratories are investigating validation of new potentially more specific tests for iron overload disorder in rhinoceros. This will require opportunistic sample collection as described below. For rhinos without clinical signs or abnormal iron profiles, samples should be collected and banked at least annually.

Current testing can be performed by:

1 ml serum (or hep plasma)	KSU Vet. Med. Center Mosier Hall Comparative Hematology 1800 Denison Avenue Manhattan, KS 66506-5605 Phone: 785-532-4424	ON, WI
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For research testing:

2 ml serum stored in four different vials (0.5 cc/vial)

200 grams frozen liver

**Please store these samples at your facility for future projects.**

**\*Indicates charge for testing – contact laboratory for current information regarding costs, submission details**

## SUMMARY OF RESEARCH PROJECT INFORMATION

*Please review shipping information carefully. Unless otherwise noted, all shipping is paid by submitter. Some tests may require additional lab fees – contact investigator PRIOR to shipping.*

### **Black Rhinoceros SPP Serum and Tissue Bank**

These materials are vital to maintaining a resource for research on all rhinoceros species. However due to a lack of a centralized bank, each facility will need to store samples at their location.

Shipping information for samples: Samples may be requested for specific research projects through the SSP/TAG. Shipping arrangements should be made directly between the institution and researcher.

### **Investigation of Encephalomalacia Cases**

Researcher: Dr. Eric Miller, St. Louis Zoo

Although encephalomalacia has occurred primarily in young animals, it should be considered in all deaths of unknown cause. Symptoms can vary from hyperexcitability to hyperthermia, to a nearly comatose state.

Shipping information: Ship to address listed in investigator directory (Dr. Eric Miller). Frozen samples should be shipped overnight on dry ice. Formalin tissues can be sent by US mail.

### **Sperm Rescue and Banking**

Researcher: Dr. Terri Roth, CREW, Cincinnati Zoo

Dr. Roth has developed successful methods for cryopreserving epididymal sperm samples and is now actively establishing a rhino sperm bank. Genetically valuable (under-represented) males of all species are especially important.

Shipping Information: Ship to Dr. Terri Roth, Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 (see contact information in investigator directory). Remove testes from animal as soon as possible following death. Place in zip-lock baggies with saline-moistened gauze. Surround plastic bag with paper towels for insulation then place in Styrofoam cooler on one or two frozen cold packs **making sure the tissue is not in direct contact with the cold packs** and ship overnight.

### **Genetic Bank on All Rhino Species**

Researcher: Dr. Oliver A. Ryder, Institute for Conservation Research, San Diego Zoo Global

Dr. Ryder and Marlys Houck are interested in receiving samples from any species of rhinoceros for genetic analysis, cell culture and banking. Any or all of the following samples would be welcomed.

1. Skin biopsy in biopsy media – shipped the same or next day at room temperature; vials with media are available from the researchers upon request. Can place bx in sterile vacutainer (no additives) if no vials on hand.
2. ACD or heparinized whole blood, 2 x 10 ml tubes – shipped same as for skin biopsy.
3. 2 gm heart muscle – ship overnight on dry ice.

If additional information is needed, please contact Marlys Houck, Senior Researcher, Cytogenetics, at San Diego Zoo Global's Institute for Conservation Research (formerly CRES); Email: [mhouck@sandiegozoo.org](mailto:mhouck@sandiegozoo.org).

**VETERINARY AND SCIENTIFIC ADVISORS  
RHINOCEROS SSPs AND TAGs**

**Rhinoceros Taxon Advisory Group (TAG)**

TAG Chair: Steve Shurter  
Research Advisor: Dr. Terri Roth  
Veterinary Advisor: Dr. Michele Miller  
Co-Advisor: Dr. Eric Miller

**Black Rhinoceros SSP**

SSP Coordinator: Lisa Smith  
Veterinary Advisor: Dr. Michele Miller  
Pathology Advisor: Dr. Mary Duncan  
Nutritional Advisor: Katie Sullivan

**White Rhinoceros SSP**

SSP Coordinator: Adam Eyres  
Veterinary Advisor: Dr. Jeff Zuba  
Pathology Advisor: Dr. Michael Kinsel

**Greater Asian One-Horned Rhinoceros SSP**

Species Coordinator: Randy Rieches  
Veterinary Advisor: Dr. Barb Wolfe  
Pathology Advisor: Dr. John Trupkiewicz  
Nutritional Advisor: Kerrin Grant

**ALPHABETICAL DIRECTORY OF ADDRESSES OF  
RESEARCHERS AND SCIENTIFIC ADVISORS**

Dr. Scott Citino  
White Oak Conservation Center  
White Oak Plantation  
3823 Owens Road  
Yulee, Florida 32097-2145  
Phone: (904) 225-3387  
Email: scottc@wogilman.com

Dr. Pam Dennis  
Cleveland Metroparks Zoo  
4200 Wildlife Way  
Cleveland, OH 44109  
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Dr. Mary Duncan  
St. Louis Zoo  
Veterinary Hospital  
One Government Drive  
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Phone: 314-646-4634  
FAX: 314-646-5534  
Email: duncan@stlzoo.org

Adam Eyres  
Fossil Rim Wildlife Center  
Phone: 254-898 4230  
Email: adame@fossilrim.org

Kerrin Grant, M.S.  
Wildlife Care Director/Nutritionist  
The Wildlife Center  
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Española, NM 87532  
Phone: 505-753-9505  
Email: kerrin@thewildlifecenter.org

Barbara Henry, M.S.  
Cincinnati Zoo & Botanical Garden  
3400 Vine Street  
Cincinnati, OH 45220  
Phone: 513-487-3323  
FAX: 513-559-7773  
Email: barbara.henry@cincinnati-zoo.org

Dr. Michael Kinsel  
University of Illinois  
Zoological Pathology Program  
LUMC Bldg. 101 Rm. 0745  
2160 South First Ave.  
Maywood, IL 60153  
Phone: 708-216-1185  
FAX: 708-216-5934  
Email: mkinsel@lumc.edu

Ship tissues to:  
Brookfield Zoo  
Attn: Dr. Kinsel  
3300 Golf Road  
Brookfield, IL 60513-1060

Dr. Eric Miller  
St. Louis Zoo  
1 Government Drive  
St. Louis, MO 63110-1396  
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Dr. Robin Radcliffe  
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Cornell University  
C2-508 Clinical Programs Center  
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Dr. Terri Roth  
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Center for Conservation and Research of Endangered Wildlife (CREW)  
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Cincinnati, OH 45220  
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FAX: 513-569-8213  
Email: terri.roth@cincinnati-zoo.org

Dr. Oliver Ryder  
Attn: Marlys Houck, Senior Researcher, Cytogenetics  
Institute for Conservation Research (formerly CRES)  
San Diego Safari Park (formerly Wild Animal Park)  
15600 San Pasqual Valley Road  
Escondido, CA 92027-7000  
Phone: 760-291-5454 or 760-747-8702 x5716  
Email: mhouck@sandiegozoo.org

Steve Shurter  
Phone: 904-225-3290  
FAX: 904-225-3395  
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Lisa Smith  
Great Plains Zoo and Delbridge Museum of Natural History  
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Katie Sullivan M.S.  
Disney's Animals, Science and Environment  
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Dr. Jeff Zuba  
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Email: jzuba@sandiegozoo.org