VETERINARY RESEARCH AND NECROPSY PROTOCOL FOR RHINOCEROS

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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

Veterinary Advisor, Rhinoceros TAG

Phone: 314- 646-4557

FAX: 314- 647-7969

Email: remiller@stlzoo.org

RHINOCEROS SAMPLE SUBMISSION FORM

(MAKE EXTRA COPIES AS NEEDED)

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):

OTHER RELEVANT INFORMATION:

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

Researcher/Purpose Sample Time of Shipment How to Ship

Rhino TAG/future research Serum and \*Please store at current N/A hep plasma facility Freeze (2-4 ml aliquots; at least 2-4 aliquots) (-80°C)

Dr. Oliver Ryder/ 10-20 ACD or Ship same or next day ON, room Genetics hep whole blood temp

**GENETICS/SKIN BIOPSY SAMPLES**

Researcher/Purpose Sample Time of Shipment How to Ship

Dr. Oliver Ryder/ Skin biopsy in bx Ship same or next day ON, room

Genetics media (vials provided) temp

# REPRODUCTIVE/SPERM BANK SAMPLES

Researcher/Purpose Sample Time of Shipment How to Ship

Dr. Terri Roth/ Both testicles Ship day of collection ON, WI

Sperm Rescue with epididymus

and vas deferens

**NECROPSY TISSUES**

Researcher/Test Sample Time of Shipment How to Ship

Dr. Mary Duncan/ Full tissue set in Ship at zoo’s convenience Regular US

Pathology formalin plus mail

\*Black rhino only sections of main

organs in

glutaraldehyde

Dr. Mike Kinsel/ Full tissue set in Ship at zoo’s convenience Regular US

Pathology formalin mail

\*White rhino only 50-100 g of Freeze (-80°C) ON, DI

liver, spleen, Ship at zoo’s convenience

kidney, heart,

brain

Researcher/Test Sample Time of Shipment How to Ship

Dr. Trupkiewicz/ Full tissue set in Ship at zoo’s convenience Regular US

Pathology formalin mail

\*Indian rhino only

Rhino TAG 200 grams each: Freeze (-80°C) N/A

Tissue Bank liver, kidney,

spleen

Dr. Eric Miller/ ½ brain in formalin Ship at zoo’s convenience Regular US

Encephalomalacia mail

½ brain frozen (-80°C) ON, DI

Dr. Oliver Ryder 2 g heart Freeze (-80°C) ON, DI

Genetics Ship at zoo’s convenience

**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)**

## Sample Volume Investigator Shipping Project

Serum 4-10 ml Rhino TAG N/A Resource bank

(2-4 aliquots of 2 mls serum – freeze at -80°C and store at current facility)

Heparinized 20 ml Dr. Oliver Ryder ON, room temp Genetics

whole blood (2 x 10 ml ACD or hep blood tubes – leave as whole blood)

**NECROPSY TISSUE CHECKLIST**

**All Rhino Species**

## Tissue Amount Investigator Shipping Project

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 Dr. Oliver Ryder ON, room temp Genetics

In bx media (vials provided)

Testicles-fresh/cooled

Both testes Dr. Terri Roth ON, WI Sperm Bank

**Black Rhinoceros Only**

## Tissue Investigator Shipping Project

Complete set of formalinized tissues Dr. Duncan US Mail Pathology

or slide set (see below for tissue list)

1 mm squares of major tissues Dr. Duncan US Mail Pathology

in glutaraldehyde

Liver, kidney, spleen, heart, duodenum- Rhino TAG N/A Store at own

200 grams of each, frozen at -80°C facility

**Greater Asian One-Horned Rhinoceros Only**

## Tissue Investigator Shipping Project

Complete set of formalinized tissues Dr. Trupkiewicz US Mail Pathology

**White Rhinoceros Only**

## Tissue Investigator Shipping Project

Complete set of formalinized tissues Dr. Kinsel US Mail Pathology

Liver, kidney, spleen, heart, brain- Dr. Kinsel ON, DI Pathology

50-100 grams of each, frozen at –80°C

**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.

⁪**Adrenal** ⁪**Kidney** ⁪Penis ⁪Thymus

⁪Blood ⁪Large intestine ⁪Pituitary ⁪Tongue

⁪Bone with marrow ⁪**Liver**  ⁪Prostate ⁪Trachea

⁪Bulbo-urethral gland ⁪**Lung**  ⁪Salivary gland

⁪Brain ⁪Parathyroid ⁪Seminal vesicles

⁪Cecum ⁪Mammary gland ⁪Skin ⁪Ureter

⁪Diaphragm ⁪**Muscle**  ⁪Small intestine ⁪Urinary bladder

⁪Esophagus ⁪Nerve (sciatic) ⁪Spinal cord ⁪Vagina

⁪Eye ⁪Ovary/testis ⁪**Spleen** ⁪Uterus

⁪Epididymus

⁪**Heart/aorta** ⁪**Pancreas** ⁪Stomach ⁪ Thyroid

⁪**Lymph nodes** (sampling of head/neck, thoracic, abdominal, and peripheral)

⁪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)

**Tissue Investigator Shipping**

#### Encephalomalacia

Brain – ½ in formalin Dr. Eric Miller US Mail

100-200 grams frozen 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 ON, WI

Mosier Hall

Comparative Hematology

1800 Denison Avenue

Manhattan, KS 66506-5605

Phone: 785-532-4424

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.

**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

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White Oak Plantation

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Cleveland Metroparks Zoo

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St. Louis Zoo

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Adam Eyres

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Wildlife Care Director/Nutritionist

The Wildlife Center

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Barbara Henry, M.S.

Cincinnati Zoo & Botanical Garden

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Cincinnati, OH 45220

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FAX: 513-559-7773

Email: barbara.henry@cincinnatizoo.org

Dr. Michael Kinsel Ship tissues to:

University of Illinois Brookfield Zoo

Zoological Pathology Program Attn: Dr. Kinsel

LUMC Bldg. 101 Rm. 0745 3300 Golf Road

2160 South First Ave. Brookfield, IL 60513-1060

Maywood, IL 60153

Phone: 708-216-1185

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Email: michelemiller128@gmail.com

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Dr. Terri Roth

Cincinnati Zoo & Botanical Garden

Center for Conservation and Research of Endangered Wildlife (CREW)

3400 Vine Street

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Phone: 513-569-8220

FAX: 513-569-8213

Email: terri.roth@cincinnatizoo.org

Dr. Oliver Ryder

Attn: Marlys Houck, Senior Researcher, Cytogenetics

Institute for Conservation Research (formerly CRES)

San Diego Safari Park (formerly Wild Animal Park)

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Escondido, CA 92027-7000

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Email: mhouck@sandiegozoo.org

Steve Shurter

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