
**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 current and future research projects regarding the health of rhinoceroses. To date, your cooperative efforts have supplied a variety of researchers 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 recipient.
3. Store or ship the samples as outlined in the “Summary of Tests and Samples”. Addresses for express shipments are listed in the Directory at the end of the document.
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 recipient prior to shipment of samples to check availability for receipt or additional instructions. Names, phone numbers, and email addresses are listed in the Directory at the end of the document.
6. If you have specific questions, consult with the individual included in the description of each project. If you have additional questions, please contact the TAG/SSP pathology or veterinary adviser.

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

<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>
Marlys Houck/ Biodiversity Banking	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 testes with epididymes and vas deferens	Ship day of collection	ON, WI
Dr. Barbara Durrant/ Oocyte Rescue	Both ovaries with or without entire reproductive tract in saline	Ship day of collection	ON, 15°C (4° cold packs in bottom of Styrofoam box with paper between packs and tissue)

FULL THICKNESS ABDOMINAL BODY WALL & SKIN SAMPLES

<u>Researcher/Purpose</u>	<u>Sample</u>	<u>Time of Shipment</u>	<u>How to Ship</u>
June Olds/Suture study	ventral midline and flank bodywall 6cm X 12 cm	Same day or next available ship date	DI, ON placed in cloth soaked in 0.9% saline (Freeze @ -80°C if necessary prior to shipment)

Insulate tissues from dry ice prior to shipment. If euthanasia is planned, please contact Dr. June Olds (515) 291-1386 or 515-291-5158, we will send shipping materials if needed.

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, including ½ brain	Ship at zoo's convenience	Regular US mail
Dr. Jaime Landolfi/ Pathology *White rhino only	Copy of FINAL necropsy report OR full tissue set in formalin**	Ship at zoo's convenience	Email for reports; regular US mail
	50-100g of liver, spleen, kidney, heart, brain	Freeze (-80C) Archive or ship at zoo's convenience	ON, DI
** Dr. Landolfi/ZPP are happy to provide histopathology review for TAG/SSP white rhinoceros submissions. However, if use of the zoo's regular pathology service is preferred a copy of the FINAL necropsy report is sufficient for archive in the TAG/SSP path database. A second set of formalin-fixed tissues for histopathology is not necessary.			
Dr. Gaffney Pathology *Indian rhino only	Full tissue set in formalin or wax blocks; necropsy report	Ship at zoo's convenience	Regular US mail; email
Rhino TAG Tissue Bank CSI-R	200 g each: liver, kidney, spleen	Freeze (-80°C) Ship at zoo's convenience	ON, DI
Dr. Oliver Ryder Genetics	2 g heart	Freeze (-80°C)	ON, DI
Dr. Terri Roth IOD research	~1 g frozen liver, kidney, spleen x 2 ~1 g formalin-fixed liver, kidney, spleen	At zoo's convenience At zoo's convenience	ON, DI Regular US mail

Dr. Budhan S. Pukazhenth
 Iron transport
 *Black rhino only

2 g each – liver,
 spleen, bone marrow
 3-4 cm each – duodenum, jejunum
 1-2 lymph nodes

Day of collection
 ON, DI

If euthanasia is planned, please contact Buddha Pukazhenth (301) 580 0833. We will ship RNA stabilization medium for sample storage.

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

Sample	Volume	Investigator	Shipping	Project
Serum	4-10 ml (2-4 aliquots of 2 ml 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

Iron overload disorder of browsing rhinos

Iron profile testing may be performed in commercial diagnostic and research laboratories.
 For research testing:
 2 ml serum stored in four different vials (0.5 cc/vial)
 200 g frozen liver

Please contact the Center for the Study of Iron in Rhinos for transfer and storage of new or any older tissue you have been holding while waiting for a centralized facility.

Dr. Kathleen Sullivan and Dr. Shana Lavin
 Disney’s Animal Kingdom
 1200 N. Savannah Circle
 Lake Buena Vista, FL, 32830

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

NECROPSY TISSUE CHECKLIST

All Rhino Species

Tissue	Amount	Investigator	Shipping	Project
Heart – frozen	2 g	Dr. Oliver Ryder	ON, DI	Genetics
Skin biopsy – fresh/room temp	Punch biopsy	Marlys Houck	ON, room temp	Biodiversity Banking
	In bx media (vials provided)			
Testes-fresh/cooled	Both testes	Dr. Terri Roth	ON, WI	Sperm Bank
Liver, spleen, kidney – frozen	~1 g x 2	Dr. Terri Roth	ON, DI	IOD research
Liver, spleen, kidney – formalin-fixed	~1 g	Dr. Terri Roth	US mail	IOD research
Full-thickness abdominal body wall samples (with skin)		Dr. June Olds	ON, DI -80°C frozen or fresh	Suture project
Ventral midline body wall at least 6 cm along the sagittal plane and 12 cm along the transverse plane with midline centered, placed in an absorbent cloth soaked with 0.9% saline to maintain hydration until time of testing.				

Black Rhinoceros Only

Tissue	Investigator	Shipping	Project
Complete set of formalized tissues or slide set (see below for tissue list)	Dr. Duncan	US Mail	Pathology
Liver, kidney, spleen, heart, duodenum-200 g each, frozen at -80°C	Rhino TAG CSI-R Dr. Katie Sullivan and Dr. Shana Lavin	ON, DI	Bank for ongoing IOD Research
Liver, spleen, bone marrow (10 g each)	Dr. Pukazhenth	ON, WI	Iron transport
Duodenum, jejunum (3-4 cm each)	Dr. Pukazhenth	ON, WI	Iron transport
Lymph node (1-2)	Dr. Pukazhenth	ON, WI	Iron transport

Greater Asian One-Horned Rhinoceros Only

Tissue	Investigator	Shipping	Project
Complete set of formalized tissues	Sent to pathologist chosen by zoo		
Completed necropsy report	Dr. Gaffney	Email	Pathology

**White Rhinoceros Only
Tissue**

	Investigator	Shipping	Project
FINAL necropsy report OR Complete set of formalized tissues	Dr. Landolfi	Email US Mail	Pathology
Liver, kidney, spleen, heart, brain- 50-100 g each, frozen at -80°C	Dr. Landolfi	Archive OR mail	Pathology ON, DI

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"/> Adrenal | <input type="checkbox"/> Kidney | <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"/> Liver | <input type="checkbox"/> Prostate | <input type="checkbox"/> Trachea |
| <input type="checkbox"/> Bulbo-urethral gland | <input type="checkbox"/> Lung | <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 | <input type="checkbox"/> Muscle | <input type="checkbox"/> Small intestine | <input type="checkbox"/> Urinary |
| bladder | | | |
| <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"/> Spleen | <input type="checkbox"/> Uterus |
| <input type="checkbox"/> Epididymis | | | |
| <input type="checkbox"/> Heart/aorta | <input type="checkbox"/> Pancreas | <input type="checkbox"/> Stomach | <input type="checkbox"/> Thyroid |
| <input type="checkbox"/> Lymph nodes (sampling of head/neck, thoracic, abdominal, and peripheral, please label to differentiate site of collection) | | | |
| <input type="checkbox"/> Any abnormal fluids | | | |

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

Coordinators: Dr. Kathleen Sullivan and Dr. Shana Lavin

These materials are vital to maintaining a resource for research on all rhinoceros species. Dr. Shana Lavin and Dr. Kathleen Sullivan are coordinating the effort for a centralized bank located at Disney's Animal Kingdom, called the Center for the Study of Iron in Rhinos (CSI-R). Please contact them prior to shipping samples.

Shipping Information for samples: Ship to Dr. Shana Lavin/Dr. Kathleen Sullivan, Disney's Animal Kingdom, 1200 N. Savannah Circle, Lake Buena Vista, FL, 32830

Samples may be requested for specific research projects through the SSP/TAG/RRC. Shipping arrangements should be made directly between CSI-R and researcher.

Sperm Rescue and Banking

Researcher: Dr. Terri Roth, CREW, Cincinnati Zoo

Dr. Roth has developed successful methods for cryopreserving epididymal sperm samples and continues to build up the 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**. Tie off the vas deferens to keep sperm from leaking out. 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.

Investigation of Iron Overload Disorder

Researcher: Dr. Terri Roth, CREW, Cincinnati Zoo

Frozen and formalin-fixed matched samples are needed for comparing mineral content and associated pathologies found within the organ tissue.

Samples:

- 1) Liver, spleen, kidney – two ~1 g pieces of tissue stored frozen and shipped on dry ice.

- 2) 2) Liver, spleen and kidney – one ~1 g piece of tissue stored in formalin and shipped when convenient.

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). Please contact her for additional details: terri.roth@cincinnati-zoo.org; office: 513-569-8220; cell: 513-300-5300.

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 g heart muscle – ship overnight on dry ice.

If additional information is needed, please contact Marlys Houck, Curator of the Frozen Zoo[®], Biodiversity Banking, at San Diego Zoo Wildlife Alliance's Beckman Center for Conservation Research (formerly the Institute for Conservation Research and formerly CRES); Email: mhouck@sdzwa.org

Molecular Pathways in Iron Transport and Immune Function Regulation

Researcher: Dr. Budhan Pukazhenti, Smithsonian Conservation Biology Institute

We are interested in characterizing molecular pathways involved in iron regulation in the black rhino. Combined with our ongoing genomic analyses, we hope to identify normal and abnormal patterns of gene and protein expression in the tissues requested. Information generated also will increase our understanding of immune function in black rhinos.

Sample: Liver, duodenum, jejunum, lymph node (any location), bone marrow

Shipping Information: Ship to Dr. Budhan Pukazhenti, Smithsonian Conservation Biology Institute, 1500 Remount Road, Front Royal VA 22630. Phone (301) 580-0833. Wearing clean gloves, please remove tissues as soon as possible following death, place in labeled zip-loc bags, and store on ice. Samples can be shipped overnight (Priority Overnight; FedEx) in a cooler with ice packs.

Ex vivo mechanical testing of various body wall suture types and patterns for use in 3 rhinoceros spp. (*Ceratotherium simum*, *Diceros bicornis*, *Rhinoceros unicornis*)

Researcher: Dr. June Olds, Iowa State University College of Veterinary Medicine

Dr. Olds and co-investigators are interested in receiving full thickness abdominal body wall samples to perform biomechanical testing to develop our understanding of the surgical challenges in abdominal body wall closures of rhinoceros species. This study is based on several foundational equine surgical studies which measured the tensile properties of the equine body wall intact and sutured, which established several recommendations on suture material, size and spacing when closing the equine body wall. This study hopes to offer similar recommendations for clinicians facing the possibility of abdominal surgery in rhinos in the future. If additional information or shipping materials are needed, please contact Dr. June Olds at jeolds@iasate.edu or 515-291-5158.

Samples requested:

1. Full thickness body wall tissue obtained during necropsy of the individual. Samples from ventral midline and flank are both desired.
2. Samples should be a minimum of 12cm wide, with midline samples having margins a minimum of 6 cm away from ventral midline. Ventral midline should be left intact in midline samples. Flank samples should be obtained in the dorsal-ventral direction and midline samples in the cranial-caudal direction with length as long as realistically feasible. *See Appendix for illustrated figures of requested tissues.
3. Samples should be wrapped in an absorbent cloth soaked in 0.9% Saline, then frozen at -80°C and shipped with dry ice to Iowa State University. Storage at -20C is acceptable, though not preferred.
4. Please contact Dr. Olds for shipping containers or pre-paid shipping label.

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

Rhinoceros Taxon Advisory Group (TAG)

TAG Chair: Adam Eyres
TAG Vice Chair: Steve Metzler
Research Advisor: Dr. Terri Roth
Veterinary Advisor: Dr. Michele Miller
 Co-Advisor: Dr. Eric Miller
Nutritional Advisor: Dr. Kathleen Sullivan

Black Rhinoceros SSP

SSP Coordinator: Lisa Smith
Studbook Keeper: Dr. Gina Ferrie
Veterinary Advisor: Dr. Michele Miller
Pathology Advisor: Dr. Mary Duncan

White Rhinoceros SSP

SSP Coordinator: Adam Eyres
Studbook Keeper: Jonnie Capiro
Veterinary Advisor: Dr. Jeff Zuba
Pathology Advisor: Dr. Jaime Landolfi

Greater Asian One-Horned Rhinoceros SSP

Species Coordinator: Scotty Wade
Studbook Keeper: Joe Hauser
Veterinary Advisor: Dr. Barb Wolfe
Pathology Advisor: Dr. Patty Gaffney

Nutritional Advising Group for all Rhinoceros SSPs: Dr. Kathleen Sullivan, Dr. Ellen Dierenfeld, Dr. Shana Lavin, Barbara Henry, Dr. Eduardo Valdes, Dr. Marcus Clauss