

PART II

INTENSIVE MANAGEMENT AND PREVENTATIVE MEDICINE FOR THE SUMATRAN RHINOCEROS



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Intensive Management and Preventative Medicine Protocol for the Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

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History and Background

The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is a highly endangered browsing rhinoceros that inhabits the forested regions of Indonesia and Malaysia. The Sumatran is considered a primitive rhinoceros with a characteristic coat of hair; it is closely related to the woolly rhinoceros (*Coelodonta antiquitatis*), a species once abundant throughout Asia during the Pleistocene era. In Malaysia, the Sumatran rhino is known locally as badak Kerbau while in Indonesia the local name is badak Sumatera.

Today the Sumatran rhinoceros is considered one of the most endangered large mammals on earth with an estimated 300 animals remaining. Poaching for the animal's horn has resulted in their decline with habitat loss a secondary factor contributing to population reduction and isolation. Attempts at captive propagation of the Sumatran rhinoceros have been problematic due to significant health problems and an inability to provide appropriate captive nutritional and husbandry requirements needed to meet the demands of these highly specialized browsers. This Preventative Medicine Protocol is designed to provide a basis upon which more natural captive propagation efforts for this species can proceed by providing a tool for monitoring health.

Goals of Preventative Medicine Protocol

The goals of the preventative medicine protocol are to provide a comprehensive monitoring program to assist in preventing disease and making appropriate decisions regarding health of captive animals. This protocol can be broken down into four main areas:

1. Nutrition and Diet
2. Housing and Husbandry
3. Preventative Health
 1. Parasite Control
 2. Vaccination and Disease Prevention
4. Common Diseases and Medicine

Appendix I: Anesthesia Guidelines

Appendix II: Health Monitoring Protocol

Appendix III: Pathology Guidelines

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I. Nutrition and Diet

Very generally, the recommended diet should consist of native browse for approximately 70% of the caloric requirements and a concentrated (pelleted) feed for the other 30%. However, as at the Way Kambas SRS, an all browse diet may be preferable where it seems feasible to provide most macronutrient requirements with native vegetation. Since both the pelleted ration and browse are approximately 50% digestible, estimated calorie need would be met by 5 kg pellets, and 15 to 25 kg native browse. Native fruits and other produce can be provided as needed. From the assays on mineral content of native browses, we would recommend ad lib access to iodized salt (along with water, of course); however, a trace mineralized salt block is not necessary and may actually be contraindicated with most formulations (due to the high iron content in these blocks).

Suggested Sumatran rhino diet (item name and amount in kg per day)

Feed Item		
Common Malay name	Scientific name	Quantity in kg
1. Native Browse:		
Ficus	<i>Ficus sp.</i>	40 to 60 kg browse/ animal/ day
Jadefruit Leaves	<i>Artocarpis sp.</i>	
Daun Tapai	<i>Macaranga trilobar</i>	
Tapak Gajah	<i>Macaranga gigantea</i>	
Daun Pulai	<i>Alsfoia sp.</i>	
Ficus sp.:		
Kelompong Hijau	<i>Ficus variegata</i>	
Kelompong Gatal	<i>Ficus glossolaridus</i>	

2. Pelleted Ration:

White Oak Browse Pellet 2 to 3 kg of pellets/ animal/ day

(NOTE: Pending the development of the White Oak browse pellet by Cargill in Malaysia, the Cargill horse diet is a reasonable alternative. It is also worth noting that the pelleted ration has been completely eliminated from the diet at the Way Kambas facility with apparent good results. In nutritional management, the Way Kambas facility should serve as a "model" for future captive management of Sumatran rhinoceros.)

3. Native Fruits:

Local fruits and produce as needed for training and conditioning work.

4. Salt and Mineral supplementation:

A white salt block (iodinized sodium chloride) should be provided free-choice along with fresh water. No other trace minerals should be given unless indicated by specific requirements based on your geographic location (trace mineral blocks may be contraindicated due to their high iron content).

II. Housing and Husbandry

DAILY HUSBANDRY SCHEDULE

Sungai Dusun, Malaysia

Morning

1. The first thing every morning, all rhinos should be hosed down with water so that they can be thoroughly examined for any injuries or signs of disease.
2. Scrub and clean the browse holding room before truck arrives - clean in PM.
3. Clean all browse and feces out of night stall.
4. Hose completely all the surface of inside stall floor, walls, pipes, and browse feeder.
5. Water container must be cleaned completely, scrubbed and refilled.
6. Stall floor and walls inside are to be scrubbed every day.
7. Every day hose all pipes, concrete and walls outside rhino night stall to clean off all mud.

Afternoon

1. 2:00 p.m. - lock all rhinos outside and repeat morning cleaning procedure.
2. Hose all useable browse and leave in the feeder. Any spoiled browse should be thrown away.
3. Change the water inside the water container.
4. 4:00 p.m. - Let rhinos in for the night.
5. Hose and scrub out all food buckets.
6. Hose and scrub off all tools.

Other Husbandry Tasks

1. Every day - take out all feces from all yards.
2. Every week - dig out the mud from the drain outside the night stall
5. Hose chute and chute area every morning and afternoon.
6. Hose the path leading into rhino night stalls and path to take out browse and feces daily.
7. Pellet and fruit storage room should be cleaned completely daily.
8. Give rhinos access to yards at night. Stop locking them inside the night stall to ensure that they will get more exercise (as rhinos do in the 10 acres) and they will be much happier. Feed intake has historically been a problem with giving rhinos free access to the yards so nutritional intake should be monitored closely
9. Every day hose the floor, water container, walls and pipes inside the empty stall as well as the walls and concrete outside the stall.
10. Scrub the empty stalls and water container two times per week.

III. Preventative Health

Medical Record Keeping

The maintenance of up-to-date medical records for each animal is one of the most important and fundamental tools to providing quality medical care. Only by keeping a thorough medical record system will the clinician be able to monitor changes in health parameters and disease over time. Also records of previous laboratory reports and clinical notes will enable the clinician to compare emerging disease processes to known normal parameters for each animal. This will help facilitate appropriate and timely intervention when medical care is needed.

Suggested Disease Monitoring Protocol

1. Routine Hematology and Serum Chemistry Analysis

We suggest that captive rhinos be bled a minimum of once per month to perform a complete blood count (CBC) and quarterly for serum chemistry assay. This will be essential to monitoring health in these animals.

	<u>Approximate Normal Values</u>
CBC:	
White blood cell count	7.8 - 11.0 $\times 10^3$ wbc/cmm
Red blood cell count	5.37 - 5.45 $\times 10^6$ rbc / cmm
PCV (hematocrit)	34.0 - 45.0 %
Hemoglobin	13.0 - 14.1 g/dl
MCV	62.0 - 84.0 u3
MCHC	31.0 - 38.0 %
Total protein	7.2 - 8.0 g/dl
Blood glucose	70 +/- 25 mg/dl
Serum Chemistry:	
Total protein	7.5 +/- 1.0 g/dl
Albumen	3.0 +/- 1.0 g/dl
Globulins	5.0 +/- 1.0 g/dl
Calcium	12.5 +/- 1.0 mg/dl
Phosphorus	4.5 +/- 1.0 mg/dl
Blood urea nitrogen (BUN)	10.0 +/- 5.0 mg/dl
Creatinine	1.5 +/- 0.5 mg/dl
Bilirubin	0.3 +/- 0.2 mg/dl
Alkaline phosphatase	90 +/- 50 IU/L
Creatinine Phosphokinase (CPK)	450 +/- 300 IU/L
Aspartate Transaminase (AST)	80 +/- 30 IU/L
Alanine Transaminase (ALT)	15 +/- 5 IU/L
Sodium (Na)	130 +/- 5 meq/L
Chloride (Cl)	95 +/- 5 meq/L
Potassium (K)	4.5 +/- 0.5 meq/L

2. Fecal Parasite Evaluation

Each rhino should be screened by fecal examination (both direct slide exam and fecal flotation) every 6 months (or more frequently if parasite problems are encountered) to look for any potential parasite burdens. In general, parasitism is uncommon in captive rhinos, however, it may become a problem in certain circumstances. The most important rule for prevention is to maintain strict sanitation with frequent removal of feces from enclosures and maintenance of good hygiene. The following is a short list of some common parasites of the horse.

Internal Parasites

Strongyles - Strongyles (*Strongylus vulgaris*) have been associated with verminous colic in the horse. If severe load, treat with fenbendazole or ivermectin as per dosage in the horse.

Ascarids - The large roundworm (*Parascaris equorum*) is more commonly a problem in young animals and has been associated with abdominal discomfort. In severe cases, ascarids can lead to subsequent intestinal rupture and peritonitis. Migration to the lungs can also occur with coughing and respiratory distress.

Pinworms - Pinworms (*Oxyuris equi* and *Probstmayria vivipara*) are pinworms that inhabit the large colon. They are usually of little significance, but have been associated with severe pruritis.

Threadworms - The intestinal threadworm (*Strongyloides westeri*) has been associated with diarrhea in young animals, but its role is not well understood. It is known to be transmitted from dam to offspring in the milk.

Stomach Worms - The stomach worms (*Habronema* and *Trichostrongylus sp.*) are usually asymptomatic infections, but can cause ulcers and tumor-like lesion in the stomach. The spirurids require a stable fly as an intermediate host.

Bots - Bots (*Gasterophilus sp.*) are the larval stages of bot flies that infest the stomach. These would be expected to be rare in Sumatran rhinoceros due to their extensive wallowing behavior.

Eyeworms - Eyeworms (*Thelazia lacrymalis*) are spirurid nematodes transmitted by flies. Eye infections are usually asymptomatic, but should be investigated during any ocular disease. Filarid parasites (*Onchocerca sp.*) cause skin and eye lesions by invasion of microfilaria and should be included in the differential diagnosis for parasitic ocular disease.

Lungworms - The lungworm (*Dictyocaulus sp.*) usually presents as persistent coughing. The donkey is considered the natural host for this parasite and should therefore not be housed near rhino enclosures.

Tapeworms - Tapeworms (*Anoplocephala* and *Paranoplocephala sp.*) are usually asymptomatic symbiotic hosts, however, severe infections can cause intestinal obstruction and disease. Treatment is with oral Fenbendazole.

Parasite Prevention: Strict hygiene combined with routine fecal parasite monitoring.

Parasite Treatment: For captive African rhinoceros, treatment has been routinely performed with oral Ivermectin (dose: 200 micrograms per kilogram) or oral Fenbendazole (dose: 10 milligrams per kilogram) one treatment as needed.

External Parasites

Ticks and Flies

Both ticks and flies can cause skin irritation and blood loss, but of most concern is the increased risk of these parasites causing transmission of infectious disease. Treatment is removal or topical application of a pyrethrin fly spray. Care should be taken to avoid contact with fly spray in eyes, mouth or on food items.

3. Vaccination and Disease Prevention

Probably the most important aspect of maintaining healthy captive Sumatran rhinoceroses is the provision of appropriate natural browse as the majority (if not complete) part of the animal's diet. Infectious disease is not a major problem with these forest rhinos and strict attention to nutrition and captive husbandry (see above sections).

Vaccination

Routine vaccination is not recommended unless there is a specific disease epidemic in the immediate area, or other disease concerns arise in the future. Tetanus toxoid should be administered to any rhinoceros that suffers a deep puncture wound or in areas where risk is considered high. Undue vaccination should be avoided as it can cause anaphylactic reactions that may be serious.

IV. Common Diseases and Medicine

As discussed above in more detail under the nutritional management section, the health of captive Sumatran rhinoceros is intimately linked to optimum nutritional care. Probably the most common cause of disease in captive Sumatran rhinos has been disorders of the gastrointestinal system.

Parasitic (see above)

Skin wounds and trauma

Foot pad ulcers and abscesses

Rough flooring and poor hygiene may contribute to the development of sole lesions. The sole lesions often progress to more serious sole abscesses if treatment and environmental changes are not corrected. As with Indian rhinos, Sumatran rhinos seem predisposed to development of undermining and necrosis of their feet and sole regions. Treatment should involve thorough daily cleaning and provision of rubber mat flooring. More aggressive debridement and systemic antibiotic therapy may be indicated in severe cases.

Hoof cracks

Hoof cracks may be related to inadequate environmental conditions. Provision of appropriate wallowing areas and nutrition are fundamental to prevention of hoof cracks. Hoof cracks in black rhinoceros (*Diceros bicornis*) species have been prevented by the incorporation of biotin into the diet at a dosage of 30 mg biotin orally per 1000 kg of body weight.

Myiasis

Fly strike can be a real problem with any wounds that are exposed to the environment. Prevention includes careful attention to cleaning and protection of superficial wounds as well as use of pesticide sprays as needed.

Eye problems

Corneal Opacities

Corneal lesions are not uncommon in Sumatran rhinoceros and may represent an inherent susceptibility of these strict forest inhabitants to the increased UV exposures encountered in captive environments. For numerous reasons, captive facilities for Sumatran rhinoceros should be large areas that provide good forage opportunities, appropriate shade for protection from the sun, and wallowing space. Treatment for ophthalmic disease should be based on similar care in domestic animals.

Digestive system

Salmonella Enteritis

Salmonellosis is known to have caused the death of at least one captive Sumatran rhino. Infection with salmonella species is often associated with an acute enteritis and profuse, watery diarrhea. In the black rhino, these infections have also been life threatening. Appropriate therapy should be initiated immediately and should include culture and sensitivity of the organism, elimination of stress, provision of appropriate hydration via fluid therapy and electrolyte water supplementation. Trimethoprim-sulfa has been used successfully in the treatment of Salmonellosis, but should be combined with sensitivity testing. Rhinoceroses with a known positive culture for Salmonella sp. should be placed in isolation and quarantine from other members of the herd. Foot traffic should be eliminated between areas and separate caretaker, cleaning and feeding equipment should be provided. Newly acquired animals should be tested by fecal culture with three to five cultures taken one week apart prior to entry into the facility.

Enteritis, Colitis and Intestinal Accidents

A severe enterocolitis was responsible for the acute death in a captive Sumatran rhino. Mycotoxins have been suspected based on the observation of moldy feed in the store-room. Whatever the inciting cause, the gross pathology consisted of a severe colitis with secondary renal toxicosis. It is imperative that only high quality browse be provided to captive Sumatran rhinoceros, preferably in a semi-free range situation. Any supplemental feed should be stored in an air-conditioned "dry room" to prevent formation of fungal toxins. Historically, the Sumatran rhino in captivity appears to have a propensity for intestinal accidents including large colon volvulus.

Iron Overload

Hemochromatosis and Hemosiderosis

Iron overload is a significant problem in captive browsing rhinoceros and is now recognized as a potentially serious long-term complication of captivity in these species. Progressive accumulations of iron in various tissues are insidious, without obvious signs or symptoms, and can cause organ failure and hematological disorders. This accumulation of iron is directly correlated with the time spent in captivity and represents our inability to meet the highly specialized nutritional demands of these unique animals. Routine serum iron, total iron binding capacity, transferrin saturation, and ferritin assays should be considered a fundamental part of annual health examination in the Sumatran rhino and ongoing attempts at monitoring and prevention of iron accumulation via appropriate nutrition should be considered a priority. Further research into the pathological consequences of iron overload will provide more information on this disorder of captive browsing rhinoceros.

APPENDIX I

ANESTHESIA GUIDELINES

Recommended protocols for Sumatran rhino anesthesia

NOTE: The following drug dosages are preliminary as further investigations are needed to make appropriate recommendations for captive and field use.

Standing sedation

An intramuscular combination of butorphanol and azaperone has been used extensively in captive African rhinoceros (Radcliffe, 2000). A modification of this protocol has been adapted for use in the Sumatran rhinoceros with excellent results.

<u>Drug</u>	<u>Dose</u>	<u>Total mg dose for ~ 600 kg rhino</u>
Butorphanol	40 micrograms / kg	25 mg IM
Azaperone	80 micrograms / kg	50 mg IM (If needed)*

*NOTE: Butorphanol alone will be adequate for most procedures if combined with chute restraint. Addition of azaperone should be considered if procedure will be more invasive or done outside a chute. Repeat butorphanol dose IM or IV as needed to maintain standing anesthesia.

Antagonism of butorphanol:

Naltrexone	5 x butorphanol mg dose	125 mg half IV; half IM
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Recumbent anesthesia

The recommended protocol for recumbent anesthesia is again the butorphanol / azaperone combination due to its inherent safety. Other tranquilizers may be used in place of azaperone (such as the alpha-2 agonists), but care should be exercised as hypoxia has been reported with use of these sedatives. Local anesthetics may be used for more invasive procedures, however, this may require the use of more potent narcotics such as etorphine or other drugs such as ketamine to induce surgical anesthesia.

Protocol 1 (Radcliffe)

<u>Drug</u>	<u>Dose</u>	<u>Total mg dose for ~ 600 kg rhino</u>
Butorphanol	60 - 80 micrograms / kg	30 - 50 mg IM
Azaperone	80 - 100 micrograms / kg	50 - 60 mg IM

Antagonism of butorphanol:

Naltrexone	5 x butorphanol mg dose	150 - 250 mg half IV; half IM
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Protocol 2 (Morris, San Diego Zoo)

Drug	Dose	Total mg dose for ~ 450 kg rhino
Butorphanol	55 micrograms / kg (up to 150 ug/kg)	25 mg IM
Detomidine	30 micrograms / kg (up to 50 ug /kg)	13.5 mg IM

Induction:

Ketamine* 0.5 - 1.5 mg / kg 200 mg - 600 mg IV for induction

*NOTE: Staff must be prepared to intubate the rhino and place on gas anesthesia with ventilatory support if inducing with IV ketamine.

Antagonism of butorphanol:

Naltrexone 500 micrograms / kg 225 mg half IV; half IM

Antagonism of detomidine:

Yohimbine 200 micrograms / kg 90 mg half IV; half IM

(or Atapamezole)

Field darting protocol for snare-removal or rescue of severely compromised Sumatran rhino

Sedation for snare removal

Drug	Dose	Total mg dose for ~ 600 kg	Dart volume
Butorphanol	100 ug/kg IM	60 mg IM	6.0 ml
Azaperone	130 ug/kg IM	80 mg IM	0.8 ml

OR

Recumbency for emergency treatment

Butorphanol	150 ug/kg IM	90 mg IM	9.0 ml
Azaperone	170 ug/kg IM	100 mg IM	1.0 ml

Antagonism of butorphanol:

Naltrexone 5 x butorphanol mg dose 300 - 450 mg IM
(partial dose IV if needed)

Dart volume:

If butorphanol concentration = 10 mg/ml then 60 mg is 6.0 ml

If azaperone concentration = 100 mg/ml then 80 mg is 0.8 ml

This would make a total dart volume of 6.8 ml (or 10.0 ml for recumbency)

This drug volume could be placed into a 10 ml Telinect or Daninject dart. The above dose is greater than that generally needed to sedate captive rhinos due to the increased stress and excitement of darting wild rhinoceros which can lead to overriding of drug effects. The above protocol should work well as a starting point for field rangers to have as part of a "field emergency kit" in case they need to make timely intervention as part of a conservation patrol (i.e. snare removal, etc.). Higher doses can be used to produce more marked sedation or produce recumbency as deemed necessary by the RPU team by following the guidelines outlined above.

Field darting protocol for rescue of free-ranging Sumatran rhino

NOTE: The following drug dosages are preliminary as further investigations are needed to make appropriate recommendations for field use.

Anesthesia for capture of free-ranging Sumatran rhinoceros

<u>Drug</u>	<u>Dose</u>	<u>Total mg dose for ~ 600 kg</u>	<u>Dart volume</u>
Etorphine (M99)	3 - 5 ug/kg IM	2 to 3 mg IM	0.2 - 0.3 ml
		(using 10 mg/ml M99)	
Azaperone	130 ug/kg IM	80 mg IM	2.0 ml
		(using 40 mg/ml Azap.)	
Hyaluronidase		5000 IU	Reconstitute with above

NOTE: The above protocol is provided as an alternative to use of the pit-trap for capture of the Sumatran rhinoceros when its use is not feasible. This protocol is NOT intended to be used in place of the pit-trap as the latter method is still considered the safest method for capture of free-ranging Sumatran rhinos. Since these drugs are dangerous to both the human handlers and the subject animal, this drug protocol should only be used by an experienced wildlife capture and veterinary team. However, these drugs provide the most consistent, reversible and rapid recumbency possible in order to expedite field capture of African and Asian rhinoceros species. A rapid recumbency will be critical to safe capture of a species such as the Sumatran rhino that may escape into the forest after darting and also be prone to both loss and other risks such as drowning. A capture system that employs a radiotransmitter dart, containing a transmitter that stays with the darted animal, may be important to insure that the animal can be located quickly in the thick rainforest environment.

Full Antagonism of etorphine:

Naltrexone	50 x etorphine mg dose	100 - 150 mg IM (partial dose IV if needed)
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Options for sedation / tranquilization to facilitate safe translocation

1. Partial reversal of narcotic effects with agonist-antagonist such as Nalorphine (without prior reversal with Naltrexone).
Nalorphine 5 to 10 mg IV
2. Full reversal of narcotic with Naltrexone (see below) followed by sedation with Azaperone or Butorphanol (see doses above for sedation) for crating and translocation.
Azaperone 100 to 150 mg IM (or to effect)

APPENDIX II

SUGGESTED SCHEDULE FOR HEALTH MONITORING PROGRAM

Monthly Health Checks

- 1) Body weight (**BW**) measurement and body condition score .
- 2) Complete Blood Count (**CBC** = Packed cell volume, Total protein, White blood cell count, White blood cell differential, and Cell morphology).
- 3) Update all animal medical records.
- 4) Provide monthly reports to MRF and IRF.

Quarterly Health Checks

- 1) Salmonella culture (**SC**) from stool or rectum.
- 2) Fecal parasite examination (**PE**; Direct smear and fecal flotation techniques).
- 3) Provide quarterly summary of health results and reproductive progress to be disseminated to IRF via Dr. Foose, Dr. Radcliffe, and Dr. Roth.

Annual Health Checks

- 1) Annual visit by reproductive health team to evaluate Sumatran rhino health and fertility.
- 2) Review of husbandry, health and nutritional programs.
- 3) Recommend an in-depth rhino pathology summary to be conducted by Dr. Donald Paglia using historical histopathology materials.
- 4) Comprehensive annual summary to be disseminated to IRF via Dr. Foose, Dr. Radcliffe, and Dr. Roth.
- 5) Serum iron and ferritin concentrations, total iron-binding capacity, and transferrin saturation (**Fe**) performed quarterly to evaluate iron loads.

Recommended Schedule for Rhino Health Program											
Month											
Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW
CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC
SC			SC			SC			SC		
PE			PE			PE			PE		
Fe			Fe			Fe			Fe		

APPENDIX III

Sumatran Rhinoceros Pathology Guidelines

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Donald E. Paglia, MD

Gross Necropsy General Guidelines

- 1) Document all pathology and gross findings with 35mm slide film, digital images or video.
- 2) Carefully record all gross findings on paper. See accompanying Sumatran Rhino Necropsy Protocol.
- 3) Save specimens for:
- 4) Histopathology in 10% buffered formalin at a ratio of 10 parts formalin to 1 part tissue for preservation. Tissue sections should not exceed 1 cm in diameter to allow appropriate fixation. Please collect identical, duplicate tissue sets. One tissue set can be evaluated by the UPM pathology department and the other tissue set can be forwarded (with appropriate CITES permits) to Dr. Donald Paglia in the USA for comparative study.

Dr. Donald Paglia

UCLA Hematology Research Laboratory

Building 113, Room 225

West LA Department of Veterans Affairs Medical Center

11301 Wilshire Boulevard

Los Angeles, CA 90073

Phone: (310) 312-0557

Fax: (310) 206-5178

- 5) Freeze samples of all organ systems in double whirl-pak bags. Preferably freeze the tissues at -70° C although a -20° C conventional freezer will work if that is all that is available.
- 6) Samples for bacterial culture. This is particularly important for any tissues or specimens that appear to be abnormal in appearance or if abnormal discharges are noted from any structure.
- 7) Collect feces for bacterial culture (ask lab to specifically look for Salmonella organisms) as well as examine feces for fecal parasites. Electron microscopy should also be performed on the feces to look for viruses. A coronavirus has recently been identified in captive black rhinoceros in the USA.
- 8) Collect urine for complete urinalysis.
- 9) Collect serum for disease serology.
- 10) Collect samples for toxicology. Stomach content samples from the animal and feed samples from storage should be frozen for future testing. Fresh samples of each should be forwarded to UPM for toxicological assay (ie. mycotoxins, etc.).

Gross Necropsy Protocol

See attached form that you can use to help document the information that you acquire from the gross exam.

Recommended disease serology:

Disease	Serologic Test
Equine Herpes Virus 1&2	Serum Neutralization (SN)
Leptospirosis	Macroscopic Agglutination Test (MAT)
Aspergillosis	Antigen/ Antibody Test
Equine Infectious Anemia	Agar Gel Immunodiffusion Test (AGID)
Foot and Mouth Disease	Virus Inhibition Assay (VIAA)
Malignant Catarrhal Fever	Immunoperoxidase Test (IPT)
	Serum Neutralization (SN)
	Polymerase Chain Reaction (PCR)
Vesicular Stomatitis Virus	Serum Neutralization (SN)
Bluetongue Virus	Enzyme-linked Immunosorbent Assay (ELISA)
Encephalomyocarditis Virus	Serum Neutralization (SN)
Toxoplasmosis	Hemagglutination-inhibition Test (HI)
Mycobacterium	Blood TB Test

Other disease serology based on important livestock diseases of Malaysia

List of Important Contact Information and Addresses for Sample Submission

Virology

Dr. Mike Worley
San Diego Zoo
PO Box 551
San Diego, CA 92112-0551
Phone: (619) 231-1515
Fax: (619) 231-0249

Vitamin and Mineral Assay

Dr. Ellen Dierenfeld
New York Zoological Park
Bronx Zoo
185th St and Southern Boulevard
Bronx, NY 10460
Phone: (718) 220-5100
Fax: (718) 220-7102

Foreign Animal Disease

USDA-APHIS-Veterinary Services
Foreign Animal Disease Investigation Lab
PO Box 848 Plum Island
Greenport, NY 11944
Phone: (516) 323-2500
Fax: (516) 323-2798

Sumatran Rhinoceros Necropsy Protocol

Organization_____

Address_____

City_____State_____Country_____

Veterinarian/Pathologist_____

Phone_____Fax_____

Animal Information			
Animal Name	Studbook No.		ISIS No.
Age	Date of birth		Body weight kg
Sex (circle) M / F	Origin (circle) Captive-born Wild-captured		
Date of capture		Location of capture	
Clinical History			
Date of death		Time of death	Time of necropsy
Duration of problem		Environmental conditions	
Diagnosis		Feed intake	
Therapy_____		Clinical findings/ Lab results_____	
_____		_____	
_____		_____	

Gross Necropsy Examination

General Exam (body condition, examine feet closely for any abscesses or source of infection, oral exam with special emphasis on looking for any oral lesions such as ulcers and tooth problems)

Culture of any abnormal discharges from body orifices

Sample location	Sample description
<i>Musculoskeletal System</i> (bones, joints and joint fluid, muscle mass, bone marrow, and close evaluation of digital area. Sole abscesses and necrotic lesions to feet are common problems in captive Sumatran rhino that may lead to sepsis)	
<i>Body Cavities</i> (thoracic and abdominal cavities, fat deposits - especially around the heart, kidney and abdominal organs, presence of fluids or exudates, and evaluate negative pressure in thorax)	

Respiratory System (Pharynx, larynx, nasal passages, trachea, bronchi, lungs, hilar lymph nodes, with careful evaluation for any discharges or lesions)

Cardiovascular System (pericardium, pericardial fluids, heart, heart valves, heart vessels, evaluation of pericardial fat, and observation for surface lesions such as hemorrhages, necrotic lesions, etc.)

Digestive System (oral cavity, teeth, tongue, esophagus, stomach, glandular and non-glandular stomach regions with evaluation for GI ulceration, duodenum, pancreas, liver, gall bladder, bile duct, jejunum, ileum, cecum, large colon, small colon, rectum, anus, and mesenteric lymph nodes. Careful evaluation of organs for evidence of torsion, displacement is critical based on the Sumatran rhino's apparent susceptibility to GI disease. GI content and feed from storage should be collected for toxicology)

Urinary System (kidneys, urinary bladder, ureters, urethra. Urine should be collected for gross exam - especially look for blood which would suggest a terminal hemolytic event, and complete urinalysis)

Urine exam

Color	pH	Specific gravity	Glucose
WBC	RBC	Protein	Casts

Reproductive System (Female; ovaries, oviducts, uterus, cervix, vagina, mammary gland, placenta/fetus. Male; penis, testes, accessory sex glands. We will need to develop protocols for sperm and oocyte recovery)

Endocrine System (thyroid glands, parathyroid glands, adrenal glands with evaluation for signs of stress-related illness)

Nervous System (meninges/dura mater, brain, spinal cord, and peripheral nerves. In calves, brain should be evaluated for congenital defects and leukoencephalomalacia)

Gross Diagnosis

Laboratory Test Results

Complete blood count

Serum chemistry

Serology:
Leptospirosis_____

Mycobacterium_____

Aspergillosis_____

Herpesvirus_____

Foot & Mouth Disease_____

Cytology

Microbiology

Virology

Toxicology

Parasitology

Fecal EM results_____

Other Notes

APPENDIX IV

LIST OF RHINOCEROS HEALTH PROFESSIONALS

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