

Oklahoma City Zoo Asian Elephant EEHV Protocol

January 2022



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Oklahoma City Zoo

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EEHV – OVERVIEW

Elephant Endotheliotropic Herpes Virus (EEHV) is a rapidly fatal disease affecting Asian and African elephants and is caused by several similar, but genetically distinct novel herpes viruses. EEHV is endemic within the Asian and African elephant populations and has been the cause of morbidity and mortality in elephants both in the wild and under human care. EEHV hemorrhagic disease (EEHV HD) occurs primarily in young elephants between 1-8 years of age. The onset of EEHV HD is sudden and death can occur hours after the first clinical signs are observed, without clinical signs (sudden death), or the elephant may exhibit clinical signs for a week or more prior to death. This is a particularly devastating disease for elephant managers and conservationists and is a serious threat to all populations of elephants.

In North America, EEHV 1 is the most common species associated with EEHV HD in Asian elephants. EEHV 4 and EEHV 5 have also been implicated in causing disease, but less frequently. EEHV 2, EEHV 3, and EEHV 6 have been associated with illness in African elephants.

CLINICAL SIGNS

*** Any concerns, however minor, MUST be reported to the lead caretaker and elephant curator, immediately. Veterinarians will be contacted by the lead caretaker or elephant curator. If there are questionable signs and the curator is not available, the on-duty veterinarian will be notified as soon as possible. Caretakers are the first line of defense against EEHV HD. Observing signs of EEHV HD early is what will save an elephant's life. Caretakers should not make excuses about why signs are occurring and report that the elephant is off. It is better to have fifty false alarms and be overly cautious than have one sick elephant go undetected.**

The following clinical signs are associated with EEHV HD infections:

1. Decreased food and water intake
2. Alteration in sleep pattern or activity (sleeping more or less than baseline)
3. Signs of discomfort or acute pain
 - a. Abdominal: Stretching, rolling
 - b. Musculoskeletal: Lameness, stiffness
4. Change in fecal output – diarrhea, constipation, decreased production
5. Mentation change – lethargy, disorientation, neurologic signs
6. Oral lesions
 - a. Hyperemia (dark, red oral mucosa)
 - b. Vesicle formation or ulcer formation
 - c. Petechiae/ecchymoses (hemorrhage)
7. Ocular abnormalities (scleral injection, icterus, retinal hemorrhage)
8. ****Edema** (swelling, fluid accumulation) of the head, neck or trunk
9. ****Cyanosis** (purple discoloration) of the tongue or other mucous membrane

****Presence of edema or cyanosis should warrant initiation of treatment immediately**

The following physical examination findings may be associated with EEHV infections:

1. Cardiac abnormalities
 - a. Tachycardia
 - b. Arrhythmia
 - c. Heart murmur

- d. Change in blood pressure from baseline
- e. Hypoxia (<95% measured pulse oximetry)
2. Alterations in body temperature
3. Evidence of fluid accumulation on ultrasound
 - a. Abdominal fluid
 - b. Pericardial fluid

DIAGNOSTIC TESTS

Complete Blood Count (CBC)

On presentation, affected animals often have a decreased white blood cell count (leukopenia) with a significant decrease in monocytes (monocytopenia). This may lead to a shift with neutrophils becoming the predominant white blood cell type. The presence of a decreased white blood cell count and decreased monocyte count in an animal with any level of viremia is significant and warrants immediate initiation of treatment. The presence of immature neutrophils (band cells) in an elephant is abnormal and should be considered a significant finding for possible EEHV HD or other disease process. Thrombocytopenia (decreased platelets) is usually present and anemia (decreased hematocrit, hemoglobin, and red blood cell count) is sometimes noted to varying degrees. As with clinical signs, the CBC profile may worsen for a few days even after the initiation of therapy. Follow-up CBCs are important in tracking recovery or decline of the animal's condition. As the disease progresses, a rebound leukocytosis (elevated total white blood cell count) and thrombocytosis (elevated platelet count) lasting several days to weeks may be seen.

Summary of CBC findings associated with EEHV HD:

- Leukopenia (followed by rebound leukocytosis)
- Monocytopenia
- Relative neutrophilia
- Thrombocytopenia (followed by rebound thrombocytosis)
- Anemia

Serum Biochemical Analysis (Chemistry)

Evaluation of a serum biochemistry profile is important for assessing the overall health of the animal, however, there are no specific chemistry changes that are known to be indicative of EEHV HD. Some individuals have shown an increase in serum amyloid A (acute phase protein) with EEHV viremia. Coagulation profiles may be useful, however, effect of EEHV HD on coagulation parameters has not been evaluated.

Quantitative Polymerase Chain Reaction (qPCR) Testing

This test is run on whole blood collected in EDTA (purple topped) tubes and is used to detect herpesvirus viremia. The blood sample is analyzed for evidence of any of the known species of the virus that cause EEHV HD. In addition to diagnosis, qPCR can be used to monitor response to treatment as quantitative viral load will decrease as the viremia is cleared. A shift from a positive to negative test may take between 8-14 weeks (data limited at this time).

TREATMENT

Primary treatments for EEHV HD are fluid therapy and antiviral therapy. This disease progresses rapidly with death likely resulting from shock, hypovolemia and possibly disseminated intravascular coagulation. The goal of therapy is to provide aggressive supportive care to maintain tissue and organ perfusion while the body (or antiviral medication) attempts to fight the infection. Sedation may be necessary to facilitate treatment in EEHV HD cases.

Other therapies, such as analgesics, antibiotics, anti-inflammatories and gastrointestinal protectants are often used in EEHV HD cases for additional supportive care.

Fluid therapy:

The first line of treatment is rectal fluids. Elephants are able to absorb a large amount of fluids through the rectal mucosa while excreting the rest. Lukewarm fluids administered at a slow rate via a soft hose or tubing can be very effective at keeping an animal hydrated. If condition deteriorates, intravenous (IV) fluids can be added. Asian elephants have a lower serum osmolality when compared to other mammals. Commercial crystalloid fluids are actually hypertonic for elephants therefore administration rate and dose should be adjusted accordingly and monitored closely. Colloid fluids can also be used and small amounts can be very beneficial. Plasma and whole blood can be banked or collected fresh and administered IV through appropriate blood filters. Elephants do have specific blood group antigens so cross matching is necessary before administration. Penciclovir fortified plasma can also be given to aid in treatment. Plasma is collected from a healthy animal after administration of a double dose of famciclovir anti-viral medication. The drug is metabolized to its active form, penciclovir, and plasma is collected at peak plasma concentration and stored.

Antiviral therapy:

Antiviral drugs work to inhibit viral replication, but by themselves, do not correct the damage done by the virus to the animal's cells. It is important to treat the virus as soon as possible to prevent further cellular and tissue damage. EEHV is a unique herpesvirus and data is currently unavailable to determine which antiviral drug is most effective at combating the disease. The two primary drugs that have been used to treat EEHV HD are famciclovir and ganciclovir. Famciclovir has been used in more cases and it has the convenience of being administered via oral or rectal routes with no reported side effects. Absorption in healthy animals is good through oral and rectal routes, however, animals clinically affected by EEHV HD may not have effective absorption and distribution of the drug due to hemorrhagic and cardiovascular effects of the disease. Ganciclovir is a drug used in human medicine to treat beta herpes virus infections. It is administered twice a day, intravenously over the period of 1 hour to achieve adequate blood levels but does have potentially more risk of side effects. ***At this time there is no evidence to support efficacy of either of these drugs against EEHV however, their use in the absence of adverse effects is likely to continue until more data is available.**

DAILY HERD MANAGEMENT

- Visual inspection of mouth, tongue, palate for ulcers, lesions, discoloration, or visual changes.
- Visual inspection of the elephants. Looking for swelling or abnormalities in the animals' overall appearance.
- Assessment of the animals' appetite.
- Assessment of the animals' responsiveness to cues and stimuli.
- General assessment of the elephants' overall attitude and appearance (respiration rate, locomotion, coordination, etc.).

- Weekly temperature readings on calves (fecal bolus), more often opportunistically.
- Weekly blood pressure readings on calves. Monthly readings on adults.
- Data collected will be recorded and shared between the elephant and veterinary teams (see Vital Signs Monitoring below).

BEHAVIORAL TRAINING

Successful diagnosis and treatment will depend on the ability to access the animal for visualization, sample collection, and treatment, including oral, rectal, and intramuscular injections and, intravenous catheter placement. Intensive care therapy may require isolation from the herd for potentially extended periods of time. By one year of age the following behaviors should be part of routine daily husbandry:

- Isolation from dam/other elephants
- Leg restraints
- Lay down
- Injections (IM and SQ)
- Blood collection
- Urine collection
- Body temperature measurement (fecal bolus, rectal, life chip)
- Blood pressure measurement (cuff on base of tail)
- Oral exam
- Accept oral and rectal medications
- Acceptance of rectal fluids
- Auscultation of heart w/stethoscope
- Ultrasound of heart

VITAL SIGNS MONITORING

Routine monitoring of physiologic parameters such as body temperature, respiratory rate, heart rate and indirect blood pressure will help to establish normal values for each individual elephant and give us important information for assessing any elephant that may be suspect for EEHV HD or other disease problems. Respiratory rates: baseline respiratory rates will be established for all elephants. Heart rates: the indirect blood pressure monitor gives this information; however, ultrasound can also be used in adults and auscultation in calves.

Blood pressure will be monitored at least weekly for calves and monthly for adults. The blood pressure monitor is a Cardell model 9401 with various cuff sizes. The cuff (use largest size for adults) is placed on the tail at approximately the level of the animal's heart. Consistent placement is critical to the precision and accuracy of the readings. Readings will be recorded on the handwritten log and entered into ZIMS by veterinary staff.

Body temperatures will be monitored and recorded weekly for calves using temperature measurement of a fresh fecal bolus. Temperatures in excess of 100°F should be considered elevated.

ROUTINE SAMPLING AND SURVEILLANCE

In an effort to discover subtle changes which may indicate early signs of infection, or detect viral shedding in apparently healthy animals, blood and trunk wash samples will be collected and analyzed

weekly. Serum samples from calves will be submitted periodically (ideally every 6 months) to the Baylor College of Medicine for serology screening. Other samples may be included when and if indicated. See below for more specific sampling information.

Blood and trunk wash samples will be obtained weekly from each elephant for routine monitoring, to help study the epidemiology of the EEHV virus and contribute to development of treatment protocols. Samples will be analyzed by qPCR in the Joan Kirkpatrick Animal Hospital (JKAH) laboratory. Weekly blood samples from calves 1-10 years of age will also be sent to the National Elephant Herpesvirus Laboratory (NEHL) for confirmatory testing. Any sample not immediately analyzed will be banked at the JKAH. Any positive samples may be sent to the NEHL or Johns Hopkins (JH) for sequencing.

See Appendix I: EEHV Sampling Guidelines Quick Chart for summary of information on sampling. Weekly sample collection for EEHV surveillance should include filling at least:

1 purple top tube (3 ml capacity, 3 ml)
Trunk wash

Purple Top Tubes (EDTA anticoagulant, 3 ml capacity)

- Fill all EDTA tubes at least half way with blood so the blood is not diluted with EDTA.
- 1-2 ml whole blood needed for qPCR
- Samples from all elephants are tested weekly in the JKAH laboratory; samples from calves are also shipped to NEHL weekly.
- In-house CBC performed weekly on calves and monthly on adults.

Trunk wash samples

- Collected weekly from all elephants for EEHV qPCR in the JKAH laboratory.
- Trunk washes (minimum 30 ml of fluid recovered) are collected using 60 ml sterile saline infused into the trunk, then collected into clean bags via forced exhale and transferred into 50 ml conical vials.
- Samples will be taken to the hospital on the day of collection to be processed.

PLASMA BANKING

Large volume blood collections will be attempted periodically on the adult elephants. Whole blood is collected into a sterile, closed-system 450 ml collection bag containing citrate phosphate dextrose adenine solution (CPDA-1) USP as an anticoagulant. The bag should be labeled with volume, date, time and animal ID number. If a bag is less than 1/3 full, the blood should be banked in the JKAH ultralow freezer for use as possible future research samples. If a bag is more than 1/3 full, plasma will be separated via centrifugation. The plasma will be placed in the ultralow freezer for potential future therapeutic use. Frozen plasma can be kept for up to 5 years, however storage in a frostless freezer will decrease the lifespan of the product significantly.

Fortified plasma can be collected after administration of 30 mg/kg famciclovir to a healthy adult elephant (administered PO or rectally). Blood is collected as stated above 1 hour after drug administration at peak penciclovir (active form of drug) concentration. The plasma is stored frozen for potential use as treatment during an EEHV HD case.

CLINICAL CASE SUSPECT

Because of the rapid onset and progression of the disease, treatment needs to be initiated based on clinical impression rather than absolute diagnosis. The decision whether or not to start an elephant on an antiviral medication will be made by the veterinarians with input from the lead caretaker and elephant curator. If an elephant is suspected or confirmed pregnant, this condition should be taken into consideration when deciding to treat with anti-viral medication. NOTE: Famciclovir is considered in the group B pregnancy class, meaning there is no evidence that the drug causes adverse effects on embryo fetal development in pregnant animals but specific studies have not been performed in elephants. NOTE: Ganciclovir is a Category C drug for pregnancy (Class C: Animal studies have shown an adverse effect on the fetus, but there are no adequate studies, or there are no animal reproduction studies). It has been shown to be embryotoxic in rabbits and mice following IV administration and teratogenic in rabbits. It may be teratogenic or embryotoxic at dose levels recommended for human use.

SAMPLE COLLECTION FOR A CLINICAL SUSPECT

If an elephant is showing clinical signs consistent with EEHV HD, blood and trunk washes should be collected as soon as possible for diagnostic purposes.

Blood should be collected into:

1. **Purple top tube** (EDTA for whole blood, 8-15 ml)
 - a. 1st priority 1-2 ml of blood for qPCR at JKAH laboratory and NEHL
 - b. 2nd priority is to collect 0.5 ml of blood for CBC at JKAH
 - c. 3rd priority is to collect 2-4 ml of whole blood for JH to try viral culture
2. **Light blue top tube** (Citrate for whole blood, 3-4 ml)
 - a. At least 3 ml of whole blood for fibrinogen at Antech Diagnostics
3. **Red/Grey top tube** (serum separator for serum, 8 to 400 ml of blood)
 - a. 1st priority- 2 ml of whole blood for serum biochemistry at JKAH
 - b. 2nd priority- 6 ml of whole blood for serum ELISA at NEHL
 - c. 3rd priority- 20-200 ml serum to JH for research

A trunk wash sample should be collected and processed for qPCR at the JKAH and NEHL.

Samples should be brought to the JKAH and processed for in house testing and distribution to the NEHL and Johns Hopkins University. Shipment guidelines and instructions can be found in Appendix II.

TREATMENT FOR SUSPECTED OR CONFIRMED EEHV

The decision to treat a suspect case with oral or intravenous antiviral medication will be based on the animal's clinical condition and on the confirmation of infection via positive qPCR test. In most cases antiviral medication will be started orally or rectally but if clinical signs progress despite oral treatment, then intravenous antiviral medication (ganciclovir) may be recommended.

A suspect/confirmed elephant may be locked in the barn. Calves and/or subordinate animals may be accompanied by other herd mates for companionship. Once treatment starts the suspect animal may be separated from contact with the other elephants and may be restrained with the use of leg restraints. Herd

management during treatment will be based on the decision of the elephant curator and veterinarian recommendation.

No non-essential staff will be present in the elephant area during the therapy and treatment process unless approved by the elephant curator, director of animal collections, or the zoo director.

Antiviral therapy (See Appendix VI for additional drug dosages)

Famciclovir: 15 mg/kg PO or per rectum TID-QID

Ganciclovir: 5 mg/kg IV SID-BID (give slowly over 1 hour in NaCl)

- Recipe for oral suspension (5 mg/kg PO BID) – based on recommendation from Roche chemists
 - Use 14 vials ganciclovir injectable (500 mg / vial) per dose.
 - Mix each vial with 3 ml sterile water.
 - Withdraw dissolved drug from each vial, place together in one clean beaker.
 - Add 140 ml oral sweetener solution (such as simple syrup, or Orasweet, OTC syrup at any drug store)
 - Add 2.8 ml 3% H₂O₂ (=hydrogen peroxide)
 - Mix well, add sweetener to total volume of 280 ml
 - End Product: 25 mg/ml suspension
 - Since we don't know the shelf life of the suspension, the suspension should be mixed up fresh prior to each treatment, anything left over should be discarded
 - Oral bioavailability of ganciclovir suspension has not been determined

Stem cell therapy

Autologous mesenchymal stem cells can be given IV or IM. Target dose of 150 million cells will be administered IV and potentially repeated in one week. Mesenchymal stem cells have been administered to both Achara and Kairavi with no apparent side effects.

Immune stimulation therapy

Recently, attempts have been made to investigate the effect of immune stimulation therapy in elephants. Anecdotally, immune stimulation therapy has been used in a small number of clinical EEHV cases with no apparent adverse effects. The efficacy of this therapy is still under investigation but may be a useful adjunct to other EEHV treatment modalities. The most promising candidates thus far are Zelnate® (DNA immunostimulant) and interferon alpha therapy (Intron® A and Roferan® A). Zelnate® has been administered to Kairavi at 2ml IM and repeated 3 days later with no apparent side effect.

INTENSIVE CARE OF THE EEHV PATIENT

Antiviral medications are recommended in any suspect or confirmed EEHV case to hopefully reduce or eliminate viral replication and thus reduce the viral load on the patient. However, the antiviral medications do not reverse the damage the virus has already done to internal organs. Aggressive supportive therapy and close monitoring of the patient is recommended as an adjunct to antiviral medication. In addition to rectal fluids, placement of an intravenous catheter in a large, peripheral vein is recommended for ganciclovir administration as well as fluid and colloidal support and administration of other medications. If placement and maintenance of an IV catheter is not possible under training or manual restraint, sedation may be required.

Sedation (See Appendix VI for additional drug dosages)

- Butorphanol (0.02 – 0.07 mg/kg) + detomidine (0.01-0.02 mg/kg) IM
 - Reverse butorphanol with naltrexone (2.5 – 5 X butorphanol dose) IM
 - Reverse detomidine with atipamezole (0.1-0.16 mg/kg) IM

A small-bore IV catheter can be placed in an ear vein, and a larger bore IV catheter can be placed in a cephalic vein or a saphenous vein. If possible, consider choosing one auricular vein to preserve for blood collection and make an effort to avoid using this vein for IV treatments. Also consider topical diclofenac over the auricular veins to reduce inflammation.

Intravenous Fluid Therapy

Intravenous fluids may be needed to support circulation and hydration. Physiologic crystalloids such as Lactated Ringer's or Normosol can be used for rapid rehydration or for maintenance fluids. Sodium chloride should be used if the elephant is hyponatremic or hypochloremic, and/or if the elephant is on diuretics.

Maintenance fluid therapy requirements have not been determined for elephants but are assumed to be similar to other mammals:

- Maintenance (adult) = 2ml/kg/hour = 2 liters/1000kg/ hour
- Maintenance (calf) = 4ml/kg/hour = 4 liters/1000kg/hour
- Surgical rate = 10ml/kg/hour = 10 liters/1000kg/hour
- Shock rate = 90ml/kg/hour = 90 liters/1000kg/hour
- Volume replacement fluid (liters) = Body weight (kg) x percentage dehydration

Plasma Transfusion

Colloids such as fresh or frozen plasma, or hetastarch, are often more effective than crystalloid fluids for volume expansion in viremic or seriously ill animals. The larger molecules in these fluids do not leak out of capillaries as easily, and increase plasma volume. Based on equine recommendations, plasma should be administered at an average rate of 10 ml/kg/hr. The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. Possible transfusion reactions would include fever, rash, or anaphylaxis. Mild signs can be treated with antipyretics or antihistamines and decreasing the rate of transfusion. More severe reactions should be addressed by stopping the transfusion.

Fresh plasma and whole blood are more beneficial in providing platelets than frozen plasma and should be used whenever feasible. However, frozen plasma is also be beneficial for fluid support.

Blood Transfusion

Whole blood transfusion should be considered early in treatment of EEHV HD and especially in cases where anemia develops. Blood group antigens are present in elephants and cross matching is recommended prior to transfusion; any agglutination or lysis indicates an unacceptable match. Any blood used for transfusion will be screened first by qPCR to confirm it is EEHV negative.

*See Elephant Cross Matching Protocol

Antibiotics (see Appendix VI for drug dosages)

Although antibiotics have no effect in treating EEHV, the animal's immune system will be severely compromised and the clinical situation could be complicated by secondary opportunistic infections and therefore antibiotics should be considered.

Analgesia (see Appendix VI for drug dosages)

Although EEHV is thought to be a vasculopathy as opposed to a vasculitis, antiinflammatories are indicated as part of the analgesic regime as well as reducing secondary inflammation resulting from peripheral edema and hemorrhage. Non-steroidal anti-inflammatories (NSAID's) are part of the recommendations outlined by the EEHV workshop and they play a useful part in early management of the disease. However, it should be noted that in human medicine NSAIDs are contraindicated in cases where peripheral edema or hemorrhagic diathesis is present due to the decreased glomerular filtration rate and the effects on coagulation seen when using NSAIDs. The analgesic and anti-inflammatory effects of these drugs should be weighed against these side effects. Opioids are also a useful adjunct to providing analgesia and, in some cases, mild sedation to assist in the management of animals being treated.

DAILY MONITORING OF AN EEHV CASE

Monitoring of Suspect/Confirmed Positive Elephant

If EEHV is suspected but the elephant is not clinically ill, a veterinary physical examination will be performed twice daily during the first week of treatment and should coincide with the treatment schedule. If EEHV is diagnosed via qPCR and the elephant is placed on intravenous treatment, 24 hour monitoring by veterinary and elephant staff will be instituted. Regular measurements of vital signs, including respiratory rate, heart rate, blood pressure, and body temperature are to be made starting on the first day that EEHV is suspected. A daily ultrasound of the heart will be performed to evaluate heart rate and contractility and also monitor for the development of pericardial effusion. Elephant care staff will monitor behavioral parameters.

Daily blood samples for first week of treatment (listed in order of priority):

- Whole blood (EDTA & citrate, total 20 ml daily) – JKAH (1 ml), NEHL (1-2 ml), fibrinogen (2-3 ml), JH (2-4 ml), & banking at JKAH (6-10 ml), Sonoclot
- Whole blood (no anticoagulant) – VCM coagulation profile patient side
- Serum (red/grey top, total 10-15 ml daily) – serum biochemistry (2 ml), NEHL (2 ml) & banking at JKAH (6-10 ml)
- If Ganciclovir is used, close monitoring of CBC, Creat/BUN, HCT, and urine production is recommended.

Trunk wash samples (30-50 ml): daily trunk wash samples, 30-50 ml for qPCR at JKAH

Oral swabs may need to be provided, upon request, to Virginia Pearson at Fox Chase Cancer Center (FCCC).

Surveillance of Herd Mates

If an elephant is confirmed qPCR positive, then samples should be collected from the rest of the elephant herd for EEHV testing (decision based on herd status).

- Whole blood (4-5 ml total from each) – JKAH qPCR (2 ml), NEHL (1-2ml) and CBC (0.5 ml)
- Serum (7 ml whole blood from each) – NEHL ELISA (5 ml), JKAH Chemistry (2 ml)
- Trunk washes (60-100 ml total from each) – JKAH qPCR (30-50 ml) & banking for future use

RESOURCE CONTACT LIST

Dr. Lauren Howard	Cell: 713-417-7979	Email: lhoward@sandiegozoo.org
Dr. Christine Molter	Cell: 262-909-6895	Email: cmolter@houstonzoo.org
Dr. Dennis Schmitt	Cell: 417-861-9572	Email: dennisschmitt@missouristate.edu
Dr. Ellen Wiedner	Cell: 571-228-2312	Email: ebwvmd@yahoo.com
Dr. Paul Ling	Cell: 281-460-1696	Email: pling@bcm.edu
Erin Latimer	Cell: 703-855-9611	Email: latimere@si.edu
Dr. Valerie Johnson	Cell: 603-443-2099	Email: john7670@msu.edu
Integris ProHealth Pharmacy	405-945-4426	
Integris Central Pharmacy	405-949-3747	Matthew Street
Mercy Pharmacy	405-752-3590	

APPENDIX I. SAMPLE GUIDELINES – QUICK CHART (IN ORDER OF PRIORITY)

	Routine Weekly Monitoring	Clinical Suspect or Sick Elephant	Herdmates of Sick Elephant	Daily Monitoring of Sick Elephant
Purple Top Tubes (whole blood)	Total 3 mL blood	Total 5-10 mL blood	Total 4-12 mL blood	Total 10-16 mL blood
3 mL tubes	1 mL WB NEHL for PCR	1 mL JKAH for PCR	1-2 mL JKAH for PCR	1 mL JKAH for PCR
	1 mL WB JKAH for PCR	0.5 mL JKAH for CBC	0.5 mL JKAH for CBC	0.5 mL JKAH for CBC
	1 mL WB OKC Zoo (banking)	2 mL NEHL for PCR	1 mL NEHL for PCR	2 mL NEHL for PCR
		2-4 mL Johns Hopkins for culture	6-10 mL JKAH (banking)	2 mL Johns Hopkins (research)
		2-3 mL JKAH (banking)		6-10 mL JKAH (banking)
Light blue top Tubes (Citrate, whole blood)		Total 2-3 mL blood		Total 2-3 mL blood
		2-3 mL Fibrinogen (Antech)		2-3 mL Fibrinogen (Antech)
Red/Grey Top Tubes (Serum)	Total 10 mL blood	Total 8-400 mL blood	Total 7 mL blood	Total 25-400 mL blood
4 mL tubes	2-4 mL serum JKAH (banking)	2 mL JKAH (chem)	2 mL JKAH (chem)	2 mL JKAH (chem)
		6 mL NEHL (ELISA)	5 mL NEHL (ELISA)	5 mL NEHL (ELISA)
		20-200 mL Johns Hopkins (research)		20-200 mL Johns Hopkins (research)
				6-10 mL OKC Zoo (banking)
Trunk Wash	Total 30-50 mL	Total 30-50 mL	Total 30-50 mL	Total 30-50 mL
50 mL conical vials	30-50 mL JKAH for PCR	30-50 mL JKAH for PCR	30-50 mL JKAH for PCR	30-50 mL JKAH for PCR
		30-50 mL NEHL for PCR	30-50 mL NEHL for PCR	30-50 mL NEHL for PCR
Swabs		Swab lesions (NEHL)		
Plain Red Tubes (no separators)				5 mL blood for Ganciclovir levels
Royal Blue Top (minerals)				3-4 mL MSU mineral panel, 50701

	First Priority
	Second Priority
	Third Priority

Appendix II: Shipment Guidelines for EEHV Samples

National Elephant Herpes Laboratory (NEHL)

1. In a case where EEHV is clinically suspected:
 - a. Whole blood in EDTA (1-2 ml) qPCR, sent on ice packs
 - b. Serum (> 2 ml) for EEHV ELISA, sent frozen on ice packs
2. Necropsy samples should be collected as described in Appendix VII and sent on dry ice.
3. Send samples to:
 - a. Erin Latimer, Research Assistant
Department of Pathology, Smithsonian's National Zoo
3001 Connecticut Ave, NW
Washington, DC 20008
202-633-4252 (office phone)
202-633-8717 (fax)
703-855-9611 (cell)
 - b. Email Erin Latimer (latimere@si.edu)
 1. Do not write the word "herpes" in the subject line.
 - c. Call the phone numbers above to alert the lab that a clinical or post mortem sample will be coming, and have tracking number available for Erin.
 - d. If it is after hours or on Saturday or Sunday, call Erin's cell phone to arrange for shipment and sample testing. Sample may have to be sent to Erin's house or a FedEx Office.
 1. Erin 703-855-9611 (Cell), 703-471-2168 (Home)

Johns Hopkins School of Medicine

1. In a case where EEHV is clinically suspected:
 - a. Whole blood in EDTA (2-4 ml) should be collected for virus culture, shipped unfrozen
 1. If possible, sample should be collected BEFORE initiating treatment
 - b. Serum (20-100 ml) should be collected for virus culture, shipped unfrozen
 1. If possible, sample should be collected BEFORE initiating treatment
 - c. Samples should be collected 24-48 hours after treatment as well
2. Necropsy samples should be collected as described in Appendix VII and sent unfrozen.
3. Gary Hayward should be contacted when a clinical sample is collected. He can be reached at:
Email: ghayward@jhmi.edu
Office phone (410) 955-8684
Home phone (410) 821-8197
4. Samples should be sent to
 - a. Gary Hayward
Johns Hopkins School of Medicine, Viral Oncology Program
3M09, Bunting-Blaustein Cancer Research Building
1650 Orleans St.
Baltimore, MD 21287

APPENDIX III: EQUIPMENT AND SUPPLIES

The following equipment and supplies will need to be on hand for support during therapy. One staff member will be designated to move these supplies in an organized manner into the barn. Supplies used on a daily basis in the area will be left in their normal storage locations.

Elephant Barn supplies:

- Assortment of ropes, slings and belly bands
- Flashlights
- Obstetric lubricant
- Mortar and pestle
- Towels
- Inner tubes (various sizes)/ gym mats —to be used for cushioning and support in the event of a full immobilization procedure

Clinic Supplies:

Drug administration/supportive care:

- Famciclovir powder
 - Minimum 3-day supply for Asha, which is 30,000 mg PO TID = 270 grams
- Ganciclovir 500 mg vials, 25 vials / box: 12,500 mg/ box
 - Order as needed from Integris ProHealth Pharmacy
- Gram scale, measuring cup, gel capsule
- Other drugs (see drug list appendix V)
- Ultrasound gel (for mixing with famciclovir for rectal administration)
- OB sleeves and lube
- OB suits
- Duct tape
- Orasweet OTC syrup (minimum on hand 4,500 ml)
- Exam gloves (all sizes)
- Towels (10-12)
- 1L sterile water to mix dose of ganciclovir
- 10-22 GA catheters (long & short)
- Large Animal IV (bungee type) line (3 complete sets)
- Large Animal IV extension set (3 complete sets)
- Standard IV administration set (3 complete sets)
- Standard Extension set (3 complete sets)
- Blood filters
- Alcohol
- Scrub
- 4X4 gauze (6 packages)
- Bandage scissors
- Heparin & saline
- Sterile lube (tube)
- Large animal surgery pack & sterile gauze packs
- Scalpel blades: 10, 15
- Sterile gloves (6.5, 7, 7.5)

- Lidocaine
- Drapes
- Sharps container
- Suture (0, 1, 2 prolene or similar with cutting needle)
- Tissue glue
- White tape (1-inch, 2-inch, 4 inches; 5 rolls each)
- Vetwrap (2 & 6", multiple rolls each size)
- Elastikon (2 & 4", multiple rolls each size)
- Injection caps
- T port- standard & large bore
- Tegaderm
- 1L & 5L fluids (all in stock)
- IV pump
- IV pole
- 1L & 3L pressure bags
- Ropes/wire to hang bags
- Extension cord
- Flashlights/head lamps
- Portable surgery lights
- Plasma (stored from herd, keep on ice)
- Hetastarch

Sedation/anesthesia:

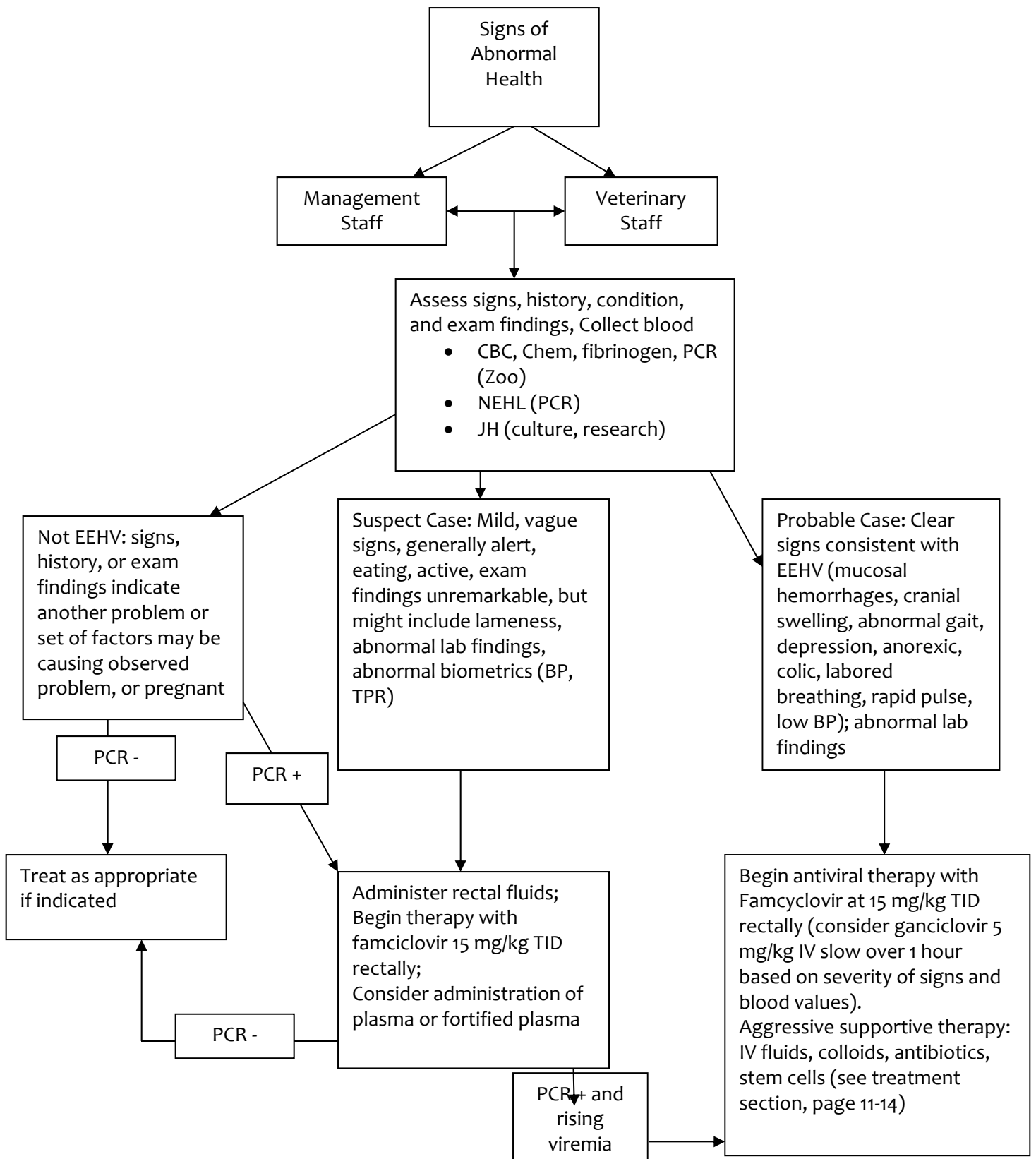
- Butorphanol (minimum 4 bottles, 5 ml/bottle, 30 mg/ml)
- Detomidine (minimum 3 bottles 5 ml/bottles, 10 mg/ml)
- Naltrexone (minimum 4 bottles, 50 mg/ml, 30 ml/bottles)
- Atipamezole (minimum 13 bottles, 10 ml/bottle, 5 mg/ml)
- Emergency drug box
- Emergency bag
- Anesthesia clip board
- Calculator
- Pole syringe
- Syringes (Box each of 60, 35, 20, 12, 6, 1 ml sizes)
- Needles (14g, 16g, 18g, 20g, 22g 1.5", 25g; one box each)
- Butterfly catheters 19g, 21g (1 box each)
- Oxygen tanks
- Portable anesthesia machine
- ET tubes (24, 22, 20, 18, 16, 14), tie gauze, & cuff syringe
- Stylets, cake spatulas, endolight
- Laryngoscope w/long blade
- Y piece (nasal administration)
- Ropes (open mouth)
- Blocks (open mouth)
- Surgivet Advisor with all leads
- Pulse oximeter
- I-STAT & cartridges
- Stethoscope
- Thermometer

- Endoscope and associated equipment (intubation)

Miscellaneous:

- ICU flow sheet, pens, clipboard, watch
- Sonosite & large tub of ultrasound gel
- Doppler
- ECG
- Ophthalmoscope (1)
- Ophthalmoscope extra battery
- 50 ml centrifuge tubes
- Lancet supplies
- Lidocaine/prilocaine cream
- Pericardiocentesis
 - 60 cc regular tipped syringes
 - 3 way stop cocks (2)
 - 5 ¼" IV catheter, smallest gauge available
 - 10" spinal needles
 - Sterile urine cup to save for culture
 - 50 ml conical vials for storage of fluid

APPENDIX IV. EEHV FLOW CHART



APPENDIX V. EEHV “FAST PLAN”

Initiate antiviral therapy if:

- Clinical signs are present
- CBC changes are evident (decreased WBC, platelet or monocyte count) as compared to individual’s normal range
- EEHV viremia present (over 5,000 vge/ml) on whole blood qPCR
- Rapidly increasing EEHV viremia vge/ml on whole blood qPCR

This is intended to be an instruction sheet to get therapy initiated as quickly as possible. Background information, details, and reasoning for these steps are present in the EEHV protocol.

1. Collect baseline information
 - TPR
 - BP
 - Blood collection (8-15 ml in purple tops, 2-3 ml in light blue top, 60 ml in tiger tops)
2. Administer rectal fluids
3. Administer 15 mg/kg Famciclovir
 - Orally or rectally (mix with OB lube)
4. Standing sedation with Butorphanol 0.06 mg/kg IM and detomidine 0.015 mg/kg IM (can reverse with 2.5 X dose naltrexone and 5 X dose atipamezole)
5. Place IV catheter in ear vein (consider multiple venous catheters if reversing sedatives immediately)
6. Administer up to 10 ml/kg fresh or frozen elephant plasma (use fortified plasma if available)
7. Administer elephant mesenchymal stem cells IV
8. Maintain IV fluids at rate of 2-4 ml/kg/hour
9. Administer ancillary therapies as indicated (antibiotic, anti-inflammatory, vitamin C etc.)
10. Consider administration of 5 mg/kg ganciclovir mixed in 1 liter of fluids over the course of an hour (based on clinical signs and viremia level severity)

APPENDIX VI. DRUG DOSAGES

	Drug	Concentration	Dosage range	Comments
Sedation	Butorphanol	10 mg/ml	0.02-0.07 mg/kg IM	Reverse with Naltrexone
	Naltrexone reversal	50 mg/ml	2.5-5 x Butorphanol mg	
	Detomidine	10 mg/ml	0.01-0.02 mg/kg IM	Reverse with Atipamezole
	Atipamezole reversal	5 mg/ml	0.1-0.16 mg/kg IM	
	Azaparone		0.024-0.038 mg/kg IM	
Antivirals	Famciclovir	500 mg/tab or bulk powder	15 mg/kg TID-QID	PO or rectally
	Ganciclovir IV	500 mg/bottle	5 mg/kg BID	Give IV with NaCl slowly over 1 hour, mix each vial with 3 ml sterile water (if lyophilized powder)
	Ganciclovir oral	500 mg/bottle	5 mg/kg BID	See recipe for mixing up oral dose
Fluids	Maintenance adult		2 ml/kg/hour IV	
	Maintenance calf		4 ml/kg/hour IV	
	Surgical rate		10 ml/kg/hour IV	
	Shock rate		90 ml/kg/hour IV	
Analgesics	Buprenorphine	0.3 mg/ml	0.004 mg/kg IV	Equine dose, given with Acepromazine or Xylazine
	Morphine analgesia		0.03-0.06 mg/kg QID IM	
	Morphine sedation		0.06-0.2 mg/kg IM	
	Butorphanol	10 mg/ml	0.01-0.03 mg/kg SQ/IV/IM Q4h	
	Xylazine	100 mg/ml	0.04-0.08 mg/kg IM/IV	May cause sedation at this dose, caution in doses >400 mg
	Medetomidine	20 mg/ml	0.003-0.005 mg/kg IM	Possible sedation
	Flunixin meglumine	50 mg/ml	0.2-0.5 mg/kg IV SID	IM administration extremely necrotizing to tissue.
	Meloxicam		0.6 mg/kg SID IV-PO	

	Drug	Concentration	Dosage range	Comments
Analgesics	Carprofen		0.7 mg/kg SID IV/PO	Anecdotal
	Ketoprofen		1-2 mg/kg Q24-48hr PO/IV	
	Phenylbutazone		2.2-4.4 mg/kg SID PO	Do not use in ear veins, Equine dose, Max 5 days
	Aspirin		10 mg/kg S-BID PO	Equine dose
	Ibuprofen		6 mg/kg BID PO	
	Firocoxib		0.1-0.2 mg/kg PO SID- BID	
Antibiotics	Amikacin	250 mg/ml	3-5 mg/kg SID IM/IV	
	Amoxicillin		11 mg/kg SID IM	
	Ampicillin		8 mg/kg B-TID PO	Amp sodium = IV, Amp trihydrate = PO
	Ceftiofur (Naxcel)		1.1 mg/kg BID IM	1.1 mg/kg SID IV (PD)
	Ceftiofur-CFA (Excede)	200 mg/ml	5 mg/kg Q7d IM	
	Enrofloxacin	Bulk powder	2.5-5 mg/kg SID PO	Caution in growing animals
	Florfenicol		20 mg/kg IM Q48hr	Cattle dose
	Penicillin-Dual	300000 IU/ml	2275-4545 IU/kg Q48hr IM	
	SMZ-TMP		20 mg/kg BID PO	
Emergency	Aminophylline	25 mg/ml	11 mg/kg BID PO/IV	IV dose should be diluted in 100 ml D5W or Saline
	Atropine (LA)	15 mg/ml	0.01-0.02 mg/kg IV 0.03-0.06 mg/kg SQ	Based on equine dosing
	Calcium gluconate 10%	100 mg/ml	100 mg/kg IV	Give IV slowly, to effect Based on equine dosing (start with ½ or less for elephant)
	Doxapram HCL	20 mg/ml	0.4 mg/kg IV/IM	
	Epinephrine 1:1000	1 mg/ml	0.01-0.02 mg/kg IV/IC/IT	Based on foal dosing
	Lidocaine 2%	20 mg/ml	0.05-0.5 mg/kg IV	To correct heart block
	Dexamethasone	2 mg/ml	0.05-0.5 mg/kg IV/IM	ONCE – Efficacy controversial

	Drug	Concentration	Dosage range	Comments
	Sodium Bicarbonate 8.4%	1mEq/ml	0.5-1 mEq/kg IV	Equine dose
Other	B-complex		5 ml SID IM	
	Furosemide x 5 days	50 mg/ml	0.8 mg/kg BID IM/PO	
	Vitamin E		2.2 IU/kg PO SID	
	Omeprazole		1 mg/kg (prophylaxis) 4 mg/kg (treatment)	SID
	Vitamin C		30-50 mg/kg PO	May be given IV slowly diluted in 1 liter of fluids
	Zelnate® (DNA immunostimulant)	5, 10 or 50 dose vials	2 ml IM (every 3-4 days)	Administer as soon as clinical signs evident or EEHV suspected
	Interferon alpha-2a (Intron® A or Roferon® A)	Intron® A (25mIU/2.5ml) Roferon® A (9mIU/0.5ml)	27-33mIU IM once daily	Administer as soon as clinical signs evident or EEHV suspected
	Pentoxifylline		8-10 mg/kg PO BID	Equine dose (*0.7 mg/kg used for Kairavi)

APPENDIX VII: NECROPSY AND POST MORTEM SAMPLE COLLECTION

Necropsy Procedures (from EEHV research and tissue protocol, April 2019)

Whole heart blood should be collected into EDTA tubes **immediately** post mortem.

- Samples should be collected for NEHL, Johns Hopkins, & FCCC and stored in the refrigerator
- As much whole blood and whole blood for serum should be collected as possible

The lesions of EEHV are nearly identical in both Asian and African elephants. Gross findings typically include hydropericardium (free fluid in membranous sac around heart), along with extensive petechial (small) and ecchymotic (large) hemorrhages within all layers of the heart. In addition, petechial hemorrhages associated with mesenteric and serosal (external surface of organs) surfaces are diffusely scattered throughout the peritoneal cavity. Cyanosis of the tongue is sometimes present as is hepatomegaly (enlargement of the liver) and ulceration of the oropharynx and large intestine. Histology correlates well with the gross findings and also demonstrates the presence of intranuclear viral inclusion bodies within the capillary endothelial cells of the heart, tongue, liver, and to a lesser extent, the intestinal tract. Electron microscopy readily demonstrates the presence of herpes virus.

It is important to perform the necropsy as soon after death as possible, to increase the chance of recovering viable virus from post mortem tissues. Timely collection of tissues samples and submission to EEHV laboratories are paramount to facilitate viral culture. However, based on the unique social requirements of elephants and need to grieve and accept the death of a herd mate, it is unlikely we will be able to remove the body immediately. The compromise between elephant social needs and need for samples will be reached by collecting the samples listed above (whole heart blood and tongue tissue) and sending them for culture immediately, then performing the complete necropsy and sample collection when herd mates are ready.

Photographs should be taken of all gross lesions.

Two complete sets of tissue samples should be collected from each organ and placed into formalin at a 10:1 ratio.

1. 1 set to go to Northwest ZooPath, for diagnostic purposes
2. 1 set to be shared with the Elephant SSP Pathologist (follow SSP necropsy protocol)

Fluid Collection

- The following fluids should be collected during post mortem examination and frozen for future use:
 - Ascites
 - Whole heart blood
 - Pericardial fluid
 - Cerebral Spinal Fluid
 - Urine
- All fluids should be placed into EDTA tubes (purple topped tubes)

Tissue Collection

The following fresh tissues should be collected in whirl pak bags or Ziploc bags: liver, heart, lung, kidney, spleen, tongue, intestine, skeletal muscle, brain, lymph nodes, and any grossly abnormal tissues or tissues with significant hemorrhages.

Five sets of each tissue listed above should be collected:

Set #1: To National Elephant Herpes Laboratory

- Each tissue should be cut in samples measuring 3 cm x 3 cm
- Each should be placed in a separate sterile whirl pak bag
- Bags should be labeled with tissue, date, and elephant name
- Suspected herpetic lesions should be swabbed with a sterile saline soaked cotton swab; place swab in 15 ml centrifuge tubes and stored at -80C until shipment
- Samples should be stored at -80C sent as early as possible on dry ice per shipment instructions in Appendix II

Set #2: To Johns Hopkins

- Each tissue should be placed in a separate sterile whirl pak bag or separate sterile plastic tubes
- Bags and/or tubes should be labeled with tissue, date, and elephant name
- Samples should be maintained FRESH and shipped on ice packs per shipment instructions in Appendix II

Set #3: To Fox Chase Cancer Center (Virginia Pearson)

- Each tissue should be placed in a separate sterile whirl pak
- Additional tissues to be collected include:
 - Salivary gland- intact
 - Trigeminal ganglion
- Bags & tubes should be labeled with tissue, date, and elephant name
- Samples should be maintained FRESH and shipped on ice packs per shipment instructions in Appendix II

Sets #4&5: To be kept at the Oklahoma City Zoo

- 4 in x 4 in sections of each tissue should be collected for future diagnostics, research and testing
 - Additional sections of any hemorrhagic tissue should be collected as well
- Tissues should be placed in separate whirl pak bags
- Bags should be labeled with type of tissue, date, elephant name and ISIS number
 - Use a freezer safe marker on the bag that won't rub off
 - Samples should be stored in -80 freezer at Oklahoma City Zoo until needed