

Houston Zoo Off-site Elephant Blood Donor Protocol

Asian elephant calves are at risk for Elephant Endotheliotropic Herpesvirus hemorrhagic disease (EEHV-HD), which can be treated with blood products, including whole blood and plasma. The Houston Zoo herd is multi-generational with both adult blood donors and at-risk calves. As the herd grows, the need for compatible blood donors also grows. To be as prepared as possible, blood donations from healthy adult Asian elephants outside of the Houston Zoo may be needed to provide lifesaving treatment. This protocol is a guideline for off-site elephant blood donations.

Blood Donor Eligibility

To be an eligible blood donor, the elephant must meet the following requirements:

1. Healthy sub-adult or adult Asian elephant on physical exam
2. Normal CBC and chemistry within 1 month of whole blood collection for donation
3. No detectable EEHV viremia at the time of donation (negative whole blood PCR)
4. Acceptable minor (for plasma donation) and/or major (for whole blood donation) crossmatch to recipient
5. Trained (or sedated) for blood donation
6. No recent health problems in herd of origin

Blood Donor Diagnostic Testing

1. Physical exam
 - a. The animal should be in good health and physical condition as determined by the donor institution.
2. CBC and chemistry within 1 month of donation
 - a. CBC = 1 EDTA (purple top) tube, non-clotted.
 - b. Chemistry = 1 serum separator (tiger top) tube.
 - c. Bloodwork may be performed in-house or sent to a diagnostic reference laboratory. Please forward results to the Houston Zoo.
 - d. If the donor institution is not able to perform diagnostic testing, arrangements may be made to send blood tubes to the Houston Zoo for analysis.
3. EEHV PCR
 - a. PCR = 1 EDTA (purple top) tube.
 - b. Do not spin blood down, send whole blood.
 - c. Blood tube should be sent to Houston Zoo, who will facilitate whole blood PCR testing at Baylor College of Medicine.
 - d. Send blood chilled (not frozen) overnight Monday, Tuesday, Wednesday, or Thursday. Weekend deliveries may be arranged on an emergency basis. This may be collected and sent with blood bags at the time of collection.
 - e. Houston Zoo will communicate with donor elephant's PCR results.
4. Crossmatching
 - a. Minor crossmatching (for plasma donation) = 1 EDTA (purple top) tube.
 - b. Major crossmatching (for whole blood donation) = 1 EDTA (purple top) tube.
 - c. Do not spin blood down, send whole blood.
 - d. Please send tube to Houston Zoo for minor and major crossmatching.

- e. Send blood chilled (not frozen) overnight Monday, Tuesday, Wednesday, or Thursday. Weekend deliveries may be arranged on an emergency basis. Ideally, crossmatching would occur prior to a donation; however, this may be collected and sent with blood bags and whole blood for PCR at the time of collection.
 - f. Houston Zoo will communicate with donor if elephant is a good match for potential recipient.
5. Summary of diagnostic testing
- a. 1 EDTA (purple top) tube and 1 serum separator tube (tiger top) for CBC and chemistry in-house or send to reference laboratory with results forwarded to Houston Zoo within 1 month of donation. Alternatively, send tubes to Houston Zoo to run.
 - b. 1 EDTA (purple top) tube to Houston Zoo at the time of blood donation for PCR.
 - c. 2 EDTA (purple top) tubes to Houston Zoo prior to donation or at the time of donation for major and minor crossmatching.

Blood Collection Procedure

1. Supplies

- a. Chlorhexidine or betadine scrub.
- b. Isopropyl (rubbing) alcohol.
- c. Gauze or cotton balls.
- d. Plasma collection bags.
 - i. 450 ml collection bags containing Citrate Phosphate Dextrose Adenine Solution (CPDA-1) anticoagulant (or equivalent) with attached plasma transfer bag have been used at Houston Zoo from ear veins.
 - ii. Larger bags, including 600 ml, 1500 ml, and 3000 ml bags, exist and may be used as well from leg veins.
- e. Hemostats (2).
- f. Scale (<5 kg limit if available).

2. Procedure

- a. Blood collection should be done with the goal to be sterile.
 - i. Blood and plasma are excellent media for bacterial growth.
- b. Tare (or get weight) of the bag set up on scale.
- c. Ensure that the line to the secondary plasma transfer bag is clamped off with a plastic clamp provided on the line or the hemostat to prevent whole blood overflow into that side.
- d. Identify vessel for donation and scrub with chlorhexidine or betadine scrub, then wipe from the center outward with alcohol. Repeat alternately as many times as needed until gauze is clean after wiping skin.
- e. Insert needle into the vein and establish good flow.
- f. Fill the primary whole blood collection bag to designated volume using scale to measure.
 - i. 1 ml of blood is equivalent to 1 g on the scale.
 - ii. Each bag size is designated to hold a measured amount of blood (i.e. 450 ml = 450 gm).

1. Different size bags may be used, but 450 ml bags are considered standard. Each bag should be labeled with its intended volume.
- iii. Do not over fill bags, as they may rupture
- iv. If bag doesn't completely fill, finish the collection process steps below. Any bag more than 1/3 full is considered useful.
- v. Mix the bag periodically during the collection to insure proper blood/anticoagulant contact.
- g. When primary whole blood collection bag is full, clamp off the blood line with the plastic clamp provided on the line or the hemostat and pull needle out.
- h. Tie blood line off with a knot close to the bag to ensure no leakage.
- i. Cut needle off end of line to remove sharps hazard. Dispose of the needle in a sharps container.
- j. Label bag with donor name (or other identifier), date, time of collection, and final weight.
 - i. If animal is sedated at time of collection, write sedative drugs on bag as well.
- k. Place bag in refrigerator immediately.

Blood Processing

There are 3 basic options for processing blood bags: leaving as whole blood, spinning for plasma, or gravity separation for plasma.

1. Whole blood
 - a. If blood is to be left whole, leave bag in refrigerator. No further processing is needed.
 - b. Transport whole blood bags chilled (not frozen) via overnight shipping or ground transport to the Houston Zoo (or meet Houston Zoo staff at designated meeting point).
2. Spinning for plasma
 - a. The Houston Zoo has a blood bag centrifuge, donated from the Gulf Coast Regional Blood Center, on site that is available for spinning bags for plasma.
 - b. Transport whole blood bags chilled (not frozen) via overnight shipping or ground transport to the Houston Zoo (or meet Houston Zoo staff at designated meeting point).
 - c. Donor bags will be centrifuged when received. Plasma will be separated from the cells using the extractor once spun down.
 - d. Ideally, to create "Fresh Frozen Plasma" the blood should be processed within 8 hours of collection.
3. Gravity separation for plasma
 - a. If spinning is not an option, hang blood bag upright (with the line and ports on the top) in refrigerator overnight and the plasma will naturally separate out.
 - b. Once separated, use a plexiglass plasma extractor device to cleanly transfer the plasma component from the primary whole blood collection bag to the secondary plasma transfer bag.
 - i. Please contact the Houston Zoo for further information if this step is needed.
 - c. Remaining red cell component may be discarded.
 - d. Freeze the plasma (-20 or -80F acceptable) and ship frozen to the Houston Zoo.

Shipping

Please let Houston Zoo staff know that you will be shipping blood products to us. Do not ship over the weekend, unless it is an emergency.

Houston Zoo, Inc.
Veterinary Clinic
1513 Cambridge St.
Houston, TX 77584

If you need assistance with shipping, please contact Kathryn Lippman, klippman@houstonzoo.org; 713-533-6632.

Storage

Whole blood: will last up to 35 days at 1-6 C (refrigerator temperature), but clotting factors reduce after 8 hours.

Non-frozen plasma: will last up to 48 hours at 1-6 C (refrigerator temperature), but clotting factors reduce after 8 hours, so it is ideally used or spun within 8 hours of collection. It is only considered “fresh” plasma if used or spun within 8 hours.

Frozen plasma: will last 1 year at -18C (regular freezer temperature) and 7 years at -80C (large scientific freezer temperature).

Contact

If there are any questions please contact Dr. Christine Molter, cmolter@houstonzoo.org, 713-533-6706 or Dr. Maryanne Tociłowski, mtocidłowski@houstonzoo.org, 713-533-6629.