Minor and Major Blood Crossmatching in Asian Elephants *(Elephas maximus)*

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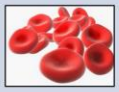
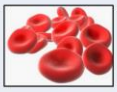
Crossmatching

- Elephants have blood groups and pre-formed blood group antibodies
- Minor crossmatch: assesses compatibility of a recipient receiving plasma products from a donor
  - Recipient red blood cells + Donor plasma
- Major crossmatch: assess compatibility of a recipient receiving whole blood products from a donor
  - Recipient plasma + Donor red blood cells
- Saline controls and recipient auto-controls
  - Rouleaux vs agglutination

Elephants have blood groups and pre-formed blood group antibodies. Consequently, crossmatching should be done in advance of transfusions.
Minor crossmatching assesses the compatibility of a recipient receiving plasma products from a donor. Recipient red blood cells are tested for reaction with donor plasma. Major crossmatching assesses the compatibility of a recipient receiving whole blood products from a donor. Recipient plasma is tested for reaction with donor red blood cells. Cross match tests that do not react have good compatibility. Saline controls and recipient auto-controls should be simultaneously tested during cross matching to help distinguish between possible red blood cell rouleaux versus agglutination reactions. With saline controls, recipient red blood cells are combined with saline and with auto-controls, recipient red cells are tested against their own plasma.

Minor and Major Crossmatching

	Minor Crossmatch -Plasma transfusion	Major Crossmatch -Whole blood transfusion -Packed red cell transfusion
Recipient		
Donor		

Crossmatching Materials

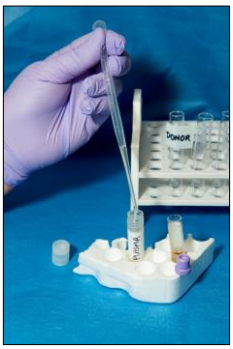
- EDTA whole blood (fresh samples are best)
- Centrifuge
- Glass, curved bottom tubes
- Cryovials or Eppendorf tubes
- 0.9% saline
- Pipettes
- Test tube rack
- Hematocrit tube + sealant
- Incubator
- Marker + paper

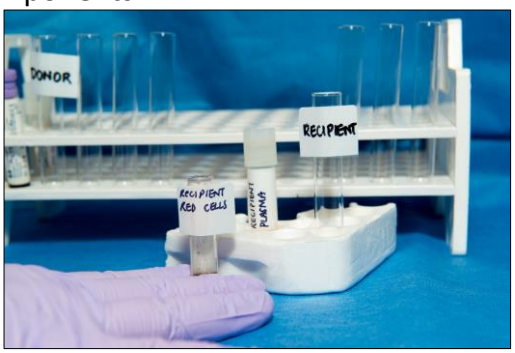
These are the materials that are needed for crossmatching.

Minor Crossmatching Steps
Part 1: washing red blood cells

Houston Zoo

1) Collect EDTA whole blood from the recipient and donor
2) Centrifuge the whole blood samples and separate the plasma from the red cells. Save both components.



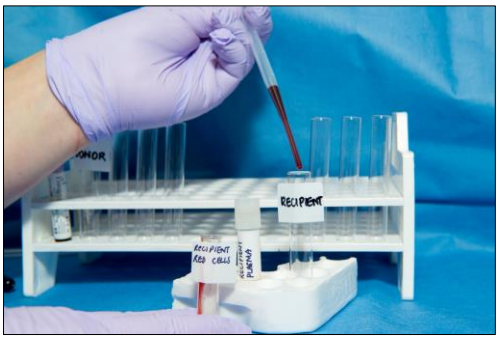


The first main part of any crossmatch requires washing red blood cells to cleanse and help remove surface elements such as surface antibodies from the red cells. First, EDTA whole blood is collected from both the recipient and the donor. The blood samples are centrifuged and the plasma is separated from the red blood cells. Both portions of the blood samples are labeled and saved. For this example we will review the steps in minor crossmatching, where recipient red blood cells are assessed for reaction with donor plasma. At the Houston Zoo, we typically prioritize conducting a minor cross match, as plasma is one of the first blood products that would be transfused to a viremic calf at the start of an aggressive treatment plan.

Minor Crossmatching Steps
Part 1: washing red blood cells

Houston Zoo

3) Place 1-2 drops of recipient red cells into a labelled glass tube
4) Add 1-2mL of saline and use the pipette to mix and wash the red cells to remove surface antigens.



One to two drops of recipient red cells is placed into a labelled glass, round bottomed tube and 1-2mL of saline is added. The solution is mixed with a pipette to wash the cells.

Minor Crossmatching Steps
Part 1: washing red blood cells

Houston Zoo

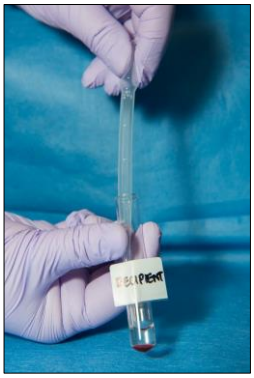
5) Centrifuge at 2500rpm for 20 seconds

The tube is centrifuged at 2500rpm for 20 seconds. The centrifuge must reach the constant speed of 2500rpm before the 20 second duration can be timed. Allow the centrifuge to naturally come to a complete stop (i.e. do not apply any brake).

Minor Crossmatching Steps
Part 1: washing red blood cells

Houston Zoo

6) Remove the supernatant
7) Repeat steps 4 – 6 three more times (4 red cell washes)

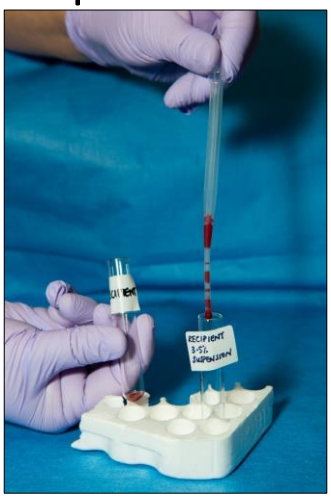


The supernatant is removed, leaving a packed red blood cell button on the bottom of the tube. Repeat these washing steps another three times for a total of 4 red blood cell washes.

Minor Crossmatching Steps
Part 2: making a 3-5% RBC suspension

Houston Zoo

8) Add 1 drop of washed red cells to a new labelled glass tube
9) Add 20-40 drops of saline and mix to resuspend the cells (this should make a 3-5% suspension)

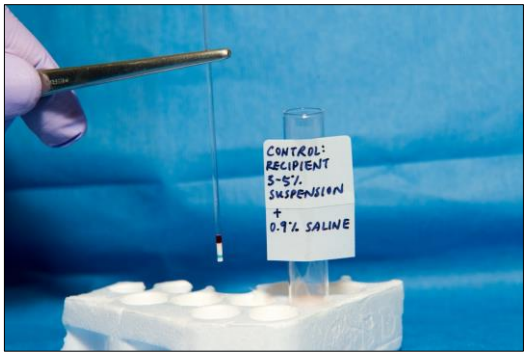


The next part involves making a 3-5% suspension with the washed red blood cells. One drop of washed red cells is pipetted into a new labelled glass tube. Then, 20-40 drops of saline is added and the solution is mixed to resuspend the cells. The suspension percentage is checked by determining it's PCV.

Minor Crossmatching Steps
Part 2: making a 3-5% RBC suspension

Houston Zoo

10) Check the suspension percentage by determining it's PCV
- If PCV < 3%, repeat steps 8 – 9 and add less saline drops; recheck PCV
- If PCV > 5%, add a few more saline drops to the suspension and recheck PCV



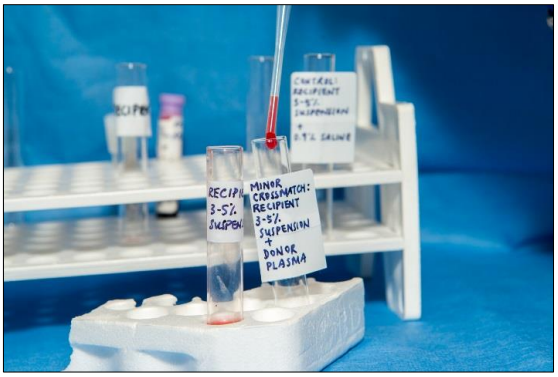
The suspension percentage is checked by determining it's PCV.

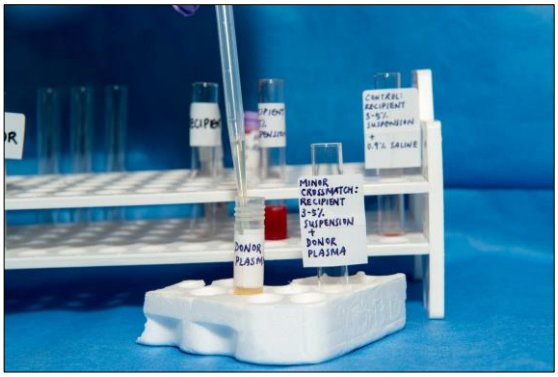
Minor Crossmatching Steps
Part 3: Crossmatch testing

Houston Zoo

11) Add 1 drop of 3-5% recipient suspension to a new glass tube

12) Add 2 drops of donor plasma and gently mix the tube





After the 3-5% suspension is made, the last part is assembling and assessing the crossmatch tests. A drop of 3-5% suspension is added to a new glass tube. Two drops of donor plasma is added and the tube is gently mixed.

Minor Crossmatching Steps
Part 3: Crossmatch testing

Houston Zoo

13) Repeat step 11 and add two drops of saline (control)

14) Repeat step 11 and add two drops of recipient plasma (auto-control)

In another clean glass tube, one drop of 3-5% red cell suspension is mixed with 2 drops of saline. This is the control test. In a third clean glass tube, one drop of 3-5% red cell suspension is mixed with 2 drops of the recipient's plasma. This is the auto-control test.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

Houston Zoo

15) Incubate at 35-37 degrees Celsius for 15 minutes

All of these tubes are incubated at 35-37 degrees Celsius for 15 minutes.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

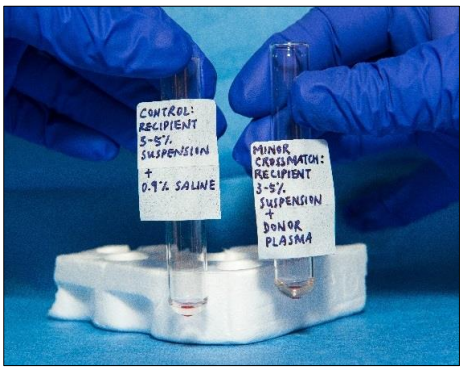
Houston Zoo

16) Remove the tubes from the incubator and centrifuge at 2500rpm for 20 seconds

After incubation, the tubes are centrifuged one more time at 2500rpm for 20 seconds.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

17) Observe the supernatant for hemolysis.
- If present in the crossmatched tube but not the control tubes, the match is not compatible

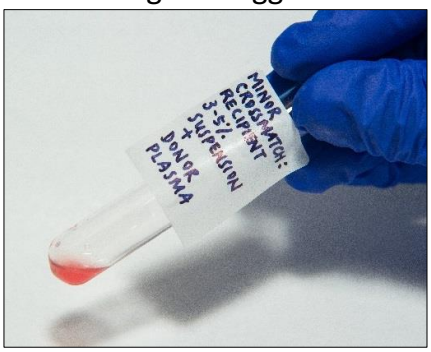


Once removed from the centrifuge, the supernatant from each tube is observed for hemolysis. If there is hemolysis in the crossmatched tube but none in the saline and auto-control tubes, the cross match is not compatible.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

Houston Zoo

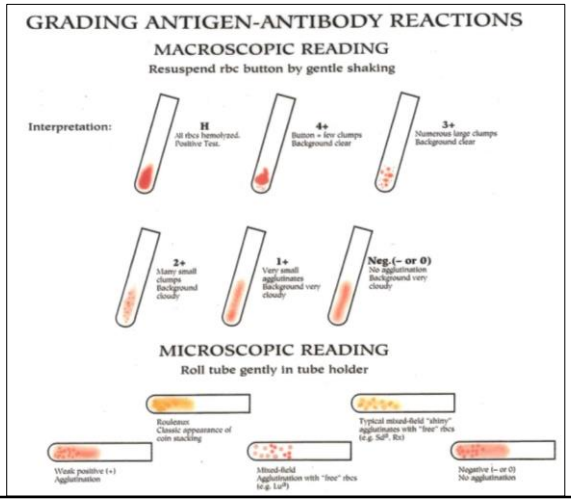
18. If no hemolysis is seen, gently rock the tube back and forth to resuspend the cell button.
19. Observe the resuspended mixture for signs of agglutination and grade on a scale of 0-4
    - 0 = no agglutination or hemolysis
    - 4 = solid agglutinated button with few clumps



If no hemolysis is seen in any of the tubes, take the cross match test tube and gently rock it back and forth to resuspend the red cell button. Once resuspended, observe the mixture for signs of macroscopic agglutination. Grade on a scale of 0-4. Repeat with the control tubes.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

- Reaction grading scale

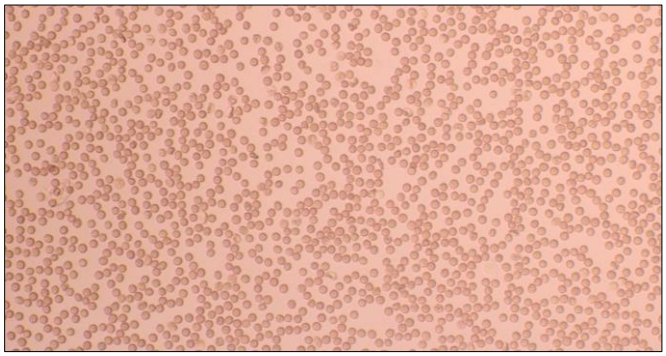


This reaction grading scale provides some extra descriptions to help categorize what you might be seeing.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

Houston Zoo

20) Place a drop of the saline control solution on a microscope slide topped with a coverslip and examine on low power (10X) and high power (40X) magnification for signs of agglutination.

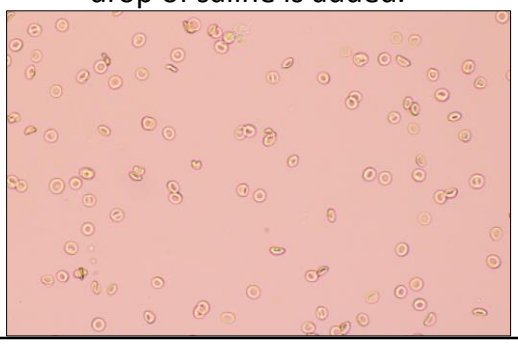


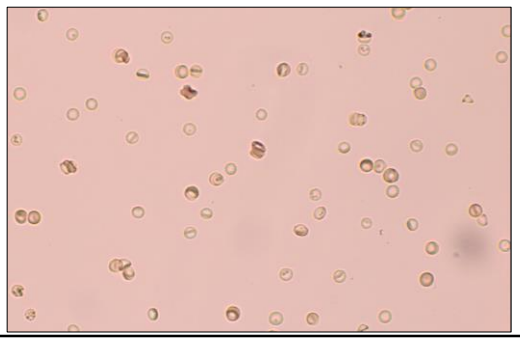
If results from macroscopic examination of the crossmatch and control tubes are not definitive, they can be examined microscopically for agglutination by strategically positioning the glass tube underneath the objective lens, or by dropping a sample of the mixture onto a slide and examining on low (10X) or high (40X) power magnification. This is an image of a saline control solution. Note how the red blood cells are evenly dispersed and cells are sitting side by side.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

Houston Zoo

21) Examine the auto-control solution microscopically for signs of agglutination (red blood cells in disorganized clumps) or rouleaux (red blood cells in stacks). Rouleaux cells should disperse when a drop of saline is added.



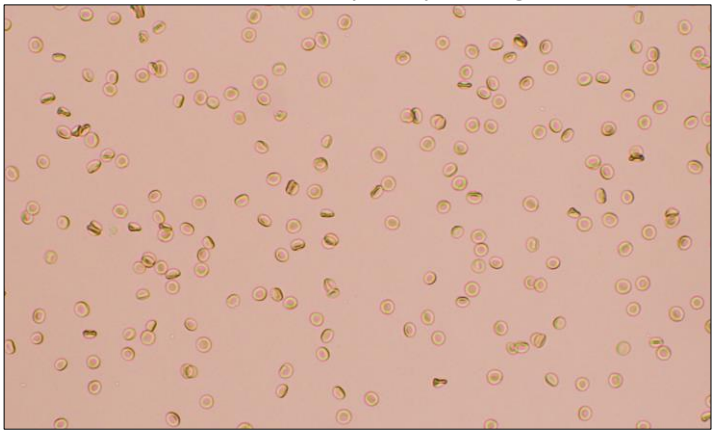


Microscopically examine the recipient auto-control solution for signs of agglutination or rouleaux. These images are of two different recipient auto-controls. Notice how the image on the right has a couple of short stacks of red blood cells. They are demonstrating rouleaux.

Minor Crossmatching Steps
Stage 3: Crossmatch testing

Houston Zoo

22) Examine the crossmatch solution microscopically for signs of agglutination.



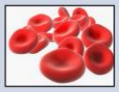
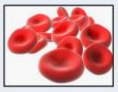
Lastly, examine the crossmatch solution microscopically for signs of agglutination. In this image of a minor crossmatch, there are several cells with rouleaux but no signs of agglutination.

Proficient crossmatching will take around 1-1.5 hours to perform. When possible, it is beneficial to cross match as a preventive measure (i.e. crossmatch in preparation for an EEHV crisis, as opposed to in response to clinical signs). Even at the Houston Zoo, our EEHV crossmatching protocols continue to advance. As our elephant keepers are able to obtain blood samples more frequently from calves earlier in their lives, we have more opportunities to preventively crossmatch calves to adult elephants and be better prepared with our plasma bank stores. If there was a situation where a preventive crossmatch could not be performed but a calf with clinical signs required treatment, our recommendation would be to administer an initial transfusion of donor plasma with a concurrent dose of diphenhydramine, followed by minor and major crossmatching as soon as possible.

Major Crossmatching Steps

Houston Zoo

To do a major crossmatch, follow the minor crossmatch protocol, except use the donor's 3-5% red cell suspension and the recipient's plasma

	Minor Crossmatch -Plasma transfusion	Major Crossmatch -Whole blood transfusion -Packed red cell transfusion
Recipient		
Donor		

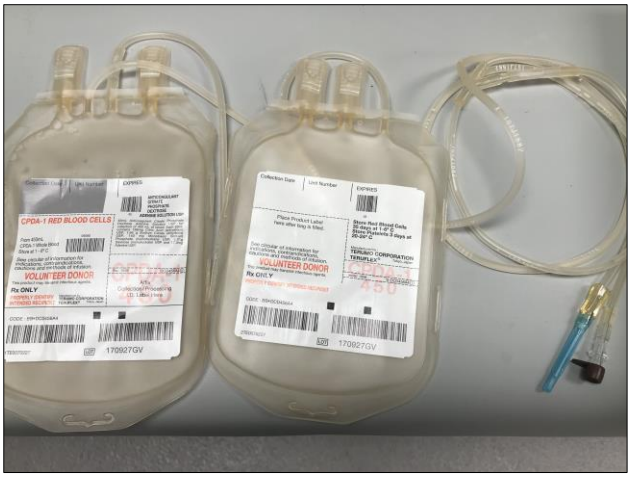
Blood Collection from a Healthy Donor

- Maintain an aseptic closed system
- Clean vasculature collection site thoroughly to minimize introduction of contaminants from collection needle into blood bag and the donor
  - Auricular vein or medial rear leg
  - Surgical skin preparation – alternating between topical antiseptic/disinfectant (e.g. iodine or chlorhexidine) and 70% isopropyl alcohol scrubs
- Prior to blood collection, place the collection bag end on a gram scale and tare the unit – as blood fills the bag, the scale will reflect the volume of blood being collected.
- Gently rock the collection bag as it fills to mix the blood with anticoagulant
- Assess general health of donor elephant (e.g. normal CBC and chemistries values and negative EEHV levels)

When blood is collected from a healthy donor it is important to maintain an aseptic closed system while handling the blood transfer bags. An ear vein or a medial rear leg vein can be used for blood collection. The vasculature site should be cleaned thoroughly to minimize introduction of contaminants from the collection needle into the donor and also the blood collection bag. Alternating scrubs of topical antiseptic/disinfectant and isopropyl alcohol will help remove debris and microorganisms. The volume of collected blood can be measured by using a gram scale which is used to weigh the blood as it fills the collection bag. As the bag fills, it should be gently rocked continuously to mix the blood with the anticoagulant. Ideally, a donor elephant's health status should be verified through normal CBC and chemistry parameters and negative EEHV qPCR levels.

Blood Bag Systems

- Blood collection bag +/- plasma transfer bag
- Collection bag
  - 16gauge collection needle
  - CPDA-1 (citrate phosphate dextrose adenine) or ACD (acid citrate dextrose) anticoagulant
  - Volume capacity = 450mL
- Transfer bag
  - No additive
  - Volume capacity = 300mL



Blood bag systems consist of a blood collection bag which may or may not be connected to a plasma transfer bag, depending on the manufactured configuration. Basic blood collection bags will have a 16 gauge needle on the blood collection line and generally contain either CPDA-1 or ACD anticoagulant. Common blood collection bag volumes are 450mL. There are also equine collection bags with 1500mL and 3000mL blood volume capacity, but it may be more challenging to separate plasma from these larger collection bags. Typical plasma transfer bags do not contain additive and have a volume capacity of 300mL.

Manual Plasma Separation

- Gravity
- Hang blood transfer bag undisturbed, overnight, in a refrigerator (4°C)
- Following plasma extraction and storage in a freezer, resulting product is **Frozen Plasma (FP)**
  - At -18°C, FP is good for 1 year
  - At -65°C, FP is good for 5 years

Plasma can be separated from blood collection bags through at least two different methods. Gravity separation is a manual separation process. Hanging the bag, undisturbed in the refrigerator overnight allows for passive separation of cellular components from plasma. Following plasma extraction protocols and storage in a freezer, the resulting product is Frozen Plasma.

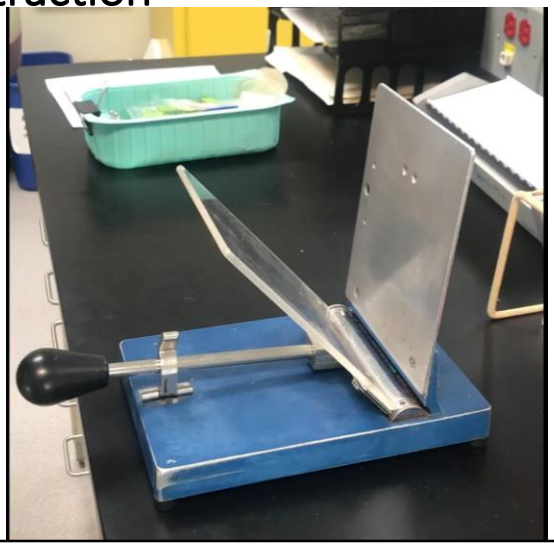
Automated Plasma Separation

- Centrifugation
- Blood collection bag is spun at recommended machine settings within 8 hours of donor blood collection
- Following immediate plasma extraction and storage in a freezer, the resulting product is **Fresh Frozen Plasma (FFP)**
  - At -18°C, FP is good for 1 year
  - At -65°C, FP is good for 5 years

Centrifugation is the automated separation process. Within 8 hours of blood collection, the collection bag set is weighed and placed in a centrifuge cup. The load is balanced within the centrifuge and spun at recommended machine settings. Following immediate plasma extraction protocols and storage in a freezer, the resulting product is Fresh Frozen Plasma. The storage life of Fresh Frozen Plasma is the same as that of Frozen Plasma.

Plasma Extraction

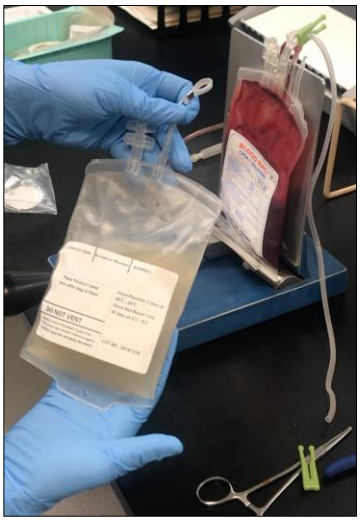
- Video clip



This video clip shows how a plasma extractor device is used to separate plasma from a blood collection bag to a transfer bag.

EEHV and Blood Product Transfusions

- As hemorrhage occurs with viremia, there is a loss of circulating blood volume, clotting factors, platelets and electrolytes
- Colloids are more effective than crystalloids for volume expansion
  - Fresh (fortified) plasma
  - Fresh (fortified) whole blood
  - Fresh frozen (fortified) plasma
  - Frozen (fortified) plasma
  - Hetastarch



As hemorrhage occurs with viremia, there is a loss of circulating blood volume, clotting factors, platelets and electrolytes.
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 help increase plasma volume. Additionally, animals with active infection are not expected to have antibody to the virus. If it is available, a plasma transfusion from a donor with a high antibody titer may help bind up virus particles in the patient. With our viremic calf, we administered a couple of treatments with fresh plasma and whole blood fortified with famciclovir. As acyclovir and ganciclovir have also been used as EEHV treatment, they may potentially be administered to a donor elephant to obtain fortified blood products for transfusion. Antiviral fortified plasma may be frozen, but this is still anecdotal.

Blood Product Transfusion

- Use a blood administration set with a blood clot filter (standard 170 – 260 microns) – do not infuse any solutions or medications through this main line
- Gently mix bag contents before using (whole blood and plasma products)
- Plasma
  - Fresh – separated and administered within 6 hours of collection
  - Frozen – if thawing with a water bath, prevent contamination of entry ports and do not warm over 37°C
- Only administer 0.9% sodium chloride with blood product transfusion
- Prophylactically administer diphenhydramine

When transfusing blood products, a blood administration set with a 170-260 micron filter must be used. The primary blood administration line must not be infused with any solutions or medications. Blood product bags should be gently mixed before use. Fresh plasma should be separated and administered within 6 hours of collection. If using fresh frozen or frozen plasma, the plasma must be completely thawed before administering. If a water bath is used to help speed up the thawing process, the entry ports on the bag should be protected and the bag must not be warmed over 37 degrees Celsius. Only administer 0.9% sodium chloride with the blood product being transfused – we recommend using a 3-way stopcock for this. Diphenhydramine can be administered as a prophylactic treatment.