

# In-House Plasma Separation Procedure for elephants.

*Based on design elaborated by Houston Zoo, Inc.*

## Materials

- Sterile blood collection bag containing anticoagulant Citrate phosphate dextrose adenine solution (CPDA-1) USP for collection of 450 ml of whole blood. \*Establish weight of the empty plasma bag prior to collection.
- Refrigerator with temperature 0-4 °C
- Scale (g)
- Plasma Extractor
- 1-2 Kelly or Crile Hemostats
- 1 smooth-jaw Hemostat
- Plasma Extractor – Handmade vs. advertised
- Hand-held Blood Bag Tube Stripper/Cutter/Sealer tool
- 4 Plastic Clamps
- Metal Clips \*\*Establish weight of a single clip.

## Procedure

1. Receive bag of whole blood with Citrate phosphate dextrose adenine solution (CPDA-1) USP coagulant.
2. Hang the bag in refrigerator for 6-24 hours to allow for gravitational separation of plasma from red cells. Temperature should be between 0-4 °C. (Figure A)
3. Carefully remove the blood bag from the refrigerator. Avoid re-suspending the separated red blood cells into the plasma (minimize abrupt motions when handling the collection bag).
4. Begin plasma separation process by inserting the blood bag into
  - a.) the “Plasma Extractor” or
  - b.) 2 pieces of Plexiglas duct-taped together. Lay the empty plasma bag beside the extraction apparatus. (Figure B)
5. Break the plastic barrier piece connecting the blood bag to the empty plasma bag. (Figure C)
6. With one hand, slowly apply gradual pressure to the Plexiglas pieces and with the other hand, use hemostats to hold the connection tubing. The plasma from the blood bag should be flowing into the plasma bag. Be cautious of disrupting the sediment. (Figure D)
7. When most of the plasma has separated into the plasma bag, quickly clamp off the connecting line with hemostats. Add secondary plastic clamps for extra security. (Figure E)
8. Using the handheld stripping tool, begin easing the remaining plasma in to the collection bag. (Figure F)
9. Using another set of hemostats, clamp the line closer to the plasma bag, leaving approximately 30 cm of tubing. Add secondary plastic clamps if necessary. (Figure G)
10. Cut the connecting line so that the plasma bag separates from the blood bag.
11. To properly seal the plasma bag for storage, tie 1-3 knots at the open end of the tubing. (Figure H)

12. Make a loop with the tubing and apply 2-3 evenly spaced metal clips. (Figure I) Slide the first metal clip as close to the bag as possible. Clamp the clips down with the multi-tool. (Figure J)
13. Weigh the full plasma bag. To determine actual plasma volume, subtract established materials weights (empty plasma bag\* and # metal clips\*\*) from the weight of the full plasma bag.
14. Label the plasma bag with animal ID number, collection date and plasma volume.
15. Store the plasma in a freezer (preferably -80 °C). However, use fresh plasma for treating EEHV-HD as freezing will activate the thrombocytes, making them useless for EEHV-HD treatment.



Figure A



Figure B



Figure C



Figure D

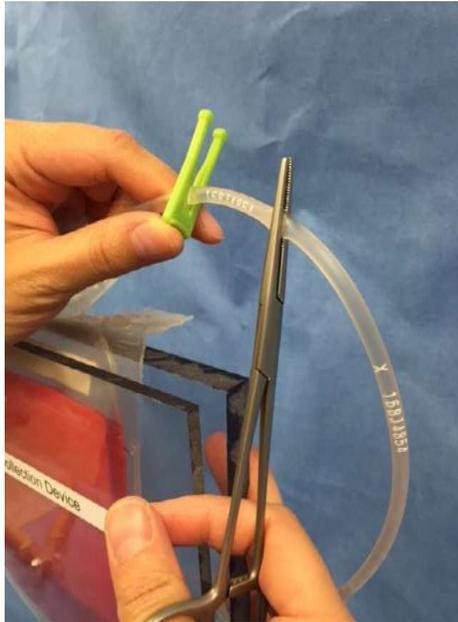


Figure E



Figure F



Figure G



Figure H



*Figure I*



*Figure J*