Minor and Major Blood Crossmatching for use as Treatment during Elephant Endotheliotropic Herpesvirus (EEHV) Viremia in Asian Elephants (*Elephas maximus*)

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Justification:

Plasma transfusions may be used to treat Asian elephants (*Elephas maximus*) during elephant endotheliotropic herpes virus (EEHV) viremia.

This may be elected when the elephant shows clinical signs, has an abnormal complete blood cell count (CBC), or when the viral load is elevated.

Plasma is a colloid that contains antibodies and clotting factors, is isotonic for the elephant, and may be advantageous to the recipient.

At the Houston Zoo, plasma transfusions have been administered to viremic elephants without complications and contributed to positive outcomes.

Minor Crossmatch:

-Assesses compatibility of a donor's serum or plasma with the red cells of a recipient

-Used when a recipient is receiving plasma from a donor

Major Crossmatch:

-Assesses compatibility of a donor's red blood cells with a recipient's plasma

-Used when a recipient is receiving whole blood or packed red blood cells from a donor

Materials:

1. EDTA (preferred) or serum tube (without the separator gel) from donor and recipient animals
2. Centrifuge
3. Small tubes (glass preferred) for separating the plasma and for testing (estimate minimum 3 tubes/animal)
4. Physiologic saline (0.9% saline without preservatives)
5. Droppers or pipettes
6. Incubator 35-37ºC
7. Markers for labeling tubes
8. Paper for recording results

Step 1: Prepare a 3-5% Red Blood Cell Suspension

1. Collect blood from both donor and recipient in EDTA.

2. Centrifuge tube and separate plasma from red cells. Save both.

3. Place 1 drop of recipient red cells into a small (2-5 ml) clean test tube.

4. Add 1-2 ml saline to the tube with the red cells (1 RBC drop:40 saline drops).

5. Centrifuge at 2500 RPM for 20 seconds.

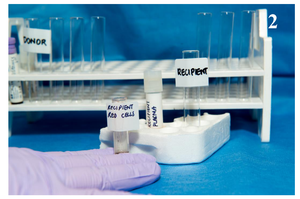
6. Remove supernatant, leaving the red cell button on the bottom.

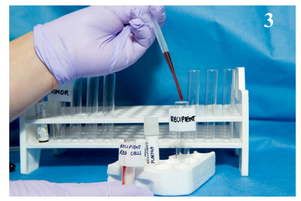
7. Repeat steps 4-6 three times (for a total of 4 washes).

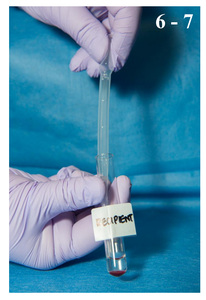
8. Add 1 drop of newly washed recipient red cells to a new test tube.

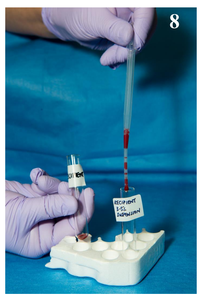
9. Add 20-40 drops of saline and mix to suspend the red cells. This should be a ~3-5% red cell suspension.

Optional: To ensure an approximate 3-5% solution, use a capillary tube, centrifuge, and packed cell volume (PCV) card to measure it. Complete this step as if it were a standard PCV. Number of drops of saline may need to be adjusted based on result.









Step 2: Minor Crossmatch

1. Add 1 drop of the recipient's 3-5% red cell suspension to a labeled test tube. Then, add 1 drop of the recipient's 3-5% red cell suspension to another labeled test tube to be used as a control.

2. Add 2 drops of donor plasma or serum to the test tube.

3. Add 2 drops of saline to the control tube.

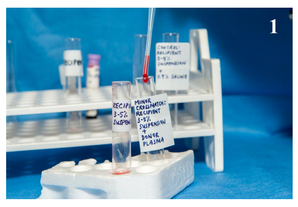
4. Incubate these tubes at 37ºC for 15 minutes (optional).

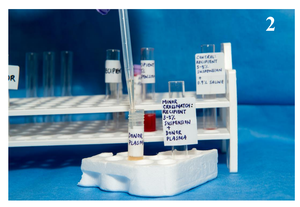
5. Centrifuge the tubes for 20 seconds at 2500 RPM.

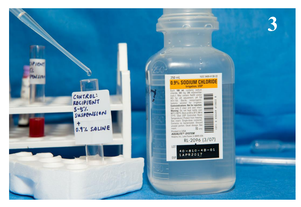
6. Observe the supernatant for signs of hemolysis.
If present in the crossmatch tube and not the control tube, the match is not compatible.
If present in both, start again with a new cell suspension.

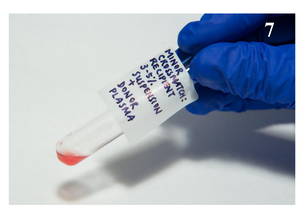
7. If no hemolysis, then gently rock the test tube back and forth to re-suspend the cell button.
Observe the cell button while rocking the tube and grade for the presence of agglutination. Grade on a 0-4 scale where 0 is no agglutination and 4 is heavy clumping.

8. Record results.









Step 3: Major Crossmatch

1. Follow minor crossmatch protocol, except use the donor's 3-5% red cell suspension and the recipient's plasma.

Acknowledgements: The authors would like to thank the Houston Zoo Veterinary Clinic and Elephant Keeper staff for their dedication to animal care.