

BLOOD COLLECTION AND PROCESSING

Purpose:

To obtain plasma, white blood cells (DNA), serum and red blood cells from cheetah blood samples for general medical profile, genetic analysis, hormonal analysis and virology

- I. Serum Tubes
 - A. Collect 3-4 serum tubes of blood from each cheetah
 - B. Centrifuge tubes at 3000 rpm for 10 minutes (serum will separate from blood sample and will be clear pink to yellow fluid on top of blood clot)
 - C. Label 1 cryovial (1.8 ml) for each serum tube collected with animal number, species and genus abbreviation (Aju, Ppa, Hbr, etc.), date of collection, sample type (serum) and animal sex
 - D. Collect the serum from each blood tube with a clean transfer pipette and dispense into a sterile pre-labeled cryovial
 - E. Place samples in freezer and record in blood log

- II. Blood Slides
 - A. *Blood smears/slides are prepared from the EDTA sample that has NOT been centrifuged
 - B. Pre-label 4 clean slides with animal number, species and genus abbreviation, date of collection and animal sex. Use the frosted slides and label with pencil.
 - C. Mix the blood in the EDTA tube gently
 - D. Using a clean capillary tube, place a small drop of mixed blood near the frosted end of a slide.
 - E. Using a second clean slide, place the edge of the slide into the blood drop until the drop spreads across the edge of the top slide
 - F. Slowly push the top slide across the full length of the labeled bottom slide to spread the drop over the surface.
 - G. Repeat for each labeled slide and stand upright to let dry
 - H. After fully dry, fix each slide in methanol fixative** Save the remaining blood in the EDTA tube utilized for making slides in the refrigerator. This tube will be sent to Golden Vet Lab for analysis.

- III. EDTA Tubes
 - A. Collect 3-4 EDTA tubes of blood from each cheetah
 - B. Set aside one EDTA tube to use for making blood smears (*see below)
 - C. Centrifuge the remaining tubes at 4000 rpm for 15 minutes
 - D. Label at least 1 cryovial (1.8 ml) with species and genus abbreviation, animal number, date of collection, sample type (EDTA plasma) and animal sex
 - E. Label at least 1 cryovial (1.8 ml) with species and genus abbreviation, animal number, date of collection, sample type (EDTA red blood cells) and animal sex
 - F. Label 1 cryovial (3.8 ml) with species and genus abbreviation, animal number, date of collection, sample type (EDTA white blood cells) and animal sex

- G. Collect the plasma from each tube (top clear layer of fluid) with a transfer pipette and dispense into the appropriate sterile pre-labeled plasma cryovial – be very careful to not disturb “buffy coat” of white blood cells underneath the plasma layer
- H. With a transfer pipette, remove the white blood cell layer (interface between the plasma and red blood cells) and dispense into the appropriate sterile pre-labeled cryovial. NOTE: the white blood cell layer is very thick and may be difficult to remove from the tube.
- I. Add an equal amount of Easy Blood to the white blood cell sample and mix; parafilm the top of the tube
- J. Collect the red blood cells from each tube (bottom layer) with a transfer pipette and dispense into the appropriate sterile pre-labeled plasma cryovial.

IV. Heparin Tubes

- A. Used to collect plasma, white blood cells and red blood cells, however, no longer routinely collected at CCF due to improved sample quality for genetic analysis from EDTA tubes.
- B. These tubes are processed in exactly the same way that the EDTA tubes are processed except that you do not use any part of this sample to make blood slides and none of the original collection tubes are sent to Golden Vet Lab.

SOME BASIC RULES TO REMEMBER:

1. Let blood tubes stand at room temperature for ~ 30 minutes after collection. Tubes can then be stored in the refrigerator after this if not centrifuged immediately; however, every effort should be made to centrifuge tubes as soon as possible.
2. When recording dates, always write out the month to avoid confusion between British and US methods of recording. i.e.: 08-05-04 is easily and frequently confused between 8th of May or 5th of August!
3. Always use a new clean pipette between animals and between collection tube types within animal (i.e.: use a different pipette for serum and plasma from same animal)
4. Slides used to make blood smears can be washed and reused; always stand blood smear slides up to dry
5. Always store WBC at room temperature after mixing with Easy Blood
6. Samples sent to Golden Vet Lab include 1 frozen serum cryovial, 1 blood slide and the EDTA tube that was not centrifuged

<u>Species Common Name</u>	<u>Genus and Species</u>	<u>Abbreviation</u>
Cheetah	<i>Acinonyx jubatus</i>	Aju
Leopard	<i>Panthera pardus</i>	Ppa
Brown Hyena	<i>Hyaena brunnea</i>	Hbr
Lion	<i>Panthera leo</i>	Ple
Caracal	<i>Felis caracal</i>	Fca
Serval	<i>Felis serval</i>	Fse
African Wild Cat	<i>Felis sylvestris</i>	Fsy
Wild Dog	<i>Lycaon pictus</i>	Lpi
Honey Badger	<i>Mellivora capensis</i>	Mca