**Field Blood Collection and Processing**

Wildlife Conservation Society, Apr 2014

Note that collection of blood samples requires a research permit from the country in which the research is being conducted. In addition all *Cyclura* species are CITES Appendix I listed. This means that you must have both an import and export permit when moving these samples from one country to another. The IUCN SSC Iguana Specialist Group has a blanket US CITES import permit for *Cyclura*, which can be used upon request and by authorized users. The format for obtaining CITES export permits varies and must be obtained from the respective country. *Iguana* species are CITES Appendix II listed and thus only require an export permit, although if importing to the United States, a USFWS 3-177 importation declaration form is required.

**Supplies**

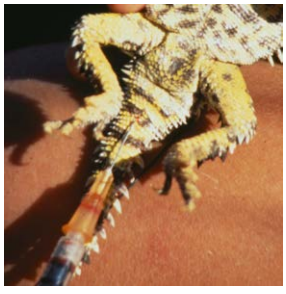
Syringes: 1 - 10 cc
Alcohol (isopropyl or similar)
Transport vials
Microscope slides
Cooler with ice or ice packs
Pencil
Needles: 20 - 27 gauge
Heparin
Pipettes
Filter paper
Blood tube rack
Lab marking pen

**Procedure**

1. Attach the appropriate size needle to a syringe, smaller needles and syringe for smaller animals, with general syringe and needle guidelines for various animals listed below:

| Iguana size | Syringe/ needle combination |
|---|---|
| <150 g | 1 ml with 27 G x 1/2" |
| 150-200 g | 1 ml with 25 G x 5/8" |
| 1-3 kg | 3 ml with 23 G x 1" |
| >3 kg | 5-10 ml with 22 or 20 G x 1-1/2" |

2. If plasma will be collected, heparinize the needle and syringe by drawing a small amount of heparin into the syringe, removing needle from the bottle, drawing the liquid all the way to the end of the syringe and then spraying any excess back out through the needle. If serum will be collected, heparinization of the syringe is not necessary. Plasma usually provides a greater yield, but serum is required for some tests.
3. Clean the tail with alcohol. Wipe excess off with swab or gauze.
4. Insert the needle between scales if possible. For a lateral approach to the tail vein hold the iguana on a flat surface and enter approximately 1/3 of the way up the side of the tail. If bone is encountered, redirect the needle slightly. For the ventral approach, restrain the animal so that the bottom of the tail can be easily accessed. Insert the needle perpendicular to the skin directly over the midline.



5. Once the blood sample (less than 1% of the body mass of the animal) has been obtained, if heparinized, gently rotate the syringe to get mixing of the blood with the heparin to prevent clotting. Rotating the syringe is not necessary if serum is being collected.
6. Place the sample into a transport tube; label the tube.
7. Using a pipette, place a small drop of blood on the edge of 2 microscope slides. Use a clean slide to make blood smears. Use a pencil to label the slides. If the sample was not heparinized, smears must be made immediately after blood collection or it will clot and smears cannot be made.
8. Using the syringe and needle, spray the leftover blood onto the filter papers, or use a pipette to distribute blood thinly on the papers.
9. Place the blood in a rack in a cooler until it can be centrifuged or until the plasma or serum can be decanted. If a centrifuge isn't readily available the blood can be left standing upright for a couple of hours and then pipettes can be used to draw off the plasma or serum being careful not to aspirate any of the red blood cells.
10. Once the plasma or serum is removed from the red blood cells it can be labeled and frozen. The red blood cells can be frozen also.
11. The blood slides should be allowed to dry thoroughly and then fixed in ethanol for several minutes.
12. The filter papers should be labeled, allowed to dry thoroughly, and placed in plastic bags with desiccant (see filter paper protocol).

**Blood Processing**

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**Supplies**

pipettes
centrifuge tubes
centrifuge tubes for freezing plasma or serum
marking pens

**Procedure**

1. Transfer blood from syringes into tubes.
2. Place the tubes into the centrifuge. Make sure to balance the centrifuge by putting equal amounts of blood in opposite tube holders, or a tube filled with water to the same level as the tube with blood that is opposite. The tops can be left on or off the tubes, but must be done the same for all tubes.
3. Centrifuge for 10 minutes.

4. Using pipettes, remove the plasma or serum being careful not to contaminate with the red blood cells. Transfer the plasma or serum into plastic freezer vials. Screw the tops tightly.
5. Place tubes into freezer.
6. The red blood cells that are left in the bottom of the tube can be saved frozen for genetics. Note that blood does not have to be separated for genetics work.
7. Keep samples cool until they can be placed into a freezer. Once frozen samples must stay frozen at all times.
8. Label tubes with species name, ID, date of collection, and note plasma or serum.