Blood Collection and Preservation for Genetics

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Note that collection of blood, tissue, bone, and egg 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 when moving samples, although if importing to the United States, a USFWS 3-177 importation declaration form is required.

Supplies

Syringes (1 cc or larger)
Needles (23-28 gauge)
Heparin
Collection tubes (2.0 ml cryotubes with o-rings and screwtops)
Buffer (EDTA-based or EtOH)
Marking Pen

Background

All iguanas have a large ventral caudal artery and vein in the tail that run parallel to the ventral medial surface of the caudal vertebrae and through a series of hemal arches, located in the proximal end of the vertebral ventral processes. It is easiest to draw blood from this caudal area.

For genetics a blood sample of 0.3 ml is plenty, but if other tests are being run then additional blood may be needed (see other protocols).

Procedure

1. It is best to use a needle and syringe that are already attached. If this is not possible attach the needle to the syringe. It is important to use an appropriately sized needle. For adults and large juveniles, a 23 or 25 gauge needle (1-inch length) attached to a 1.0 cc syringe works well. For hatchlings and small juveniles, a tuberculin syringe with a fixed 28 gauge (usually 1/2-inch) needle works best.
2. In order to reduce blood clotting in the needle and syringe, heparin sodium solution (an anticoagulant) can be used. Flush the sterile needle and syringe prior to bleeding by moving the plunger back and forth to partially fill the syringe but returning as much of the heparin solution to its bottle as possible. Spray any excess heparin out once the needle has been removed from the heparin bottle.
3. Make sure the iguana is restrained well and clean the portion of the tail where the blood will be drawn from with alcohol, removing any excess.
4. Be mindful of how you hold the syringe. It helps to brace you hand/wrist in some way and hold the syringe with one hand such that your thumb can control the plunger. Even a slight movement once blood begins to flow can stop the flow and cause you to have to begin again.
5. Insert the needle between scales and perpendicular to the skin over the midline of the ventral surface of the tail. The exact posterior distance depends on the size of the animal. In large

adults this should be at least 2 inches posterior to the cloaca. Be mindful of the hemipenes or cloacal pouch.

6. The plunger of the syringe can be withdrawn slightly to create a mild vacuum.
7. The needle should then be pushed slowly toward the exact ventral midline until blood begins to flow or the ventral surface of one of the vertebrae is reached.
8. If the vertebral column is reached without blood flow, the needle should be withdrawn ever so slightly and slowly rotated while keeping a slight vacuum in the syringe. Rotating the needle changes the orientation of its angled bore (opening) in relation to the blood vessels and this is often all that's needed to obtain blood flow.
9. Proceed very slowly and pause to allow blood flow. Sometimes it only takes a slight pause or a very slight movement of the needle to begin to see blood entering the syringe. If blood doesn't flow, the needle can be removed and inserted into a different location, but this should only be done once or twice.
10. Watch the syringe very carefully. Once you see blood be careful not to move the needle at all. It is important not to pull the syringe plunger back too far or too quickly, as creating too much vacuum can collapse the vessel, eliminating blood flow altogether.
11. Once the requisite amount of blood has been drawn, it should be preserved in a cryotube along with an equal or greater amount of field buffer solution or >70% EtOH. At San Diego Zoo Institute for Conservation Research, we use a 2x field buffer solution comprised of: 0.2 M NaCl, 0.1 M EDTA, 2% SDS, pH 8.0 (modified from: *Longmire JL, Gee GF, Hardekopf CL, Mark GA (1992) Establishing paternity in whooping cranes (*Grus americana*) by DNA analysis. Auk 109:522–529*). Make sure to add the buffer to the tube first. You may want to have a few tubes already filled and ready to go. If you do not have buffer, you can place a drop of blood on filter paper and allow it to dry until processing.
12. If vials and buffer are not available you may also simply place a few drops of blood on filter paper, or even notebook paper, though this should not be your first choice.
13. Make sure to label your sample with species, location, individual number, and date collected. Be mindful to use a marking ink that will not be affected by freezing or contact with water or EtOH. Placing tape on top of the label is always a good idea.

Note: Blood can also be obtained using a lateral puncture and some people prefer this method. With this technique, the needle is inserted straight into the side of the tail (again between scales) starting approximately one-third the distance between the tail's ventral and dorsal surfaces. It will be easiest to obtain blood shortly after capture, compared to after spending considerable time in a capture bag or other form of restraint.

Sample storage

Blood samples can be safely kept under ambient field conditions for weeks or even months. Nevertheless, to avoid possible sample degradation, efforts should be made to keep samples in the shade and, when possible, in a refrigerator or cooler. Samples to be stored for long periods should eventually be transferred to a facility with -80°C freezers. Once samples are frozen, it's best to avoid repeated freeze-thaw cycles (such as those that characterize many home freezers) as this can cause premature sample degradation. Therefore, if an ultra-cold freezer is not available, it may be advisable to simply keep samples in a refrigerator.

San Diego Zoo Institute for Conservation Research can easily store samples under the right conditions. These samples will not be used for analyses, distributed to others, or otherwise manipulated without your prior consent. Please contact Glenn Gerber to make these arrangements (ggerber@sandiegozoo.org).

Additional regional sample depositories are being sought out. If you, or anyone you know, is able to do this please contact Stesha Pasachnik (SAPasachnik@gmail.com).