An Effective Acquisition and Stable Containment Technique for *Ambystoma mexicanum* Whole Blood.

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This technique was initially developed for work on fairly large (100g.) anesthetized animals. The animals were anesthetized in a .002% benzocaine solution until the righting reflex was lost, and then placed dorsal side down on a small surgical table. The gills were brushed aside and the gill rakers exposed. Following this, one gill raker was isolated from the rest using a pair of small, hooked-end forceps. On either side of this probe were threaded 4.0 silk ligatures (untied at this point) and the branchial artery identified. This artery was exposed, but not totally isolated, by careful removal of surrounding connective tissue. A 5 cc. syringe with 23 gauge needle, prepared earlier and flushed with 3% EDTA in distilled water, was then used to remove blood. The needle was introduced bevel-side up in an oblique presentation to the artery with a deliberate smooth motion. Extreme care was taken in this step to avoid double puncturing the blood vessel. If this occurred it was remedied by employing hemostats at a site upstream of the puncture.

We were able to determine the effectiveness of needle positioning in the vessel by drawing back the plunger and observing the presence of blood in the barrel of the syringe. Once blood was observed in the syringe barrel, a smooth, steady, and slow force was applied in drawing back the plunger until the predetermined volume was attained. At times, a large resistance to flow was encountered, as well as no blood appearing to enter the syringe. It was assumed that either a blood clot had formed, the choice of needle diameter was inappropriate, or the needle was placed into the vessel wall. In any case, the procedure was aborted by clamping the wound and tying off the proximal and distal ligatures. The same clamping procedure was used to close the wound after the desired amount of blood was drawn. Extreme care was used when tying the ligatures due to the delicate nature of the blood vessels.

After blood removal and closure of the wound, the animal was placed in a fully aerated recovery aquarium until it appeared that the animal was not bleeding from the wound and was swimming around normally. Blood clots from the oral cavity were normally spit out during the recovery period and presented no cause for concern. Heightened surveillance was maintained for approximately 24 hours post surgery for leaky or incomplete closures. If this occurred the animal was immediately reanesthetized and the problem addressed. After 1-3 days recovery, the animal was reintroduced to its normal aquarium.

Following withdrawal it was important to keep all whole blood samples away from and out of glassware during experimentation unless the glassware had been coated with silicone. Uncoated glass has numerous reactive sites on its surface which can initiate clotting. We use Becton-Dickinson disposable plastic syringes for removal and plastic test tubes for containment in order to avoid clotting.

Sodium heparin has been indicated in numerous prior studies as the anticoagulant of choice (Gahlenbeck,1970; Stiffler,1990). Heparin acts to accelerate the action of the plasma protease inhibitor antithrombin III, which then inhibits clotting factor proteases. We, however, have not had much success with this anticoagulant, as the procedures we use require that the blood remain liquid for 2 to 4 hours. In exploring alternative anticoagulants, we investigated the possible use of citrate and ethylenediaminetetraacetic acid (EDTA). Both of these drugs are effective cation chelators with EDTA being the strongest. In testing the effectiveness of prolonging coagulation we concluded that EDTA was the best at a 3% solution made in distilled water. It was found that in 5ml of blood, a little less than 1/10 of a ml of 3% EDTA was needed to keep the blood from coagulating for 3 hours at room temperature. It is important to note here that thorough and complete mixing of blood with the EDTA solution must occur immediately after the blood has been drawn to insure liquidity. With proper handling, blood was able to stand at room temperature without coagulating for at least three hours.

This blood removal technique can be performed without anesthetic. This procedure was developed to avoid the possible blood vol-

ume shifts resulting from systemic application of a local anesthetic. Drawing blood from an unanesthetized animal can be difficult and requires two people. The animal must be immobilized by one individual while the other draws the blood. To immobilize the animal, it must be quickly inverted and its head held down tightly (two handed). It will struggle for a few moments and then relax. The blood drawing technique is then performed as described above.

In concluding, it is hoped that these techniques may prove useful to other investigators needing to remove a given volume of blood from an axolotl both effectively and nonlethally.

Literature Cited

Gahlenbeck, H., and H. Bartels. 1970. Blood gas transport properties in gill and lung forms of the axolotl (*Ambystoma mexicanum*). Respiration Physiology *9*:175-182.

Stiffler, D., M. Kopecky, M. Thompson, and R. Boutilier. 1990. Acid-base-electrolyte balance responses to catecholamine antagonists in *Ambystoma tigrinum*. American Journal of Physiology (Regulatory Integrative Comp. Physiol.) *27*:R1363-R1370.