New Identification System Makes 'Axolotl Life' Easier

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The IU Axolotl Colony is a breeding colony and a genetic stock center for *Ambystoma mexicanum*. Maintaining genetic stocks as well as health and production requires that we keep detailed spawning, genetic, and treatment records for each individual adult axolotl. For this reason we need to be able to positively identify all adults (approximately 500). For years we housed all the animals in individual bowls labeled with an animal's number, a very labor intensive and therefore expensive system. The transfer of animals from their resident bowls to the mating pans had been, for years, a potential source of error. There was no guarantee that an animal was the animal it was supposed to be after it left its bowl.

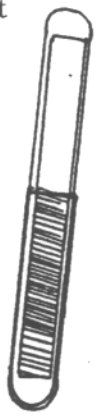
In recent years, as we have been moving to communal housing, it has become necessary to be able to mark individual axolotls, not just the containers. When we started experimenting with different means of communal housing, we also started looking for ways of marking the animals. Toe clipping, which is frequently used with frogs and other salamanders, was never an option, given the regenerative capabilities of the axolotl. Anything attached to the animals (tags, bands, bead 'necklaces') was sloughed off or overgrown. We tried tattooing the axolotls' tails for a couple of years. This approach was somewhat successful. The white axolotls were tattooed with a dark red ink, which remains visible for years. However the dark animals could only be tattooed with white ink, which fades as the animals regenerate dark tissue over top of the tattoo. Within a few months, the procedure needs to be redone. This was extremely time consuming and extremely stressful on the animals, which had to be completely anesthetized in MS-222 for the tattooing procedure. It would often take the axolotls weeks to recover completely from this procedure, making them more susceptible to disease and taking them out of the breeding population for extended periods of time.

After investigating and testing an electronic marking system, we eventually adopted serial encoded microchip transponders inserted into the animals as our identification method. For the Axolotl Colony this method provides permanent, easily read identification. It has been quick and simple to install without removing the animals from the breeding population for any period of time. We started implementing the system in the spring of 1991, and we feel that it is a great success. We are about to complete our biggest breeding season ever!

There are several electronic identification systems available. The system which fits our needs best is the Electronic Lab Animal Monitoring System (ELAMS) from Biomedic Data Systems (address below). It consists of transponders, an Electronic Notebook, a scanning wand, and static RAM cards.

The Transponder

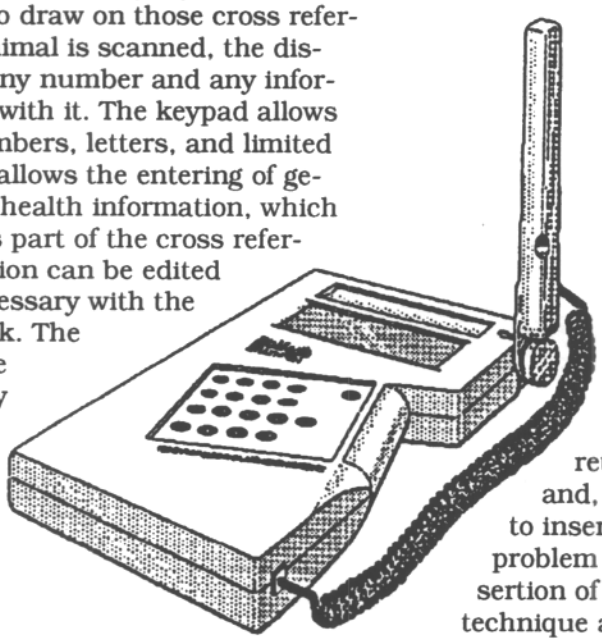
The transponder consists of a microchip permanently encoded with a 10 digit serial code hermetically sealed in a glass capsule, which is coated to prevent wandering in the tissues of the axolotl's body. It is approximately 12 mm long and must be inserted with a 12 gauge needle. We purchase transponders pre-sterilized and already loaded into sterile injection needles. For every ten loaded needles there is an injecting cartridge. This cartridge holds the needles in place and allows transponder delivery into the axolotls. The cartridge also provides a storage area for unused sterile needles and transponders.



The Electronic Notebook, Scanning Wand, and SRAM Card

The Electronic Notebook is a hand-held display/control unit. The LCD readout displays the menus of the various functions of this programmable unit. The scanning wand is attached to the Notebook by a flexible cable. Animals can be scanned without ever removing them from the water. When the transponder is inserted into the animal and the microchip number is scanned by the attached wand, the Notebook is used to correlate the transponder serial number to the animal's actual number in the colony. This information is stored on an SRAM card inserted into the

back of the Notebook. In everyday use, the Notebook is used to draw on those cross references. When an animal is scanned, the display reads the colony number and any information associated with it. The keypad allows the entering of numbers, letters, and limited punctuation. This allows the entering of genetic, mating, and health information, which will then appear as part of the cross reference. The information can be edited and deleted as necessary with the Electronic Notebook. The SRAM card and the Notebook's memory can be backed up by downloading the data to a computer.



Trial and Error

In December 1990, the colony purchased one cartridge of transponders and borrowed a reader from International Infopet Systems, Inc. Over the Christmas break we chose a set of adult axolotls from the colony representative of the different pigment groups and sexes. Our intent was to test different sites for the transponder injection, test different local anesthetics for effectiveness and ease of use, and try out a chemical sealer for the wound caused by the 12 gauge needle.

Two locations were tried. The first was intraperitoneal. I inserted the 12 gauge needle just anterior to the hind limbs with the needle directed anteriorly. This did not work well because the skin and muscle in that area are extremely elastic and tough. It took so much force to insert the needle that there was danger of sending it in too far once the needle did break through. I tried this location with only two animals. Other than the difficulties in insertion, there were no other problems with this location.

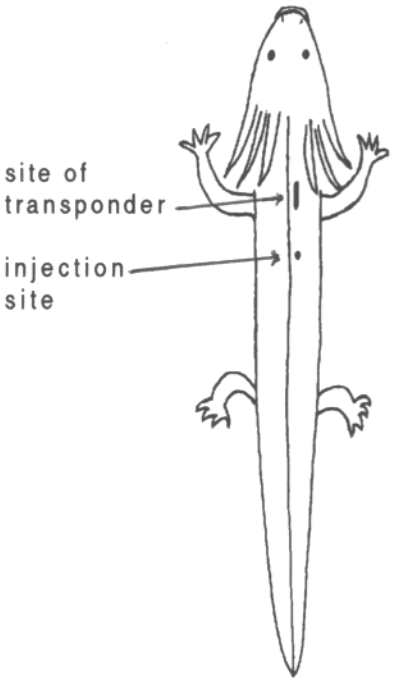
The second location, subcutaneous, parallel to the tail fin, 2 1/2 to 3 centimeters posterior to the head, worked well and is the location we currently use. There is very little flexibility in the skin and tissue, since it is supported by thick muscle and skeleton. The skin is very thick, so there is little danger of the transponder breaking through the skin even though it is a close fit. The transponder should be embedded as far as possible by inserting the needle completely. This way muscle action will not move the glass capsule back out of the wound. Placing the transponder on the back of the axolotl not only makes it convenient for scanning but also easy to inspect healing and possible dislocation. The transponders have worked themselves back out in three cases, all from some of my earlier insertions on small young animals. I assume that it was due to my not having inserted the needle in far enough, as I had a tendency to do on small animals. This reticence is not really necessary, and, since I don't hesitate any more to insert the needle all the way, the problem has not happened again. Reinsertion of transponders follows the same technique as above, only the transponder and needle need to be disinfected in 70% ethanol for 20 minutes before reinsertion.

The use of a local anesthetic is necessary given the size of the needle and depth of insertion. I tried a number of chemical anesthetics including benzocaine, an anaesthetic cream, and ethyl chloride. These all had a combination of drawbacks. Benzocaine acted systemically even though applied locally. The cream was too difficult to apply to moist skin. Ethyl chloride fumes built up in the vicinity of use and were too noxious to consider safe. White and albino animals showed skin irritation from the application of all three. All three involved several steps and too much time to insert transponders efficiently into several hundred axolotls.

Frustated, I returned to simplicity and decided to try ice packs. I laid an ice pack over the animal for a few minutes and then inserted the transponder. There was no thrashing and no 'obvious' pain to the animal. When it was returned to the room temperature water, it immediately recovered. There was no bleeding or behavior denoting sick animals. There was no skin damage on white animals. Females and males would both breed as usual. I eventually upgraded to an ice bath, which chills the animals even more quickly and is much more convenient for me, since I can prepare many axolotls for injection at one time. The animals still recover within seconds of being returned to room temperature water and eat and breed as if nothing ever happened.

We had been given samples of formulated cyanoacrylate (Nexaband) to use to help close

Transponder Removal from Adult Axolotls



For those researchers who order adult axolotls we may ask that you remove a transponder and send it back to us after the animal has been euthanized or dies naturally. Here are some instructions to aid in removal.

The transponder is approximately 1.2 cm long. It is in a glass capsule which is half covered with a plastic sheath to help prevent wandering in the tissue. Care should be taken when using a scalpel so that this plastic coating is as little damaged as possible.

The transponder should be located within or just under the first layer of muscle on the back. It should lie parallel to the backbone, approximately 1.0 - 1.5 cm away from it. Freezing the animal, then partially thawing it, will make removal much easier, as the tissues can be removed a little at a time until the transponder is spotted.

Clean the transponders off with water and a paper-towel. Place them in a plastic bag and mail to:

IU Axolotl Colony
Jordan Hall 407
Bloomington, IN 47405.

the wound. This is a sort of veterinary super-glue. It works well on wet animals and stays on after the animal is returned to the water. It is easy to use, and there are no obvious side effects. I now massage the skin around the wound before I apply the Nexaband, so that the wound closes up to a smaller diameter. This has seemed to help a great deal to speed the healing process.

At the end of a year...

We are extremely pleased with our new method of identification. It involves much less work for us and continued health of the animals. This form of identification has helped us more fully utilize our facility for communal housing (Axolotl Newsletter 19: page 3) while allowing us the security of absolutely positive identification. Housing arrangements and matings are now more flexible, more efficient, and less frustrating. Our available breeding pool is larger, hence the incredible number of spawns we have gotten this year (350 between July 1, 1991 and April 30,1992).

ADDRESSES

BioMedic Data Systems
255 West Spring Valley Avenue
Maywood, New Jersey 07607
Phone: (800) 526-2637.

International Infopet Systems, Inc.
31264 La Baya Dr. Suite A
Westlake Village, CA 91362
Phone: (800) 463-6738.

Nexaband
distributed by Tri-Point Medical L.P.
5265 Capital Blvd.
North Carolina 27604
Phone : (800) 334-8560.