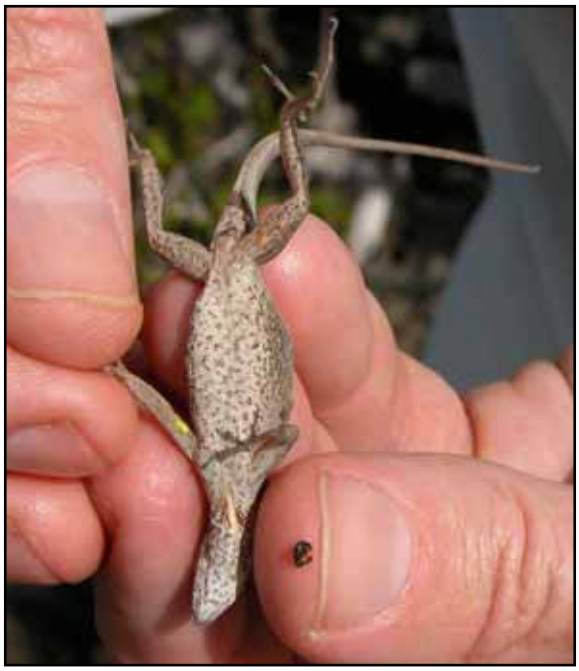
Tagging Reptiles and Amphibians

Application Note APG02

In the past, the most widely used method of marking amphibians and reptiles has been toe clipping. Although the potential detrimental effects have been well documented, the removal of one to eight digits is used extensively because it is inexpensive and quick. Toe clipping can reduce survival (Clarke 1972; McCarthy & Parris 2004), and cause infection at the wound site (Golay and Durrer 1994). Furthermore, toe clipping is unreliable because digits may regenerate (Donnelly et al. 1994), and larvae and the adults of certain species lack limbs. In a recent article in *Nature*, May (2004) questioned whether this practice is ethical.

Northwest Marine Technology specializes in biocompatible tags that are injected under the skin so that the tag is completely internal but externally detectable. Our coded wire, visible implant alphanumeric, and especially visible implant elastomer tags are being used more and more as an alternate approach to identifying reptiles and amphibians. All three tag types are injected beneath the skin with little effect on the host animals. The visible implant tags can usually be seen in ambient light and tag detection is greatly enhanced by fluorescing the tags with NMT's VI Light.



Anolis sagrei tagged with VIE for a study of evolution in character traits. (Photo courtesy of Melissa Losos)

Visible Implant Elastomer (VIE) Tags

Species which have been successfully tagged with Visible Implant Elastomer (VIE) tags include *Ambystoma maculatum, Anolis sagrei, Ascaphus truei Plethodon cinereus , Rana sylvatica, Xenopus tropicalis* and many others. Please see the references tab of our website, www.nmt.us for details on how VIE has been used with reptiles and amphibians. There are also some very good websites describing tagging techniques in detail. Please see the links on the support tab of our website.

Visible Implant Elastomer is the most widely used alternative to toe clipping for identifying reptiles and amphibians. It is a two-part silicone-based material that is mixed immediately before use. VIE tags are injected as a liquid using a 28 gauge needle and cure into a pliable rubber-like material. Excess material is then wiped from the skin of the animal allowing the opening to heal and the tag to remain internal. Many researchers do not anesthetize amphibians, while others prefer the ease of handling that anesthetic

provides, and still others have developed techniques in which the animal is placed in a plastic bag with some water and they tag through the bag.

Correct injection provides high retention and readability. Because the material is fluorescent it can be seen under pigmented skin and at night with the help of the VI Light. Anholt et al. (1998) state that the consistency and biocompatibility of the VIE tags allows for the tagging of small animals, including larvae, that could not be tagged using other methods. In a novel approach to monitoring the development of salamander egg masses, Regester and Woosley (2005) used VIE to identify and track the egg masses.

Depending on temperature, the working time of the mixed elastomer is about 1-2 hours. While the elastomer will not be completely cured in that time, it will become very difficult to push it out of the injection syringe. The working time can be extended if the elastomer is stored in a freezer or on ice in a cooler after mixing.

VIE tags are an excellent choice for batch identification, and they also offer a surprising number of individual codes if colors and body locations are combined. For example, Jung *et al*. (2000) used three colors and four body locations to create 255 individual codes in his study of salamander. Successful tagging sites include between the toes in frogs, on the upper hind leg of frogs, and at the base of the limbs on the ventral side of salamanders. Lizards have been tagged in the upper legs and in the tail.

Some amphibians lack septa between the skin and underlying tissue. VIE tags injected into these animals can therefore migrate from the original tagging location, making it impossible to use those tagging locations to create individual codes. In such cases, we recommend the use of Visible Implant Alpha tags.

Above: Combinations of tag colors and tag locations can be used to create individual and batch codes. Below: This frog, tagged as a tadpole, retained it's VIE tags through metamorphosis. Photo courtesy of S. Hopkins.

Visible Implant Alpha (VI Alpha) Tags

Species that have been successfully tagged with VI Alpha include *Elgaria coerulea*, *Gegeneophis ramaswamii*, *Rana aurora*, *Xenopus tropicalis,* and many others. Please see the references tab of our website, www.nmt.us for details on how VI Alpha has been used with reptiles and amphibians. There are also some very good websites describing tagging techniques in detail. Please see the links on the support tab of our website.

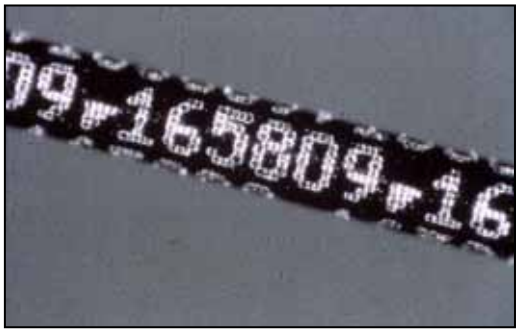
VI Alpha tags are made of the same biocompatible, flexible material as the VIE tags but are pre-cured with individual alphanumeric codes printed on one side. VI Alpha tags are available in two sizes: standard (1.0 mm x 2.5 mm) and large (1.5 mm x 3.5 mm) and in a variety of colors. VI Alpha tags are injected under the skin in areas with little or no pigmentation so that the codes can be read without removing the tag. As with the VIE tags, the use of the VI Light dramatically improves the detection and readability of VI Alpha tags, sometimes even through highly pigmented skin.

VI Alpha tags provide individual identification as in this frog. The tags are most successful when placed under transparent skin.

VI Alpha tags have not been used as extensively as VIE tags in amphibians and reptiles. Measey et al. (2001) found there to be no negative effects of VI Alpha tags in a legless amphibian (*Gegeneophis ramaswamii),* and further stated that when individual identification is necessary that these tags are the "most promising" of the investigated tags. Buchan et al. (2005) tested VI Alpha in a number of species, and concluded that VI Alpha "appears to be an effective and low cost method for individually marking and identifying amphibians". Researchers tagging *Xenopus tropicalis* have found that the VI Alpha tags with a black background and red letters are much more readable through the highly mottled skin than tags with a fluorescent background. In both cases, the VI Light is very helpful for illuminating the tags.

Decimal Coded Wire Tag (DCWT)

Species that have been successfully tagged with CWT include *Lampropholis guichenoti, Rana aurora, Malaclemys terrapin,* and *Chelonia mydas.* Please see the references tab of our website, www.nmt.us for details on how CWT has been used with reptiles and amphibians.



A standard DCWT (left) is a tiny length of magnetized stainless steel wire 1.1 mm long x 0.25 mm diameter. Tags half this length can be used for very small animals, and tags one and a half or double length may be used for larger specimens. DCWT are marked with rows of numbers that must be read under magnification, denoting a specific batch or individual codes. Single tags are cut from rolls of wire and injected hypodermically into suitable tissue. The small size and biocompatibility of these tags allow for very high retention with no reported effect on the animal.

If the experiment requires reading the tag code, the animal is killed to retrieve the tag. However, if the presence or absence of a tag will provide the necessary information, the tag can be externally detected without harming the animal. It is also possible to use the location of the tag in the animal for a limited number of individual or batch codes without killing the animal. In a novel approach to evaluating the prey choices of snakes, Downes (2000) identified individual skinks with CWT using several body sites, then used a fluoroscope to identify the skinks while in the digestive system of snakes. For suggestions on deploying small numbers of sequential tags for individual identification please see the application note "Individual identification of small numbers of organisms using sequential coded wire tags - an inexpensive option" on the support tab of our website.

Citations

- Please see our website (www.nmt.us) for an extensive list of published references related to tagging reptiles and amphibians.

Anholt, B. R., Negovetic, S., and C. Som. 1998. Methods for anaesthetizing and marking larval anurans. Herpetological Review 29(3):153-154.

Buchan, A., L. Sun and R. S. Wagner. 2005. Using alpha numeric fluorescent tags for individual identification of amphibians. Herpetological Review. 36 (1):43-44.

Clarke, R. D. 1972. The effect of toe-clipping on survival in Fowler's toad (*Bufo woodhousei fowleri*). Copeia 1972:182-185.

Donnelly, M. A., C. Gutoad, J. E. Juterbock, and R. A. Alford. 1992. Techniques for marking amphibians. In W. R. Heyer, M.A. Donnely, R. W. McDiarmid, L.-A. D. Hayek, and M. S. Foster (eds.). Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, pp. 275-276. Smithsonian Institution Press, Washington, D.C.

Downes, S. 2000. The use of wire microtags to identify small individual prey in snakes. Amphibia-Reptilia 21:126-131.

Golay, N. and H. Durrer. 1994. Inflammation due to toe-clipping in natterjack toads (*Bufo clamita*). Amphibia-Reptilia 15:81-96.

Jung, R., S. Droege, J. R. Sauer, and R. B. Landy. 2000. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah national park. Environmental Monitoring and Assessment 63:65-79.

McCarthy, M. A. & K. M. Parris. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. Journal of Applied Ecology 41:780-786.

May, R. M. 2004. Ethics and amphibians. Nature 431:403.

Measey, G. J., K. J Gower, O. B. Oommen, and M. Wilkinson. 2001. Permanent marking of a fossorial caecilian, *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). J. South Asian Nat. Hist. 5(2):109-115.

Regester, Kurt J., Woosley, Lori B. 2005. Marking Salamander Egg Masses with Visible Fluorescent Elastomer: Retention Time and Effect on Embryonic Development. The American Midland Naturalist 153(1):52-60