

Manual Elastomer Injection Systems

Instructions for 10:1 Visible Implant Elastomer

Introduction

Visible Implant Elastomer (VIE) tags are implanted beneath transparent or translucent tissues but remain externally visible. VIE tags are widely used for marking fish, crustaceans, reptiles, and amphibians. VIE is a biocompatible, two-part material that is mixed immediately before use and then injected as a liquid that cures to a pliable solid. VIE tags are ideal for batch identification, but by combining different colors, multiple tags per animal, and multiple tag locations, many individual codes can be created.



VIE is available in six fluorescent colors (red, orange, green, yellow, pink, blue) and four non-fluorescent colors (brown, black, purple, white). The visibility of the fluorescent colors is greatly enhanced with Northwest Marine Technology's (NMT) VI Light.



VIE in ambient light (left) and illuminated by the VI Light (right)

Proper color selection is a vital part of good experimental design. Your choice depends on how much contrast you need with the background pigmentation and how many different colors you require. Certain color combinations can be difficult to distinguish. In particular, we do not recommend that green and yellow be combined in a study because they are difficult to distinguish when fluoresced or when placed under pigmented tissue. Please contact Biological Support (email: biology@nmt.us) if you would like assistance.

Before using Visible Implant Elastomer, available reference material should be reviewed. Many references are listed on our website (www.nmt.us). The biological staff of Northwest Marine Technology is available to advise users in this regard. If references are lacking, experiments to evaluate suitable tag locations, retention rates, and tag visibility should precede applied uses.



Most clear or translucent tissue is a suitable target, if there are no associated pores or cavities through which the material can exit. The adipose eyelids of salmonids and some other fishes, as well as the spaces between fin rays are examples of potential targets. Other possible targets are along fin margins of flatfish, and the abdominal area of shrimps. Elastomer tags can be placed under pigmented skin where they are difficult to see in ambient light, but quite visible when fluoresced.

Storing VIE

The shelf life of unmixed elastomer is at least 12 months from the date of purchase when properly stored. To maximize shelf life, NMT recommends that unmixed VIE be stored in a refrigerator to ensure constant temperature. If this is not available, store the unmixed VIE in a cool, dark place. Do not freeze unmixed VIE. Exposure to fluctuating temperatures, such as on the dashboard of a car, will decrease the shelf life. Prolonged exposure to sunlight may fade the color. The VIE Color Standard should also be stored out of direct sunlight.

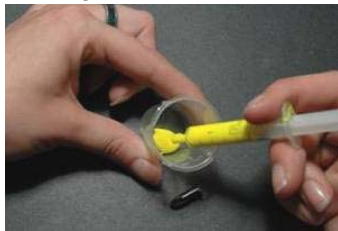
Elastomer components older than one year may still be useable. You can test this by mixing a small quantity and observing whether it cures properly. Thoroughly mix a small quantity of the two components. If it has cured to a rubbery solid within 24 hours, the material is useable. Otherwise, discard it and contact NMT about purchasing a refill kit. If you have any questions, please contact NMT.

Mixing Instructions

For complete curing to occur, the two elastomer components (colored part and curing agent) must be thoroughly mixed in a 10:1 ratio. The suggested mixing procedure is as follows:

Step 1

Remove the black cap from the syringe containing the colored component. Using the calibrations on the syringe, dispense the desired amount into the bottom of a mixing cup. Replace the black cap.



Step 2

Remove the clear cap from the syringe containing the curing agent, the clear component. Using the calibrations on the syringe, dispense 1/10 of the amount



as the colored part into the cup. **Caution:** The curing agent will dispense with much less pressure than the colored component. Be careful not to dispense too much!

Be very careful not to contaminate the curing agent syringe with the colored component as this will begin curing the elastomer in the syringe, making it unusable. You will know you contaminated the clear component if any colored material is on the syringe tip. If this happens, immediately dispense enough of the clear material to clean out the colored portion and wipe the tip clean. Replace the clear cap.

Step 3

Mix thoroughly! Stir and scrape the mixing cup walls and bottom for one full minute, but not longer, to ensure complete mixing. After mixing, the user will have about 1 hour of working time (depending on temperature) during which the tags can be injected.



Step 4

Remove the white cap from a 0.3 cc injection syringe. Remove the plunger.

Use a new 1 ml syringe to slowly draw up a small amount of the mixed elastomer, and then wipe off the tip.

The tip of the 1 ml syringe will fit tightly into the opening of the 0.3 cc injecting syringe. Fill the injection syringe about one third full. Filling the syringe more than this makes it difficult to push out the elastomer and may result in a broken plunger. For best results, deposit the mixed elastomer into the syringe so that no air pockets form between the



plunger and elastomer. More than one injection syringe can be loaded from a single mixing. The working time can be extended by storing the mixed material on ice or in a refrigerator.

Step 5

Replace the plunger in the injection syringe and remove the orange cap covering the needle. Push the plunger forward until a small bead of elastomer appears at the needle tip. Install the injection syringe into the body of the Manual Elastomer Injector, then put the back piece of the Manual Elastomer Injector in place. The

Injector can be held with the thumb on the bevel as shown, or with the bevel down to allow a shallow angle of approach for tagging.



NMT recommends you verify that your elastomer cured properly. This can be done by retaining the mixing cup for 24 hours, although the elastomer may cure faster depending how much material is left in the cup. If the elastomer is not fully cured within these time frames, please contact NMT immediately.

Mixing Small Quantities of VIE

In some applications, a researcher may wish to tag only small numbers of fish each day, over a period of many days. As the elastomer marking system uses a two-part mixture, which has a limited life after mixing, this can be wasteful of material unless very small quantities can be mixed. Accurate measuring of the two components, especially the curing agent, can be difficult. This has typically resulted in waste, sub-optimal mixing ratios, imperfect mixing, and running out of curing agent. NMT is aware of these problems and we offer the following suggestions.

(1) There is a minimum quantity of material that can sensibly be handled and mixed; we suggest that this is 0.1 ml. We recommend the following procedure for mixing this small amount:

(a) To ensure accurate measuring of the curing agent, dispense slightly more than 0.01 ml of curing agent into a 0.3 cc injection syringe. Remove any air bubbles that appear after you replace the plunger. Note: You must not leave the curing agent in this syringe for more than a day as the rubber stopper will react with the curing agent and keep the elastomer from curing. VIE kits are packaged with more curing agent that would be required for the 6 ml of colored portion.

(b) Using the injection syringe from step (a), put 0.01 ml of the curing agent into the barrel of a clean injection syringe. Put the curing agent as far down into the barrel as the needle will reach. Take care to keep the needle off of the lip of the barrel so that you do not contaminate the elastomer during step (c).

(c) Press the tip of the syringe with the colored elastomer into the barrel of the injection syringe. Using the graduations on the side of the 3 ml syringe, dispense 0.1 ml of the colored portion on top of the 0.01 ml of curing agent.

(d) Use a flat toothpick (rather than round to minimize wasted elastomer) to mix the elastomer in the barrel of the syringe for 1 minute. Scrape the elastomer off of the toothpick, insert the plunger and begin tagging. You should have about 0.09 ml of elastomer, enough for about 20 to 50 tags.

(2) One way to reduce waste is to maximize the usable life of the mixed material. The curing process starts as soon as the two components are mixed and is heavily temperature-dependent. At room temperature the material remains usable for about an hour. If the minimum volume that can be mixed is more than can be used in an hour, storing mixed material that is not actually in use the freezer or on ice in a cooler may extend its life up to 3 days.

(3) We cannot guarantee the above working time estimates for mixed material, as performance is dependent upon local conditions. We recommend that you undertake your own trials.

Tagging with VIE

The Manual Elastomer Injector is designed for the tapered front piece to be held in the thumb and forefinger with the back piece against the palm. Most users inject elastomer with the bevel of the needle up. A sponge or paper towel is useful to clear the needle of excess elastomer before each injection. Insert the needle to the farthest point where you would like the elastomer to be, and then begin injecting elastomer as the needle is pulled back.



Care should be taken to stop dispensing the elastomer before the needle is completely withdrawn to avoid material trailing from the tagging wound. Trailing material will keep the wound from healing and increase tag loss. Any excess material should be gently wiped off towards the tag.

Combining four colors with different body locations offer the potential for developing a range of unique identifications. For example, experiments with bull trout indicate that there are eight suitable VIE tag locations. Using a single mark per fish would produce $8 \text{ (no. of body locations)} \times 4 \text{ (no. of colors)} = 32$ unique marks. However, use of two different marks per fish, in combination with these same body locations and colors, results in the potential for hundreds of unique marks.

Tag retention varies between species, among taggers, and with tag location. In many animals, long-term retention of VIE tags exceeds 90% if done properly. However, if the tagged animals are vigorously handled before the material cures or the wound heals, the material can be forced back out of the needle wound. Merely dropping the animal into water or subjecting it to heavy current could increase tag loss. Tagged animals should be handled with care for at least 10 days after tagging.

The possibility of spreading disease with tagging equipment concerns our customers and NMT. To disinfect, rinse the surfaces of the hand injectors with tap water to remove extraneous material. Place the equipment on a clean, disinfected surface and spray liberally with a chlorine solution, made from 1 part household bleach and 250 parts water (one ounce of bleach to 2 US gallons of water) or similar disinfecting solution. Use water to thoroughly rinse away disinfectant.

Please dispose of used syringes properly in a sharps collection container to reduce the risk of needle stick injury.

Tag Detection

Although VIE tags can usually be seen with the naked eye under normal daylight or interior lighting, their visibility is greatly enhanced with NMT's VI Light. Our VI Light will fluoresce green, yellow, orange, red, pink, and blue tags without the need for amber filters. When fluoresced, a small spot of elastomer can be seen at considerable distance. Tags obscured by pigmentation in ambient light are often detected with the VI Light.

To maximize tag identification:

- Choose distinct colors for tagging.
- Tag in clear tissue whenever possible.
- Train your samplers – let them practice with the tag colors they will encounter before they start collecting data.
- Use the VIE Color Standard to correctly identify colors.
- Fluoresce poor or obscured tags with the VI Light.



Seahorses can be tagged with VIE to identify individuals. Photo © D. Harasti.

Using the VIE Color Standard



NMT's VIE Color Standard presents the ten VIE colors on a clear card. This allows the sampler to place the color sample directly beside a tag for comparison, either under or over the tagged tissue.



Using the VI Light

CAUTION!

- ✿ Never look directly into the VI Light.
- ✿ The VI Light must be kept out of the reach of children.

Turn on the VI Light and verify that it does not need fresh batteries. The VI Light has a built in regulator to



insure the beam intensity is constant throughout the life of the batteries. When the batteries are weak, the VI Light will flash to let you know it's time for a fresh set. Shine the light directly on the area where the tag is, or is thought to be. If you are working in direct sunlight, you will need to fluoresce the tags in the shade – even the shade of your body is probably enough. Very faint tags are best seen when fluoresced in darkness.

Continuing Projects

When the supplies in your kit have been exhausted, NMT offers Refills containing elastomer, and mixing and injecting supplies.

The Manual Elastomer Kits are designed for projects that require tagging up to several thousand individuals. Larger elastomer tagging operations may be best conducted using an Air Driven Elastomer Injection System. These injectors have been used to tag Pacific salmon where groups approach a million fish. Please contact NMT for further information.

Contents of Manual Elastomer Injection System Kits

60 ml Kit

- 60 ml of elastomer (customer selects up to 10 colors)
- 2 Manual Elastomer Injectors
- mixing and injection supplies
- 1 VI Light
- 1 VIE Color Standard
- field carrying case
- written and video instructions

24 ml Kit

- 24 ml of elastomer (customer selects up to eight colors)
- 1 Manual Elastomer Injector
- mixing and injection supplies
- 1 VI Light
- 1 VIE Color Standard
- field carrying case
- written and video instructions

6 ml Kit

- 6 ml of elastomer (customer selects up to two colors)
- 1 Manual Elastomer Injector
- mixing and injection supplies
- 1 VI Light
- 1 VIE Color Standard
- field carrying case
- written and video instructions

6 ml Refill

- 6 ml of elastomer (customer selects up to two colors)
- mixing and injection supplies
- written and video instructions

VIE Trial Pack

- 1 ml of elastomer (customer selects one color)
- mixing and injection supplies
- written and video instructions

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