



## Turtle PIT Tagging Protocol for the Northeastern U.S.

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As more researchers use passive integrated transponder (PIT) tag technology, there is an interest in standardizing methodology among teams working with turtles in the Northeast in order to facilitate collaboration and the effectiveness of potential law enforcement efforts. This PIT tagging protocol was developed by collecting information from biologists who have PIT tagged turtles, and by surveying veterinarians with reptile expertise. This protocol is **not** an instructional on *how* to PIT tag turtles. It is the responsibility of all those that intend to carry out PIT tagging to consult with experienced veterinarians and/or researchers in order to obtain the expertise necessary. All necessary governmental permits and Institutional Animal Care and Use Committee (IACUC) permits should be obtained prior to commencement of any research involving turtles.

### Tagged Species:

- Biologists in the Northeast are using PIT tags to mark turtle species including Diamondback Terrapins (*Malaclemys terrapin*), Northern Red-bellied Cooters (*Pseudemys rubriventris*), Wood Turtles (*Glyptemys insculpta*), Blanding's Turtles (*Emys blandingii*), Spotted Turtles (*Clemmys guttata*), and Box Turtles (*Terrapene carolina*).
- Depending on the goals of the project, any species of turtle may be appropriate for PIT tagging.
- Species that are particularly vulnerable to illegal collection may warrant priority.

### Equipment Specifications:

- Necessary supplies include PIT tags, injection syringes, tweezers, PIT tag readers, latex/nitrile gloves, disinfection materials, and liquid bandage.
- The NEPARC Turtle Working Group conducted a study in April/May 2019 to assess use and current investment by teams PIT tagging turtles in the Northeast. A total of 9 parties responded to the survey. Seven of those 9 parties used exclusively BioMark equipment, with others using Avid Inc. products.
- More important than the manufacturer of the equipment though, is the frequency and code structure of the PIT tags themselves and the ability of the PIT tag reader being used to detect tags of a certain frequency and code structure. Towards this end, we have devised the following recommendations:

<p><b>PIT tags should have a frequency of 134.2 kHz.</b> <b>PIT tags should have an ISO code structure of FDX-B.</b></p>
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- The ability of a reader to detect and read a tag depends on its ability to read different frequencies and code structures. The majority of readers available do not have “universal” capability and are designed to

detect a limited number of frequencies and code structures. Check the manufacturer specifications of the readers to learn about their capability and compatibility.

- However, both BioMark and Avid produce “universal” readers that can detect and read virtually any previously manufactured PIT tag. In both cases, the readers will detect and read any frequency (125, 128, or 134.2 kHz) with any code structure (e.g., AVID, FECAVA, HDX, FDX-A, FDX-B, TROVAN, etc.). The Avid model is called the *MiniTracker3*, is partially waterproof, and currently sells for \$349. The current Biomark models are the *GPR Plus* (not waterproof) for \$545, and the *HPR Lite* (waterproof) for \$800. The higher price of Biomark equipment reflects several convenience features such as proprietary software that allows for easy download.
- FDX-B information can be displayed in either a 15-digit decimal (123.456789012345) or 13-character hexi-decimal (1A2.3B4CDEFGH5) format. While it is possible to convert between the two, we recommend recording data in 15-digit decimal format.

### Tagging Restrictions:

- Turtles with recent injuries or that appear ill in any way should not be tagged.
- Strict minimum size guidelines remain elusive, but both **tag size and syringe size should be minimized** when implanting small turtles. Both BioMark and Avid manufacture tags that are approximately 8mm long; these are the smallest tags currently available. They are injected using a 16 gauge (0.165 mm) needle, and a 15 gauge (0.183 mm) needle, respectively.
- There was no consensus among veterinarians as to whether PIT tagging hatchlings is harmful (see Question 6). Turtles less than 50mm in carapace length or 50g are not tagged by some teams (e.g., bog turtles in NC). Other teams use a minimum size of 65mm (Munscher et al. 2015). Other teams have implanted hatchlings weighing as little as 6.7g (Duncan 2013, Duncan & Burke 2016).
- **As long as PIT tags are not placed into the body cavity, the majority of veterinarians do not consider PIT tagging gravid females to result in increased health risk (see Question 5).**

### Tag Placement:

There have been two general approaches for tag placement used in the Northeast:

- Method 1: Injection of tag subcutaneously into the hind limb, parallel with the femur (researchers using this method include M. Jones, J. Regosin, S. Buchanan).
- Method 2: Injection into the body cavity lateral to the bridge, no deeper than the bevel (Buhlmann and Tuberville 1998; researchers using this method include G. Johnson, B. Ruhe, K. Oxenrider).
- **The veterinarian survey suggests Method 1 results in less risk to the turtle (see Question 3): placing the tags subcutaneously, parallel with the femur.**
- When done correctly, there should be very little or no bleeding. When bleeding does happen, pressure is applied until bleeding has stopped, and that animal is observed to ensure it is not injured.

### Disinfection/Biosecurity:

- Latex or nitrile gloves should be worn by the person(s) injecting the PIT tag and/or handling the turtle.
- Check the PIT tag with the reader prior to injection to confirm the tag is functioning properly.
- Injection site should be prepped with disinfectant/antiseptic (e.g., hydrogen peroxide, chlorhexidine, or isopropyl alcohol).

- Both BioMark and Avid offer all PIT tag models in pre-loaded, gas sterilized syringes. **Gas sterilized syringes and tags are the preferred equipment whenever possible. A majority of veterinarians surveyed selected this method over topically disinfected equipment (see Question 2).**
- After injection, the wound should be treated with liquid bandage antiseptic solution. This helps seal the injection site and prevent infection. Animals should be observed after the PIT tag is injected to ensure that it is not pushed out immediately, that there is no bleeding, and that the animal has no sign of injury.
- Scan the PIT tag one more time to ensure placement and functionality.

#### Further Regional Coordination:

- Development of a multi-state PIT tag database to track all individuals tagged.
- Development of a list of all those with “universal” readers to use as a reference in the event one is needed (e.g. large-scale law enforcement confiscation).
- Further study elucidating questions about retention rates and safety of PIT tags with emphasis on small turtles.

#### Literature Cited:

- Buhlmann, K.A. and T. Tuberville. 1998. Use of passive integrated transponder (PIT) tags for marking small freshwater turtles. *Chelonian Conservation Biology* 3: 102–104.
- Duncan, N. 2013. *Note*. Using a handheld PIT scanner and antenna system to successfully locate terrestrially overwintering hatchling turtles. *Herpetological Review* 44: 233–235.
- Duncan, N.P. and R.L. Burke. 2016. Dispersal of newly emerged diamond-backed terrapin (*Malaclemys terrapin*) hatchlings at Jamaica Bay, New York. *Chelonian Conservation and Biology* 15: 249–256.
- Munscher E., A. Walde, J. Daren Riedle, E. Kuhns, A. Weber, and J. Hauge. 2015. Population structure of the Florida softshell turtle, *Apalone ferox*, in a protected ecosystem, Wekiwa Springs State Park, Florida. *Chelonian Conservation and Biology* 14: 34–42.

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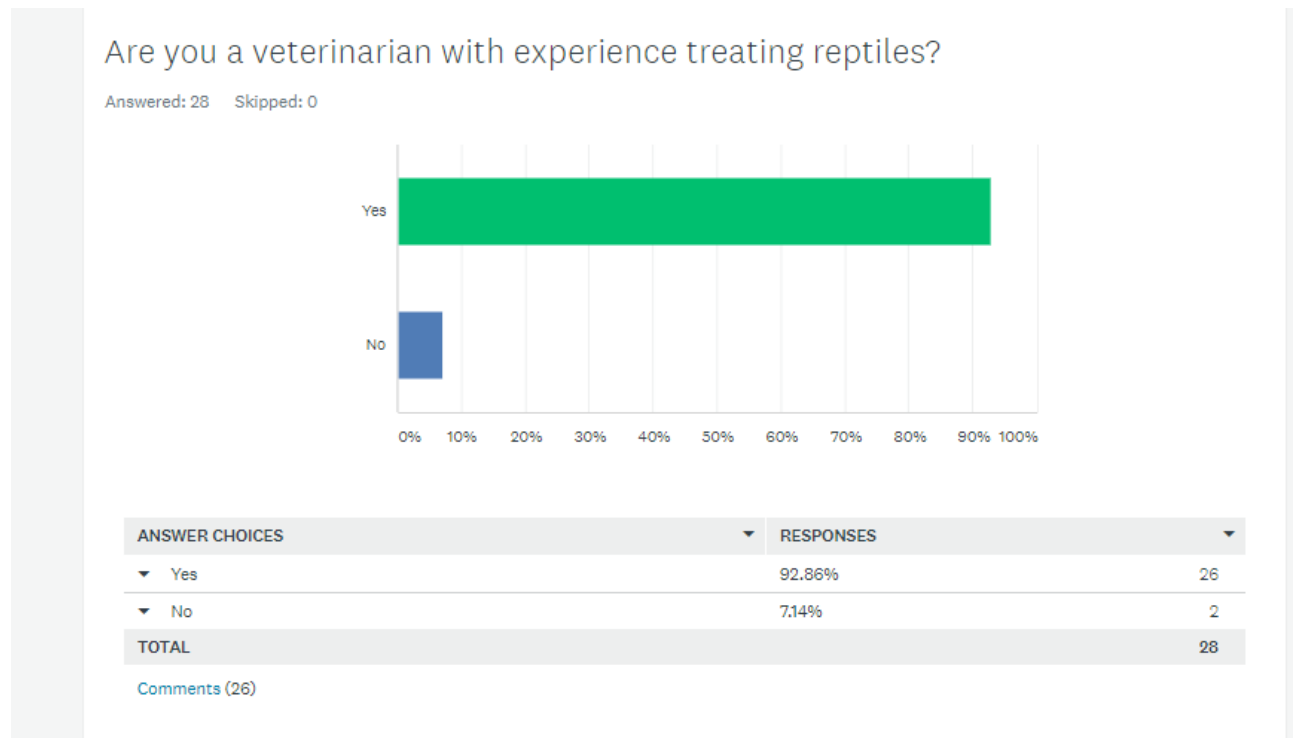
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### Veterinarian Turtle PIT Tagging Survey - Results

The following survey was distributed to veterinarians with experience treating reptiles. Our primary objective with the survey was to inform this PIT tagging protocol in the development of both practical and safe methods that could be used by wildlife biologists in the field for the turtle species under study in the Northeast. The survey was conducted using Survey Monkey and took place during October/November 2019. All data and comments are included below.

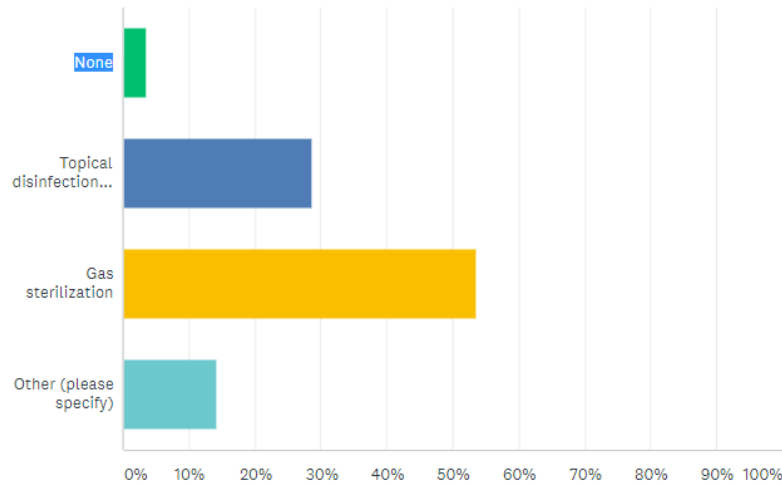
Question 1:



Question 2:

What level of decontamination do you consider appropriate for implantation syringes and PIT tags intended for permanent implantation in a wild turtle?

Answered: 28 Skipped: 0



ANSWER CHOICES	RESPONSES
None	3.57% 1
Topical disinfection (e.g., isopropyl alcohol)	28.57% 8
Gas sterilization	53.57% 15
Other (please specify)	14.29% 4

Comments:

I think topical disinfection (isopropyl alcohol) is fine when the equipment is used within the same population. But gas sterilized or new equipment should be used between different populations.

11/14/2019 5:36 PM

Topical disinfection with chlorhexidine scrub, all implantation materials going under the skin should be sterile

11/14/2019 11:11 AM

I have never implanted pit tags

11/13/2019 9:20 PM

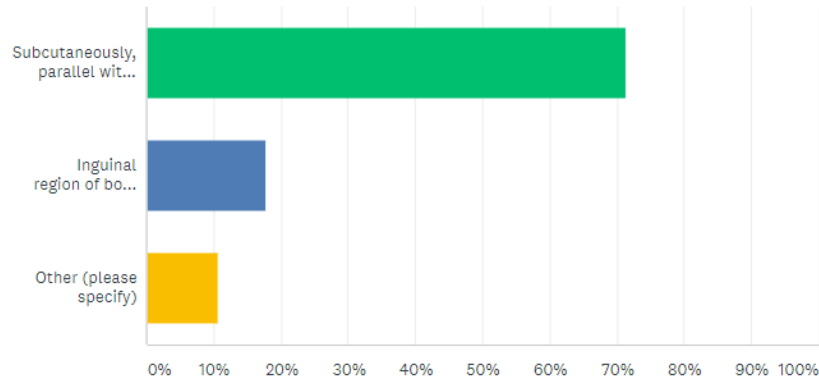
Gas, steam, or minimal cold/ chemical sterilization as per manufacturer's instructions- purchased preloaded in sterile syringes or sterilized and loaded into sterile syringes prior to field activities.

10/24/2019 7:07 PM

Question 3:

What placement of a PIT tag introduces the least amount of risk to a wild turtle?

Answered: 28 Skipped: 0



ANSWER CHOICES	RESPONSES
Subcutaneously, parallel with femur, in between skin and muscle	71.43% 20
Inguinal region of body cavity	17.86% 5
Other (please specify)	Responses 10.71% 3
<b>TOTAL</b>	<b>28</b>

Comments:

Never implanted Pit tags  
11/13/2019 9:20 PM

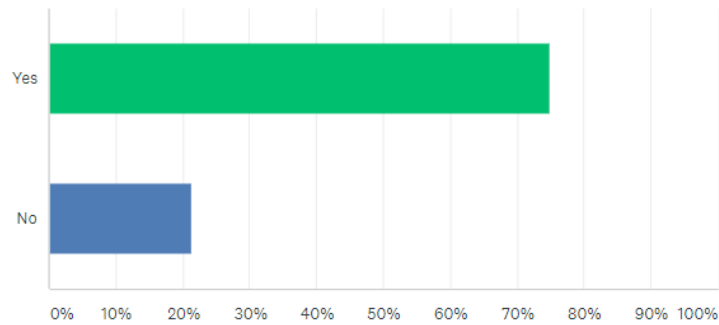
I have never placed a PIT tag so my opinion would be SQ, parallel with the femur, but I am unaware if this is truly the best site  
11/7/2019 7:11 PM

Inguinal region between skin and muscle layer along surface of plastron cranial and ventral to leg- not in body cavity.  
10/24/2019 7:07 PM

Question 4:

Should nitrile gloves be worn during implantation?

Answered: 28 Skipped: 0



ANSWER CHOICES	RESPONSES
Yes	75.00% 21
No	21.43% 6
<b>TOTAL</b>	<b>28</b>

[Comments \(19\)](#)

Comments:

If the syringe and needle and tag and turtle skin are disinfected, the human hands have little impact  
11/15/2019 9:12 PM

prevent human skin bacterial contamination  
11/15/2019 4:14 PM

The turtle's skin should be disinfected with isopropyl alcohol prior to implantation. Glove use by the person doing the implanting prevents bacteria (Staph species) from her/his hands from being introduced to the turtle along with the PIT tag.  
11/14/2019 5:36 PM

Gloves are always a good idea!  
11/14/2019 11:11 AM

Harder to feel what you're doing, just wash hands before and after  
11/14/2019 2:22 AM

Gloves are ideal but not essential.  
11/14/2019 12:36 AM

Helps reduce human hand micro-flora contamination of implantation site, as well as reduces cross-contamination from animal to animal.  
11/14/2019 12:18 AM

A Northeast Partners in Amphibian and Reptile Conservation Product. NEPARC publication 2020-1. Turtle PIT Tagging Protocol – December 2019

Sterile gloves would be best

11/13/2019 9:50 PM

In general, I would say yes.

11/13/2019 9:20 PM

to protect the implanter as well as the subject

11/13/2019 8:52 PM

I do not see the benefit.

11/13/2019 6:17 PM

If done in clinic, I prefer aseptic/sterile technique whenever possible.

11/13/2019 5:10 PM

As long as the instrument is sterilized and the patient's skin prepped with chlorhexidine

11/13/2019 4:15 PM

Gloves should be worn to prevent spread of infectious disease from one individual to another. Human hands are fomites

11/7/2019 7:11 PM

Reduced risk of contamination

10/25/2019 10:31 AM

It's cleaner

10/25/2019 1:10 AM

This is not essential, since the gloves are not sterile, but they are cleaner than bare hands. Ideally hands (gloved or not) should not be touching the implant or insertion needle, just the plastic syringe.

10/24/2019 7:52 PM

Only if the human has immunosuppression, latex allergy, or if the turtle is infested with parasites.

10/24/2019 7:33 PM

Change between turtles. Helps increase biosecurity for handler and turtle- minimize risk of pathogen transfer.

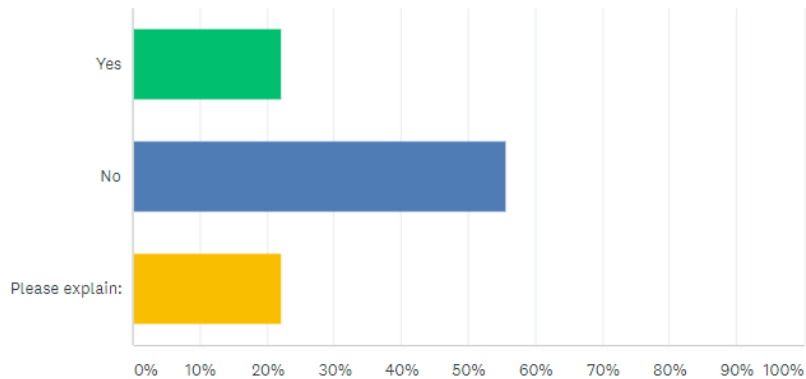
10/24/2019 7:07 PM



Question 5:

Does PIT tagging gravid females put them at greater risk of health complications than PIT tagged non-gravid females or males?

Answered: 27 Skipped: 1



ANSWER CHOICES	RESPONSES
▼ Yes	22.22% 6
▼ No	55.56% 15
▼ Please explain:	<a href="#">Responses</a> 22.22% 6
<b>TOTAL</b>	<b>27</b>

Comments:

If done properly, the tag should be subcutaneous and not through the body wall  
11/15/2019 9:12 PM

Yes if tags are placed in the coelom; no if tags are placed subcutaneously.  
11/14/2019 5:36 PM

No, as long as you aren't implanting into the coelom.  
11/14/2019 12:36 AM

I am unaware of the impact of the minimal stress associated with tag implantation on health of gravid females  
11/13/2019 6:17 PM

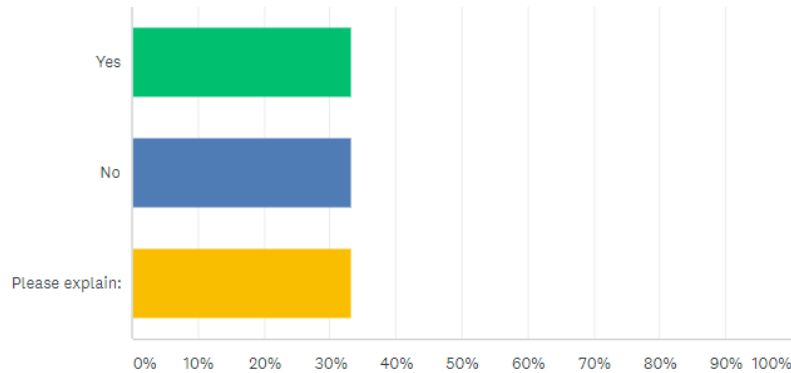
Depending on how large the tag is, if it's the size of a microchip it's fine.  
11/13/2019 4:15 PM

Minimal increased risk to gravid females with injection site described in answer above (between skin and muscle). Moderate to major risk to gravid females if injecting into body cavity.  
10/24/2019 7:07 PM

Question 6:

Does PIT tagging hatchlings put them at greater risk of health complications than PIT tagged turtles of older age classes?

Answered: 27 Skipped: 1



ANSWER CHOICES	RESPONSES
▼ Yes	33.33% 9
▼ No	33.33% 9
▼ Please explain:	<a href="#">Responses</a> 33.33% 9
<b>TOTAL</b>	<b>27</b>

Comments:

It must be done very carefully to avoid trauma to internal organs. For very small turtles (e.g. <50g), I prefer to place tags surgically under anesthesia.

11/15/2019 9:12 PM

Maybe slightly due to the immune systems in hatchlings not being less developed. But it's hard to say whether this risk is significant. It's definitely worth tracking any complications in this age class for comparison to older turtles if they are PIT tagged.

11/14/2019 5:36 PM

Depends; tiny Bog Turtle hatchlings may be too small; other hatchlings may not.

11/14/2019 12:36 AM

Even though small tags can be purchased, many hatchlings are so small that I'd have to anticipate mild to moderate mobility issues with a SC femoral site.

11/14/2019 12:18 AM

depends on the size of the tag and the hatchling (and placement location) I would think - so I'm a maybe

11/13/2019 8:52 PM

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Again, unaware of any studies indicating impact

11/13/2019 6:17 PM

Difficulty placing tag

11/6/2019 7:51 PM

possibly

10/25/2019 2:27 AM

in general, yes in a recent hatchling of a small species- this is most dependent on physical size of the animal.

10/24/2019 7:07 PM