

## Amphibian Specimen Collection & Submission Guidelines

### Determine the condition of the carcass(es):

*Freshly Dead Specimens* (e.g., no signs of scavenging, decomposition, fly eggs, or bloating; skin and eyes appear normal; no slime or mold; minimal damage). Collect, chill (**do not freeze**), and ship immediately. If they cannot be shipped immediately, follow the directions for “Not Freshly Dead Specimens” below.

*Not Freshly Dead Specimens* (e.g., not very freshly dead but not yet scavenged or decomposing; this will be most cases). Preserve half of the individuals frozen and half fixed in preservative. If there is only one individual, fixing is preferred, but could be frozen alternatively.

**Fixing:** About half the dead amphibians should be immediately placed into 70-80% ethanol for gross and histologic examinations. When possible, the freshest carcasses (those with the least amount of decomposition) should be selected for fixation.

- Allow specimens to soak in fixative 3-4 days before shipping. For the first 3-4 days of fixation, the volume of fixative to volume of carcasses should be 10:1. Add more fixative if volume drops owing to evaporation.
- After 3-4 days of fixation, the carcasses may be transferred to a minimal amount of fresh fixative that prevents drying of the specimen and shipped. A small amount of the fixative should remain to keep the specimen from drying out, but there should not be so much that the liquid sloshed in the bottom of the bag.

**Freezing:** About half the carcasses should be promptly frozen. The preferred freezing temperature is -40°C (-40°F), but any freezing temperature is preferable to a chilled carcass. **Do NOT freeze amphibians in water.** Frozen carcasses can be used for virus cultures, toxicological examinations, and molecular (DNA) tests. If freezing in a commercial freezer with a freeze-thaw cycle, surround it with ice packs to keep frozen during the thaw cycle.

**Decomposed Carcasses.** Decomposed carcasses may have some diagnostic usefulness for molecular testing and toxicological analyses. If fresher carcasses are not available, very decomposed carcasses with fluffy growths of fungus on the skin; maggots in the mouth, vent, and body cavity; or those that consist of just skin and bones, should be frozen and shipped.

# Guidelines for Packaging Specimens

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Each individual should be individually bagged and labeled. Individual specimen bags can then be placed into a larger bag for each collection site by date.

- 50mL centrifuge tubes (polypropylene preferred) and Whirl-Pak® 4.5"x9" Sampling Bags are best, and a pack of 8 can be provided upon request if necessary.
- These can also be purchased online from Amazon or other commercially available locations (example provided [here](#)).
- Ziploc® brand freezer bags or other leakproof freezer plastic bags will work to hold the specimen as a primary container if other containers are not available and the specimen will be frozen but are not preferred if chilled or fixed with ethanol. These have a higher chance of leaking during shipment. If using as a primary container with liquid, please triple bag instead of double bagging.
- Plastic storage or food jars (8 oz or other smaller or larger sizes) can work in place of 50 mL tubes, though are not guaranteed to be leak-resistant or leak-proof or freeze-tolerant.

***Chilled (Found freshly dead or euthanized and never frozen):*** Place in a cooler with plenty of ice packs and wrap the specimen bags in a paper towel or similar to insulate slightly and keep from freezing against ice packs.

***Fixed (Preserved):*** Once specimens have been fixed for 3-4 days, the preserved samples should be mailed in a minimal amount of preservative that prevents drying. **Do not mail large volumes of ethanol.** If the specimen is larger than 8-10 inches and requires a large volume of ethanol for fixing, wrap fixed carcasses in a paper towel or other absorbent material that is moistened with the ethanol and place inside the specimen container. Double bag the specimen. Include a few ice packs.

***Frozen:*** Frozen specimens should be mailed with plenty of ice packs. If possible, ship frozen carcasses in separate coolers from fixed or chilled carcasses. If frozen and fresh dead (or fixed specimens) must be mailed in the same cooler, separate the shipping container into two compartments with Styrofoam panels or other insulating material and place most of the ice packs at one end of the container next to the frozen samples.

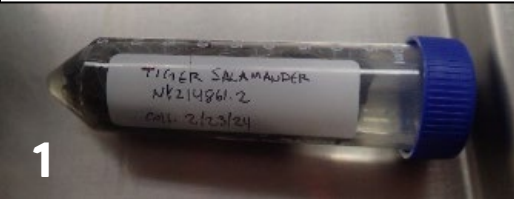
## Reference

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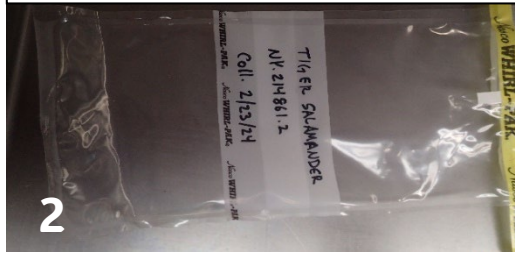
USGS National Wildlife Health Center. (2018, December 11). Collection, preservation, packaging, and shipping of amphibians for diagnostic examinations. USGS.  
<https://www.usgs.gov/media/files/collection-preservation-packaging-and-shipping-amphibians>

# Amphibian Specimen Packaging Steps

Place the specimen in an appropriate individual container and label it with the species name, identification number, and date collected.



Label the first bag with same information.



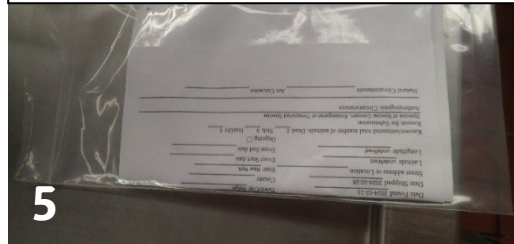
Place specimen container in first bag.



Place all bagged specimens in a second bag.



Fill out paperwork and place it in an individual bag to protect from spills.



Place bagged paperwork inside second bag with specimens, and seal second bag.



*\*See Specimen Submission Guidelines for additional information.*