

SOP: Amphibian and Reptile Specimen Fixation and Preservation

A. What this SOP covers

Use this SOP if you have amphibian or reptile specimens that you need to fix and preserve, or if you need to mix up neutral buffered formalin, or correct concentration of ethanol. Fixation involves the preservation and stiffening of tissues so that they do not decompose and so that the specimen remains in an easy-to-study posture. Preservation involves the storage of the specimen in a medium that allows for its safe study for many years.

B. What you need before you start

- Good quality specimens
- Plastic fixing tray and pins
- Syringe with needle
- Rinsing tray
- Water, ethanol, paraformaldehyde, sodium carbonate
- Stir plate and beakers
- Electronic balance

C. Warnings

Paraformaldehyde (a powder) and neutral buffered formalin (an aqueous solution) are known to cause cancer. It is important to minimize exposure to these chemicals when using them. Work with both chemicals in the fume hood and wear gloves when handling them. These two practices are sufficient to protect yourself. You can refer to the MSDSs for paraformaldehyde, sodium carbonate, formalin, and ethanol at the links below this paragraph. You can also obtain these MSDSs from Dr. Bergmann. These are the only chemicals that we regularly use in the lab.

- MSDS for paraformaldehyde: <http://www.sciencelab.com/msds.php?msdsId=9927382>

- MSDS for sodium carbonate: <http://www.sciencelab.com/msds.php?msdsId=9927263>

- MSDS for formalin:

[http://www.allinahealth.org/ahs/allinalabs.nsf/page/surgipath609.pdf/\\$file/surgipath609.pdf](http://www.allinahealth.org/ahs/allinalabs.nsf/page/surgipath609.pdf/$file/surgipath609.pdf)

- MSDS for 95% ethanol: <http://www.sciencelab.com/msds.php?msdsId=9923956>

The use of syringes with needles is another hazard associated with this protocol. Syringes are used to inject specimens with formalin (a carcinogen). Handle syringes carefully. Feel free to place the specimen being fixed in a tray to inject it instead of holding it in your other hand to minimize the risk of sticking yourself with the needle. If stuck by a needle, rinse with cold water for 15 minutes and wash area with soap and water. Report any such injury promptly to Dr. Bergmann or Frank Abell, the Chemical Safety Officer.

The final hazard associated with fixation and preservation of specimens themselves. Since these are dead animals, they may carry zoonoses and parasites. To minimize the hazard of being exposed to these pathogens, always handle specimens with gloves.

D. Procedure

1. Making neutral buffered formalin solution
 - a. Weigh out 16 g of paraformaldehyde
 - b. Weigh out 4 g of sodium carbonate
 - c. Combine paraformaldehyde and sodium carbonate with 400 mL of distilled water to a 1000 mL beaker
 - d. Place beaker on the stir plate in the fume hood and turn on stirrer until solution is being mixed vigorously but not violently. Do not heat the solution.
 - e. Once the chemicals are dissolved in the water (can take several hours), transfer the neutral buffered formalin to a jar, close the lid, and store in the fume hood.

2. Making correct concentrations of ethanol for specimen preservation
 - a. Reptiles are stored in 70% ethanol and amphibians are stored in 55% ethanol. Stock ethanol is 95%.
 - b. Dilute 95% ethanol with distilled water to the desired concentration.
 - c. Use the equation $C_1V_1 = C_2V_2$, rearranged to $V_1 = C_2V_2/C_1$
 - C_1 is 95%, C_2 is 70% or 55%, V_2 is the volume of ethanol you want to mix up, and V_1 is the volume of 95% ethanol that you need.
 - d. Add V_1 of 95% ethanol to a beaker, then add enough distilled water to make a total volume of V_2 .
 - e. Mixing ethanol and water generates a mild exothermic reaction, so always add water to ethanol.
 - f. We have a large plastic beaker in the lab with lines drawn on it to mix up ~4 L batches of 70% and 55% ethanol.
 - g. Store clean 70% ethanol in the carboy in the lab.

3. Fixing specimens
 - a. Fixation of specimens involves injection of formalin into the specimen, so should be done with caution and in the fume hood.
 - b. Specimens should be fresh or thawed (if they were frozen).
 - c. Place specimen in fixing tray, ventral side up.
 - d. Use syringe to inject formalin into the body cavity, limbs and tail base.
 - i. Use a fine needle so as not to damage specimen.
 - ii. Inject in two places in body cavity – in the abdomen and near the heart.
 - iii. Inject enough formalin to mostly fill but not over fill the cavities – if the body starts to distend, stop injecting.
 - iv. Larger specimens can also be injected superficial in the limbs and tail, but volumes injected should be small, as there is not cavity in these body parts.
 - v. Snakes should be injected in multiple places along the body (5-8, depending on size).
 - vi. Turtles should be injected near where the limbs enter the shell.
 - e. Use the needle to prick through the skin of the limbs and tail to allow formalin to enter. One or two pricks per appendage is sufficient.
 - f. The fixing tray has Styrofoam covered in paper towels that are wetted with formalin in the bottom.

- g. Position the specimen in the desired position, using sewing pins as needed to ensure that limbs don't get displaced. Do not put pins through the specimen.
 - i. Lizards & Salamanders: straight body; elbows, knees and ankles at 90°, tail looped to one side.
 - ii. Snakes: coiled into a spiral with the head inside, spiral can be oval to ensure that specimen fits in jar.
 - iii. Frogs: straight body, elbows at 90°, knees and ankles <90°.
 - iv. Turtles: limbs and neck stretched out of shell, as possible.
 - h. Place a specimen label on or beside specimen, and note the label number and the experimental specimen number for the specimen. E-mail this to Dr. Bergmann.
 - h. Cover specimen with a paper towel soaked with formalin and close tray.
 - i. Let specimen fix for 24-48 hours.
4. Rinse specimen to remove formalin.
 - a. Remove pins and specimen from fixing tray.
 - b. Tie specimen label to the specimen using a butcher's knot.
 - i. Lizards, salamanders, frogs: tie around waist, just anterior to hind limbs.
 - ii. Snakes: tie around the body at mid body.
 - iii. Turtles: tie around a limb.
 - c. Place specimen in a basin of cold water.
 - d. Let soak for 24-48 hours.
 5. Place specimen in used alcohol.
 - a. When the specimen is freshly fixed and rinsed, it contains a lot of water, which will dilute alcohol in a jar.
 - b. Place the specimen in a jar with used 70% or 55% ethanol. These jars are on the lab bench.
 - c. Leave specimens in the used ethanol for 1 to 8 weeks.
 6. Place specimen in its permanent jar with new alcohol.
 - Always remember: 70% ethanol for reptiles and 55% ethanol for amphibians!