Fluid preservation protocol for avian specimens: collecting to long-term storage

The information presented here was compiled by:

Mark B. Robbins, Univ. of Kansas and Christopher Milensky, Smithsonian Institution

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This document suggests basic guidelines for fluid preservation of bird specimens. Sources include publications (e.g., Simmons 2014), and long-term experience from museum staff. It should be underscored that this is based on current knowledge and may change with results from on-going research.

Formalin: Note that 10% formalin solution that you purchase in the United States is 3.7% formaldehyde. This is the standard strength for preserving fluid specimens. Full strength (=100%) is 37% formaldehyde. Thus, if you purchase **full** strength, you will need to dilute it 9 parts water with one-part formaldehyde. If you purchase formalin abroad, it may not be buffered and thus you will need to add the following salts to the formalin to preclude acidification: Salt recipe: for 1 L of 10 % formalin, combine 4 g Monobasic and 6.5 g Dibasic Sodium phosphate. Mix these two salts together with the liquid and test using pH test strips. These salts dissolve best in warm water so you may want to use one-part warm water to dissolve the salts before adding the remainder. If transporting the salts abroad, pre-measure and heat seal the salts into individual packets (see below). It is always a good idea to carry the MSDS sheets and original labels for both salts with you to avoid confusion. If you have access to seawater this acts as a great buffering agent without the need for salts (just make sure it is clean water).

Formalin may be purchased from a chemical supply company, such as Fischer Scientific, or prepared in-house. Purchasing concentrated formalin then diluting it in-house may save some money.

https://www.fishersci.com/shop/products/formalin-buffered-10-phosphate-buffer-certified-fisher-chemical-3/sf10020

At any state/stage, **DO NOT** store formalin in direct sun or in the heat. Formaldehyde turns to gas as temperature rises, which may result in reduced potency of formalin. Some people recommend that you use higher concentrations (20% formalin) in hot environments to ensure fixation. However, higher concentrations can be difficult to obtain in foreign countries.

Specimen choice: Ideally, specimens that have been collected using a non-destructive method or live capture (netted) are preferred, as shot from firearms, both lead and steel, have been shown to interfere with modern imaging techniques.

A fluid specimen should be preserved as quickly as possible. Decay begins immediately after death and specimens should be injected within one hour of death at a minimum and preferably within 30 minutes. Beyond 2 hours, other preparation types should be considered. Be sure to record the time after death a specimen was preserved.

If saving tissues for genetics, take those samples before injecting. In addition, wash the bird with soapy water before injecting to reduce water repelling properties which will cause the bird to float and will impede formalin from reaching the skin. Do not use laundry soap!

Injection: Methods may vary slightly depending on the size of the bird, but in general, specimens are injected in the major muscle areas with a 10% buffered (see above) formalin solution using the appropriate Personal Protective Equipment (nitrile gloves, protective eyewear, etc.).

Using a syringe and small gauge needle (number 22 or 23 seem to work best for birds), inject formalin into **each** breast muscle, leg, wing, the neck, the lower body cavity and then through the furcula. Injecting enough into the lower body cavity until it bulges is a critical step to stop bacterial decomposition of the gut. Do not inject directly into the brain cavity as this can cause damage. However, injecting between the 1st vertebrae and the occipital foramen will help with brain preservation. On large birds some effort should be made to inject behind the eyes to assist in formalin penetration of the brain. Once injected, you should wrap the bird in cheesecloth and place it in a tray with formalin so that it can set up properly. It does not need to be completely submerged, but there should be plenty of formalin in the tray at all times. In a field setting, one may simply choose to place specimens directly into a Nalgene or other container, where it will be completely submerged. The length of time in formalin will vary based on the size of the bird. There is no magic number for the length of time needed to properly 'fix' a specimen, and many people refer to leaving it in formalin until it 'feels firm'. We suggest 3-5 days for small passerines (e.g., parulid, *Passerina*), 5-10 days for medium-sized birds (e.g., *Turdus*), 10-20 days for large birds (jay to ptarmigan). Anything larger than a *Buteo*, use your best judgement. The idea is to get the muscles and tissue firm. Many experienced preparators refer to a firmness test using your fingers, checking the specimen periodically to make sure it is firm, but not too rigid. Roughly, 10cc are needed to inject small birds, 30cc for medium birds, and 60-100cc for large birds. Very large birds like loons or cormorants might require 1-2 liters.

Transporting salts for formalin purchased abroad:

If transporting abroad, include the label below as the salts might get misconstrued as illegal drugs

For Scientific Research

Contents: Biological specimen preservative buffer

10.5g Sodium phosphate monobasic

CAS#: 7558-80-7

Formula: HNa2O4P

Not a DOT controlled material (United States)

Not regulated under 49 CFR 172

IATA Classification: Not A Hazardous Substance

Not a Controlled Substance

If taking paraformaldehyde in powder form to mix abroad or as a pre-mixed liquid keep in mind that it is considered a hazardous material when shipped by air. See IATA regulations for paraformaldehyde packaging instructions for more details as packaging must follow their regulations and one must be a certified shipper.

Surprisingly, 10% formaldehyde in liquid form is unregulated by IATA and can be carried on a plane under special provision A189, although this can only practically be done in checked baggage (due to TSA liquid requirements) and you should take extra precautions for packaging by heat sealing your primary rigid container within three layers of 2-4 mm plastic bags. Special Provision A189 requires that containers be labeled (substance, concentration and UN number 3334) and include the phrase **"A189, not restricted".** It is also a good idea to carry a copy of the MSDS for formalin in case of inspection. It should be kept in mind however, that formaldehyde could be considered a noxious substance and as

such a pilot has the authority to refuse these goods on a plane. It is possible that you may be denied boarding with formalin at any stage in your journey.

A far better solution is to purchase formalin in pharmacies or at medical supply companies in the country where work is conducted. This option should be researched to determine availability before traveling. Keep in mind that you may need to add the buffer salts if it is not pH neutral.

Long-term storage: Many people will soak specimens in water prior to stepping up to the long-term storage in 70 % ETOH. There are a number of opinions on this, but it is recommended that rinsing or soaking specimens in water be done to remove the majority of surface formalin before switching to the first ETOH bath. Some even suggest soaking in water for up to 2 days. When switching from formalin to 70% ETOH, you should use 3 stages, 25%, 50%, 70%, or 4 stages as 20%, 40% 60%, 70% for large specimens. It is important to keep measuring the alcohol concentration once or twice a day to ensure that the concentration levels out before advancing to a higher step. A hydrometer that goes from 0-100% alcohol or a digital density meter are ideal for this purpose. If cost allows, fluid should be discarded between each stage so that you start with fresh ETOH at each concentration. It is recommended that you use formaldehyde test strips to check the concentration. Ideally, you will end up with about 1-2% formaldehyde in the final ETOH solution, ensuring that there will continue to be an equilibrium reaction for maintaining fixation. If you end up with a higher concentration, a final transfer to a fresh alcohol solution will usually take care of it.

Specimens should be stored in 70% ethanol in areas with good air circulation and low light levels, i.e., not in rooms where there is direct sunlight through windows. Periodically, both fluid levels and ethanol concentrations should be checked.

Simmons, J.E., 2014. Fluid preservation: a comprehensive reference. Rowman & Littlefield