Methods for obtaining *Batrachochytrium dendrobatidis* (Bd) samples for PCR testing

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General considerations

In terms of PCR samples, bleach and flame destroy DNA, while alcohol preserves DNA. Collection of samples for PCR testing requires that equipment used to collect samples not inhibit DNA detection while also not contaminating the current sample with DNA from a previous sample. In addition, equipment should be decontaminated so that it does not spread Bd (or other pathogens) from one animal to another.

The PCR sample collection methods described here have been used for obtaining samples from Colorado amphibians for Bd (*Batrachochytrium dendrobatidis*) detection. These methods should apply generally to collection of samples for Bd testing from amphibians.

Steps for obtaining samples

Collect animals

Animals should be collected with clean, decontaminated equipment, individually handled with fresh disposable gloves, and placed in individual containers prior to obtaining the samples. Although using Purell or other hand decontamination solutions may prevent the spread of live Bd from one animal to another, it is likely to allow contamination of samples with Bd DNA (in other words, if you handle a Bd-negative animal after handling a Bd-positive animal, the PCR samples you obtain may both appear to be positive for Bd).

Do not place multiple animals in the same container prior to sampling. In this situation, a single infected animal could infect others, and PCR tests could have inflated numbers of positive test results.

Equipment (such as individual containers for holding animals) can be cleaned and bleached so that they can be reused. However, this equipment must be rinsed well and allowed to dry prior to reuse so that there is no residual bleach (note that even parts per million bleach in/on/around a sample could possibly destroy all of the DNA in a sample over the course of a few weeks.)

Obtaining samples

Skin swabs are the preferred method of collecting samples from live individuals as the same individual can be tested repeatedly over time.

Obtain the PCR sample before doing other procedures with the animal (for example, before weighing, checking PIT tags, and so on). Samples require the following equipment:

- Swabs: use cotton swabs on 2mm-diameter wood without adhesive (such as Puritan Cotton-Tipped Applicators, #VWR 10806-005, or equivalent) cut to lengths (ca. 3-cm) that fit into 2-ml tubes.

- 2-ml screw-cap tubes containing 1 ml of 70 percent ethanol (2.0 ml screw cap tube with cap/500 per bag, VWR catalog # 20170-217, or equivalent)

To obtain the sample, hold the animal (using fresh gloves) in one hand, and gently but firmly swab the ventral surface 25 times; for large animals, you may swab the ventral surface 20 times and the feet and webbing 5 times.

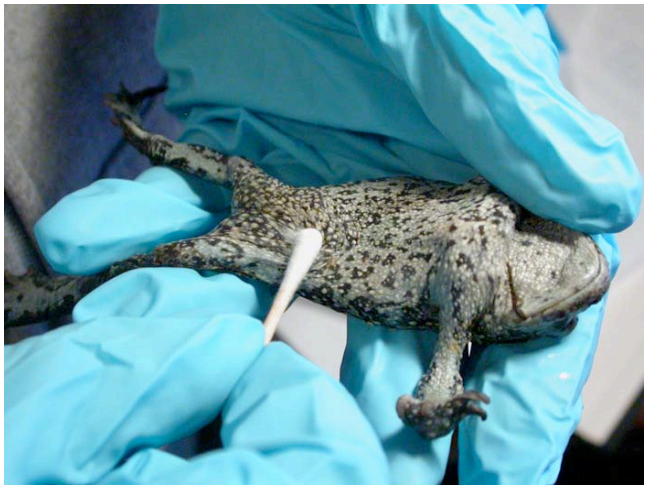

FIGURE 1. Swabbing ventral surface of amphibian.

Place the swab (cotton side down) in the tube. Secure the lid and place in a rack or other container so that the tube remains upright. (Leakage from one tube with BD may get on other tubes and result in contamination of your samples.)

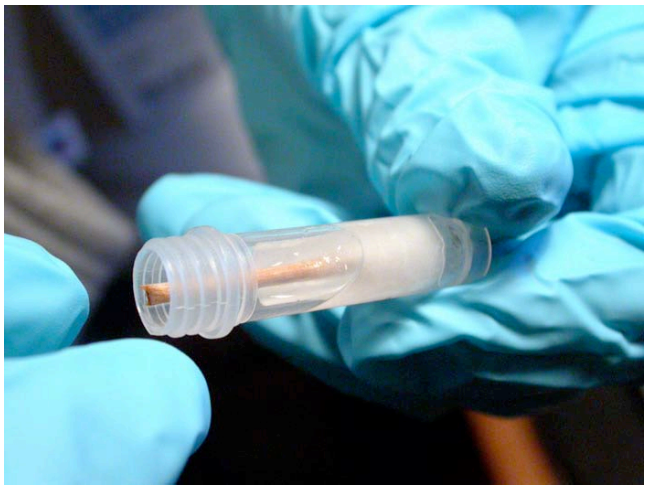

FIGURE 2. Insert swab or stick into tube with sample at bottom of tube.

Other skin tissues (such as toe clip samples or samples of ventral skin from dead animals) may also be collected for PCR testing. Use fine scissors to obtain the tissue. Between each sample, clean the scissors with an ethanol-soaked swab or tissue, and then hold the blades over an open flame to destroy any DNA from the previous sample. Place each sample in a 2 ml tube containing 1 ml of 70 percent ethanol.

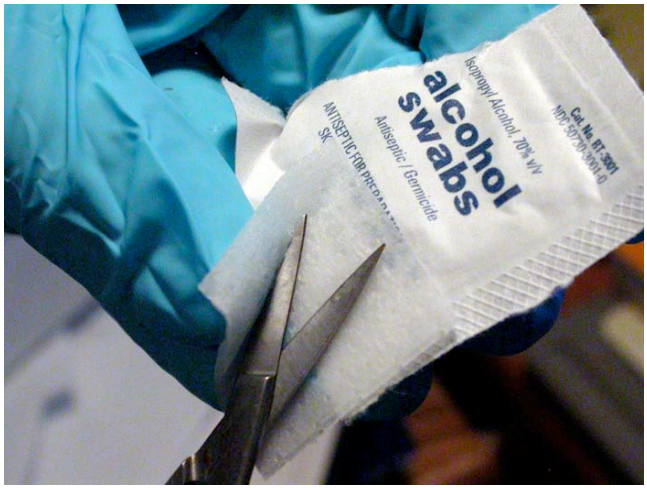

FIGURE 3. Cleaning scissor blades with alcohol.

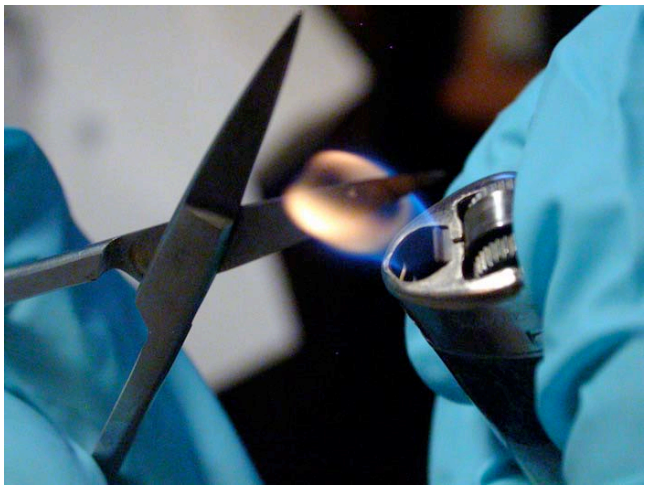

FIGURE 4. Passing scissor blades through flame to destroy residual DNA.

Toe clipping: If you collect toe clips from live individuals, use fine scissors to amputate the toe tip. When selecting a toe to amputate, you should avoid especially important digits such as the thumb, to avoid having an undue effect on the ability of the animal to feed, reproduce, and so on. Toe clips have rates of false-negative results similar to skin scrapes, but may have more potential for false-positive results through contamination. To obtain a toe clip, cut off the toe tip with the scissors. If bone protrudes from the wound, trim the bone further back (preferably to a joint) so that skin covers the wound, then dab a drop of Vet-bond or other sealant on the wound. In past studies, I selected the right rear toe, and continued to encounter individuals with this digit missing 2 to 3 years after the initial sampling.

Labeling samples

Label each tube with a unique sample number. Solution (for example, 70 percent ethanol) and sample type (for example, skin swab, skin scrape, pelvic patch sample, or toe clip) should also be associated with each sample:

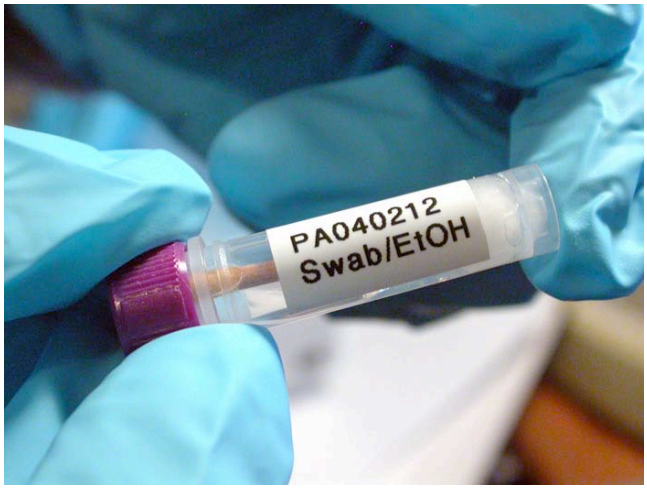

FIGURE 5. Label on tube.

Do not place sample information inside the tube. (It can be difficult to extricate, may contaminate other samples through handling, and as paper may contain bleaching agents, may inhibit detection of the target DNA).

Designate a plastic bag for the disposal of gloves and other materials (for example, alcohol wipes) to minimize the possibility of contamination.

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