

UC Berkeley Briggs NIH Group  
**Swab Protocol (2004-2007)**

This protocol was developed to allow field biologists to non-destructively sample amphibians in the field for the presence of *Batrachochytrium dendrobatidis*. This document was produced for the Briggs NIH research group based on Boyle et al 2004. Contact Vance Vredenburg ([vancev@berkeley.edu](mailto:vancev@berkeley.edu)) or Cherie Briggs ([cbriggs@berkeley.edu](mailto:cbriggs@berkeley.edu)) if you have questions.

**Supplies:**

Screw cap 1.5ml microcentrifuge tubes, available through Fisher (catalog number 05-669-12) and sterile fine tip swabs, available through Advantage Bundling SP. The swabs are made by Medical Wire (catalog number MW113). Advantage Bundling can be reached either by phone, 1-866-Bundling or orders can also be placed through email, [sales@advantagebundlingsp.com](mailto:sales@advantagebundlingsp.com). All microcentrifuge tubes used out in the field should be sterilized either by autoclaving before use or can be purchased pre-sterilized (catalog number 05-669-17). We use cyro- babies labels on the tubes, much sturdier than regular labels and are made by Diversified Biotech, catalog number 9188-2000. Their phone number is 1-800-LAB-TIPS. Please use ethanol- proof black markers to label your vials as they tend to withstand the time best.

**Procedure:**

1. Preferably, capture amphibians by hand. Wear gloves when swabbing animals and change gloves between animals. If you are using a dip net, be aware that *B. dendrobatidis* zoospores could be caught on the net and transferred between individuals, therefore, use different nets whenever possible, or disinfect the net as often as you can (there is no perfect solution to this problem).
2. Swab the underside or ventrum of adult/metamorphs 20-30 times. Remember you are in effect scraping small amounts of tissue from the skin. Some pressure must be applied, but this does not mean that you must squash the animal. Areas to target are the drink patch, thighs and webbing between the toes.
3. Air dry the swab for approximately 5 minutes, avoid direct sunlight if possible.
4. Break swab ~3cm from tip and drop into empty screw cap tube. The swab stick should not touch or bump against the top of the vial. Screw the cap on the vial and store in the shade.
5. Samples can be kept a room temperature for a week or maybe longer, but it is best to keep the samples cool and placed as soon as possible in a 4 degree C freezer (the kind you have at home is fine). Avoid extreme high temperature and direct sunlight. Samples may be stored in a freezer for many months without problems.
6. Analysis of swabs is no easy task. We use the quantitative PCR methodology as described by Boyle et al 2004 (reference included).

**Labeling:**

Tubes should be labeled with the collectors first and last initials followed by an "S", for swab, followed by a three-digit number, starting at 001. Example for Cherie Briggs first swab: should be labeled CBS001, etc... We are not assigning any other ID number to these vials so do not reuse numbers. Other data should also be collected along with the

swab reference # such as: Site ID, Site Name, Observer, Time, Species, Location (inlet, marsh or stream, pool), Life stage (larva, subadult, adult), Gosner Stage, Weight, SVL, Sex, and Behavior (lethargic, ok), pit tag number, and notes on animal condition (i.e., lethargic, righting reflex response, etc.).

#### Analysis of swabs:

Unfortunately, the Briggs Lab does not have the resources to run swabs for other research groups. Samples typically cost about \$10 each not including labor costs. As quantitative PCR machines become more common, we believe costs will fall and the technique described by Boyle et al. (2004) will become more widely applied to measure and monitor the spread of chytridiomycosis.

#### Reference:

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* **60**:141-148.

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