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Chytrid Swab Protocol

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@sfsu.edu) or Cherie Briggs (briggs@lifesci.ucsb.edu) if you have questions.

Supplies:

Swabs: These can be ordered directly or from a distributor:

1. Directly: Medical Wire and Equipment: go to <http://mwe-usa.com/mwe/mwe.php>
The product code is MW113.
2. Distributor: Advantage Bundling SP. (catalog number MW113). Advantage Bundling can be reached either by phone, 1-866-Bundling, or orders can also be placed through e-mail, sales@advantagebundlingsp.com.

Vials: Screw cap 1.5 ml microcentrifuge tubes. Available through many companies, such as Fisher (catalog number 05-669-12). All microcentrifuge tubes used should be sterilized either by autoclaving before use or they can be purchased at a higher price as pre-sterilized (Fisher catalog number 05-669-17).

Marking pen: When possible, use ethanol-proof black markers to label your vials as they tend to withstand time best. Some people prefer to scratch the sample id on the vial because it cannot then be washed off or erased by accident.

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 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 (if conditions are too humid to air dry then store in 95% EtOH).
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: We use quantitative PCR methodology as described by Boyle et al (2004).

Labeling:

Tubes should be labeled with the collector's 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:

Additional information 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/tadpole, subadult, adult), Gosner Stage, Weight, SVL, Sex, PIT tag number, and notes on Animal Condition (i.e., lethargic, righting reflex response, etc.).

Analysis of swabs:

Unfortunately, neither the Briggs Lab nor the Vredenburg Lab has the resources to run swabs for other research groups. Samples typically cost about \$4-10 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., Boyle, D. B., Olsen, V., Morgan, J. A. T., and Hyatt, A. D. (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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