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Collection and Analysis of Amphibian Skin Swabs for qPCR Analysis of Bd Load

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Sample Collection

The purpose of collecting skin swabs from amphibians is to provide information on *Batrachochytrium dendrobatidis* (Bd) infection intensity (i.e., Bd "load"). To avoid moving Bd from one animal to another, catch one animal, conduct the swabbing and related data collection, and release the animal. Do not hold multiple animals together (e.g., in a net) prior to swabbing. Prior to capturing each animal, clean your net and hands (e.g., sweep the net vigorously through the water, scrub hands in water) to remove as much Bd from previously-captured frogs as possible. Before swabbing an animal, also make sure that the animal's skin is free of detritus, mud, and other extraneous materials. Such contaminants can impair the PCR reaction and cause spurious results.

To collect a skin swab sample, remove a sterile swab from its packaging and swipe the animal 30 times. It is essential that the swabbed animal be wet (not just damp) when swabbed as this allows the swab to pick up skin cells. Therefore, dip the animal in water (from the collection site) immediately before swabbing. This also serves to clean the animal of any detritus. If water is not available at the site, bring sufficient sterile water with you and rinse the animal using a squirt bottle.

On post-metamorphic frogs and toads, swipe the left and right sides of the abdomen five times each, left and right thighs five times each, and foot webbing of the left and right rear feet five times each. For tadpoles of Gosner stage <42 (no front legs), make all 30 swipes on the mouthparts. For tadpoles of Gosner stage ≥42 (front legs present), swipe the mouthparts 10 times, left and right sides of the abdomen five times each, and left and right thighs five times each. After making 30 swipes, allow the swab to dry (at least 15 minutes on a dry, warm, breezy day; at least 1 hour on a cloudy day or if late in the day) before putting it into a swab vial. To dry swabs, push the swab stick (not the rayon swab tip) into the ground and through the loop of the tether that connects the vial to the cap. This will ensure that each swab is associated with the correct vial (and swab id). When swabs are dry, insert the swab ~3/4 of the way into a vial, break off the swab stick against the rim of the vial (using your finger or swab cap to ensure that the swab stays in the vial), and close vial cap securely. Swabs need to be completely dry before they are sealed into a vial; damp swabs will become moldy and results compromised. If swabs are being collected from multiple widely-separated locations, it is helpful to have a headband that allows swabs to be transported securely with their associated vial (contact us for details). Swab vials submitted to our laboratory must be labeled with a pre-printed swab_id, as described below. Use swab vials in order of their swab_id (see labeling information below), and place them in tube boxes in numerical order. Because swabs are analyzed in numerical order, shipping them in numerical order ensures that the lab processing can proceed as efficiently as possible. Efficient lab processing is essential for us to keep the costs of swab analysis low. If this protocol is not adhered to, costs for swab analysis will increase accordingly.

During swab collection and subsequent transport, keep collected swab samples cool. Hot temperatures will degrade DNA. When in the field, putting the samples into a resealable plastic bag and out of the sun

will usually suffice. If working in hot conditions, putting the samples in a small cooler with icepacks may be necessary. **After arranging the shipment with us**, ship samples to the Sierra Nevada Aquatic Research Laboratory using FedEx or UPS overnight delivery: Molecular Laboratory, Sierra Nevada Aquatic Research Laboratory, 1016 Mount Morrison Road, Mammoth Lakes, CA 93546. Ship samples in a small cooler containing ice packs. Do not ship samples on a Thursday or Friday. Doing so may result in the samples sitting in a hot location over the weekend. Also, at the time of shipment please provide us with the tracking number.

Supplies

- Gloves (non-powdered, sterile, disposable – optional)
- Swabs (only use Medical Wire, MW113; available from Advantage Bundling)
- Swab vials (use only Fisher Scientific 1.5 mL microcentrifuge tubes, 02707353) – must be labeled with pre-printed Tough Spot (USA Scientific: 9185-1000).
- Sterile water (if no water is available on site)
- Squirt bottle (for sterile water)
- Swab headband (optional)
- Resealable plastic bags (separate bags for sample vials and trash)
- 96-place tube box (for convenient storage of collected samples – optional)

If you will be collecting only a small number of swabs, purchasing the minimum order size of swabs, vials, etc. may not be economical. In such cases, we can supply you with swabs and labeled vials at cost.

qPCR analysis

Skin swab samples will be analyzed using standard qPCR methods as described in Boyle et al. (2004). Unless requested otherwise, samples will be run singly instead of in triplicate (see Kriger et al. 2006, for details). Every precaution will be taken to ensure that samples will be processed using established protocols and that assay results are accurate. However, we are not liable for lost samples or for results that are inaccurate for reasons that we were not aware of, including improper sample collection or problems encountered during the qPCR analysis. When testing is complete, results will be shared with the client via a Google Doc file, and clients are invoiced for the analyses conducted. Invoices are generally paid using a credit card, and payment details are provided with the invoice. Current per-sample cost is $7.00 and $10.96 for UC-based and non-UC clients, respectively.

Sample disposition

Following analysis, DNA extracts are either discarded or returned to the client. Prior to shipping samples, please contact us to discuss which of these options you would like us to use.

References

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.

Kriger, K. M., J.-M. Hero, and K. J. Ashton. 2006. Cost efficiency in the detection of chytridiomycosis using PCR assay. Diseases of Aquatic Organisms 71: 149–154.