Wildlife Epidemiology Laboratory

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Diagnostic and Research Submission Sample Preparation

The Wildlife Epidemiology lab is designed to be a collaborative research diagnostic lab for new or ongoing studies in free-ranging and captive amphibians, reptiles, birds, and mammals. We encourage collaboration with our lab and joint projects will allow for discounts on the assays. The following document is meant as a guide for quick reference, but is not all-inclusive and exceptions may occur. The utility of these assays must be interpreted based on the clinical or research question. Samples that are derived outside the validation parameters (species, sample type, storage type) may and are likely to affect the sensitivity of the test. If an assay doesn't exist below, then please inquire as new tests are being developed.

Ship samples overnight to:

University of Illinois
Veterinary Diagnostic Lab
2001 S. Lincoln Ave.
Urbana, IL 61802
217-333-1620

DO NOT SHIP AFTER WEDNESDAY AS THE UNIVERSITY OF ILLINOIS DOES NOT ACCEPT SATURDAY DELIVERIES AND IT IS LIKELY TO DAMAGE YOUR SAMPLE IF THAWING OCCURS.

Quantitative and Qualitative PCR

Polymerase Chain Reaction (PCR) is a highly sensitive assay that detects the presence of DNA in the sample. We currently offer 3 quantitative (real-time) and 1 qualitative PCR assays, but are continually developing more. PCR does not detect infection, it only detects presence, and thus needs to be interpreted in a clinical setting. Please communicate with the lab to improve the usefulness of your results. All samples should be stored frozen (ideally -80C) and shipped overnight.

Quantitative PCR:

Ranavirus (FV3-like virus):	*Antemortem*: Recommended to utilize dual testing strategy that includes two of the following: whole blood (0.2 ml in lithium heparin), oral swab (dry, preferably nylon flocked), and cloacal swab. Or a biopsy of kidney, spleen, liver will produce reliable results.

Post-mortem: Kidney has the highest viral copies in experimental studies in red-eared sliders, but millions of copies are found in liver, spleen, heart, lung. Avoid bone unless marrow is harvested.

This assay has been validated and tested for several tissue types and species of turtles and amphibians and is 100 times more sensitive than conventional PCR. The sensitivity and specificity of the assay when using whole blood is 100% and 100%, respectively. The sensitivity and specificity in swab samples is 83% and 100%, respectively. However, in surveillance studies, oral swabs have been positive, while whole blood is negative and thus the recommendation for a dual testing strategy. In experimental studies, viremia occurs 7-10 days after inoculation and presence in oral and cloacal swabs initially is observed 307 days after seen in blood. Furthermore, during re-infection, viremia spikes early and then is cleared in the blood prior to the clearance in oral epithelium.

Box Turtle Mycoplasma

Antemortem: Recommended to sample oral epithelium using swab (dry, preferably nylon flocked) or nasal flushed (not been validated).

Post-mortem: Upper respiratory epithelium.

This assay has been validated and tested for swabs of the oral cavity in box turtles. Nasal flushes can be effective, but have not been tested in a research or clinical setting because in preliminary studies, DNA yield is low. Thus, the sensitivity of detection may not be as good if no DNA is present in the sample.

Ophidiomyces (Formerly Chrysosporium):

Antemortem: Recommended to use a dry nylon or cotton-tipped applicator of the lesion and apply deep pressure. If a scab is present, swabbing under the scab is preferred. In venomous snakes, swabbing of the nasolabial pits is recommended.

Post-mortem: Sample grossly abnormal skin, underlying, muscles, and/or internal organs. Fresh or formalin-fixed paraffin embedded tissues.

This assay has been validated and tested for nylon flocked swabs, cotton-tipped applicators, and post-mortem skin tissue. In venomous snakes, the pattern of disease is to remain within the skin and only deep local invasion at that site (underlying muscle and bone). In non-venomous snakes, systemic involvement of the liver, spleen, and kidneys has been observed. This assay has been used successfully on formalin fixed paraffin embedded tissues (FFPE). Testing of non-clinical snakes has a low yield of positives, thus surveillance at this time should most definitely target snakes with lesions.

Batrachochytrium dendrobatidis (Bd-chytrid):

Antemortem: Recommended to use a dry nylon or cotton-tipped applicator of the lesion and apply deep pressure. Swab the ventral surface of the animal.

Post-mortem: Sample grossly abnormal skin.

SAMPLES CAN ONLY BE ACCEPTED FROM ILLINOIS CURRENTLY. This assay has been validated and tested for cotton-tipped applicators, and post-mortem skin tissue.

Batrachochytrium salamdrivorans (Bsal-chytrid):

Antemortem: Recommended to use a dry nylon or cotton-tipped applicator of the lesion and apply deep pressure. Swab the ventral surface of the animal.

Post-mortem: Sample grossly abnormal skin.

SAMPLES CAN ONLY BE ACCEPTED FROM ILLINOIS CURRENTLY. This assay has been validated and tested for cotton-tipped applicators, and post-mortem skin tissue.

Qualitative PCR:

Chelonian herpesvirus: *Antemortem*: Recommended to sample oral epithelium using swab (dry, preferably nylon flocked).

Post-mortem: Upper respiratory epithelium.

The current assay will detect presence but does not allow for relative quantitation. Due to conventional nature, sequencing is required to confirm the pathogen identity as other host of pathogen gene segments may produce a similar size band.

Virus Isolation

This can be performed on whole blood, swabs, or tissues. Recommend to store in cryovial (or similar) at -80C until analysis.

This assay utilizes Terrapene heart cell lines and is the preferred media for ranavirus. Terrapene herpesvirus is difficult to cultivate and numerous false negatives may occur. A snake cell line is available, but call lab prior to submission.