Shipping Amphibians for Diagnostic Testing and Biosecurity Precautions

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Diseases, Pathogens and Parasites Task Team

What does Biosecurity mean?

1) Protecting biologists from zoonotic pathogens.
2) Protecting wildlife from pathogens or contaminants that could be introduced by biologists or the public.
   (decontamination, translocation)

World Organization for Animal Health

Guidelines: International Transport of Animals

Notifiable Diseases: OIE Aquatic Code

(1) Chytridiomycosis
(2) Ranaviral disease

Regulate Commercial Transport
“Bd” or “Ranavirus”-Free Status

Pathogen Pollution:
- Salamanders as Bait
- Introduction of Novel Strains
  Jancovich et al. (2005), Picco et al. (2007)

Minimize Overland Transmission
- Regulate Amphibian Translocations Among Watersheds
- Decontaminate Surfaces
Wear Disposable Gloves

- Latex
- Vinyl
- Nitrile
- Gloves Rinsed with Distilled H₂O by Cashins et al. (2008)
- $12/box of 100

Wear Personal Protective Equipment

- Non-porous or disposable

Disinfecting Equipment

Scrape Mud and Scrub
Disinfecting Equipment
Spray Bottle or Immersion

- Bleach >4%
- EtOH >70%
- Virkon >1%
- Nolvasan >0.75%

$50/bottle

Johnson et al. (2003), Bryan et al. (2009)

Bd Surveillance

Non-lethal Techniques: Brem et al. (2007)
Swabbing Preferred

Adults:
- Swab 5 times in 5 locations
  - Rear feet (webbing)
  - Inner thighs
  - Ventral Abdomen

Larvae:
- Swab Oral Cavity 5 times

Store in 70% EtOH

A. Cressler, USGS

Ranavirus Surveillance

Lethal Collection:
Liver Preferred
St-Armour & Lesbarrères (2007)

Non-lethal Techniques: Gray et al. in review

Misclassification Decreases as Disease Progresses
Greer and Collins (2007)

False negative = 7%
False positive = 3%
St-Armour & Lesbarrères (2007)

n = 96 tadpoles

Lethal followed by Tail

A. Cressler, USGS
What do you do if you observe diseased amphibians or a die-off?

(1) STOP!!
Are other animals dead?

(2) RECORD
• Number of animals dead
• Number collected animals
• Possible stressors
• Water quality & sample

Basic Collection Supplies

(1) Disposable Gloves
(2) Nets
(3) Distilled Water
(4) Sealable Plastic Bags
(5) Permanent Marker
(6) Cooler or EtOH
(7) Disinfectant

Collect Amphibian within 24 hrs of Death
Live Animals Ideal!

Transporting Amphibians

Most Diagnostic Labs Prefer Fresh Specimens if Possible

Tent Design
Dry Ice
Sample w/ Cushioning Paper Towel Dry Ice

No Direct Contact with Dry Ice
Preserving Animals

- 95% EtOH
- 10% neutral buffered formalin

Separate Containers for Each Specimen!

Euthanizing Animals

Amphibians:

- Tricaine Methanesulfonate
  - 100–250 mg/L
  - (Finquel®, Argent Laboratories)

- Benzocaine hydrochloride
  - >250 mg/L
  - 20% Benzocaine OTC Gel

Shipping Animals

1. Call the Diagnostic Lab for Specific Instructions
2. Follow Courier Guidelines
Shipping Specimens
Triple Packaging
First Layer

Label Each Layer!

Shipping Specimens
Triple Packaging
Second Layer

Do not use Biohazard Bags (unless known to be infected with a BSL-2 agent)

Absorbent Paper Towel

Shipping Specimens
Triple Packaging
Third Layer

Place Cooler in Cardboard Box

Only Use Dry Ice for Frozen Samples

ThermoSafe® Polar Pack
**List of Contents & MSDS if Needed**

**MSDS Required**
- EtOH or Formalin

- Detailed list of all contents
- Description and location of die-off
- Contact information of the shipper

**Requested services**
- General Pathological Screening
- Specific Pathogen Testing

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**Labeling**

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**Amphibian Diagnostic Labs in U.S.**

**Southeast:**
1. University of Georgia Veterinary Diagnostic Lab, Tifton
2. Southeastern Cooperative Wildlife Disease Study, Athens
3. University of Florida, College of Veterinary Medicine

**Elsewhere:**
4. USGS National Wildlife Health Center, Madison
5. Pisces Molecular, Boulder
6. Colorado State University Veterinary Diagnostic Lab, Fort Collins

Cost per Sample = $20 – $100 / animal

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Questions??