Instructions for Submitting a Fish Sample to the Aquatic Diagnostic Laboratory for Pathogen and Disease Diagnostics

Disease and parasite diagnostics are fee-based services, but the Aquatic Diagnostic Laboratory (ADL) only charges costs for materials, media, and assays used during the diagnostic. At the bottom of this document there is a 2016 fee schedule (procedure costs). This fee schedule contains an example of the number and type of tests performed for most "common" diagnostic cases including the test and final costs. However, this is only an example. When conducting diagnostic testing, test results must be followed along the path to where each subsequent test leads the investigation, so tests and prices do often vary.

Step 1. Work Order. Complete a work order form for the ADL and submit by email to kathrine.bradshaw@tamu.edu.

- A new disease diagnostic case cannot be started without the signed work order.

Step 2. Shipping. After receiving the work order, the ADL principle investigator will contact you to arrange sample shipment or personal delivery date and time and provide shipping address.

- All samples must be shipped overnight or hand delivered.
- Samples must be shipped in an insulated container with sufficient ice or freezer packs to keep the samples below 40F for at least 36 hours
- In cases where dry ice must be used, such as during high summer temps or long shipping times, place a folded towel between the fish and the dry ice
    - If the fish directly contacts the dry ice, it will freeze ruining the possibility of bacterial isolation from frozen tissues
- Never ship samples on a Friday or prior to a Federal Holiday - any delay in the delivery process will render the samples unusable
- Never ship samples unannounced or without first contacting the ADL to arrange a sample delivery date (ADL is not staffed full time)

Step 3. Veterinarian. Contact your veterinarian prior to sample collection.

- If you do not have a veterinarian willing to treat fish, it is strongly suggested to develop a relationship with a veterinarian that will treat fish
    - Most vets will have to make a mandatory facility inspection before they will write a prescription for medicated fish feed
    - Most vets do not have training or experience with fish disease and parasite issues, but they still play a vital role (see below)

- If at all possible, but not required, your veterinarian should collect or be present at the collection of the fish sample (If they cannot be present, document sample collection and fish through photography and make available to your veterinarian).
    - The ADL is staffed by personnel with a Ph.D. in fish stress and disease physiology, and 16 years in fish disease research and diagnostic testing, but ADL personnel cannot write a prescription necessary for medicated feed. The ADL will provide a case write up with confirmatory diagnostics for the pond owner to share with their veterinarian. Their veterinarian will have to write a prescription for any medicated feed the ADL recommends as treatment for the disease issue. Most veterinarians require a mandatory pond/facility inspection before they will write a prescription for medicated fish feed.

Step 4. Fish Sample Collection. One disease sample submission is comprised of 1-3 fish, and 3 fish are highly suggested.

- More than 3 fish submitted means another sample, and associated costs are doubled for a second sample.
- A sample may consist of up to 3 different species, but should only consist of mixed species when multiple species are clearly sick/dyeing with similar symptoms.
- If at all possible, your veterinarian should collect or be present at the collection of the fish sample.
- The sample fish should be obviously sick and demonstrating symptoms, but alive
    - Dead fish do not make good samples for several reasons
        - External parasites and internal and external bacteria will begin to leave the fish or die within as little as 15 minutes of fish death, and therefore it becomes increasingly difficult to isolate the pathogen or parasite that is responsible for the mortality.
        - Dead fish that are not immediately refrigerated begin to decay quickly at warmer temperatures, which mean rapid invasion and colonization by secondary pathogens and heterotrophic bacteria decaying the fish tissue, which means the presence of many types of bacteria that make isolation and identification of the original bacterial pathogen almost impossible.
        - For these reasons, any samples submitted showing and signs of bloating, decay, or white or grey colored gills will not be examined and will be discarded.
    - If dead fish must be used, they must be fresh dead – within 1 hour of death.
        - To determine if a dead fish is suitable for use, check the gills. If the gills are still bright, blood-red, then the sample should be okay to use. If the gills are any shade of light-red, pink, white, or grey, then the fish has been dead for too long and is no longer acceptable as a sample.

- Cast nets and seines are an excellent way to quickly collect live fish samples for ADL submission.
    - Other methods include long-handled dip nets and fishing. Long-handled dip nets are only useful in situations where numerous sick, lethargic fish are found at the surface or along the bank in a semi-incapacitated state. Fishing is typically not a good method for sample collection, because with most pathogenic infection the first symptom of sick fish is that they stop feeding, so it may be difficult to catch sick fish.
- Collected fish specimens should be double bagged in ziplock bags without water, placed immediately in an insulated container with sufficient ice to keep the samples below 40F for at least 36 hours, and shipped overnight.

Step 6. Water Sample Submission. In most cases, it is suggested that a water sample be submitted for basic water chemistry testing along fish sample submission so that water quality can be ruled out as the cause or a contributing factor to fish mortalities.

- Samples should be collected in a new clean, plastic bottle with a screw cap. Purchased 16-20 ounce drinking water bottles can be reused if you rinse the bottle three times with the water source to be submitted to the laboratory. Insure the cap is tight prior to shipping. Please note that the lab does not test for bacteria, pesticides, or petrochemicals unless these services are clearly requested on the work order and these services are not included in the price of the basic water test. Clearly identify each bottle with a simple sample I.D. using the last name, date, source format (i.e. Smith, 4/19/2012, well). Samples in glass containers have higher potential for breakage and therefore will not be accepted and tests will not be conducted.
- When collecting a water sample, be sure not to disturb any sediments prior to or during sample collection. Sediments picked along with the water sample will potentially change the water chemistry and results of the water test will not be accurate. The water sample is acceptable “as is” if sediment is already suspended in the water column despite no disturbance from the collection process. Also, make sure the sample bottle contains no vegetation, insects, snails, tadpoles, small fish or other organisms after collecting the sample, as they will change the water chemistry and the result of the water test will not be accurate. To collect a sample, place a thumb over the mouth of the empty collection bottle, place the bottle 6” to 24” below the surface of the water, and remove your thumb allowing water to fill the sample bottle. Make sure all the air has been removed from the bottle and place the cap on the bottle before removing it from the water. Check the sample to determine that no air is trapped inside the bottle. If air is trapped inside the bottle, empty the bottle and repeat the process again.
- Fisheries (ponds and lakes) and Aquaculture - Provide as much information as possible about the condition of the tank (flow through or recirculation) or pond. If fresh water is running into the tank or pond, collect the sample in the area least affected by the fresh

water. When samples are taken from salt-water systems or ponds where fresh water may be added, gather water from both the top and bottom of the pond.
    - The ADL cannot test for dissolved oxygen or free carbon dioxide, even though these criteria all affect fish mortality. These substances must be tested for on-site, and kits for conducting these tests are commercially available.
- Well Water - Let the pump operate ten minutes to an hour before taking the sample. Take the sample as close to the pump as possible.
- Assessing Waters Problems - Two separate water samples may be required to address water related problems due to plumbing and/or fixtures. One sample should be collected at the point of entry (well or water service) and another at point of use (tank, pond, etc.). This sampling method will help pinpoint problematic plumbing.

Step 7 (optional). Toxicology Testing. If a toxin(s) is suspected in a fish mortality case, then a water sample and fresh fish samples should be immediately collected and frozen to prevent decay or breakdown of the potential toxins. The samples should be shipped on sufficient dry ice to keep them frozen, and a work order must be submitted clearly identifying that toxicology testing is requested. The ADL will then contact you about specific compounds or categories are suspected or you would like tested. The toxins that can be tested by the ADL:

Arsenic
Boron
Chlorine (free, total)
Chromium
Copper (free, total)
Cyanide
Detergents
Ethylene glycol (anti-freeze)
Hydrazines [herbicides (triazole, metribuzin, paclobutrazol, credazine, pyridafol, pyridate), systemic fungicides (triadimefon, propiconazole) anti-fungal and anti-bacterial compounds]
Hydrogen sulfide
Iron (ferrous)
Iron (total)
Manganese
Metals bundle (copper, manganese, iron)
Metals plus heavy metals bundle (copper, lead, manganese, arsenic, chromium, iron)
Nitrate
Nitrite
Pesticides bundle (triazoles, hydrazines, phenols, detergents)
Phenols [coal tar, petroleum, disinfectants, antiseptics, auxin herbicides (MCPA; 2,4-D; 2,4,5-T), detergents, endocrine disruptors]
Sulfates
Pospherous

Sulfites
Total ammonia nitrogen and un-ionized ammonia
Triazoles (3-Amino-1,2,4-triazole herbicides, fungicides)