

SUBMITTING SAMPLES TO APL

From all around the globe, shrimp and shrimp related samples and products make their way to our lab for testing. The Aquaculture Pathology Laboratory has served the shrimp industry since the 1980s under the guidance and direction of former P.I. and Director Dr. Donald V. Lightner. Upon his retirement in 2017 Dr. Arun K. Dhar was appointed the title of P.I. and Director of the laboratory. The Aquaculture Pathology Laboratory (APL) works around the clock to perform diagnostic testing and vital research studies. APL is divided between two campuses - Main Campus and West Campus at The University of Arizona. The Main campus is comprised of three major testing units: Polymerase Chain Reaction (PCR), Histopathology and Microbiology. The West Campus is the home of all challenge studies and live animal trials.

Because we receive many different varieties of samples, there have been numerous occasions where we have come across some discrepancies in sample submission that prevent us from moving forward with diagnosis. This causes delays in processing and could be detrimental to clients who need rapid results for timed shipments. Many of these discrepancies arise at the accessioning station- where the initial logging in of samples occurs. It is extremely important that the receiving process goes smoothly so that we can deliver results as quickly as possible.

It has been requested that we provide some guidance for submitting samples to prevent these delays. We believe it is important for clients to see why we prefer and require things a certain way. This article was constructed to provide some insight for submitting samples to the Aquaculture Pathology Laboratory here at The University of Arizona, as well as to clarify why certain forms are used and why we have very specific guidelines to follow.

What to know about shipping samples:

There are many limitations and guidelines to follow when shipping samples. If you are shipping outside of the country, please know the rules and regulations where you are located prior to shipping anything. Depending on the courier you are using, there are specific guidelines to follow when shipping biological or biohazardous materials. Please visit your courier's website for more details. For international samples that come to our lab, we have very specific USDA import permits that allow us to receive samples from all over the world. There are 2 import permits—one for tissue and live animals, and another for feed material. Ask a lab member of APL for the appropriate permit for your sample.

Additional documentation is required for international samples. Please see the “Restrictions and Guidelines” portion of the import permits for more information. Samples that don't have an original signed document from the sending company will most likely be rejected or held in US customs.

We can only send a sample received confirmation email to “PERSON/COMPANY REQUESTING TESTING” and release a final report to the contact of “REPORTING INFORMATION” on the 1st page of our submission form based on our policy.

Preparing samples:

Both PCR and Histology testing have different sample preparation methods.

Sending PCR samples:

PCR samples can be sent dried (i.e., dry feed, water treatments, etc.), frozen (shrimp tissue, extracted hemolymph or DNA/RNA) or preserved in RNA later or 95% ethanol (shrimp tissues). For the USDA pooling guideline, please check page 3 of our submission form and make sure the submission of the samples is proper. Please remember—we are in Arizona. Temperatures here can reach upwards of 110-115 degrees (°F). If you are sending PCR samples frozen, please include enough dry ice/ice packs to keep them cold until they reach our lab. The temperature of all frozen samples is checked upon arrival. If the temperature of the sample is above 8 °C, we cannot process the sample. If your sample doesn't have adequate amount of dry ice or ice packs, it will

thaw and degrade quickly. Samples that are rejected for processing are discarded as biohazard waste. Clients will receive notification that their sample was rejected for testing and will be encouraged to send a new sample. If you are sending samples in 95% ethanol, please use a leak proof container. We suggest sealing the top of each tube or container with parafilm to prevent leakage. That container should also be placed into a secondary container (like a plastic bag) in case the liquids escape.

If you are sending dried samples, please make sure the container is sealed properly. We also recommend including multiple (at least two) layers of bags around the sample to reduce possible spillage. Samples that are not sealed properly can spill into the package contaminating other samples – these will not be accepted, as this could compromise results.

We cannot proceed with the samples for testing if the quantities are below 250 mg (tissue sample), 100 g (dried samples) for each sample.

Sending HISTO samples:

Histological samples must be preserved prior to shipping. Samples for histological examination at APL should be fixed in Davidson's Fixative for an appropriate amount of time (depending on size of sample), and then transferred over to 70% ethanol. Please contact us for a more detailed fixation procedure.

Davidson's Fixative

(Please use in a well-ventilated area and use gloves and eye protection)

330 ml 95% Ethyl Alcohol

220 ml Formaldehyde

115 ml Glacial Acetic Acid

335 ml Tap Water

Shipping histology samples:

Once the sample has been fixed, you will need to ship samples moistened to prevent them from drying out. Because of the limitation of how much ethanol or liquid can be shipped, we have a method for histology samples that keep them damp until they arrive at our lab where we can submerge them in 70% ethanol until the testing process begins. Use a paper towel and pour 70% ethanol to dampen it. Wrap your shrimp in the damp paper towel. Place the shrimp wrapped in the towel in a plastic bag and pour an additional amount of 70% ethanol over the top of your wrapped shrimp. Seal tightly. Place into a secondary Ziploc bag to prevent leakage. Make sure you write the lot and ID number of your sample on the bag for us to ID. Some clients prefer to send samples through regular postal mail. However, we recommend sending your sample overnight via FedEx/UPS/DHL to prevent your sample from drying out. Samples that have been improperly fixed or that have dried out during shipping are of little to no use in histology and will be rejected.

Sending MICRO samples:

Micro samples typically come in the form of slants and or culture plates. Please ship these at room temperature and with the proper padding to ensure they arrive at the lab safely. Improper packaging can result in broken slants or plates that are of no use for testing and could potentially contaminate other samples in the package. Please parafilm slant tubes, and close culture plates with a rubber band.

Our Sample Submission Form:

Each sample we receive must come with our Sample Submission Form (SSF) sealed in its own plastic bag (preventing damage to the form due to samples leaking) to proceed with testing. Our form is 5 pages long (6 pages with the price list included) and provides us with the information necessary to perform testing and bill for the requested tests. Our form is an approved USDA and ISO/IEC 17025 submission form and must accompany each sample submission to be tested. Our newest version of the form requires a signature and cannot be altered

in any way. This signature ensures that clients understand the cost of testing and are promising to pay for these services.

Please fill out each portion of the form with as much detail as possible.

Any changes to your current original form will need to be revised and re-sent to us. Due to ISO/IEC 17025 requirements, no changing of dates, exclusion of tests on the final report, or change in sample name and lot number can be made.

Our current turnaround time for results are as follows:

PCR RUSH: 3-5 business days

PCR Routine: 7-10 business days

HISTO RUSH: 8 business days for up to 5 adult shrimp

HISTO Routine: 14-21 days

- For histology cases, the quantity of samples can change turnaround times significantly. If you submit more samples than we can process in the above timeframes, we will contact you and let you know the expected turnaround time before we begin work.

MICRO cases: Because of the nature of this testing, we cannot RUSH Micro cases. Turnaround times depend on size of sample but is typically within 2 weeks' time.

All RUSH cases have an additional fee.

Types of samples and target tissues-

Different pathogens require specific target tissues for pathogen detection. Pathogens are split into systemic and enteric. Systemic pathogens require systemic tissue-Enteric pathogens require enteric tissue. Below is a chart for reference that shows which target tissues are needed for each pathogen, whether they are systemic or enteric, and whether that pathogen is DNA or RNA.

| Pathogen/Disease | Pathogen Type | Target tissue |
|---|--------------------------------|--|
| White Spot Syndrome Virus (WSSV) | DNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph (*Gills for IHNV when available) HP, Feces if Enteric requested |
| *Infectious Hypodermal and Hematopoietic Necrosis Virus (IHNV) or (IHNV-integrated) | DNA Systemic virus | |
| Spawner Mortality Virus (SMV) | DNA Enteric /Systemic virus | |
| Infection with Decapod iridescent virus 1 /Shrimp Hemocyte Iridescent Virus (DIV1/SHIV) | DNA Enteric/Systemic Virus | |
| <i>Spiroplasma penaei</i> (Spir) | DNA Systemic bacteria | Pleopods, Gills, Post Larvae, Muscle |
| Taura Syndrome Virus (TSV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph |
| Yellow Head Virus (YHV) | RNA Systemic virus | |
| Infectious Myonecrosis Virus (IMNV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph, Muscle |
| Gill Associated Virus (GAV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph |

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| Penaeus vannamei Nodavirus (PvNV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Muscle | |
| Laem-Singh Virus (LSNV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph | |
| Mourilyan Virus (MoV) | RNA Systemic virus | | |
| White Tail Disease (MrNV) | RNA Systemic virus | Pleopods Post Larvae, Muscle | |
| Extra Small Virus (XSV) | RNA Systemic virus | | |
| Covert Mortality Nodavirus (CMNV) | RNA Systemic virus | Pleopods, Gills, Post Larvae, Hemolymph, Muscle | |
| Baculovirus Penaei: Tetrahedral Baculovirus (BP) | DNA Enteric virus | HP, Feces, or Post Larvae | |
| Monodon Baculo Virus: Spherical Baculovirus (MBV) | DNA Enteric virus | | |
| Hepatopancreatic Parvo Virus (HPV) | DNA Enteric virus | | |
| <i>Vibrio parahaemolyticus</i> (Vp) | DNA Enteric bacteria | | |
| <i>Vibrio harveyi</i> (Vh) | DNA Enteric bacteria | | |
| <i>V. parahaemolyticus</i> causing Acute Hepatopancreatic Necrosis Disease/Early Mortality Syndrome (AHPND/EMS) | DNA Enteric bacteria | | |
| <i>Enterocytozoon hepatopenaei</i> (EHP) | DNA Enteric fungus | | |
| Necrotizing Hepatopancreatitis: <i>H. penaei</i> (NHP-B) | DNA Enteric bacteria | | |
| <i>A. astaci</i> (Cp) | Mold | | Pleopods, Gills, Post Larvae, Cuticle/Exoskeleton |
| <i>Rickettsia</i> (Rick) | DNA Systemic/Enteric | | Pleopods, Gills, Post Larvae, Cuticle/Exoskeleton, HP, Feces, PLs |

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Don'ts for sample submissions and the reason for importance:

| DO | DON'T | Reason |
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| Use a chemical resistant marker to label samples | Use a ball point pen or sharpie | Ball point pen/sharpie rub off with liquid leakage especially when in contact with alcohol. If your samples arrive unmarked and we are unable to distinguish each tube's lot/ID for separate testing, it will be rejected. |
| Use a leak proof container with parafilm if possible | Use any container and wrap in duct/scotch tape | This always results in fixative leakage. It also leaves your sample dry. This could lead to a rejected sample if your sample does not arrive in good condition. |

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| Package your form in a Ziploc bag | Place your form in with your sample without a protective barrier | Leakage and condensation could cause the ink on the form to run, making it very difficult or impossible to read what your form says. We will be asking you for a new form while we put your sample on hold. This causes a delay in your results. |
| Make sure your sample name and lot numbers are on each sample and match your form | Label only the sample or only the form | If we cannot match your sample to your form, you will receive an email to clarify. This will delay your results. |
| Fill out the sample submission form completely | Leave parts blank | If there is missing information on your form that we need, we will put your sample on hold until we receive the information. This will delay your results. |
| Pack your frozen samples with enough dry ice/ice packs and send overnight | Pack your sample with minimal dry ice or few ice packs | More times often than not, samples packed with just ice packs do not make it to us within temperature. If your sample arrives above 8 degrees, thawed, and already starting to decompose, you will receive an email notifying you that your sample arrived, but is not suitable for testing. |
| Make sure your point of contact is easily available should we have questions about your sample and get back to us as soon as possible | Provide an incorrect phone number or email where we can't get a hold of someone to clarify answers for us | If we are unable to contact anyone, your sample will not be processed. The countdown for your results will not start until the discrepancy is resolved and your sample is able to be processed. |