

Institution: _____ **Veterinarian/Primary Contact:** _____
Email for Billing: _____ **Email for Results:** _____
Phone #: _____ **Common/Scientific Name:** _____
Sample Type: _____ ☐ Rush/Stat ☐ Necropsy Case ☐ Free ranging animal ☐ Human exposure concern
Animal ID: _____ **Sample ID:** _____
Collection Date: _____ **Submission Date:** _____ **Storage Temp:** _____ **Transport Temp:** _____
Conditions Suspected/ Special Instructions: _____

Fungal PCR Tests:

- ☐ Fungal Generic*
☐ *Aspergillus* sp.
☐ *Aspergillus fumigatus*
☐ *Blastomyces dermatitidis*
☐ *Candida albicans*
☐ *Candida glabrata*
☐ *Coccidioides immitis*
☐ *Cryptococcus neoformans*
☐ *Emydomyces testavorans*
☐ *Histoplasma capsulatum*

Bacterial PCR Tests:

- ☐ Bacterial Generic*
☐ *Brucella* sp.
☐ Strain typing
☐ *Leptospira* sp.
☐ *Mycobacterium* sp.
☐ *Mycoplasma* (see submission guidelines)
☐ *Nocardia* sp.
☐ Sequencing for speciation if positive PCR
☐ *Staphylococcus aureus*
☐ *S. aureus* Methicillin-resistance gene
☐ *Streptococcus* sp.
☐ *Streptococcus phocae*

Protozoal PCR Tests:

- ☐ Systemic *Isospora* (*Atoxoplasma*)
☐ Determination of fecal shedding (see submission guidelines)

Viral PCR Tests:

Specific:

- ☐ California sea lion Adenovirus
☐ Canine Distemper Virus
☐ Hawaiian monk seal herpesvirus
☐ Mustelid herpesvirus
☐ N. elephant seal herpesvirus
☐ Otarine herpesvirus 1
☐ Phocine herpesvirus-1

Family (Pan):

- ☐ Adenovirus*
☐ Astrovirus*
☐ Calicivirus*
☐ Coronavirus*
☐ Enterovirus*
☐ Herpesvirus*
☐ Influenza virus*
☐ Morbillivirus for cetaceans*
☐ Morbillivirus for pinnipeds*
☐ Papillomavirus*
☐ Paramyxovirus*
☐ Parapoxvirus*
☐ Poxvirus*
☐ Unknown virus detection (contact lab to discuss)

Panels:

- ☐ Cetacean Respiratory Panel (includes *Aspergillus* sp., *Aspergillus fumigatus*, *Candida albicans*, fungal generic*)
☐ Primate Respiratory Panel (includes human metapneumovirus, Influenza A, respiratory syncytial virus, *Streptococcus pneumoniae*)

*Unless requested otherwise, positive samples will be sequenced. Additional charges will be applied (see fee schedule).

To ensure prompt processing, please email zpplaboratory@vetmed.illinois.edu prior to shipping with tracking number and expected date of arrival. If you have any questions, please contact us via email or at 312-585-9050.

The submission sheet must be completely filled out and accompany each animal being submitted for testing. Please address sample shipments as follows: Zoological Pathology Program c/o Chicago Zoological Society 3300 Golf Rd., Brookfield, IL 60513

Submission Guidelines for Zoological Pathology Program Molecular Diagnostic Lab

Submissions should be shipped in a leak-proof primary receptacle, absorbent material, leak-proof secondary receptacle, and outer packaging. The submitting institution is responsible for following **all** USDOT and IATA shipping requirements. If you have any questions regarding sample submission to the ZPP molecular diagnostic lab, please contact us by email: zpplaboratory@vetmed.illinois.edu.

Body fluids: A minimum of 500 µl is required for testing. See the Systemic *Isospora* section below for appropriate sample submission for this specific test. Fresh samples should be shipped on ice; previously frozen samples (-80°C) should be shipped on dry ice. For whole blood, either heparin or EDTA samples are acceptable. Blood tubes should be packaged carefully to avoid breakage.

Cultures: Slants and plates can be shipped at room temperature. Ice packs can be employed if there is concern about agar melting in high ambient temperatures.

Feces: A minimum of 500 mg is required for testing. See the Systemic *Isospora* section below for appropriate sample submission for this specific test. Fresh samples should be shipped on ice; previously frozen samples (-80°C) should be shipped on dry ice.

Formalin-fixed paraffin-embedded (FFPE) tissue: A total of 45 microns of sample is required for testing. Blocks must be cut with a sterile unused blade. Samples can be shipped at room temperature in a cryogenic vial. Entire blocks can also be submitted, however, ZPP sectioning of blocks will add up to 72 hours to turnaround time and additional fees for processing and return mailing apply. PLEASE denote on submission form the estimated length of time tissue was in formalin (optimal time is ≤ 5 days). Consultation with ZPP pathologists regarding tissues with longer formalin fixation/storage times is welcome.

RNA-Stabilizing Solutions: Samples may be submitted for PCR tests in RNA preserving solutions such as RNAlater®; however, please note presence of preservative under “special instructions”.

Swabs: Place sterile swab in cryovial or red top tube and ship on ice. **DO NOT** submit swabs in culturettes as the transport media can inhibit PCR testing.

Tissues: A minimum of 50 mg is required for testing. Small biopsies or endoscopic brush samples subject to desiccation should be placed in a small quantity of sterile saline (just enough to keep the sample moist) in a sterile vessel (cryogenic vial or microfuge tube). All samples should be shipped on dry ice and in separate packages from any formalin fixed tissues.

Turnaround time: For routine cases, please note that samples may be batched and run weekly depending on the number of samples received. Samples received on Friday will be processed the following Monday. Typical turnaround is 5 business days from receipt. Turnaround time for RUSH/stat cases is 1-2 business days from receipt. Samples that contain bone, shell, or FFPE have prolonged DNA/RNA extraction protocols and will therefore have a longer processing time. Sequencing confirmation adds an additional 48-72 hours from preliminary results.

To optimize diagnostic results please consider contacting the laboratory if you have any questions on the appropriate samples to submit for testing or when planning diagnostic procedures.

For ANY sample, regardless of estimated delivery time, **PLEASE email (zpplaboratory@vetmed.illinois.edu) that a sample is in route and include your tracking number** to expedite timely acquisition of the sample upon arrival.

Fee Schedule per sample:

Individual bacterial, fungal or viral pathogen PCR test	\$50.00
Family (pan) viral PCR	\$60.00
<i>Brucella</i> strain typing	\$100.00
Cetacean and Primate respiratory panel	\$150.00
Systemic <i>Iso</i> spora fecal shedding screen (5 samples)	\$100.00
RUSH (STAT) Case Processing	\$50.00
Sequencing, per sample *required for all pan viral PCR	\$40.00
Sectioning and return mailing, 1 st block	\$10.00
Sectioning, additional block	\$2.00
Novel virus identification (consult with lab)	Price varies

Additional explanation of tests:

Generic bacterial and fungal PCR: PCR tests designed to detect genomic DNA from any bacterial or fungal organism. Sequencing for definitive organism identification will proceed immediately for positive samples **unless submitter specifically requests otherwise**.

Sequencing cannot distinguish between multiple organisms in the same sample, therefore, these tests are not ideal for samples where a mixed infection (e.g. ≥ 2 of the same class of organism) is suspected or for samples of tissues that are normally colonized by commensal bacteria (gut, blowhole, etc.). Please contact ZPP if you have additional questions.

The generic fungal PCR is the best test option if a **zygomycete** is suspected. Because there are a diverse number of zygomycete organisms, specific PCR tests are considered of lesser utility, and current reliance is on the generic PCR with sequencing.

Fungal Tests:

Aspergillus: The *Aspergillus* sp. test utilizes primers that will detected many species of *Aspergillus*. The test for *A. fumigatus* provides a more sensitive and specific test that will detect *A. fumigatus*, but not other species of *Aspergillus*.

Candida: Testing is offered for detection of *C. albicans* and *C. glabrata*. Each test utilizes primers and probes sensitive and specific to each individual species, but will not detect other species of *Candida*.

Bacterial Tests:

Brucella: This test utilizes primers that will detect many marine mammal and terrestrial *Brucella* species. This test uses broadly reactive screening primers as a first step to minimize the possibility of false negatives due to the diversity of marine mammal *Brucella* species. For an additional fee, positive tests can be followed by strain typing to more definitively classify organisms if desired by the submitter. Equivocal results will require sequencing for final confirmation and will proceed **unless submitter requests otherwise**.

Mycobacterium: This test utilizes screening primers that will identify DNA of *Mycobacterium* spp., but not similarly related bacteria. Some isolates of *M. celatum*, *M. heckeshornense* and *M. leprae* are not consistently amplified by this assay. There is no follow up sequencing with this test and exact species identification is not possible.

Mycoplasma: Test utilizes primers and probe that will identify members of the *M. hominis* group which includes most relevant animal (including marine mammal) pathogens, but will not amplify *M. mycoides* or members of the *M. pneumoniae* group.

Nocardia: This test utilizes screening primers that will identify samples as being positive for *Nocardia* sp., but not similarly related bacteria of the actinomycetales group. Positive samples can be further characterized through sequencing.

Staphylococcus aureus / MRSA: Testing can be requested for *Staphylococcus aureus* alone or in combination with testing for the *mec* gene that confers methicillin resistance. Any samples positive for the *mec* gene will be further evaluated to confirm that the resistance gene is associated with *S. aureus* (other bacteria can carry the methicillin resistance gene), and thus a MRSA.

Streptococcus sp. / *S. phocae*: Testing can be requested for *Streptococcus* genus or for *S. phocae* specifically. *Streptococcus* genus primers will detect *Streptococcus* sp. including but not limited to *S. pneumoniae*, *S. phocae*, *S. agalactiae*, *S. marimammalium*, *S. bovis*, and *S. canis* but will not differentiate among *Streptococcus* sp..

Protozoal Tests:

Systemic *Isospora* (*Atoxoplasma* spp.): This test utilizes primers that detect a wide variety of reported *Isospora* spp.. This test does have limited cross-reactivity with some *Eimeria* sp., therefore any positive fecal samples will be sequenced for confirmation at no extra charge. Positive blood samples will be presumed *Isospora*, as *Eimeria* sp. of birds are not known to infect blood cells. A single sample may be submitted for testing. **Please submit 50 µl of heparinized blood or 500 mg of feces.** For individual samples, a single individual test will be charged. **For evaluation of fecal shedding, it is recommended that 5 consecutive daily fecal samples (submitted as 5 separate and not pooled samples) be submitted.** These 5 samples will be run at a reduced charge (rather than 5 individual tests) with a single result for the submission.

Viral Tests:

Specific viral PCR tests: These PCR tests detect the specific virus indicated but may not detect other viruses in the same viral family.

Family (Pan) viral tests: These broad assays detect a wide range of viruses in the specific family indicated. Sequencing is required for specific identification.

Pan Coronavirus: Broad assay to detect a wide range of Coronaviruses. Positive samples will be sequenced for specific identification. For SARS-CoV-2 testing, contact laboratory for further submission instructions.

Pan Morbillivirus for Cetaceans: Tests for morbillivirus in cetaceans using universal primers and utilizes additional steps (hemi-nested PCR) to rule in/out dolphin morbillivirus. Positive samples are confirmed via sequencing.

Pan Morbillivirus for Pinnipeds: Tests for morbillivirus in pinnipeds and utilizes additional steps (nested PCR) to rule in/out Phocine Distemper and Canine Distemper viruses. Positive samples are confirmed via sequencing.

Panels:

Cetacean Respiratory Panel: Includes regular PCR testing for generic fungal and real-time PCR testing for *Aspergillus* sp., *Aspergillus fumigatus*, and *Candida albicans*. If the entire panel is not required, instead select from the individual regular and real time tests offered.

If desired, please contact the laboratory for additional specific information on the primers and techniques utilized for specific assays. References can be provided upon request.