

Institution: \_\_\_\_\_

Veterinarian/Primary contact: \_\_\_\_\_

Email for Billing: \_\_\_\_\_

Email for Results: \_\_\_\_\_

Phone #: \_\_\_\_\_

Common/Scientific Name: \_\_\_\_\_

Sample type: \_\_\_\_\_

Rush Stat case     Necropsy case     Free ranging animal     Human exposure concern

Animal ID: \_\_\_\_\_

Sample ID: \_\_\_\_\_

Collection Date: \_\_\_\_\_

Submission Date: \_\_\_\_\_

Storage Temp: \_\_\_\_\_

Transport Temp: \_\_\_\_\_

**Regular PCR tests:**

Bacterial Generic PCR

Fungal Generic PCR

**Real time PCR Tests**

*Aspergillus fumigatus*

*Mycobacterium sp.*

*Aspergillus sp.*

*Mycoplasma* (please see submission guidelines)

*Blastomyces dermatitidis*

*Nocardia sp.*

*Brucella sp.*

Sequencing for speciation if positive PCR

Strain typing

*Staphylococcus aureus*

*Candida albicans*

*S. aureus Methicillin-resistance gene*

*Candida glabrata*

*Streptococcus sp.*

*Coccidioides immitis*

*Streptococcus phocae*

*Cryptococcus neoformans*

Systemic *Isospora (Atoxoplasma)* qPCR (please see submission guidelines)

*Emydomyces testavorans*

Determination of fecal shedding (please see submission guidelines)

*Histoplasma capsulatum*

*Leptospira spp.*

**Other**

Canine Distemper Virus Reverse Transcriptase PCR

**Cetacean Respiratory Panel** (includes *Aspergillus sp.*, *Aspergillus fumigatus*, *Candida albicans*, fungal generic)

Conditions Suspected / Special Instructions (no sequencing, etc.):

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To ensure prompt processing, please email [zpplaboratory@vetmed.illinois.edu](mailto: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.

This 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 UIUC Zoological Pathology Program Molecular Diagnostic Lab

Submissions should include a leak-proof primary receptacle, absorbent material, leak-proof secondary receptacle, and outer packaging. Submitting institution is responsible for following **all** USDOT and IATA shipping requirements.

**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/or 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 and sections submitted in a cryogenic vial. Samples can be shipped at room temperature. Entire blocks can be also submitted: 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  $\leq 5$  days. Consultation with ZPP pathologists regarding tissues with longer formalin fixation/storage times is welcome.

**Swabs:** Place sterile swab in cryovial or red top tube and ship on ice. **DO NOT submit swabs in culettes 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 (sterile cryogenic vial or microfuge tube). All samples should be shipped on dry ice.

**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 from receipt will be 5 business days. RUSH stat cases turnaround will be 1-2 business days from receipt. Certain samples such as those that contain bone, shell or FFPE have prolonged DNA/RNA extraction protocols and may be delayed. If samples require sequencing confirmation, turnaround will be on average an additional 48-72 hours from preliminary results.

For ANY sample, regardless of estimated delivery time, **PLEASE inform the lab via email ([zplaboratory@vetmed.illinois.edu](mailto:zplaboratory@vetmed.illinois.edu)) that a sample is in route and include your tracking number** as this knowledge will expedite our timely acquisition of the sample upon arrival at our institution.

## Additional explanation of tests:

### Regular PCR tests:

**Generic bacterial and fungal PCR:** Standard PCR tests designed to detect genomic DNA from any bacterial or fungal organism.

The generic bacterial and fungal PCRs can be utilized with either culture isolates or biological samples. These are not ideal tests for samples where a mixed infection (e.g.  $\geq 2$  of the same class of organism) is suspected or the sample is tissue that normally is colonized by commensal bacteria (gut, blowhole, etc.) as sequencing cannot distinguish between multiple organisms in the same sample. Please contact the ZPP if you have additional questions.

Generic PCRs are designed to be utilized with sequencing for definitive organism identification. If generic PCR is negative, the test ends at that point (thus the separate charge for sequencing in the Fee Schedule); for positives, sequencing will proceed immediately **unless submitter specifically requests otherwise**.

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.

**Real time PCR:** Advantages of Real-time PCR (qPCR) include increased sensitivity, specificity, rapid resulting and quantitative analysis.

*Aspergillus*: The *Aspergillus* sp. test utilizes primers that will detect many species of *Aspergillus* sp. This can be selected individually. *A. fumigatus* provides a more sensitive and specific test that will detect *A. fumigatus* but not other species of *Aspergillus*.

*Brucella*: Test utilizes primers that will detect many marine mammal and terrestrial *Brucella* sp. Because of diversity among marine mammal *Brucella* sp and the possibility of as yet undiscovered species of medical importance, this test uses broadly reactive screening primers as a first step to minimize the possibility of false negatives. Positive tests can be followed by strain typing to more definitively ID organisms.

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

*Canine distemper virus Reverse Transcriptase PCR*: Designed to detect CDV RNA in fresh, frozen, RNA-stabilizing preserved, as well as formalin-fixed paraffin-embedded tissue.

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

*Mycobacterium*: 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. If species identification is needed, check with the laboratory on a case by case basis to see if additional testing/sequencing can be pursued.

*Mycoplasma*: Test utilizes primers & 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*: Similar to the Brucella test, 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*: Test 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*: Test 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.*.

*Systemic Isospora (Atoxoplasma spp)*: Test utilizes primers that detect a wide variety of reported *Isospora spp.* The 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 *Atoxoplasma* as *Eimeria sp.* of birds are not known to infect blood cells. A single sample may be submitted to 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 and these 5 samples will be run at a reduced charge (rather than 5 individual tests) with a single result for the submission.

**Fee Schedule per sample:**

Individual test (regular or qPCR)	\$50.00
RUSH stat case (additional per sample)	\$50.00
Brucella strain typing	\$100.00
Cetacean respiratory panel	\$150.00
Canine Distemper Reverse Transcriptase PCR	\$50.00
Systemic Isospora fecal shedding screen (5 samples)	\$100.00
Sequencing, per sample	\$40.00
Sectioning and return mailing, 1 <sup>st</sup> block	\$10.00
Sectioning, additional block	\$2.00

If you have any questions regarding sample submission or the ZPP molecular diagnostic lab, please contact us by email: [zpplaboratory@vetmed.illinois.edu](mailto:zpplaboratory@vetmed.illinois.edu).