

Institution:
Address:

Veterinarian/contact:
Email:
Phone #:
Fax #:

Common/Scientific Name: _____

Sample type: _____

Sample ID: _____

Collection Date: _____ **Submission Date:** _____

Storage Temp: _____ **Transport Temp:** _____

Suspected Pathogen:

- Bacterial
- Fungal
- Unknown
- None suspected, routine sample/screening

Test Requested:

Regular PCR tests

- Bacterial Generic PCR (Cx isolates or biological samples)
- Fungal Generic PCR (Cx isolates or biological samples)

Bacterial qPCR Tests

- Staphylococcus aureus
 - S. aureus Methicillin-resistance gene
- Streptococcus sp.
- Streptococcus phocae
- Brucella sp.
 - Sequencing for speciation if positive PCR
- Nocardia sp.
 - Sequencing for speciation if positive PCR
- Leptospira spp.
- Mycobacterium sp.
- Mycoplasma (please see submission guidelines)

Fungal qPCR Tests (for "panel" please also check specific tests)

- Aspergillus sp.
- Aspergillus fumigatus
- Blastomyces dermatitidis
- Candida albicans
- Candida glabrata
- Coccidioides immitis
- Cryptococcus neoformans
- Histoplasma capsulatum
- Fungal panel

Other

- Canine Distemper Virus Reverse Transcriptase PCR
- Atoxoplasma sp. qPCR

Special Instructions (no sequencing, etc.): _____

To ensure prompt processing please email zpplaboratory@vetmed.illinois.edu prior to shipping with tracking number and expected date of arrival.

This sheet must be completely filled out and accompany samples being submitted for testing. Please address sample shipments as follows:

**Zoological Pathology Program
c/o Chicago Zoological Society
3300 Golf Rd.
Brookfield, IL 60513
312-585-9050**

Submission Guidelines for UIUC Zoological Pathology Program Molecular Diagnostic Lab

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 *Atoxoplasma* 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.

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). Fresh samples should be shipped on ice; previously frozen samples (-80°C) should be shipped on dry ice.

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.

Swabs: Place sterile swab in cryovial or red top tube and ship on ice. Do not submit swabs in culettes as the media can impact testing.

Formalin-fixed paraffin-embedded 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 ≤ 5 days. Consultation with ZPP pathologists regarding tissues with longer formalin fixation/storage times is welcome.

Brief explanation of available tests and turnaround time:

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

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 sequencing to more definitively ID organisms.

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.

Leptospira: Test uses primers that will detect all pathogenic serovars of *Leptospira sp.* including *but not limited to* *Leptospira kirschneri grippotyphosa* and *Leptospira interrogans pomona, canicola, bratislava, hardjo,* and *icterohaemorrhagiae*. There is no follow up sequencing with this test.

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.*

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.

Fungal qPCR: Tests for specific fungi:

Candida albicans
Aspergillus fumigatus
Aspergillus sp.

Blastomyces dermatitidis
Candida glabrata
Coccidioides immitis

Cryptococcus neoformans
Histoplasma capsulatum

Tests can be ordered individually or as a "panel" of any combination of the 8 tests. If the panel is requested, please specify which fungi are of concern. If the panel is ordered and the specific fungal qPCR are negative, then a generic fungal PCR will also be run and, if positive, PCR products sequenced for identification. Ordering 2 or more individual fungal qPCR tests will automatically be considered as an order for the fungal panel for optimal fee discount although only selected fungi will be tested. Utilizing the specific organism tests versus relying solely on the generic primers affords rapid resulting since sequencing adds days to the turnaround time for a final result and the qPCR assays are more sensitive. If one or more of the specific organisms are suspected, ordering specific test(s) is strongly encouraged. Ordering the panel (specific tests in combination with the generic fungal) can rapidly rule in/out suspect organisms while maintaining surveillance for any fungal organism.

The generic fungal real time 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.

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. ≥ 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**.

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.

Atoxoplasma: Test utilizes primers that detect a wide variety of reported *Atoxoplasma sp.* 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. **Please submit 50 μ l of heparinized blood or 500 mg of feces.**

Turnaround time: For uncomplicated cases, 48 hours for PCR/qPCR, and 48-72 hours for post-PCR sequencing. Please note that sequencing may be batched and run twice weekly dependent on number of samples received. Samples received on Friday will be processed the following Monday.

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

Fee Schedule:

Generic bacterial PCR	\$75.00
Generic fungal PCR	\$75.00
Bacterial qPCR (<i>Nocardia</i> , <i>Leptospira</i> , Methicillin resistance, etc.)	\$75.00
Individual Fungal qPCR	\$75.00
2 or more Individual Fungal qPCR (Fungal Panel) [†]	\$145.00
Canine Distemper Reverse Transcriptase PCR	\$75.00
Atoxoplasma qPCR	\$75.00
Sequencing, per sample	\$40.00
Sectioning and return mailing, 1 st block	\$10.00
Sectioning, additional block	\$2.00

[†]For ≥ 2 individual fungal PCRs fee is \$145, **if ordered simultaneously**

If you have any questions regarding sample submission or the ZPP molecular diagnostic lab, please contact us by email:
zplaboratory@vetmed.illinois.edu.