

Discover better insight

# Health Monitoring for your zebrafish colonies



The clear choice for a comprehensive  
health monitoring program.





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# Introduction

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A growing number of researchers are choosing zebrafish as models for biomedical research because of the advantages zebrafish offer over other animal models for certain studies. First, their small size and ease of breeding make zebrafish relatively inexpensive to maintain, which allows researchers to perform experiments using zebrafish that would be cost prohibitive using larger animal models. Secondly, embryos are transparent, which allows easy visualization of cell and organ development and permits experimental manipulations involving DNA or mRNA injection, cell labeling and transplantation.

Zebrafish are now commonly employed as models in a diverse range of bio research fields, such as immunology, infectious disease, cardiac and vascular disease research, chemical and drug toxicity studies, reproductive biology and cancer research to name a few. As with other vertebrate models used in research, undetected infections can alter, confound or invalidate experimental results. Therefore, it is important to develop and utilize a health monitoring program to detect infectious agents that may affect the animal and the research outcomes.

IDEXX BioResearch has developed sensitive molecular diagnostic assays to improve health monitoring for zebrafish colonies. These newly available molecular panels, along with histopathology and microbiology, provide laboratory animal veterinarians, facility managers and investigators with the tools to design and optimize health monitoring programs.

To discuss implementing a zebrafish health monitoring program, please contact us at **1-800-544-5205, option 1.**

# *Edwardsiella ictaluri*

**Classification** Bacteria

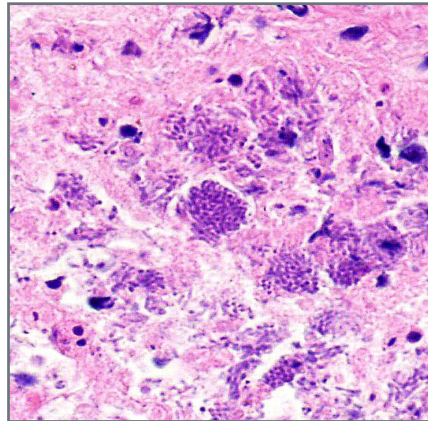
**Common name** Enteric septicemia of catfish (ESC)

**Prevalence and pathogenicity** Economically important as a catfish pathogen, *Edwardsiella ictaluri* is an uncommon but serious primary pathogen of zebrafish colonies, causing high-mortality epizootics.

**Impact on research** *E. ictaluri* causes severe disease characterized by behavioral changes, loss of appetite and acute mortality. Histologically, zebrafish display severe acute tissue necrosis with large numbers of intracellular and extracellular bacteria.



Naturally infected zebrafish displaying severe cranial hemorrhage.



H&E-stained section of the same *E. ictaluri*-infected zebrafish exhibiting severe acute necrosis with macrophages containing numerous intracellular bacterial rods.

# Flavobacterium columnare

**Classification** Bacteria

**Common name** Tail rot, columnaris disease

**Prevalence and pathogenicity** *Flavobacterium columnare* is highly prevalent in both the aquarium trade and commercial aquaculture, and it can cause clinical or subclinical infections in zebrafish colonies. Pathogenicity depends on multiple factors, including stress, temperature and bacterial strain.

**Impact on research** Columnaris disease is highly contagious and causes increased mortality, severe gill disease, frayed fins and necrosis of the epidermis and underlying tissues.



Gross photo of a zebrafish exhibiting severe disease from natural infection with *Flavobacterium columnare*. Notice frayed fins and the dull, whitish appearance of the tail from epidermal necrosis.

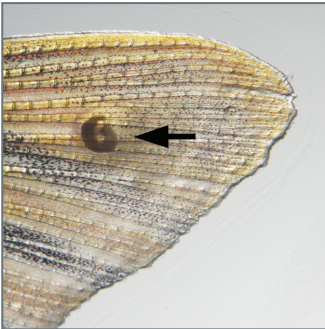
# *Ichthyophthirius multifiliis*

**Classification** Ciliate

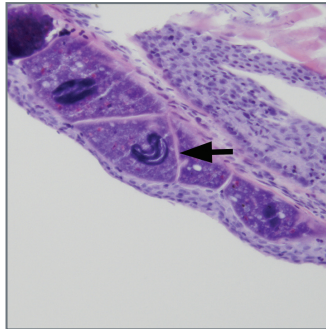
**Common name** White spot or ich

**Prevalence and pathogenicity** Extremely prevalent in the aquarium trade, *Ichthyophthirius multifiliis* infects the epithelium of skin and gills, causing increased mucus production and labored breathing.

**Impact on research** *I. multifiliis* causes increased mortality and clinical disease characterized by respiratory difficulty, epithelial hyperplasia and mucus production. Moreover, *I. multifiliis* may act as a vector to transmit bacteria to fish, alter the immune response and enhance the severity of bacterial infections.



Sub-gross image of an *Ichthyophthirius multifiliis* trophont with horseshoe-shaped macronucleus in the epidermis of a zebrafish.



H&E-stained section showing multiple trophonts embedded beneath the epidermis of an infected zebrafish.



*Ichthyophthirius multifiliis*-infected zebrafish showing variably-sized, opaque, white, encysted trophonts in the skin.



# Infectious Spleen and Kidney Necrosis Virus (ISKNV)

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**Classification**

Virus

**Prevalence and pathogenicity**

Infectious spleen and kidney necrosis virus (ISKNV) infects an extremely diverse range of fish hosts. Zebrafish have been shown to be experimentally susceptible, exhibiting increased mortality, the presence of enlarged cells (megaloocytes) and necrosis in the spleen and kidney, scale protrusion and widespread petechial hemorrhage.

**Impact on research**

ISKNV alters host gene expression, suppresses interferon beta (IFN- $\beta$ ) and causes apoptosis.

# *Mycobacterium abscessus*

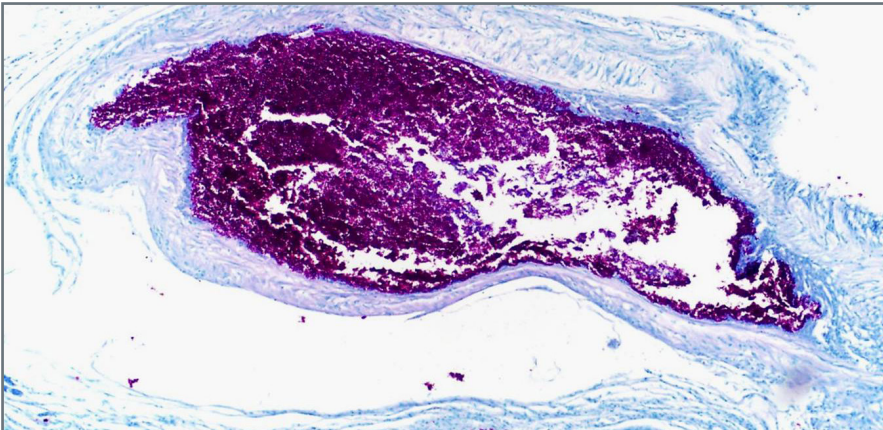
**Classification** Bacteria

**Common name** Fish TB

**Prevalence and pathogenicity** *Mycobacterium abscessus*, a close relative of *Mycobacterium chelonae*, is moderately pathogenic to zebrafish and has been identified in zebrafish colonies from multiple institutions.

**Zoonotic** Potentially zoonotic, *M. abscessus* can cause pulmonary and cutaneous disease primarily in immunocompromised individuals. Unlike some other mycobacteria, *M. abscessus* can grow at human core body temperature.

**Impact on research** *M. abscessus* causes chronic granuloma formation in multiple tissues.



Ziehl-Neelsen-stained section displaying severe aerocystitis with numerous acid-fast *Mycobacterium abscessus* bacilli.

# *Mycobacterium chelonae*

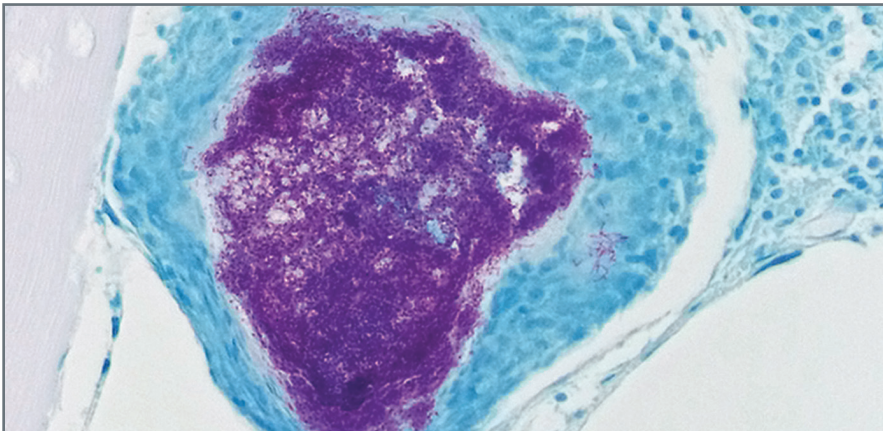
**Classification** Bacteria

**Common name** Fish TB

**Prevalence and pathogenicity** *Mycobacterium chelonae* infections in zebrafish are extremely common and typically associated with chronic inflammatory lesions and granuloma formation in many tissues.

**Zoonotic** Potentially zoonotic, causing cutaneous and soft tissue infections primarily in immunocompromised individuals. Mycobacterial infections require prolonged antibiotic therapy.

**Impact on research** Most infections in zebrafish are chronic and subclinical, characterized by aerocystitis and/or granuloma formation in a variety of tissues. Chronic inflammation is problematic for almost any research area.



Ziehl–Neelsen-stained section showing many acid-fast *Mycobacterium chelonae* bacteria in a zebrafish granuloma.

# *Mycobacterium fortuitum*

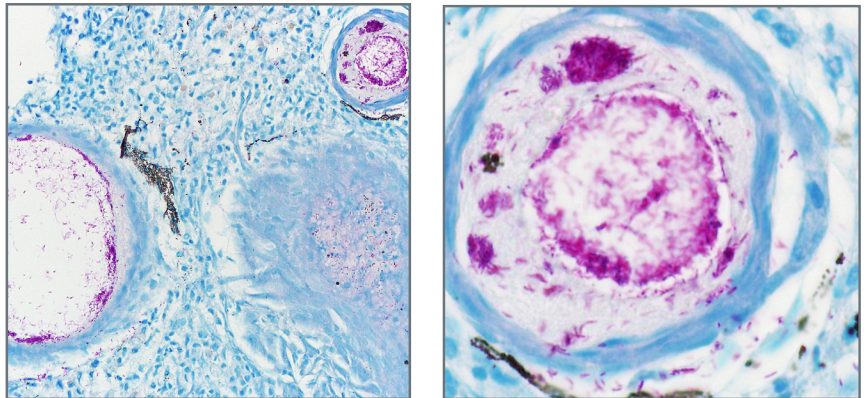
**Classification** Bacteria

**Common name** Fish TB

**Prevalence and pathogenicity** *M. fortuitum* is a common pathogen of many fish species; and has been identified in zebrafish colonies from multiple institutions, but is more frequently identified in environmental samples than in sentinel zebrafish. Infections may be subclinical, but are also associated with increased colony mortality, decreased reproductive performance and clinical signs.

**Zoonotic** Potentially zoonotic, *M. fortuitum* is primarily a risk to immunocompromised individuals.

**Impact on research** *M. fortuitum* has been associated with poor fecundity, increased mortality, hepatic necrosis, dropsy and emaciation in zebrafish.



Ziehl–Neelsen-stained section displaying acid-fast mycobacteria in well- and poorly-demarcated granulomas in a naturally *M. fortuitum*-infected zebrafish.

# *Mycobacterium haemophilum*

**Classification** Bacteria

**Common name** Fish TB

**Prevalence and pathogenicity** *Mycobacterium haemophilum* infections are typically associated with chronic progressive disease and ongoing mortality in zebrafish colonies.

**Zoonotic** Potentially zoonotic, *M. haemophilum* is primarily a risk for immunocompromised individuals. *M. haemophilum* can cause skin and joint infections in immunocompromised hosts.

**Impact on research** *M. haemophilum* causes increased colony mortality and clinical disease. Clinically infected fish may exhibit dropsy, lethargy or anorexia resulting in severe emaciation. Histologically, granulomas may be evident in multiple tissues, but diffuse systemic disease can also occur without the formation of prominent granulomas.



A zebrafish naturally-infected with *Mycobacterium haemophilum* displaying dropsy (diffuse edema and scale protrusion).

# *Mycobacterium marinum*

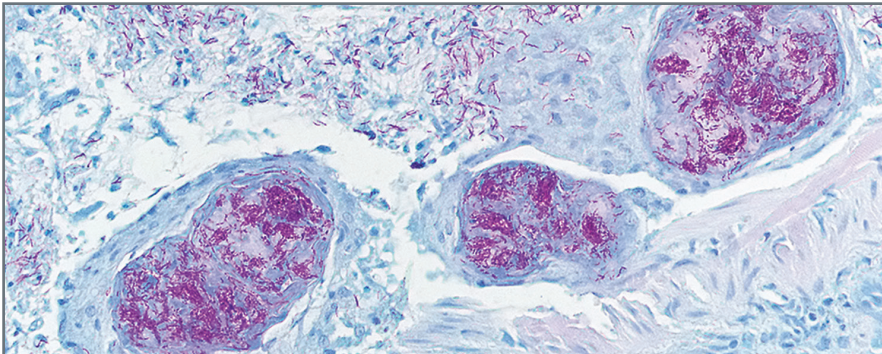
**Classification** Bacteria

**Common name** Fish TB

**Prevalence and pathogenicity** *Mycobacterium marinum* infections in zebrafish are associated with ulcers, dropsy and acute mortality. Pathogenicity of this species varies by strain.

**Zoonotic** *M. marinum* is well-characterized as a zoonotic pathogen and infects immunocompetent as well as immunocompromised individuals. The human disease, known as aquarium or fish handlers' granuloma, typically presents as a nonhealing hand lesion that may exhibit sporotrichoid spread. It may cause granulomas or deep tissue infections of tendon and bone. Because of poor growth at 37°C, it is seldom systemic. *Mycobacterium* infections are difficult to treat and require prolonged antibiotic therapy.

**Impact on research** *M. marinum* causes increased mortality, reduced fecundity and systemic clinical disease, including the development of ulcers, granulomas and organ failure, in addition to altered cytokine levels and gene transcription.



Ziehl–Neelsen-stained section showing multifocal to coalescing zebrafish granulomas containing many acid-fast *Mycobacterium marinum* bacteria.



# *Mycobacterium peregrinum*

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**Classification**      Bacteria

**Common name**      Fish TB

**Prevalence and pathogenicity**      *M. peregrinum* infections are less common than for other species of *Mycobacterium* that are associated with disease in zebrafish; however, pathogenicity in zebrafish colonies can be high.

**Zoonotic**      Potentially zoonotic, *M. peregrinum* is a risk primarily to immunocompromised individuals.

**Impact on research**      Most infections in zebrafish are likely to be chronic and subclinical although high pathogenicity has also been reported. Both clinical and subclinical infections can confound research.

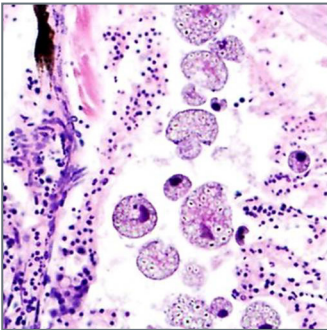
# *Piscinoodinium pillulare*

**Classification** Dinoflagellate

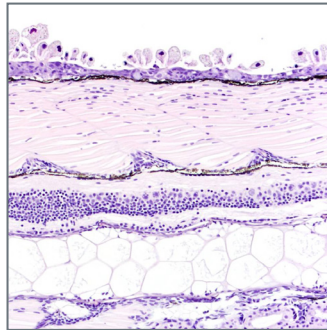
**Common name** Velvet or gold dust disease

**Prevalence and pathogenicity** *Piscinoodinium pillulare* is uncommon in research colonies and is usually detected in quarantine. This agent can be very pathogenic when zebrafish are crowded or stressed.

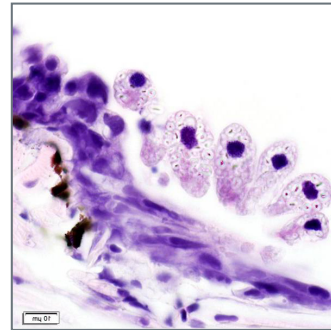
**Impact on research** This ectoparasite infects the gills and epidermis, causing mortality, respiratory difficulty, inflammation and an increased susceptibility to bacterial infection.



H&E-stained section showing the gills of an adult zebrafish that was heavily-infected with *Piscinoodinium pillulare*.



H&E-stained section showing the dorsum of a naturally-infected larval zebrafish, including the hyperplastic epidermis with numerous *Piscinoodinium pillulare* trophonts.



*Detail of pear-shaped *Piscinoodinium pillulare* trophonts attached to zebrafish epidermis with characteristic refractile cytoplasmic starch granules.*



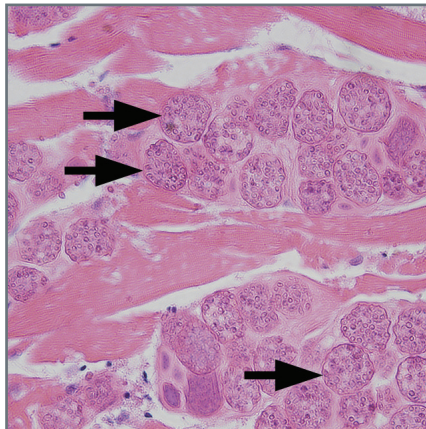
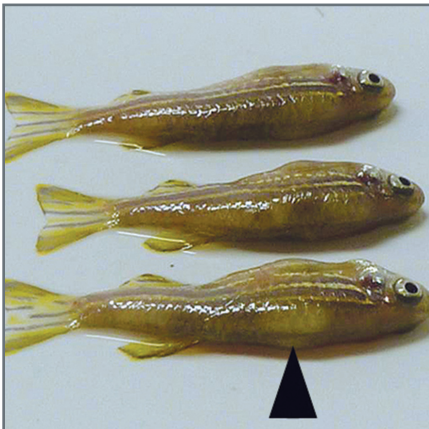
# *Pleistophora hyphessobryconis*

**Classification** Microsporidia

**Common name** Neon tetra disease

**Prevalence and pathogenicity** This parasite of skeletal muscle is extremely common in the aquarium trade and is occasionally present in research colonies. Pathogenicity is greater in irradiated or immunocompromised zebrafish lines.

**Impact on research** *Pleistophora hyphessobryconis* causes clinical or subclinical disease characterized by necrosis of skeletal muscle and accompanying inflammation.



Far left: Gross image of *Pleistophora hyphessobryconis*-induced lesions (arrows) in a naturally infected laboratory zebrafish.

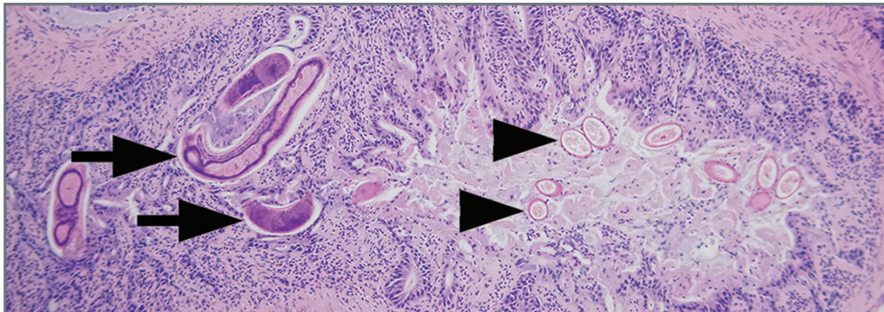
Left: H&E-stained section showing numerous *P. hyphessobryconis* sporophorocysts (arrows) in zebrafish skeletal muscle.

# *Pseudocapillaria tomentosa*

**Classification** Nematode

**Prevalence and pathogenicity** *Pseudocapillaria tomentosa* is a common parasite in zebrafish colonies and is associated with anorexia and chronic emaciation.

**Impact on research** Experimental outcomes may be impacted by chronic disease, including chronic inflammation, anorexia, emaciation and poor nutritional absorption, causing electrolyte imbalance or failure to thrive. One confounded carcinogenicity study suggests that *P. tomentosa* infections can promote intestinal neoplasia.



Top: Subgross image of an adult *Pseudocapillaria tomentosa* female showing numerous eggs in the uterus.

Bottom: H&E-stained section showing *P. tomentosa* infection of the zebra-fish intestine, including eggs in the intestinal lumen.

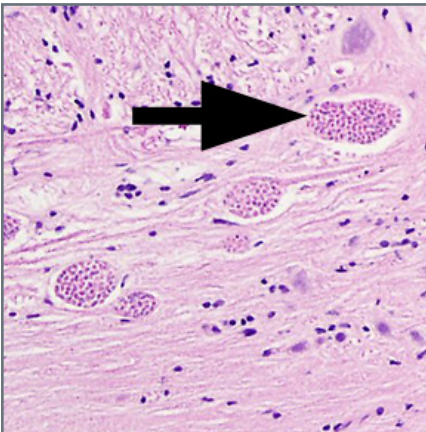
# *Pseudoloma neurophilia*

**Classification**      Microsporidia

**Common name**      Formerly known as “skinny disease”

**Prevalence and pathogenicity**      *Pseudoloma neurophilia* is the most prevalent pathogen in zebrafish colonies. Most infections are chronic and subclinical.

**Impact on research**      *Pseudoloma neurophilia* infections are associated with poor fecundity, reduced growth, emaciation and musculoskeletal deformities in zebrafish. *P. neurophilia* infections in the central nervous system are associated with scattered neuronal necrosis that is sometimes accompanied by encephalitis or myelitis, and are thus likely to confound some behavioral experiments. The central nervous system is most affected, although infections of skeletal muscles, ovary, vertebral bodies, and other tissues are not uncommon. *Pseudoloma* spores are resistant to bleach and can be vertically transmitted by intraovum transmission.



H&E-stained section showing numerous *Pseudoloma neurophilia* spores in the ovary of a naturally-infected zebrafish.

# Profiles and pricing

## ■ Zebrafish PCR Profiles

	<i>Mycobacterium</i> Profile	Essential Profile	Comprehensive Profile
Profile Contents	\$99.75	\$224.00	\$329.60
<i>Mycobacterium abscessus</i>	•	•	•
<i>Mycobacterium chelonae</i>	•	•	•
<i>Mycobacterium fortuitum</i>	•	•	•
<i>Mycobacterium haemophilum</i>	•	•	•
<i>Mycobacterium marinum</i>	•	•	•
<i>Mycobacterium peregrinum</i>	•	•	•
<i>Edwardsiella ictaluri</i>		•	•
<i>Pseudocapillaria tomentosa</i>		•	•
<i>Pseudoloma neurophilia</i>		•	•
<i>Flavobacterium columnare</i>			•
<i>Ichthyophthirius multifiliis</i>			•
ISKNV (Infectious spleen and kidney necrosis virus)			•
<i>Piscinoodinium pillulare</i>			•
<i>Pleistophora hypessobryconis</i>			•

Pooling Fee \$10.00 (up to 5 fish can be pooled.)

	Add to fee
<b>Histopathology (Add-on to profile)</b>	
H&E slide preparation (per fish)	\$17.30
Shipping of histologic slides and paraffin block to client (per case)	39.00
<b>Pathology (Add-on to profile)</b>	
Pathology service	65.75

## ■ Histopathology and Pathology

### Histologic Slide Preparation Service

\$22.75

For clients who read their own slides.

Includes H&E and acid-fast stains, no charge for necropsy.

🔴 Formalin-fixed or frozen fish

### Pathology Service

76.00

For clients who want a pathologist's evaluation and report. Includes necropsy and H&E and acid-fast stains.

🔴 Formalin-fixed or frozen fish

## ■ Micro Services

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Individual Micro Tests

**23.25**

## ■ Individual Zebrafish PCR Tests

<i>Edwardsiella ictaluri</i>	<b>\$66.95</b>
<i>Flavobacterium columnare</i>	<b>66.95</b>
<i>Ichthyophthirius multifiliis</i>	<b>66.95</b>
ISKNV (Infectious spleen and kidney necrosis virus)	<b>66.95</b>
<i>Mycobacterium abscessus</i>	<b>66.95</b>
<i>Mycobacterium chelonae</i>	<b>66.95</b>
<i>Mycobacterium fortuitum</i>	<b>66.95</b>
<i>Mycobacterium haemophilum</i>	<b>66.95</b>
<i>Mycobacterium marinum</i>	<b>66.95</b>
<i>Mycobacterium peregrinum</i>	<b>66.95</b>
<i>Piscinoodinium pillulare</i>	<b>66.95</b>
<i>Pleistophora hypohessobryconis</i>	<b>66.95</b>
<i>Pseudocapillaria tomentosa</i>	<b>66.95</b>
<i>Pseudoloma neurophilia</i>	<b>66.95</b>

# Sample preparation and shipping

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**For submission of zebrafish for histopathology only:** Please submit fish fixed in 10% buffered formalin. To ensure proper fixation, open up the coelom (abdomen). This can be accomplished by making a lengthwise incision through the body wall or by removing a small piece of the body wall. Samples may be shipped at ambient temperatures by overnight courier.

**For submission of zebrafish for histopathology and PCR:** Please submit fish that have been frozen in liquid nitrogen or at -80°C. Samples should be shipped on dry ice by overnight courier.

**For submission of zebrafish for PCR only:** Please submit frozen fish with sufficient ice packs to ensure that fish remain frozen during transit. Dry ice is not required. You can pool up to 5 fish in the same tube to be tested as one sample. We do not recommend pooling fish of different health statuses or across different systems, like quarantine and a main system, for example.

**For submission of zebrafish embryos for PCR:** Please euthanize embryos and submit frozen on ice or cold packs. Dry ice is not required.

**For submission of live feed cultures for PCR:** Please submit at least 1 mL of live feed cultures (e.g., rotifer or Paramecium) frozen on ice or cold packs. Dry ice is not required.

You can pool up to 5 fish in the same tube to be tested as one sample. We do not recommend pooling fish of different health statuses or across different systems, like quarantine and main system, for example.

**Ship samples to:**  
IDEXX BioResearch  
4011 Discovery Drive  
Columbia, MO 65201

# Additional resources

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**Contact IDEXX BioResearch for additional resources including the following:**

- Zebrafish health monitoring webinars
- Approaches to setting up a periodic zebrafish health monitoring program
- FAQ's about Zebrafish health monitoring

To learn more, call **1-800-544-5205, option 1** or go to **[idexbioresearch.com](http://idexbioresearch.com)**

