Health Monitoring of Laboratory Zebrafish

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What is health monitoring?

Program to detect infectious agents capable of confounding research and endangering personnel, and limit their spread

Composed of:

- Quarantine
- Disease surveillance
- Disease investigation

Why perform health monitoring?

ZEBRAFISH
Volume 13, Supplement 1, 2016
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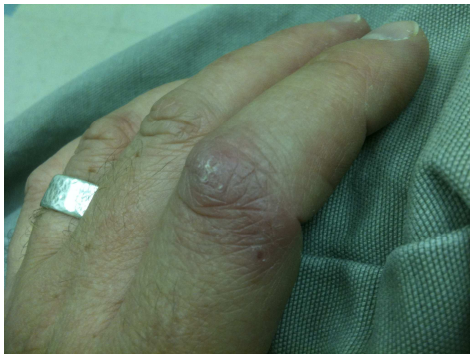
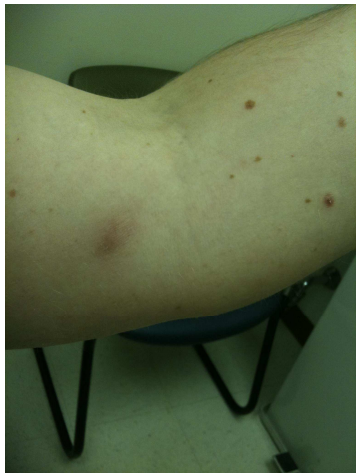
Strategies to Mitigate a *Mycobacterium marinum* Outbreak in a Zebrafish Research Facility

Timothy Mason,[1] Kathy Snell,[1] Erika Mittge,[2] Ellie Melancon,[3] Rebecca Montgomery,[1] Marcie McFadden,[1] Javier Camoriano,[1] Michael L. Kent,[4] Christopher M. Whipps,[5] and Judy Peirce[3]





Photos courtesy of Erik Sanders

ZEBRAFISH
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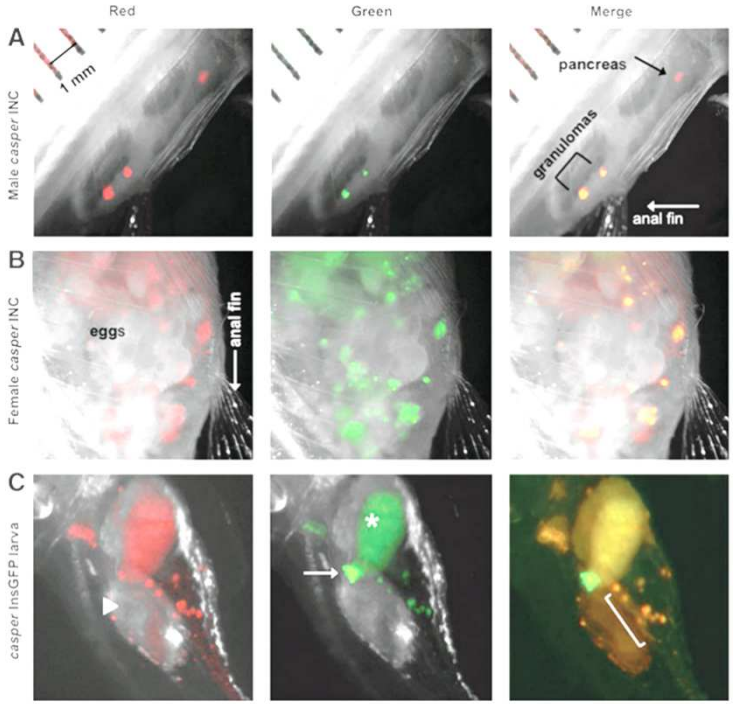
DOI: 10.1089/zeb.2012.0863

Detection of Autofluorescent *Mycobacterium Chelonae* in Living Zebrafish

Christopher M. Whipps,[1] Larry G. Moss,[2] Dana M. Sisk,[3] Katrina N. Murray,[4] David M. Tobin,[3] and Jennifer B. Moss[2]

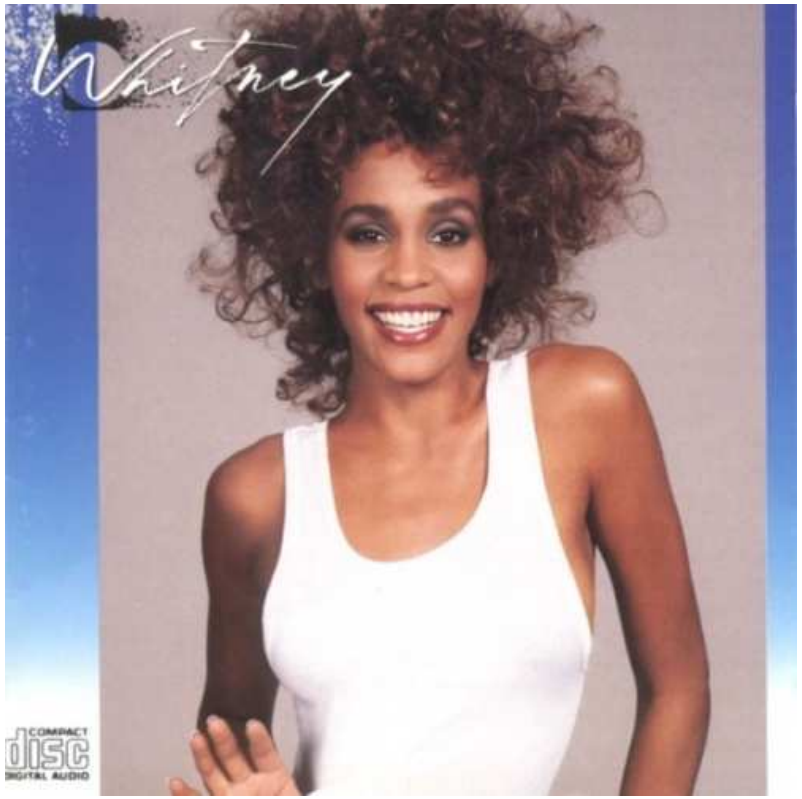


Before you build your health monitoring program…

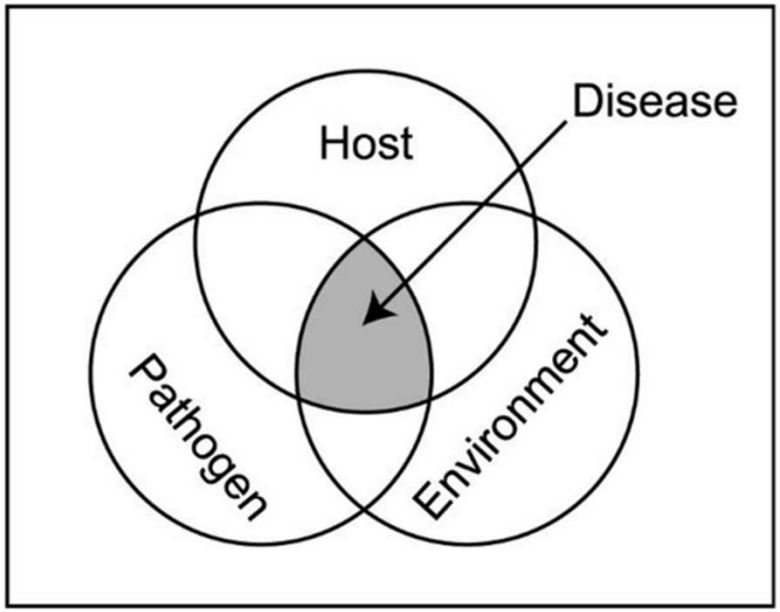
Staff

You have nothing, nothing… without trained staff

Invest in their training!



Great husbandry = optimal fish health



Disease rarely results from simple contact between the fish and a potential pathogen. Environmental problems, such as poor water quality, or other stressors often contribute to the outbreak of disease.

Consistent, appropriate water quality

Regular cleaning and disinfection

Life support system maintenance

Health Monitoring

Health Monitoring Program

What diseases should you test for? → What samples should you collect? → What tests will you run on the collected samples? → How often will you test your colony? → How will you disseminate the results of testing?

Infectious agents

Determine which agents are of concern to your program

- Are you working with immunodeficient fish?
- How often will you import fish?
- What kind of research are you supporting?
- What is your water source?
- Do you have 1 life support system or many within your rooms?
- Are other species housed in the room/on the same systems?
- What agents are *currently* present on your LSS?

Decide what diseases you want to exclude

Zoonotic agents

Devastating pathogens (e.g., *Edwarsiella ictaluri*)

Agents that may impact research

Published in final edited form as:
J Fish Dis. 2017 March ; 40(3): 443–446. doi:10.1111/jfd.12512.

The common neural parasite *Pseudoloma neurophilia* causes altered shoaling behavior in adult laboratory zebrafish (*Danio rerio*) and its implications for neurobehavioral research

Sean Spagnoli[1], **Justin Sanders**[2], and **Michael L. Kent**[3]

Test samples

Testing Samples

Fish

- Imported animals
- Colony animals
- Sentinel fish (pre- and post-filtration)
- Sump fish
- Sick fish

Environmental

- Water and LSS surfaces
- Feces
- Embryos

Sources of pathogens

Food

Shared equipment

Sentinel Fish

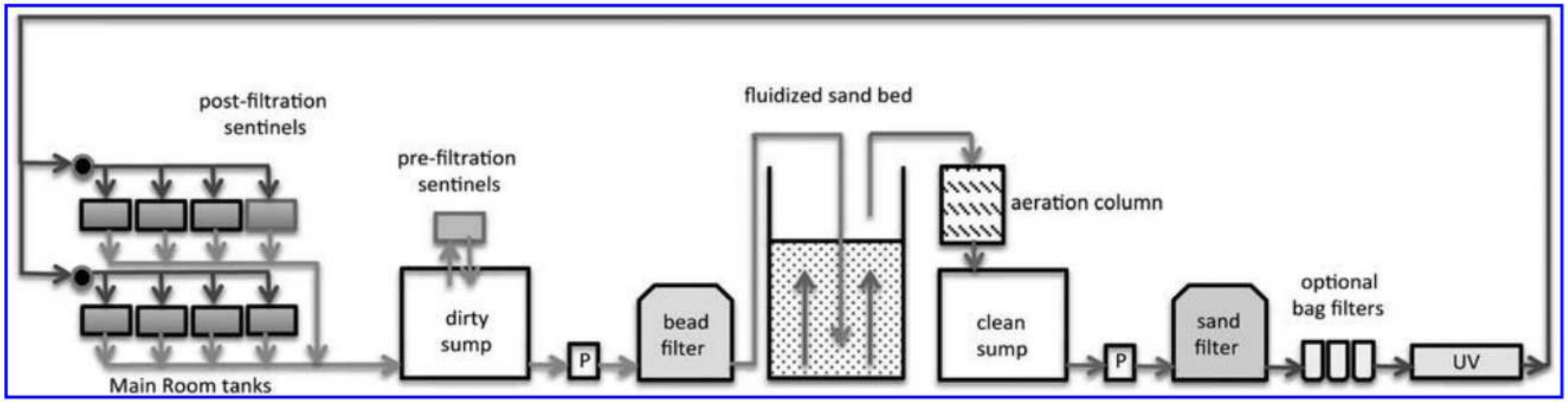
Pre-filtration fish provide information on disease status of fish on system

Post-filtration fish provide information on efficacy of UV irradiation, water treatment

Remain on system for at least 3 months

Should be raised on system

MURRAY 2016



Other Fish

Imported animals

- After spawning
- Embryos

Colony animals

- Retired breeders

Sump fish

- Should be removed regularly

Sick fish

- For disease investigation

Environmental Samples

Sump swabs**

PCR to detect *Mycobacterium* spp.

Sludge/detritus samples

Light microscopy to detect *P. tomentosa*

Not reliable for *P. neurophilia*

TABLE 2. PCR IDENTIFICATION OF *MYCOBACTERIUM* SPP. IN FISH AND IN SUMP SWABS IN 2015 AT MH

83 fish tested			*14 sump swabs*	
Positive	*%*	*Mycobacterium* spp.	*%*	*Positive*
6	7	*M. chelonae*	57	8
3	4	*M. haemophilum*	29	4
0	0	*M. fortuitum*	71	10
0	0	*M. peregrinum*	7	1
0	0	*M. abscessus*	0	0
0	0	*M. marinum*	0	0

Percentage is obtained by dividing the number of positive results for each species by the number of tested samples. Note that this does not include the samples taken to confirm *M. haemophilum* infection in fish during the system 2 outbreak.
PCR, polymerase chain reaction.

TABLE 4. DETECTION OF *PSEUDOCAPILLARIA TOMENTOSA* IN AQUARIUM A

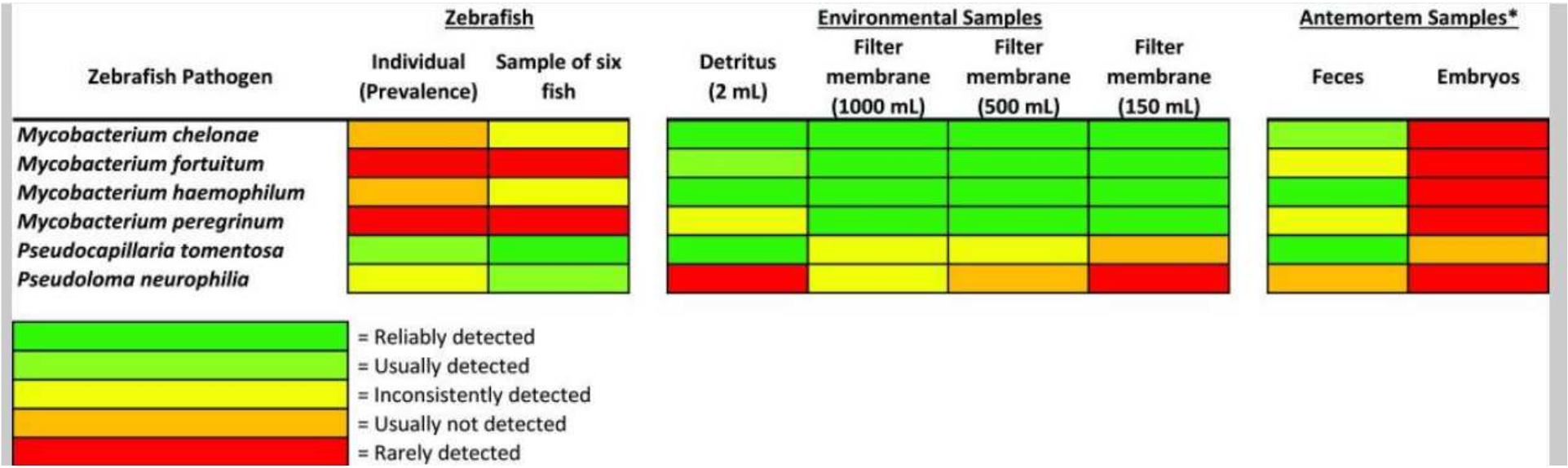
Fish tested by PCR			*Tank sludge analyses*		
Number	*Positive*	*%*	*Number*	*Positive*	*%*
11	3	27	14	13	93

Percentage is obtained from number of positive results divided by number of tests.

Environmental samples

Filtered water

Samples of 150-1000 mL vacuumed through a 0.2 micron filter



CRIM 2017

Other Samples

Food

Mycobacterium spp. can be cultured from live feed

Shared Equipment

Nets, breeding tanks, etc.

Mycobacteria	Dose per fish per day	Days fed	Duration (wk post-exposure)	No. of fish examined	Total fish infected	Intestinal acid-fast bacilli
M. marinum (OSU 214)						
Paramecia	3.4×10^5	14	8	45	21 (47)	12 (34)
High dose	6.1×10^5	14	8	56	0 (0)	20 (41)
Low dose	3.6×10^4	14	8	42	2 (5)	18 (47)
Control	0	14	8	60	0 (0)	31 (51)
M. marinum (CH)						
Paramecia	3.6×10^5	14	8	19	9 (47)	8 (42)
High dose	4.6×10^7	14	8	22	0 (0)	14 (64)
Low dose	3.8×10^6	14	8	21	0 (0)	10 (48)
Control	0	14	8	20	0 (0)	18 (90)
M. chelonae (H1/E2) 8 wk						
Paramecia	3.4×10^5	14	8	13	5 (38)	12 (92)
High dose	8.3×10^7	14	8	14	0 (0)	11 (78)
Low dose	3.5×10^6	14	8	16	0 (0)	11 (69)
Control	0	14	8	11	0 (0)	5 (45)
M. chelonae (H1/E2) 16 wk						
Paramecia	3.4×10^5	14	16	14	3 (21)	11 (79)
High dose	8.3×10^7	14	16	16	0 (0)	12 (75)
Low dose	3.5×10^6	14	16	15	0 (0)	6 (40)
Control	0	14	16	12	0 (0)	9 (75)

PETERSON 2013

Testing Methodologies

Testing Methodologies

Gross necropsy

Histology

Bacterial culture

PCR

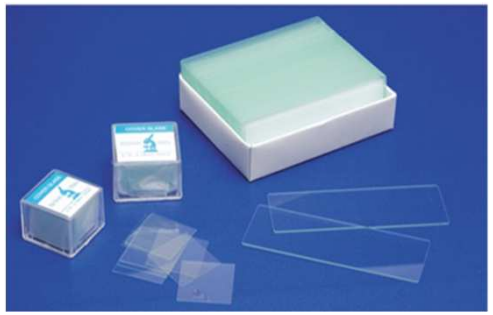
Gross Necropsy

Excellent for external and internal parasites

Perform gill and fin clips, intestinal squash preparations

Requires minimal equipment

Microscope, slides, dissection instruments



Histology

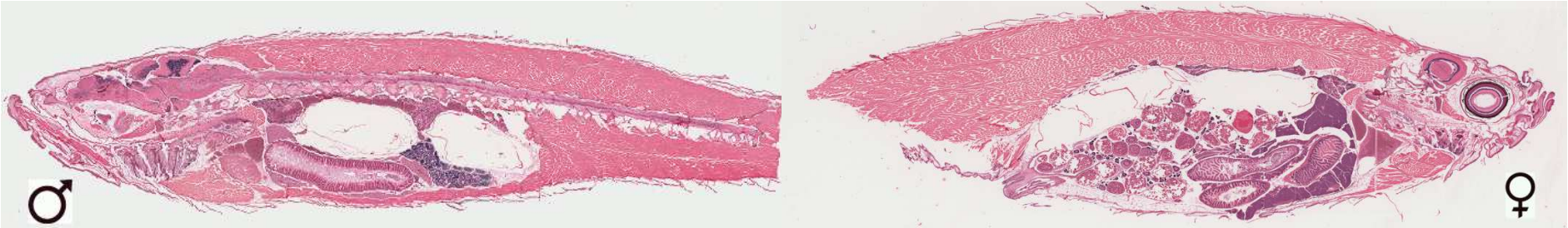
Whole fish may be examined

Detect infectious and non-infectious diseases

Help identify new/unknown pathogens

Excellent for general screening

Sensitive but not necessarily specific



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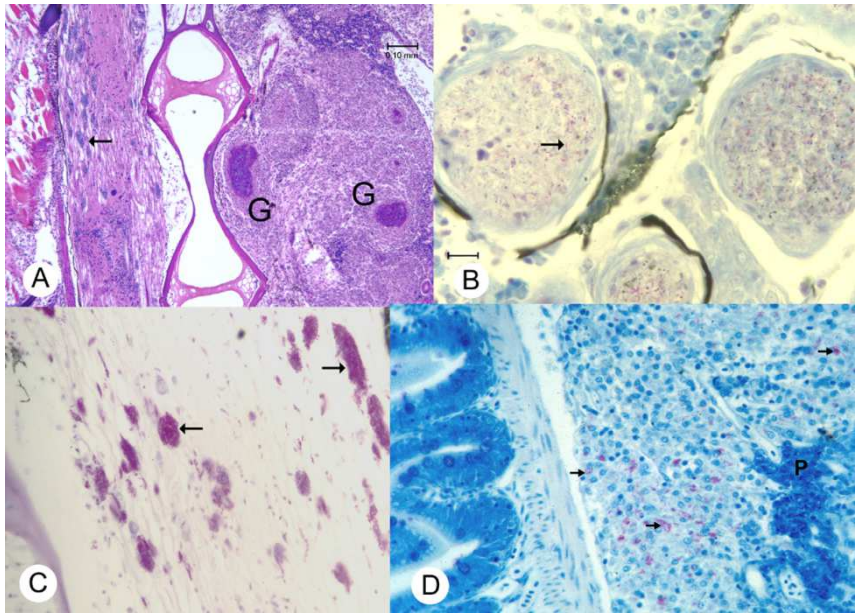
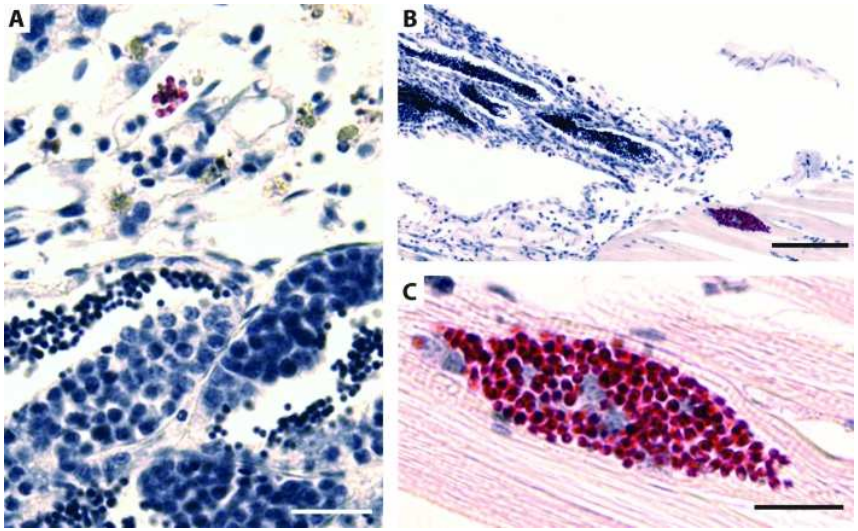
Special Stains

ZIEHL-NEELSEN



LUNA STAIN



ZEBRAFISH.ORG/HEALTH; MURRAY 2011; PETERSON 2011

Bacterial Culture

May be taken from kidneys of fish

Swabs of environment

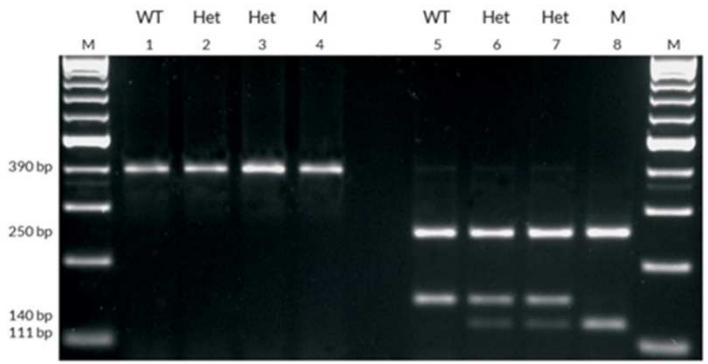
Should be grown at 28°C

PCR

Good for detection and surveillance of specific pathogens

Very specific, not sensitive

Excellent for analyzing environmental samples



	Advantages	Disadvantages
Gross necropsy and histology	Can look at entire animal Greatest sensitivity for widest range of diseases Potential to do follow-up PCR from blocks	Can miss low burdens Lacks specificity Long turn-around time Requires rapid evaluation upon death or euthanasia Cost
PCR	Sensitive and Specific Fast turn-around time Wide variety of samples Can be evaluated at later time Can pool fish samples to decrease cost	Frozen samples give best results Testing for specific pathogens only Only detects nucleic acid Need to evaluate appropriate samples Non-infectious conditions not detected

Testing Frequency

Quarantine fish

- Each group should be tested, especially if no health reports are provided

Recommend quarterly for main colonies

- Depends on the research programs
- Sentinel fish should be used

In the Rodent World

Table 3. Recommended infectious agents to monitor and frequencies of monitoring for laboratory mice (*Mus musculus*).

	Every 3 months	Annually
Viruses		
Mouse hepatitis virus	x	
Mouse rotavirus	x	
Murine norovirus	x	
Parvoviruses:		
Minute virus of mice	x	
Mouse parvovirus	x	
Theiler's murine encephalomyelitis virus	x	
Lymphocytic choriomeningitis virus		x
Mouse adenovirus type 1 (FL)		x
Mouse adenovirus type 2 (K87)		x
Mousepox (ectromelia) virus		x
Pneumonia virus of mice		x
Reovirus type 3		x
Sendai virus		x
Bacteria		
Helicobacter spp.	x	
If positive, speciation for *H. hepaticus*, *H. bilis* and *H. typhlonius* is recommended		
Pasteurella pneumotropica	x	
Streptococci β-haemolytic (not group D)	x	
Streptococcus pneumoniae	x	
Citrobacter rodentium		x
Clostridium piliforme		x
Corynebacterium kutscheri		x
Mycoplasma pulmonis		x
Salmonella spp.		x
Streptobacillus moniliformis		x
Parasites		
Endo- and ectoparasites (reported to the genus level)	x	

How many fish to sample?

- Number depends on:
  - Number of fish on system
  - Prevalence of pathogens (if known)
- Formulas exist to determine number of fish to test
  - Good in theory
  - Not always useful in practice
    - *Presumes fish all have same risk of exposure, which is not true of all current systems*
- Many facilities have limited funds for testing

Quarantine

Prior to Importation

Does the exporting facility have health reports?

Do they have a health monitoring program and description of zebrafish husbandry?

If not, can they provide extra fish for diagnostic screening?

Quarantine

Physically separate from main life support system

Restricted to essential personnel only

Have its own dedicated equipment for fish care

- Nets, buckets, feeding devices, etc.

Flow-through to avoid spread onto system

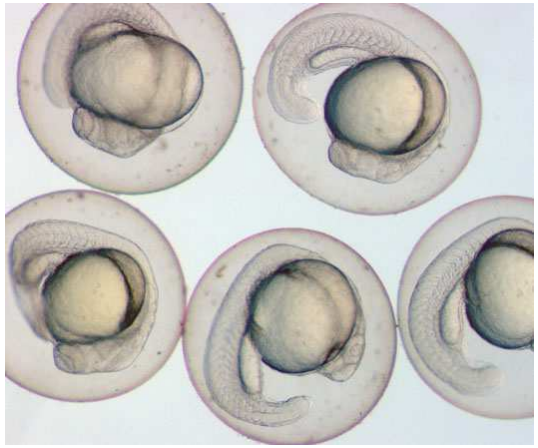
Quarantine

1st choice: import surface disinfected embryos

2nd choice: import adult zebrafish

Minimum 4 week acclimatization

Leaving quarantine

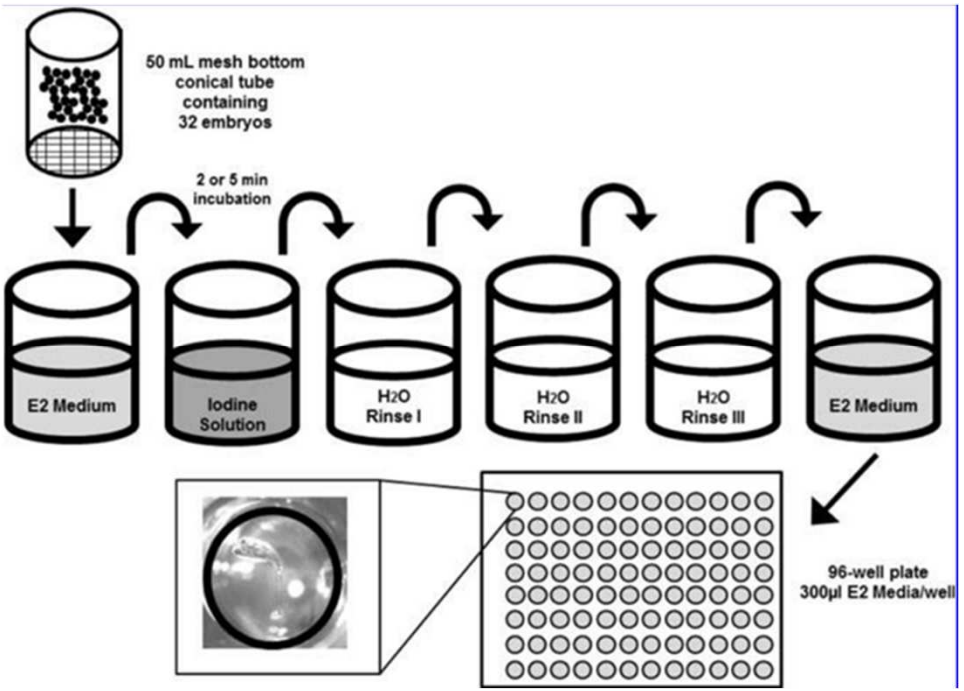


Embryo Surface Disinfection

Eggs < 30 hpf

Surface disinfect with chlorine or iodine

Rinse thoroughly



KENT 2014; ZEBRAFISH.ORG

CHANG 2016

Disease Investigation

Observation is Key

Daily observation and recording of morbidity and mortality on the system

- Note abnormal behavior or physical signs of disease

Sick and dead fish should be isolated/removed immediately

- Reduces chance of disease spread
- Allows for closer monitoring of the sick animal
- Identify potential adverse environmental conditions
- Sick animals are excellent for testing

Older fish should be euthanized

Disease Investigation

Moribund/sick fish are ideal samples for investigating infectious disease outbreaks

- May identify pathogens not detected in sentinels

Method for identifying sick fish

- E.g., bright sticker labels

Personnel must be able to identify sick fish and collect specimens when required

Non-infectious Causes of Illness

- Water quality testing
- Toxin screening
  - Chlorine
  - Heavy metals
  - Chemicals
- Environmental changes
  - Abnormal light cycle
  - Excessive noise/vibrations

Reporting

Reporting

Health Monitoring Report for Zebrafish

Institution: Fish Facility ZF2015
Microbiological Unit: (choose level suitable for your facility)
Building: 5
Room: 520
System: 1 (Main Colony)
Rack:
Contact person: (name, e-mail, phone) Fish facility manager email@institution.org (XXX) XXX-XXX

Date: November 18, 2015
Unit type: (mark the correct type)
Re-circulating: X
Flow through:
Static:
Other:

Testing Results:	Tested Subjet (sentinel, colony fish, sump fish, environmental, etc.)	Sampling Location (pre-filtration, post-filtration, main colony, quarantine, etc.)	Age of Fish	Exposure Time	Testing Frequency	Testing Method	Testing Laboratory	Recent Testing: Sampling Date	Recent Testing: # Positives/ # Tested	Historical Results: Collected over ___ months: # Positives/ # Tested
Bacteria										
Aeromonas hydrophila	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	aerobic culture	In-house	Oct, 2015	0/6	0/12
Edwardsiella ictaluri	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	aerobic culture	In-house	Oct, 2015	0/6	0/12
Flavobacterium columnare	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	aerobic culture	In-house	Oct, 2015	0/6	0/12
Mycobacterium spp.	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Mycobacterium abscessus	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Mycobacterium chelonae	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	3/12
Mycobacterium fortuitum	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Mycobacterium haemophilum	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Mycobacterium marinum	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Mycobacterium peregrinum	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/12
Microsporidia										
Pseudoloma neurophilia	Sentinel/AB	pre-filtration, main colony	12 months	12 months	quarterly	Histology	In-house	Oct, 2015	0/6	3/24
Pleistophora hyphessobryconis	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	Histology	In-house	Oct, 2015	0/6	0/12
Protozoa										
Ichthyophthirius multifiliis	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	Skin scrape	In-house	Oct, 2015	0/6	0/12
Flexicodinium piliatore	Sentinel/AB	pre-filtration, main colony	12 months	12 months	bi-annually	Skin scrape	In-house	Oct, 2015	0/6	0/12
Fungi										
Saprolegnia brachydanis	x	NT	x	x	x	x	x	x	x	x
Parasites										
Pseudocapillaria tomentosa	Sentinel/AB	pre-filtration, main colony	12 months	12 months	quarterly	PCR	Commercial Diagnostic Laboratory	Oct, 2015	0/6	0/24
Additional Agents										
Spring Viremia of Carp Virus (SVCV)	Colony Fish/ *tnf3* $^{mf2/+}$	post-filtration, main colony	6 months	6 months	as needed for exports	PCR	OIE-approved Diagnostic Laboratory	Nov, 2015	0/6	0/6
Infectious Spleen and Kidney Necrosis Virus (ISKNV)	Sentinel/AB	pre-filtration, main colony	12 months	12 months	as needed for exports	PCR	Commercial Diagnostic Laboratory	Sept, 2015	0/6	0/6

Pathology: Occasional egg-associated inflammation in sentinels > 6 months

Additional comments:

FIG. 1. Example of a health monitoring report.

Template can be found here: http://online.liebertpub.com/doi/suppl/10.1089/zeb.2015.1210

Should be accompanied by a husbandry description

May be required for export of fish

New template from FELASA/AALAS to come

COLLYMORE 2016

Building the Program

1. Determine what agents are present on your system(s) and in your fish
2. Decide what agents you want/need to exclude
3. Select the appropriate test samples and testing methodologies
4. Develop SOPs for performing health monitoring and quarantine