

## Preparing and sending fish samples for histology

### Protective equipment

Fixing specimens for histology involves the handling of potentially dangerous chemical products. **Always wear gloves and goggles (or protective glasses)**, when handling fixatives (and fixative components) and throughout the fixation process.

### Materials

- Disposables gloves
- Goggles
- Vials (tubes) of the appropriate size relevant to the size of specimens to be fixed
- Fixative solution (*e.g.* 10% neutral buffered formalin, ethanol *etc.*)
- Dissecting instruments (scalpel, scissors, tweezers, *etc.*)
- Labels
- Pencil

### Sampling

This is one of the most critical steps in the process. If the sampling and the sample fixation steps are not properly done, then the final results may be poor or inconsistent.

### Two types of sampling

1. Random sampling: Sampling for screening a population;
2. Non-random sampling: Sampling for diseases diagnosis.

#### ***Random sampling for population screening***

Determine the size of the sample that is needed using the following table (see **Table 1**). The table gives the size of the sample for a population, depending on the prevalence of a disease (at 95% confidence).

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**Table 1.** Sample size determination

Population Size	Size of sample needed to detect at a prevalence of						
	2%	5%	10%	20%	30%	40%	50%
50	50	35	20	10	7	5	2
100	75	45	23	11	9	7	6
250	110	50	25	10	9	8	7
500	130	55	26	10	9	8	7
1,000	140	55	27	10	9	9	8
1,500	140	55	27	10	9	9	8
2,000	145	60	27	10	9	9	8
4,000	145	60	27	10	9	9	8
10,000	145	60	27	10	9	9	8
>/=100,000	150	60	30	10	9	9	8

***Non-random sampling - sampling for diseases diagnosis***

Only live or moribund specimens with apparent signs of disease should be fixed for histology examination. Do not sample specimens that already dead.

**Fixing fish samples for histology**

- Anaesthetize **one** fish at a time in an appropriate anaesthetic (e.g. clove oil in 95% ethanol administered at 40-100 mg/l) and process it, before anaesthetising and processing a second fish. It is recommended to use a new scalpel for each batch of fish (or every 10 specimens). All specimens should be fixed **immediately** in chilled 10% buffered formalin. It is recommended that at least ten volumes of fixative for each volume of tissue sample should be used and that the samples are allowed to fix for at least 24 hours before processing.
- **Fish fry**, *i.e.* those measuring less than 3 cm can be placed whole in fixative.
- For **fish that are larger than 3 cm** in body length, open up the body cavity to allow fixative to penetrate into the deeper tissues, *i.e.* use a pair of scissors to open up the belly to expose the internal organs.
- For **fish that are larger than 10 cm**, send samples of a fin, an eye, a muscle block including the lateral line (block should be no larger than 7 mm x 7 mm by 5 mm deep), the second gill arch, heart, a piece of the spleen, liver, kidney with surrounding muscle, and, representative pieces of the intestinal tract (fore, mid and hind). All the tissue samples from one fish should be placed in a vial (with a leak proof lid).
- Carefully label each sample (use pencil, **do not use** ink or marker pens), including the date of fixation, the fixative used, species and information regarding the culture unit.

### **Keep the formalin cool and exchange the formalin for better results**

Better tissue and organ fixation is achieved when formalin is maintained at cooler temperatures. Keep all formalin solutions in a refrigerator before use. When travelling and working in the field keep the formalin in a polystyrene box with ice to keep the temperature cool. Tissues should be fixed in a ratio of 1 part tissue to at least 9 parts formalin. When working in the field, it is advised that the formalin in each pot is exchanged for fresh cool formalin 6 – 12 hours after initially fixing the tissue and organ samples.

**Caution:** When working with formalin wear disposable rubber gloves and protective eye glasses. Work in a well-ventilated area and avoid breathing in formalin fumes.

### **Sending samples by post to FVGAL**

To reduce the weight of samples and the risks of sending formalin fixed material in the post, once the samples have been fixed for a minimum of 24 hours in formalin, the fixative can be poured off and carefully disposed of, and then either:

- 1) Return them to their original sample tube with a tiny bit of liquid in the bottom of the tube to ensure the samples do not dry out during postage. Ensure the lid is securely fastened. Place the samples in a strong plastic bag with some tissues in the bottom to catch any liquid that may leak out of the tubes. Ensure this bag is securely closed, then place all the samples in a strong cardboard box to ensure that none of the samples get crushed and damaged during postage.
- 2) Place the sample inside a sealable zip lock plastic bag, and then place this inside a second sealable zip lock plastic bag. Then place all your samples in a strong cardboard box to ensure that none of the samples get crushed and damaged during postage.

We advise these samples are couriered to us as quickly as possible (**please use the postal address and labelling instructions on the next page**). Please ensure that all packages have your contact telephone number and FVGAL's clearly number marked on the outside.

### **Fixatives**

**If you require fixative, then please contact the Fish Vet Group Office.**

#### **To make one litre of 10% Neutral Buffered Formalin**

Sodium di-hydrogen orthophosphate (monobasic)	4.0 g
di-sodium hydrogen orthophosphate (dibasic)	6.5 g
Formaldehyde*	100 ml
Water	900 ml

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\*Please be aware that formaldehyde is a known carcinogen and should be handled with extreme care. Store formalin at room temperature in a brown glass bottle with a secure fitting lid.

**Fish Vet Group Asia Limited postal address**

Fish Vet Group Asia Limited  
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Chaengwattana Road  
Kwaeng Toongsonghong  
Khet Laksi  
Bangkok  
10210  
Thailand

Office: +66 (0) 2 575 1680-2

Fax: +66 (0) 2 575 1683

**Labelling instructions**

Please label the parcel or package to be couriered to Fish Vet Group Asia Limited as follows:

*"This package contains specimens for scientific analysis. The specimens are non-infectious, non-hazardous and of no-commercial value."*

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