

## Verification of Barriers - Sampling of skin, gills and guts Revised March 2019

**NOTE: The barriers of fish have *living* cells on their surfaces.**

**Tools:** plastic histocassettes, scalpel, extra scalpel blades, forceps, scissors, pencil for labels, about 2 L of 10% phosphate buffered formalin (Merck®, HT501320); pre-labelled wide-mouth jars for storing formalin or biopsy containers (Biopsafe® contains formalin) and histocassettes with samples; notebook.

**Sampling:** The salmon should be only very freshly dead or terminally anesthetized (waiting time makes the tissue degrade, as can be quickly seen on the gills). **Do not touch the area to be sampled because the mucous cells will wipe off.** Carry the anesthetized fish carefully to the sampling table, avoiding areas to be samples – we usually carry flat and hold with open hands on the flank side which will not be sampled.

### Overview of Sampling procedure:

1. Fill in **Project form** (see page 4).
2. Pre-label histocassettes with fish nr, and tissue (pencil on sloping end). \* See Appendix for guidelines on labelling and shipping.
3. Pre-label widemouth 90 ml jars or biopsy containers with project name and date (fish number(s) and tissue).
4. Cut out the desired tissues section using a scalpel (see below for details)
5. Transfer tissues to individual histocassettes by touching only the ends of the section. If the section is too broad to fit in without crushing, cut away some tissue or gill arch. Remember one sample is for one histocassette.
6. **With pre-labelled wide-mouth jars:** Place the labelled histocassettes containing all tissues for an individual fish into labelled jar and fill with 10% buffered formalin. Rule of thumb: fill to the top. Tape jar closed to prevent leakage.  
**With pre-labelled biopsy containers** (read Biopsafe® instructions): a) Unscrew the lid. Place 3 labelled histocassettes with samples for an individual fish into labelled containers. b) Close Biopsafe by screwing the lid onto the container. The lid must be screwed all way on. c) Release the formalin by pressing the top of the lid.
7. Fill in **Fish identification form** (see page 5).
8. Pack securely and ship to Quantidoc (\*see Appendix for details).



Left: an example of histocassette size and labelling for gills, dorsal skin, hindgut and foregut from one fish (number 12). Middle: an example of labelled formalin jar for multiple samples of dorsal skin from 5 fish. Right: an example of Biopsy container (Biopsafe®) with 3 histocassettes.

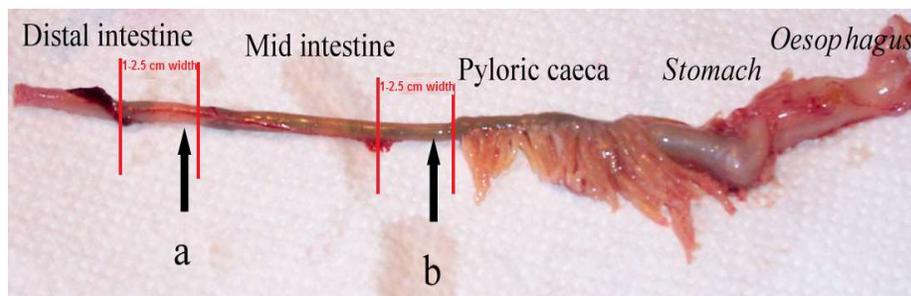
**For the SKIN:** using a sharp scalpel, make a rectangle about 1-2 cm x 1-2.5 cm just below the dorsal fin (line up with the front edge of the dorsal fin, see figure). Lift one edge carefully and remove the skin square WITH a bit of the underlying muscle. Place carefully into marked histocassette, close cassette and pop into Biopsafe container or a jar with 10% phosphate buffered formalin. More skin sections can be cut, according to need. The head and tail skin sections are taken in that order because the tail is hardest

to hold still. It is unlikely that you will get more than about 1.5 x 1.5.cm slices from head and tail. Put head and tail skin sections carefully into properly labelled histocassettes and fix in buffered formalin.



If the sample flips over on itself or wipes along the table or body, the mucous cells will be disturbed, so start again. A few practice rounds are useful. If nonetheless the sample is potentially disturbed, make a note of this in the comments section of the overview.

**For the GUT:** Cut a piece of the hindgut after about “a” of the picture and a piece of the foregut at “b” of the picture. Flush it with seawater *if necessary* to get out any remaining food but do not cut it open. Place in marked histocassette and pop into 10% phosphate buffered formalin. At this point, note fish gender by checking **gonads (sex makes a difference)**. It is important that all samples are from standardized places because the mucous epithelium has zones which are anatomically distinct.



**For the GILLS:** remove much of the second gill arch (**the one below the outermost one**) with the gill filaments and lamellae, without touching the soft tissue. *It is often easiest to hold the arches spread by going through the mouth.* Cut the arch at the top and bottom of the “V” of the gill arch, leaving the outermost soft parts untouched by you and intact. Lift by the bony arch, place carefully in marked histocassette and pop into 10% phosphate buffered formalin.



## APPENDIX

### Labelling

Abbreviations for sampled tissues: DOR-dorsolateral skin, GIL-gills, HIN-hindgut, FOR- foregut, MID-midgut, HEAD-head skin, CD-caudal dorsal skin,

**Use one serial sequence of numbers for all fish**, like 1, 2, 3.....

An example of labelling is “DOR 12”, “GIL 12”, “HIN 12” or “FOR 12”, where “FOR 12” means a foregut sample from fish 12.

If you have more than 1 sample per fish, keep all samples from one fish in one jar, please. If you have only 1 sample per fish, please, place 3-6 histocassettes in one jar/biopsafe container and **label this jar with tissue**. It is supposed to be up to 3 histocassette in one Biopsy container (Biopsafe®). Label jars/containers with paper labels using a pencil or waterproof marker. Seal labels with tape.

### Packaging and sending

Pack all histocassettes with samples in sealed, leak-proof jars with formalin. Surround these with absorbent material (paper towel) and seal in leak-proof zip-lock bags. Finally, pack the bag and some bubble wrap in an adequately strong container, such as a stiff cardboard or styrofoam shipping box.

All formalinized samples are supposed to be sent with TNT. In accordance with TNT guidelines, a formalin safety data sheet (in a water-proof bag) is to be attached to the side of the outer packaging. In addition to that, 2 copies of formalin safety data sheet (see attachments) are to be given to TNT agent.

### Shipping Address:

Att: Grigory Merkin, Quantidoc AS  
c/o Reception 2<sup>nd</sup> floor- Thormøhlensgate 53 A  
5006 Bergen, Norway

### Contact information:

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