

Mollusc processing for diagnosis by cytology (imprints)

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Editions

Edition	Date	Updated part
N° 1	08/2021	Creation

1. Scope

This procedure describes the preparation of mollusc samples for cytological examination (technical preparation of imprints).

2. Reference

- OIE. Manual of Diagnostic Tests for Aquatic Animals, current edition, Paris, France.
- Howard D.H., Lewis J.L., Keller B.J. & Smith C.S. (2004). Histological Techniques for Marine Bivalve Mollusks and Crustaceans, NOAA Technical Memorandum NOS NCCOS 5, 218 p.

3. General information

Cytology allows a quick diagnosis and can be useful in case of **suspicion of some parasites** (*Marteilia* sp., *Bonamia* sp.,) or when some **lesions** are visible on animals (such as pustules).

4. Equipment, reagents and environment

4.1. Equipment

- Razor blades
- Tweezers
- Gloves
- Paper towelling
- Hanging baskets and reagent tanks for colouring baths
- Histological slides and cover glasses
- Racks for histological slides
- Sink (to rinse the slides)
- Fume hood
- Timer

4.2. Reagents

- Hémacolor® solution 2
- Hémacolor® solution 3
- Absolute Ethanol (replace the Hémacolor® solution 1)
- Xylene
- Mounting medium: Eukitt®

Each new Hemacolor lot should be checked on few slides.

Each bath should be regularly renewed

For the fixation and coloration step:

- The bath of ethanol should be renewed every week of use
- Hemacolor baths should be renewed every month of use

For the mounting of the slides step:

- Bath of xylene and ethanol should be renewed every 3 months of use

4.3. Environment

- Well ventilated laboratory
- Fume hood (for mounting slides)

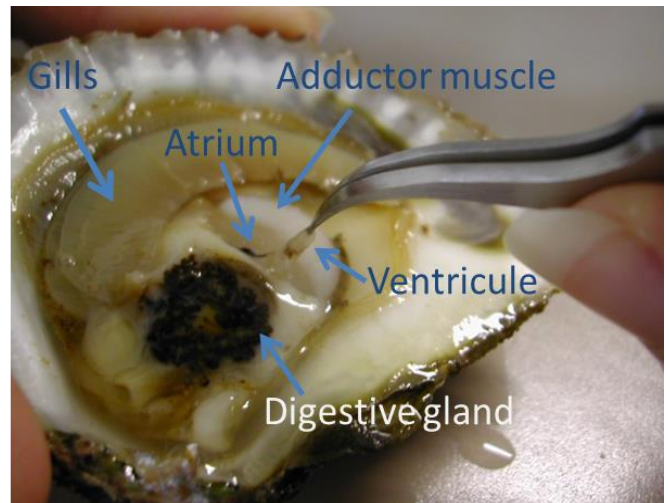
5. Procedure

5.1. Imprints

1-After mollusc opening, take a piece of targeted organs (heart, gills, digestive gland...)

Bonamia Suspicion: take a piece of heart or gills
For heart imprints, gently take the ventricle (white colour), in the pericardial cavity with tweezers (see picture)
Marteilia suspicion : take a piece of digestive gland

Abnormal lesions: take a piece of the lesion (example pustule)

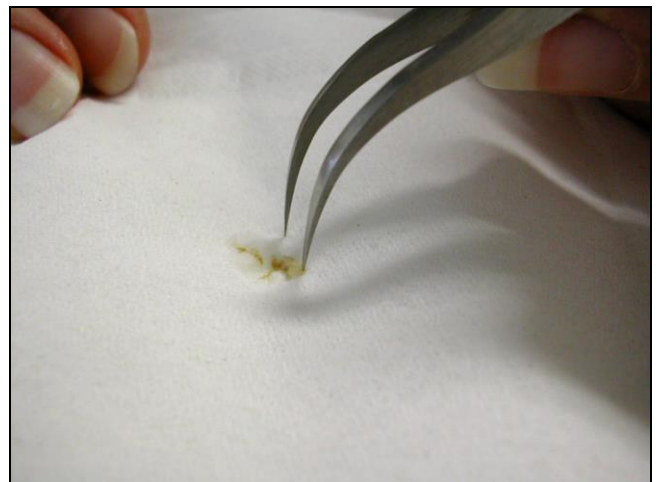


Preparation of heart imprints in a flat oyster

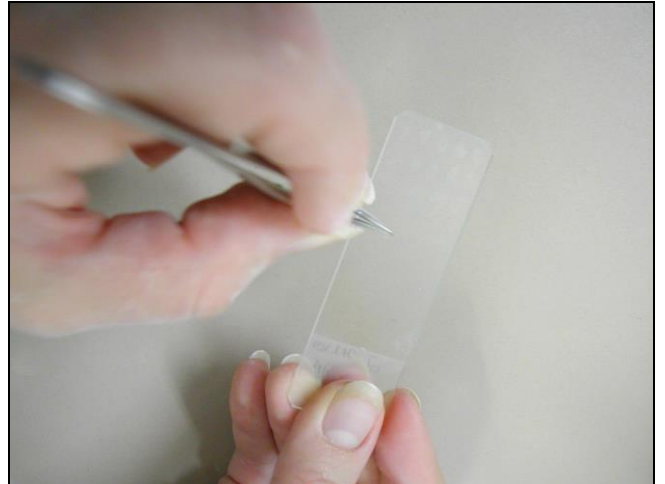


Preparation of digestive gland imprint in a mussel

2-Remove the liquid in excess by rolling the organ on paper towel. This step is important as too much liquid in the organs could prevent cell fixation on the slide.



3- Slice a small piece of organ with scissors or razor blade (For heart imprint, take back the whole ventricle). Take the small piece of organ with tweezers and make several successive imprints by gently pressing the organ on the slides. Check that there no residual water on the slide.



5.2. Fixation and coloration

Slides, previously air-dried, are placed in a hanging basket and soaked in the following baths:

- 1) Absolute ethanol (fixation): 1 minute minimum
- 2) Hémacolor® solution 2 : 1 min
- 3) Hémacolor® solution 3 : 1 min
- 4) Running water : about 5 minutes or until water clarification

Once dried, slides can be observed directly under the microscope, or can be mounted (see section 5.3 below).

5.3. Mounting the slides

If slides are completely dried, they are directly placed in a xylene bath. If not, they are soaked in ethanol for few minutes in order to have a proper dehydration before being placed in the xylene bath. Slides can stay in xylene without time limit.

Slide mounting consist in soaking coloured tissues with mounting liquid (quite viscous) and to place them between slide and cover glass, while avoiding air bubbles in mounting liquid.

The protocol below describes one possible way to do so:

- Put one drop of Eukitt mounting liquid on a cover glass using a clean Pasteur pipette
- Place the cover glass horizontally above the coloured tissues slide (freshly taken away from xylene)
- Gently apply the cover glass on the slide
- Remove potential bubbles by slightly pushing at the center of the cover glass
- Dry overnight under a fume hood

One day of drying at room temperature is recommended before slide examination. Drying can be hastened by placing the slides in an oven.

Refer to the following SOPs for slide examination:

Diagnosis by histo-cytopathology of *Marteilia* spp. in the flat oyster *Ostrea edulis* and the mussels *Mytilus edulis* and *M. galloprovincialis*

Diagnosis by histo-cytopathology of *Bonamia* spp. in flat oysters *Ostrea* spp.

Diagnosis by histo-cytopathology of *Mikrocytos* sp. in oysters

6. Safety information

Hazardous chemicals used for cytology come in containers with special labels identifying their hazard characteristics. Information on MSDS (material safety data sheets) can be found on Internet (for example: <http://www.chemexper.com/>). The **flash point** of a flammable product is the lowest temperature at which it can form an ignitable mix with air. Use of protective gloves is recommended.

Absolute Ethanol (*use under a fume hood*)

Eye: Causes severe eye irritation.

Skin: Causes moderate skin irritation.

Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea.

Inhalation: Vapours may cause dizziness or suffocation.



H225 - Highly flammable liquid and vapor

Flash point: 16.6°C

Xylene (*use under a fume hood*)

Eye: Causes severe eye irritation.

Skin: Exposure may cause irritation. Prolonged contact may cause dermatitis.

Ingestion: May cause central nervous system depression, kidney damage and liver damage.

Inhalation: High concentrations may cause central nervous system effects characterised by nausea, headache, dizziness, unconsciousness and coma. Vapours may cause respiratory tract irritation. Irritation may lead to chemical pneumonitis and pulmonary oedema.



H332 - Harmful if inhaled
H315 - Causes skin irritation
H312 - Harmful in contact with skin
H226 - Flammable liquid and vapor

Flash point: 32°C
