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

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

1. Scope

This procedure explains the diagnostic tests used for the protistan *Marteilia refringens* presumptive diagnosis in the flat oyster *Ostrea edulis* and the mussels *Mytilus edulis* and *M. galloprovincialis* following histological or cytological processing of samples.

2. References

- Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals.
- OIE. Manual of Diagnostic Tests for Aquatic Animals (last edition).
- **Howard, D.W., E.J. Lewis, B.J. Keller, and C.S. Smith,** 2004. Histological techniques for marine bivalves mollusks and crustaceans. NOAA Tech. Memo. NOS NCCOS 5, 218 p.
- **Franck C.J.B, Le Roux F., Adlard R.D. and Figueras A.,** 2004. Marteiliosis in molluscs: A review. Aquat. Living Resour. 17: 433–448
- **Bower, S.M.**, 2007. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: Marteiliosis (Aber disease) of Oysters and Marteilia *refringens/maurini* of mussels.

3. General information

In Europe marteiliosis is a disease caused by *Marteilia refringens* in the European flat oyster *Ostrea edulis* and the mussels *Mytilus edulis* and *M. galloprovincialis*. *Marteilia refringens* is a lethal parasite of the flat oyster *Ostrea edulis* and can also be lethal for the mussels. The parasite sporulates in the epithelia of the digestive gland. Infection is associated with poor condition index, emaciation of the mollusc and consumption of its reserves of energy (glycogen), discoloration of the digestive gland, cessation of growth and mortalities (particularly in oysters). Mortality appears to be related to sporulation of the parasite. Presporulation stages occur in the epithelia of the palps, stomach, digestive ducts and gills. Period of infection is confined to spring and summer, when water temperature is greater than 17°C. Some other oyster species are susceptible to the disease: *Ostrea angasi, O. puelchana and O. chilensis*.
Other unidentified *Marteilia* (given as *Marteilia* sp.) have been observed in *Cardium edule*, *Tapes rhomboides*, *T. pullastra*, *Ensis minor*, *E. siliqua*, *Modiolus modiolus*, *Argopecten gibbus*, *Tridacna maxima*, *Saccostrea forskali* and *Pinctada margaritifera* (Berthe et al., 2004).
Other information is available on the CRL website.

4. Equipment and environmental conditions

Binocular microscope for histological and cytological examination, equipped with different normal dry objectives (10X, 20X, 40X and/or 60X) and a 100X (oil) objective with immersion oil. Microscope should be set with Koehler illumination technique.

5. Operating procedure

5.1. Imprints examination

Slides with tissue imprints (digestive tract and digestive gland) must be observed first with 10X or 20X dry objectives to find areas with many epithelial cells of the digestive gland or the digestive tract.

Marteilia refringens early stages occur within the digestive tract epithelia and sporulating stagesor sporangia within the digestive gland epithelia.
The parasite is 5-8 µm in size in the early stages and reaches up to 40 µm during sporulation. The cytoplasm of the cells stains basophilic, the nucleus is eosinophilic (i.e. respectively blue and red with the Hemacolor® kit for example).

The secondary cells of sporangia or sporoblasts are surrounded by a bright halo (**Figure 1** and **Figure 2**). Refringent bodies usually appear grey on imprints.

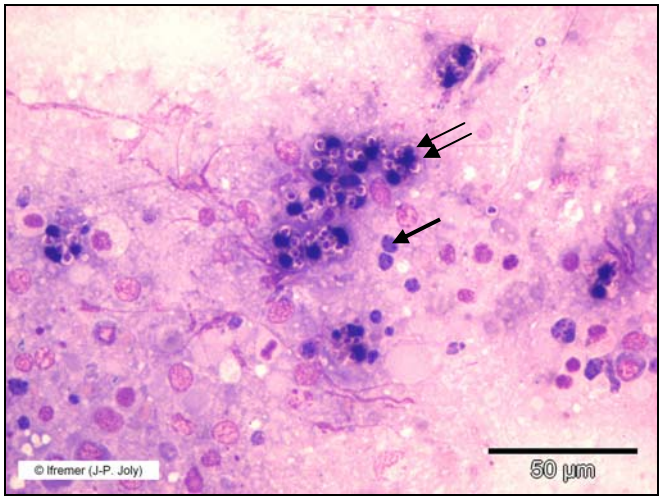

Figure 1 : *Ostrea edulis* digestive gland imprint observed with young stages (arrow) and sporangia (double arrow) Hemacolor® staining (X40 objective).

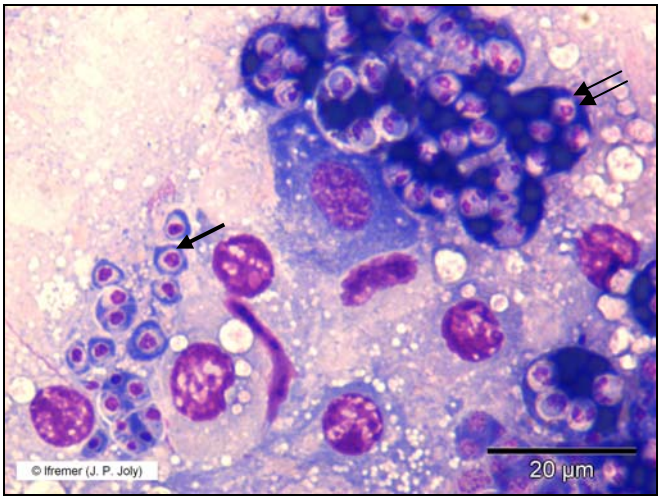

Figure 2 : *Mytilus galloprovincialis* digestive gland imprint with numerous young stages (arrow) and sporangia (double arrow). Hemacolor® staining (X100 objective).

5.2. Histological examination

The young stages of *Marteilia refringens* are present in the epithelia of the stomach, intestine and digestive tracts (**Figure 3**) and sometimes in digestive tubules. Sporulating stages (or sporangia) can be found in the epithelia of the digestive tubules (**Figure 4**). Lesions of the digestive tubules epithelium can occur. Free sporangia can also be observed in the lumen of the intestine.

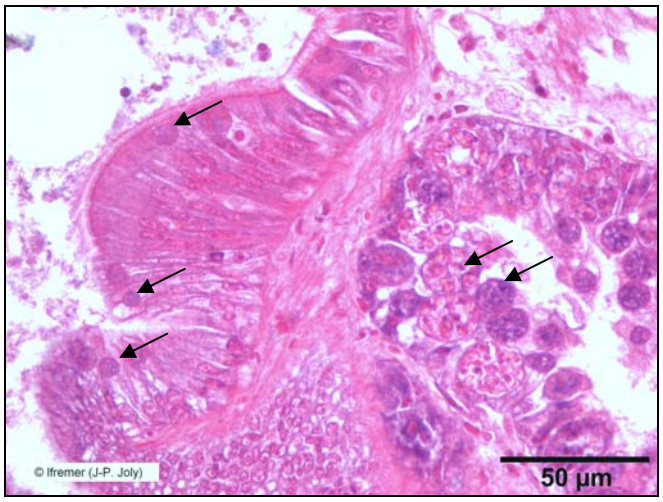

Figure 3 : Young stages of *Marteilia* refringens within the epithelium of *Mytilus edulis* digestive tract (on the left) and advanced sporulating stages in the digestive diverticulum (on the right). H&E staining (X40 objective).

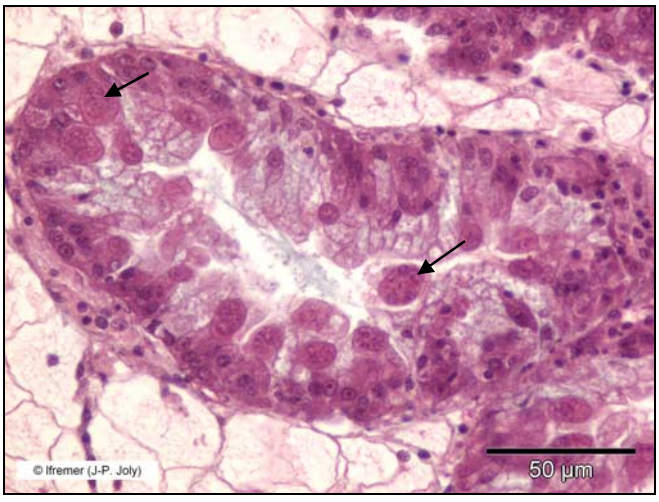

Figure 4 : *Marteilia refringens* sporangia in a digestive tubule epithelium of *Ostrea edulis*. H & E staining (X40 objective).

5.3. Results statement

Results are expressed **qualitatively** (infected = **positive** / non-infected = **negative**) and only at the genus level in histo-cytology (i.e. *Marteilia* sp.). *They can also be expressed according to* ***semi-quantitative*** *scales for histology and imprints (high, medium or low infection).*