

**Diagnosis by histopathology of *Perkinsus* sp. in molluscs**

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## Diagnosis by histopathology of *Perkinsus* sp. in molluscs

### 1. Scope

This procedure explains the diagnostic test used for the protistan *Perkinsus* sp. presumptive diagnosis in molluscs after histological processing of samples (Howard et al., 2004). The Eastern oyster *Crassostrea virginica*, the carpet shell clam *Ruditapes decussatus*, the Manila clam *Ruditapes philippinarum*, the Pacific oyster *Crassostrea gigas* and several species (other clams, abalone, scallops...) can be tested the same way.

### 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 & C.S. Smith, 2004. Histological techniques for marine bivalves mollusks and crustaceans. NOAA Tech. Memo. NOS NCCOS 5, 218 p.
- Villalba A., Reece K.S., Ordás M.C., Casas S.M. & Figueras A., 2004. Perkinsosis in molluscs: A review. *Aquat. Living Resour.* 17: 411–432
- Villalba, A. (Ed.), 2008. Workshop for the analysis of the impact of Perkinsosis to the European Shellfish Industry, Vigo, Spain, September 12-14<sup>th</sup> 2007. 163 p.
- Bower, S.M., 2007. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Perkinsus* of Clams and Cockles. URL: [http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/perkincc\\_e.htm](http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/perkincc_e.htm)
- Bower, S.M., 2006. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Perkinsus marinus* ("Dermo" Disease) of Oysters. URL: [http://www-sci.pac.dfo-mpo.gc.ca/shelldis/pages/pmdoy\\_e.htm](http://www-sci.pac.dfo-mpo.gc.ca/shelldis/pages/pmdoy_e.htm)
- Bower, S.M., 2007: Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Perkinsus olseni* of Abalone. URL: [http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/perkolab\\_e.htm](http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/perkolab_e.htm)

### 3. General information

Perkinsosis have been reported from many parts of the world (Europe, America, Asia, Australia) in different mollusc species including abalone, oysters, clams, scallops, etc. (Garcia et al. in Villalba, 2008).

*Perkinsus olseni* is a pathogenic protistan infecting different species of clams in Europe (mainly *Ruditapes decussatus* and *R. philippinarum*). It was also reported as an important pathogenic organism of the abalone *Haliotis rubra* in Australia.

*Perkinsus marinus* causes disease of economic importance in *Crassostrea virginica*. *Crassostrea gigas* can be infected to a lesser extent.

*Perkinsus chesapeaki* is a species commonly observed in USA in several clam species (e.g. *Mya arenaria*) and the oyster *C. virginica*.

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

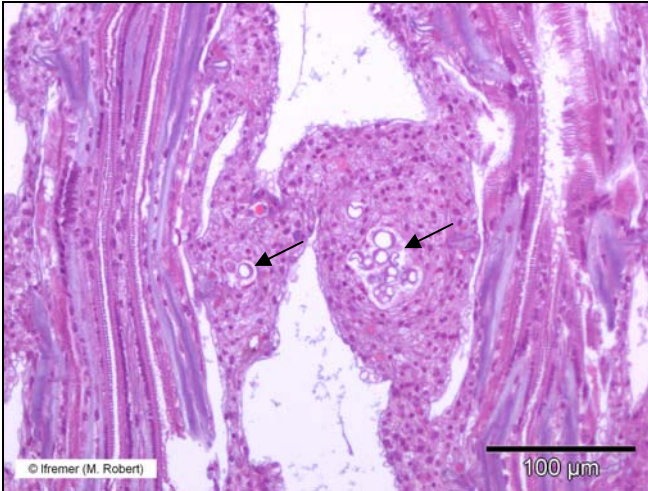
#### 5.1. Histological examination

We describe here the two most important *Perkinsus* species encountered in the world: *Perkinsus olseni* and *P. marinus*

##### 5.1.1. *Perkinsus olseni*

Trophozoites vary from 2 to 40 µm (usually 3 - 15 µm) and can easily be visualised by histology as a rounded cell with a large vacuole and an eccentrically displaced nucleus ("signet ring" appearance). In most clams, infection is associated with huge haemocytes infiltration into the surrounding tissues (**Figure 1**). Encapsulation and phagocytosis are common. In clams gills (**Figure 1**), connective tissue of the digestive gland (**Figure 2**) and the foot are the most highly infected tissues. In light infections there is often no visible host reaction.

The host can react to the infection by encapsulation of the parasites by haemocytes. These haemocytes produce lysosomal enzymes and ROS (reactive oxygen species) that can degrade the parasites (Soudan et al. *in* Villalba, 2008), so partially or totally lysed *Perkinsus* cells (easily visible as eosinophilic matter) are often observed in the tissue (**Figure 2**).



**Figure 1** : Gill from a Manila clam *Ruditapes philippinarum* infected by several trophozoites of *Perkinsus olseni*. Note the heavy inflammatory reaction around the parasites, H&E staining.

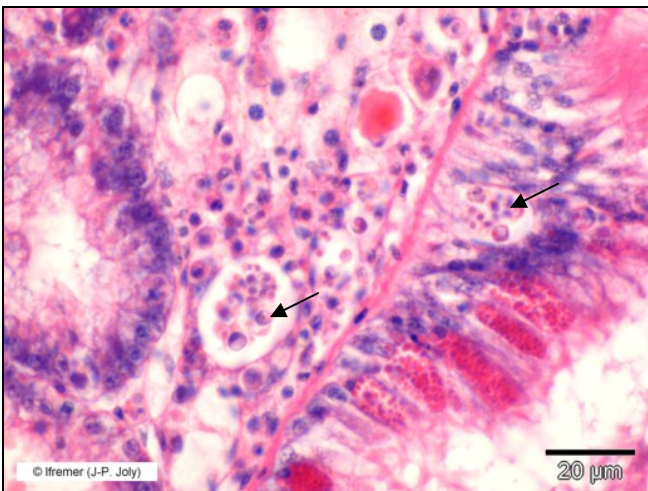


**Figure 2** : Degradation of *Perkinsus olseni* Trophozoites encapsulated by haemocytes in the digestive gland connective tissue from a clam (*Ruditapes philippinarum*), H&E staining.

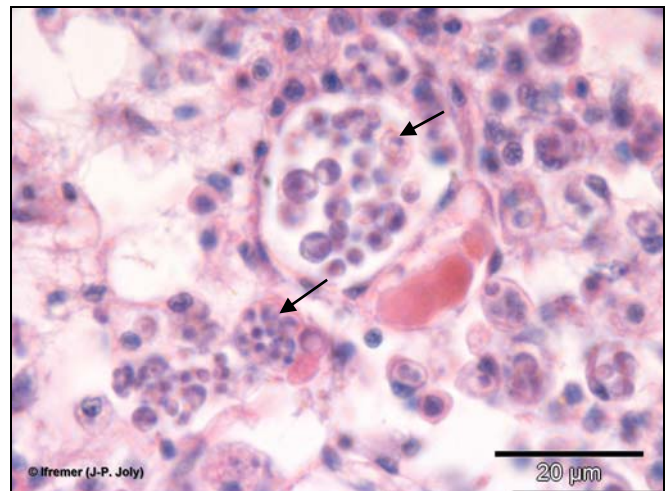
### 5.1.2. *Perkinsus marinus*

Trophozoites are spherical cells, 2-10 µm in diameter (smaller than *P. olseni*), with a large vacuole and an eccentrically displaced nucleus (**Figure 3**). Parasites can be observed within oyster haemocytes. In light infections *P. marinus* occurs only in intestine epithelia.

In heavy infection, the connective tissue of all organs (**Figure 4**) can contain immature trophozoites (2-3 µm), mature trophozoites 3-10 µm in diameter each containing a large eccentric vacuole ("signet-ring" stage) and tomites (sporangia or schizonts) 4 -15 µm in diameter and containing up to 32 immature trophozoites.



**Figure 3** : *Perkinsus marinus* cells in the connective tissue of the digestive gland and in the gut epithelium of an infected *Crassostrea virginica* oyster, H&E staining.



**Figure 4** : Different stages of *Perkinsus marinus* trophozoites in the connective tissue of the digestive gland (*Crassostrea virginica*), H&E staining.

## 5.2. Results statement

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