

# WSSV diagnostic manual

- The present EU regulations for surveillance and diagnostics of WSSV is described in Commission Implementing Decision (EU) 2015/1554.
- As a supplement to the new health law (Regulation (EU) 2016/426) the Commission Delegated Regulation (EU) 2020/689 replaces 2015/1554.
- The new regulation applies from April 21, 2021.

# WSSV surveillance program

*Table 6. A*

**Scheme for Member States, zones and compartments for the 2-year control period which precedes the achievement of status free from infection with WSSV**

Year of surveillance	Number of health visits per year to each establishment or group of establishments	Number of laboratory examinations per year	Number of crustaceans in the sample
Year 1	1	1	150
Year 2	1	1	150

# WSSV surveillance program

Table 6. B

## Scheme for Member States, zones or compartments to maintain status free from infection WSSV

Risk level <sup>(1)</sup>	Number of health visits to each establishment/group of establishments	Number of laboratory examinations	Number of crustaceans in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

<sup>(1)</sup> Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

A high-risk farm or mollusc farming area is a farm or mollusc farming area which:

- (a) has a high risk of spreading diseases to or contracting diseases from other farms or wild stocks;
- (b) operates under farming conditions which could increase the risk of disease outbreaks (high biomass, low water quality), taking into account the species present;
- (c) sells live aquatic animals for further farming or restocking.

A medium-risk farm or mollusc farming area is a farm or mollusc farming area which:

- (a) has medium risk of spreading diseases to or contracting diseases from other farms or wild stocks;
- (b) operates under farming conditions which would not necessarily increase the risk of disease outbreaks (medium biomass and water quality), taking into account the species present;
- (c) sells live aquatic animals mainly for human consumption.

A low-risk farm of mollusc farming area is a farm or mollusc farming area which:

- (a) has a low risk of spreading diseases to or contracting diseases from other farms or wild stocks;
- (b) operates under farming conditions which would not increase the risk of disease outbreaks (low biomass, good water quality), taking into account the species present;
- (c) sells live aquatic animals for human consumption only.

A high-risk farm or mollusc farming area is a farm or mollusc farming area which:

- (a) has a high risk of spreading diseases to or contracting diseases from other farms or wild stocks;
- (b) operates under farming conditions which could increase the risk of disease outbreaks (high biomass, low water quality), taking into account the species present;
- (c) sells live aquatic animals for further farming or restocking.

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- (c) sells live aquatic animals for human consumption only.

# Risk level assessment

- New risk level assessment system under development  
(based on a combination of risk of disease spread from and risk of disease contraction to a given establishment)

# Diagnostic manual 2015/1554 for WSSV:

- Pleopods, legs, gills or mouthparts stored in EtOH, RNAlater or snap frozen at -80
- Put in G2 buffer with 10 µl Proteinase K at 1:10 w/v
- Fastprep in Lysing matrix A for 2 min
- 56 °C 4 hours to over night
- Vortexed, centrifuged 9000 rpm 2 min
- ~50 µl used for DNA extraction on EZ1 Advanced XL Biorobot (Qiagen)
- Extraction evaluated with nanodrop
- 2.5 µl used in nested PCR with GoTaq (Promega) and 146F1 + 146R1 primers in first round and 146F2 + 146R2 in second round using this program: 1 x 94°C 2', 30 x 94°C 30'' + 62°C 30'' + 72°C 30'', 1 x 72°C 2'
- Electrophoresis on 2 % agarose gel. Bands cut out and purified using Promega SV Gel and PCR clean-up system and sequenced using Big Dye Terminator Kit v3,1
- In case of a positive PCR result the infection should as far as possible be supported by histology and TEM.

# Diagnostic manual 2020/689 for WSSV:

- Pleopods, legs, gills or mouthparts stored in EtOH
- PCR using method approved by EURL followed by sequencing
- Histology and TEM may be used to support diagnosis



# OIE methods for WSSV diagnostics

Method	Pros	Cons
Nested PCR	<ul style="list-style-type: none"> <li>No need for Real Time PCR machine</li> <li>High sensitivity</li> <li>Sequencing possible</li> </ul>	<ul style="list-style-type: none"> <li>Risk of false positives</li> <li>Gel electrophoresis needed</li> </ul>
Real Time PCR	<ul style="list-style-type: none"> <li>Quantitative</li> <li>Gel electrophoresis not needed</li> <li>High sensitivity</li> </ul>	<ul style="list-style-type: none"> <li>Real Time PCR machine needed</li> <li>Probes are expensive</li> <li>Sequencing difficult</li> </ul>
LAMP	<ul style="list-style-type: none"> <li>No need for PCR machine</li> <li>Quick</li> </ul>	<ul style="list-style-type: none"> <li>Gel electrophoresis needed</li> <li>Sequencing difficult</li> <li>Low sensitivity</li> </ul>

# EURL Diagnostic manual for WSSV

- Real Time PCR for surveillance (alternatively nested PCR) and after first identification
- Conventional PCR followed by sequencing for first identification of outbreak
- We have not tried LAMP – is it worth including?
- Pooling of individuals? (e.g. 5 – 10 animals)

# Questions or comments?

DTU

