

## CHAPTER 2.2.5.

# INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

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### 1. Scope

Infection with infectious myonecrosis virus means infection with the pathogenic agent infectious myonecrosis virus (IMNV) that is tentatively assigned to the Family *Totiviridae*.

### 2. Disease information

#### 2.1. Agent factors

##### 2.1.1. Aetiological agent

IMNV is tentatively assigned to the family *Totiviridae* (Lightner, 2011; Nibert, 2007; Poulos *et al.*, 2006; Wickner *et al.*, 2011).

IMNV particles are icosahedral in shape and 40 nm in diameter, with a buoyant density of 1.366 g ml<sup>-1</sup> in caesium chloride. The genome consists of a single, double-stranded (ds) RNA molecule of 8226–8230 bp (Loy *et al.*, 2015; Naim *et al.*, 2015). Sequencing of the viral genome reveals two non-overlapping open reading frames (ORFs). The first ORF (ORF1, 470–5596 nt) encodes a putative RNA-binding protein and a capsid protein. The coding region of the RNA-binding protein is located in the first half of ORF1 and contains a dsRNA-binding motif in the first 60 amino acids. The second half of ORF1 encodes a capsid protein, as determined by amino acid sequencing, with a molecular mass of 106 kDa. The second ORF (ORF2, 5884–8133 nt) encodes a putative RdRp (Poulos *et al.*, 2006). The most variable region of IMNV genome is located in the first half of ORF1, coinciding with a region which probably encodes the capsid protrusions (Dantas *et al.*, 2015).

The complete genomes of IMNV types originating from Brazil and Indonesia have been sequenced and found to be 99.6% identical at the nucleotide level (Poulos *et al.*, 2006; Senapin *et al.*, 2007). The 99.6% full genome sequence identity (and anecdotal information on the introduction of *Penaeus vannamei* stocks from Brazil) indicate that the disease was introduced from Brazil to Indonesia in 2006. A new genotype was analysed in infected samples in 2018 in Indonesia, including an isolate that contains a deletion of 622 amino acids (Mai *et al.*, 2019).

##### 2.1.2. Survival and stability in processed or stored samples

No data.

##### 2.1.3. Survival and stability outside the host

No information available.

For inactivation methods, see Section 2.4.5.

#### 2.2. Host factors

##### 2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IMNV according to Chapter 1.5 of *Aquatic Animal Health Code (Aquatic Code)* are: brown tiger prawn (*Penaeus esculentus*), banana prawn (*P. merguensis*), and whiteleg shrimp (*P. vannamei*).

### 2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with IMNV according to Chapter 1.5 of the *Aquatic Code* are: giant tiger prawn (*Penaeus monodon*) and blue shrimp (*P. stylirostris*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: southern brown shrimp (*P. subtilis*).

### 2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Juveniles and subadults of *P. vannamei*, farmed in marine, brackish, and low salinity brackish water, appear to be most severely affected by infection with IMNV (Lightner, 2011; Lightner *et al.*, 2004; Nunes *et al.*, 2004; Poulos *et al.*, 2006).

### 2.2.4. Distribution of the pathogen in the host

The principal target tissues for IMNV include the striated muscles (skeletal and less often cardiac), connective tissues, haemocytes, and the lymphoid organ parenchymal cells (Lightner, 2011; Lightner *et al.*, 2004; Poulos *et al.*, 2006; Tang *et al.*, 2005).

### 2.2.5. Aquatic animal reservoirs of infection

Some members of populations of *P. vannamei* that survive IMNV infections or epizootics may carry the virus.

### 2.2.6. Vectors

Experimental studies have demonstrated that brine shrimp *Artemia franciscana* can act as a vector for IMNV (da Silva *et al.*, 2015).

## 2.3. Disease pattern

### 2.3.1. Mortality, morbidity and prevalence

In early juvenile, juvenile, or adult *P. vannamei* in regions where infection with IMNV is enzootic, outbreaks of IMNV infections associated with sudden high morbidity and mortality may follow 'stress' events such as capture by cast-netting, feeding and sudden changes in water salinity or temperature (Lightner, 2011; Lightner *et al.*, 2004; Nunes *et al.*, 2004; Poulos *et al.*, 2006). Feed conversion ratios of affected populations can increase from a normal value of ~ 1.5 up to 4.0 or higher (Andrade *et al.*, 2007). Mortalities from infection with IMNV can range from 40% to 70% in cultivated *P. vannamei*.

In regions where infection with IMNV is enzootic in farmed stocks of *P. vannamei*, its prevalence may reach 100% (Andrade *et al.*, 2007; Nunes *et al.*, 2004).

### 2.3.2. Clinical signs, including behavioural changes

Affected shrimp present with visibly white tails. Such severely affected shrimp may have been feeding just before the onset of stress and may have a full gut. High mortality can occur suddenly and continue for several days. A sudden onset of clinical signs may occur following stress events (e.g. capture by cast-netting, feeding, and sudden changes in temperature or salinity).

Only shrimp in the acute phase of disease present behavioural changes. Typically, severely affected shrimp become lethargic during or soon after stress events such as capture by cast-netting, feeding, sudden changes in water temperature, sudden reductions in water salinity, etc.

### 2.3.3 Gross pathology

Shrimp in the acute phase of disease present focal-to-extensive white necrotic areas in striated (skeletal) muscles, especially in the distal abdominal segments and tail fan, which can become necrotic and reddened in some individual shrimp.

Exposing the paired lymphoid organs (LO) by simple dissection will show that they are hypertrophied (3–4 times their normal size) (Lightner *et al.*, 2004; Poulos *et al.*, 2006).

#### 2.3.4. Modes of transmission and life cycle

IMNV has been demonstrated to be transmitted horizontally by cannibalism (Lightner, 2011; Poulos *et al.*, 2006). Transmission via water probably occurs. Although vertical transmission is suspected from anecdotal evidence, it is not known whether this occurs via transovarial mechanism or by surface contamination of newly spawned eggs.

#### 2.3.5. Environmental factors

Temperature and salinity effects are likely predisposing factors to disease outbreaks, but no experimental data are available (Nunes *et al.*, 2004).

#### 2.3.6. Geographical distribution

Infection with IMNV has been reported to occur in some countries in the Americas, Asia and Africa (Aly *et al.*, 2021; Andrade *et al.*, 2007; Lightner *et al.*, 2004; Naim *et al.*, 2014; Nunes *et al.*, 2004; Poulos *et al.*, 2006; Sahul *et al.*, 2017).

See WAHIS (<https://wahis.woah.org/#/home>) for recent information on distribution at the country level.

### 2.4. Biosecurity and disease control strategies

#### 2.4.1. Vaccination

No effective vaccines for infection with IMNV are available.

#### 2.4.2. Chemotherapy including blocking agents

Ctn[15-34], a cathelicidin-derived eicosapeptide was found to demonstrate antiviral activity against IMNV in primary haemocyte cultures (Vieira-Girao *et al.*, 2017).

#### 2.4.3. Immunostimulation

No data.

#### 2.4.4. Breeding resistant strains

There are anecdotal reports of some selected lines of *P. vannamei* having better survival and culture performance in farms where infection with IMNV is enzootic. During a 20-day controlled laboratory study in which the shrimp were challenged with IMNV, some domesticated lines of *P. vannamei* were found to survive better than other lines (White-Noble *et al.*, 2010).

*Penaeus monodon* and *P. stylirostris*, for which there is incomplete evidence of susceptibility (see section 2.2.2), are considered to be more resistant to infection with IMNV than *P. vannamei* (Tang *et al.*, 2005).

#### 2.4.5. Inactivation methods

No data.

#### 2.4.6. Disinfection of eggs and larvae

While IMNV is believed to be transmitted vertically, there are no scientific data confirming this route of transmission. Disinfection of eggs and larvae (Chen *et al.*, 1992) is a good management practice recommended to reduce the potential for transmission of a number of penaeid shrimp diseases from female spawners to their eggs or larvae, and the practice may reduce IMNV contamination of spawned eggs and larvae produced from them.

### 2.4.7. General husbandry

Management practices in endemic areas principally involves exclusion of IMNV from shrimp farms. Broodstock or their spawned eggs or nauplii are PCR-tested and those that test positive are discarded (Andrade *et al.*, 2007). Following and restocking of affected farms or entire culture regions with IMNV-free stocks of *P. vannamei* most suited to local culture conditions has proven to be the most successful for preventing and controlling other virus diseases of shrimp, and should be applicable to control and prevent infection with IMNV (Lee & O'Bryen, 2003; Lightner, 2005; Lightner *et al.*, 2009; Moss & Moss, 2009).

## 3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

### 3.1. Selection of populations and individual specimens

Specimens suitable for testing for infection with IMNV using molecular methods (e.g. RT-PCR, nested RT-PCR, real-time RT-PCR, etc.) include postlarvae (PL), juveniles, subadults and adults. While IMNV may infect all life stages, infection severity, and hence virus load, may be below detection limits in spawned eggs and in larval stages, so these life stages may not be suitable for demonstrating freedom from infection with IMNV unless validated for those life stages.

### 3.2. Selection of organs or tissues

IMNV infects tissues of mesodermal origin. The principal target tissues in the acute phase of infection with IMNV are the striated muscles (skeletal and less commonly cardiac muscle), connective tissues, haemocytes, and the lymphoid organ tubule parenchymal cells. In chronic infections, the lymphoid organ may be the principal target tissue.

### 3.3. Samples or tissues not suitable for pathogen detection

IMNV replicates systemically but does not replicate in enteric tissues (e.g. the hepatopancreas, the midgut, or its caeca). Hence, enteric tissues are inappropriate samples for detection of IMNV infection.

### 3.4. Non-lethal sampling

Haemolymph or excised pleopods may be collected and used when non-lethal testing of valuable broodstock is necessary.

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

### 3.5. Preservation of samples for submission

Several factors can affect specimen quality during collection, handling and storage, such as exposure to light, heat, desiccation, and incomplete preservation. Hence, standard operating protocols or recommended practices should be followed at all steps of the diagnostic process.

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

#### 3.5.1. Samples for pathogen isolation

Not applicable.

#### 3.5.2. Preservation of samples for molecular detection

Tissue samples (pleopods, cephalothorax, muscle, haemolymph) for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower

grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

### 3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.3.0 *General information* (diseases of fish).

### 3.5.4. Samples for other tests

Not applicable.

## 3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose should only be recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger shrimp should be processed and tested individually. Small life stages such as PL or fry can be pooled to obtain the minimum amount of material for molecular detection.

## 4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

**Ratings for purposes of use.** For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

**Validation stage.** The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

**Table 4.1. WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals**

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis <sup>1</sup> of a suspect result from surveillance or presumptive diagnosis			
	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV	Early life stages <sup>2</sup>	Juveniles <sup>2</sup>	Adults	LV
Wet mounts												
Histopathology					++	++	++	2				
Cell culture												
Real-time RT-PCR	+	++	++	1	++	++	++	2	++	++	++	2
Conventional RT-PCR	+	++	++	1	++	++	++	1				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	1
<i>In-situ</i> hybridisation					+	+	+	1	+	++	++	1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods												
Other methods												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification;

Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.

<sup>1</sup>For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). <sup>2</sup>Susceptibility of early and juvenile life stages is described in Section 2.2.3.

Shading indicates the test is inappropriate or should not be used for this purpose.

#### 4.1. Wet mounts

Stained or unstained tissue squashes of affected skeletal muscle or of the LO may show abnormalities. Tissue squashes of skeletal muscle when examined with phase or reduced light microscopy may show loss of the normal striations. Fragmentation of muscle fibres may also be apparent. Squashes of the LO may show the presence of significant accumulations of spherical masses of cells called lymphoid organ spheroids (LOS) amongst normal LO tubules.

#### 4.2. Histopathology and cytopathology

Infection with IMNV in the acute and chronic phases can be presumptively diagnosed using histology (Bell & Lightner, 1988; Lightner, 2011; Lightner *et al.*, 2004; Poulos *et al.*, 2006). However, the lesions in striated muscles and LO are not pathognomonic for infection with IMNV. White tail disease of penaeid shrimp caused by the *P. vannamei* nodavirus (PvNV) can mimic infection with IMNV (Tang *et al.*, 2007).

Haematoxylin and eosin stained tissue sections from shrimp with acute-phase infection with IMNV present myonecrosis with characteristic coagulative necrosis of striated (skeletal) muscle fibres, often with marked oedema among affected muscle fibres. Some shrimp may present a mix of acute and older lesions. The affected muscle fibres appear to progress from presenting coagulative necrosis to liquefactive necrosis, which is accompanied by moderate infiltration and accumulation of haemocytes. In the most advanced lesions, haemocytes and inflamed muscle fibres are replaced by a loose matrix of fibrocytes and connective tissue fibres that are interspersed with haemocytes and foci of (presumed) regenerating muscle fibres (Lightner *et al.*, 2004; Poulos *et al.*, 2006).

Significant hypertrophy of the LO caused by accumulations of LOS is a highly consistent lesion in shrimp with acute or chronic-phase infection with IMNV lesions. Often, many ectopic LOS are found in other tissues not near the main body of the LO. Common locations for ectopic LOS include the haemocoelom in the gills, heart, near the antennal gland tubules, and ventral nerve cord (Lightner *et al.*, 2004; Poulos *et al.*, 2006).

#### 4.3. Cell culture for isolation

No crustacean cell lines exist, but IMNV was observed to propagate in C6/36 subclone of *Aedes albopictus* cell line (Kumar *et al.*, 2020). Performance of the test should be confirmed before being recommended.

#### 4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

##### *Extraction of nucleic acids*

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

Published methods are available for the molecular detection of IMNV by *in-situ* hybridisation (ISH), nested RT-PCR and quantitative real-time RT-PCR (Andrade *et al.*, 2007; Poulos *et al.*, 2006; Tang *et al.*, 2005). A nested RT-PCR kit for detection of the virus is available commercially.

##### 4.4.1. Real-time RT-PCR

A real-time RT-PCR method was developed to detect and quantify IMNV in shrimp tissue. The method which can detect as few as 10 IMNV RNA copies  $\mu\text{l}^{-1}$  total RNA (Andrade *et al.*, 2007) is summarised below.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Andrade <i>et al.</i> , 2007; GenBank Accession No.: AY570982			
IMNV Capsid protein gene	Fwd IMNV412F: GGA-CCT-ATC-ATA-CAT-AGC-GTT-GCA Rev IMNV545R: AAC-CCA-TAT-CTA-TTG-TCG-CTG-GAT Probe: CCA-CCT-TTA-CTT-TCA-ATA-CTA-CAT-CAT-CCC-CGG	300 Nm 200 nM	40 cycles of: 95°C/3 sec and 60°C/30 sec

#### 4.4.2. Conventional PCR

The nested RT-PCR method to detect IMNV uses two PCR primer sets that produce a 328 bp one-step amplicon and 139 bp two-step amplicon. The 1-step PCR can detect as little as 100 IMNV RNA copies and the 2-step PCR can detect in the order of 10 IMNV RNA copies (Poulos & Lightner, 2006).

Pathogen/ target gene	Primer (5'–3')	Concentration	Cycling parameters
Method 1: Poulos & Lightner, 2006; GenBank Accession No.: KJ636783.2; amplicon size: 328/139 bp			
IMNV Capsid protein gene (nested-PCR)	Primary Fwd 4587F: CGA-CGC-TGC-TAA-CCA-TAC-AA Rev 4914R: ACT-CGG-CTG-TTC-GAT-CAA-GT	200 nM	45 cycles of: 95°C/45 sec; 60°C/45 sec; 60°C/7 min
	Nested Fwd 4725 NF: GGC-ACA-TGC-TCA-GAG-ACA Rev 4863 NR: AGC-GCT-GAG-TCC-AGT-CTT-G	620 nM	39 cycles of: 95°C/30 sec, 65°C/30 sec, 72°C/30 sec; 72°C/2 min

#### 4.4.3. Other nucleic acid amplification methods

None.

#### 4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

#### 4.6. *In-situ* hybridisation

##### *DNA probe for ISH detection of IMNV*

A cDNA library was generated from RNA extracted from purified IMNV. A IMNV-specific ISH DNA probe is prepared from clone IMNV-317 by PCR labelling with digoxigenin-11-dUTP (DIG). The PCR primers used for amplification of the 993 bp probe are IMNV993F (5'-AAC-ACA-AAA-TCT-GCC-AGC-AA-3') and IMNV993R (5'-CCC-AAC-CAC-CCA-AAT-TCA-TA-3'). Following PCR, the DIG-labelled DNA probe is precipitated with ethanol, re-suspended in water and stored at -20°C until used. The ISH procedure for detecting IMNV follows that outlined by Tang *et al.* (2005). Negative and positive controls should be sourced from PCR-confirmed uninfected and infected shrimp, respectively.

#### 4.7. Immunohistochemistry

Monoclonal antibodies have been generated using recombinant IMNV capsid protein fragments to immunise mice (Kunanopparat *et al.*, 2011). Immunohistochemical analysis demonstrated strong reactivity in muscle, gill, heart, LO and connective tissue derived from IMNV-infected *P. vannamei* similar to that demonstrated by *in-situ* hybridisation (Tang *et al.*, 2005). There was no cross-reactivity to tissues derived from uninfected shrimp or shrimp infected with other viral pathogens such as WSSV, YHV, TSV among others.

#### 4.8. Bioassay

Not applicable.

#### 4.9. Antibody- or antigen-based detection methods

None are recommended, however an immunochromatographic strip test has been developed (Chaivisuthangkura *et al.*, 2013) using the monoclonal antibodies developed by Kunanopparat *et al.* (2011). While the test is simple, fast and low-cost it is approximately 300-fold less sensitive than one-step RT-PCR (Chaivisuthangkura *et al.*, 2013).

#### 4.10. Other methods

A chromatographic method for detection of PCR amplicons has been developed (Koiwai *et al.*, 2018).

### 5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time RT-PCR is the recommended test for surveillance to demonstrate freedom of infection with IMNV in apparently healthy populations as described in Section 4.1.1.

### 6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAHP Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free. There are currently no WOAHP Reference Laboratories designated for IMN.

#### 6.1. Apparently healthy animals or animals of unknown health status<sup>1</sup>

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

##### 6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with IMNV shall be suspected if at least one of the following criteria is met:

- i) Positive result by real-time RT-PCR
- ii) Positive result by conventional RT-PCR

##### 6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with IMNV is considered to be confirmed if the following criterion is met:

- i) Positive result by real-time RT-PCR and positive result by conventional RT-PCR followed by amplicon sequencing

<sup>1</sup> For example transboundary commodities.

## 6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

### 6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with IMNV shall be suspected if at least one of the following criteria is met:

- i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality
- ii) Positive result by conventional RT-PCR
- iii) Positive result by real-time RT-PCR
- iv) Histopathology consistent with the presence of the pathogen or the disease

### 6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with IMNV is considered to be confirmed if at least one of the following criteria is met:

- i) Positive result by real-time RT-PCR and a positive result by conventional RT-PCR followed by amplicon sequencing
- ii) Positive result by *in-situ* hybridisation and a positive result by real-time RT-PCR
- iii) Positive result by *in-situ* hybridisation and a positive result by conventional RT-PCR followed by amplicon sequencing

## 6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with IMNV are provided in Tables 6.3.1. and 6.3.2 (no data are currently available). This information can be used for the design of surveys for infection with IMNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

### 6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe ( <i>n</i> )	DSp ( <i>n</i> )	Reference test	Citation
Real-time PCR	Diagnosis	Experimentally infected SPF <i>P. vannamei</i>	abdominal muscle	<i>P. vannamei</i>	100 ( <i>n</i> =30)	100 ( <i>n</i> =30)	Histopathology	Andrade <i>et al.</i> (2007)

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR = polymerase chain reaction.

### 6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe ( <i>n</i> )	DSp ( <i>n</i> )	Reference test	Citation
Real-time PCR								

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of samples used in the study, PCR = polymerase chain reaction.

## 7. References

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**NB:** At the time of publication (2023) there was no WOA Reference Laboratory for infection with infectious myonecrosis virus (please consult the WOA web site: <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

**NB:** FIRST ADOPTED IN 2009. MOST RECENT UPDATES ADOPTED IN 2023.