**Annex 54. Item 9.1.4. – Chapter 2.2.9. Infection with yellow head virus genotype 1**

Chapter 2.2.9

infection with   
yellow head virus GENOTYPE 1

1. Scope

Infection with yellow head virus genotype 1 means infection with the pathogenic agent yellow head virus genotype 1 (YHV1) of the Genus *Okavirus* and Family *Roniviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Yellow head virus genotype 1 (YHV1; species *Yellow head virus*) is one of eight known genotypes in the yellow head complex of viruses and is the only known genotype that causes yellow head disease. YHV1 forms enveloped, rod-shaped particles 40–50 nm × 150–180 nm (Chantanachookin *et al.,* 1993; Wongteerasupaya *et al*., 1995). Envelopes are studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids appear as rods (diameter 20–30 nm) and possess a helical symmetry with a periodicity of 5–7 nm. Virions comprise three structural proteins (nucleoprotein p20 and envelope glycoproteins gp64 and gp116) and a ~26 kb positive-sense single-stranded RNA genome. The nucleotide sequence of the ORF1b region of the viral genome has been used to determine the phylogenetic relationships of YHV1 and other yellow head virus genotypes (Dong *et al*., 2017; Mohr *et al*., 2015; Wijegoonawardane *et al.,* 2008a).

YHV1, yellow head virus genotype 2 (YHV2; species *Gill-associated virus*) and yellow head virus genotype 8 (YHV8; species *Okavirus 1*) have been formally classified by the International Committee on Taxonomy of Viruses (Walker *et al*., 2021). Four other genotypes in the complex (YHV3–YHV6) occur commonly in healthy *Penaeus monodon* in East Africa, Asia and Australia and are rarely or never associated with disease (Walker *et al.,* 2001; Wijegoonawardane *et al.,* 2008a). Of the remaining two yellow head virus genotypes, YHV7 was detected in diseased *P. monodon* in Australia (Mohr *et al*., 2015) and YHV8 was detected in *P.* *chinensis* suspected of suffering from acute hepatopancreatic necrosis disease (Liu *et al*., 2014). There is evidence of genetic recombination between genotypes (Wijegoonawardane *et al.*, 2009).

2.1.2. Survival and stability in processed or stored samples

YHV1, identified by either transmission electron microscopy (TEM) (Nunan *et al.,* 1998), or molecular methods (Durand *et al.,* 2000; McColl *et al.,* 2004), has been detected in frozen commodity prawns with infectivity demonstrated by bioassay.

2.1.3. Survival and stability outside the host

YHV1 remains viable in aerated seawater for up to 72 hours (Flegel *et al.*, 1995b).

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 YHV1 according to Chapter 1.5 of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~blue shrimp (~~*~~Penaeus stylirostris~~*~~), dagger blade grass shrimp (~~*~~Palaemonetes pugio~~*~~), giant tiger prawn (~~*~~Penaeus monodon~~*~~), jinga shrimp (~~*~~Metapenaeus affinis~~*~~) and whiteleg shrimp (~~*~~Penaeus vannamei~~*~~).~~

| **Family** | **Scientific name** | **Common name** |
| --- | --- | --- |
| Palaemonidae | *Palaemonetes pugio* | dagger blade grass shrimp |
| Penaeidae | *Metapenaeus affinis* | jinga shrimp |
| *Penaeus monodon* | giant tiger prawn |
| *Penaeus stylirostris* | blue shrimp |
| *Penaeus vannamei* | whiteleg shrimp |

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 YHV1 according to Chapter 1.5 of the *Aquatic Code* are:

| **Family** | **Scientific name** | **Common name** |
| --- | --- | --- |
| Palaemonidae | *Palaemon serrifer* | carpenter prawn |
| *Palaemon styliferus* | Pacific blue prawn |
| *Macrobrachium sintangense* | Sunda river prawn |
| Parastacidae | *Cherax quadricarinatus* | red claw crayfish |
| Penaeidae | *Metapenaeus brevicornis* | yellow shrimp |
| *Penaeus aztecus* | northern brown shrimp |
| *Penaeus duorarum* | northern pink shrimp |
| *Penaeus japonicus* | kuruma prawn |
| *Penaeus merguiensis* | banana prawn |
| *Penaeus setiferus* | northern white shrimp |

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the

following species, but an active infection has not been demonstrated: ~~acorn barnacle~~~~(~~*~~Chelonibia patula~~*~~),~~~~blue crab (~~*~~Callinectes sapidus~~*~~),~~~~cyclopoid copepod (~~*~~Ergasilus manicatus~~*~~)~~*~~,~~* ~~gooseneck barnacle (~~*~~Octolasmis muelleri~~*~~),~~~~Gulf killifish~~~~(~~*~~Fundulus grandis~~*~~)~~~~and paste shrimp (~~*~~Acetes~~* ~~sp.)~~*~~.~~*

| **Family** | **Scientific name** | **Common name** |
| --- | --- | --- |
| Chelonibiidae | *Chelonibia patula* | acorn barnacle |
| Ergasilidae | *Ergasilus manicatus* | cyclopoid copepod |
| Fundulidae | *Fundulus grandis* | gulf killifish |
| Poecilasmatidae | *Octolasmis muelleri* | gooseneck barnacle |
| Portunidae | *Callinectes sapidus* | blue crab |
| Sergestidae | *Acetes sp.* | paste shrimp |

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

*Penaeus monodon* are susceptible to YHV1 infection beyond PL15 (Khongpradit *et al.*, 1995). Lightner *et al*. (1998) YHV1 challenge caused disease in juveniles of *Penaeus aztecus, P. duorarum, P. setiferus,* and *P. vannamei* but postlarvae appeared resistant (Lightner *et al*. 1998). YHV1 infections are usually detected only when disease is evident, however infections have been detected in healthy wild populations of *P. stylirostris* (Castro-Longoria *et al.,* 2008). Natural YHV1 infections have been detected in *P. japonicus*, *P. merguiensis*, *P. setiferus*, *M. ensis*, and *P. styliferus* (Cowley *et al.,* 2002; Flegel *et al.,* 1995a; 1995b).

2.2.4. Distribution of the pathogen in the host

YHV1 targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al*., 1993; Lightner, 1996).

2.2.5. Aquatic animal reservoirs of infection

YHV1 was detected by reverse-transcription polymerase chain reaction (RT-PCR) in clinically normal wild *P. stylirostris* collected for surveillance purposes in the Gulf of California in 2003 (Castro-Longoria *et al*., 2008). ~~The infectious nature of the YHV1 detected was confirmed by experimental infections. There is also evidence that YHV1 can persist in survivors of experimental infection (Longyant~~ *~~et al.~~*~~, 2005; 2006).~~

2.2.6. Vectors

There are no known vectors of YHV1.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

In farmed *P. monodon*, YHV disease can cause up to 100% mortality within 3–5 days of the first appearance of clinical signs (Chantanachookin *et al.,* 1993). Mortalities can be induced by experimental exposure of *P. monodon* to YHV1 (Oanh *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

Shrimp from late postlarvae (PL) stages onwards can be infected experimentally with YHV1. In cultured shrimp, infection can result in mass mortality occurring, usually in early to late juvenile stages. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax. However, these disease features are not particularly distinctive, and gross signs are not reliable even for preliminary diagnosis of YHV1.

Exceptionally high feeding activity followed by an abrupt cessation of feeding may occur within 2–4 days of the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface (Chantanachookin *et al.,* 1993).

2.3.3 Gross pathology

The yellow hepatopancreas of diseased shrimp may be exceptionally soft when compared with the brown hepatopancreas of a healthy shrimp (Chantanachookin *et al.,* 1993).

2.3.4. Modes of transmission and life cycle

YHV1 can be transmitted horizontally by ingestion of infected tissue, immersion in membrane-filtered tissue extracts, or by cohabitation with infected shrimp (Walker &Sittidilokratna, 2008). YHV1 replicates in the cytoplasm of infected cells in which long filamentous pre-nucleocapsids are abundant and virions bud into cytoplasmic vesicles in densely packed paracrystalline arrays for egress at the cytoplasmic membrane (Chantanachookin *et al.,* 1993).

2.3.5. Environmental factors

Elevated virus infection levels accompanied by disease can be precipitated by physiological stress induced by sudden changes in pH or dissolved oxygen levels, or other environmental factors (Flegel *et al*., 1997).

2.3.6. Geographical distribution

YHV1 has been reported in South-East Asia (Walker *et al.*, 2001). YHV1 has also been detected in *P. stylirostris* and *P. vannamei* in the Americas (Castro-Longoria *et al.,* 2008; Sanchez-Barajas *et al.*, 2009).

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

None available.

2.4.2. Chemotherapy including blocking agents

No effective commercial anti-viral product is yet available.

2.4.3. Immunostimulation

A multi-target dsRNA for simultaneous inhibition of YHV1 and white spot syndrome virus demonstrated inhibition of the two viruses when administered to shrimp by injection (Chaimongkon *et al.,* 2020)

2.4.4. Breeding resistant strains

Not reported.

2.4.5. Inactivation methods

YHV1 can be inactivated by heating at 60°C for 15 minutes (Flegel *et al.*, 1995b). Little information is available on other inactivation methods but the virus appears to be susceptible to treatment with chlorine at 30 parts per million (0.03 mg ml–1) (Flegel *et al.,* 1997).

2.4.6. Disinfection of eggs and larvae

Not reported.

2.4.7. General husbandry

The focus is to exclude YHV1 from entering production systems; for example, by using specific pathogen-free (SPF) stock, batch testing stock and biosecurity measures to reduce entry into culture systems.

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

For diagnosis during a disease outbreak, moribund shrimp collected from pond edges are the preferred source of material for examination. Apparently healthy shrimp should also be collected from the same ponds. For surveillance in populations of apparently healthy shrimp, life stages from PL stage 15 onwards can provide tissue sources useful for testing.

3.2. Selection of organs or tissues

In moribund shrimp suspected to be infected with YHV1, pleopods, gill and lymphoid organ are the most suitable sample tissues. For screening or surveillance of juvenile or adult shrimp that appear grossly normal, pleopods or gills are preferred.

3.3. Samples or tissues not suitable for pathogen detection

Not determined.

3.4. Non-lethal sampling

Haemolymph can be used for non-lethal sampling.

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

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for bioassay

The success of bioassay depends strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (absolute) 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 can be frozen at –20°C or below for 1 month or less; for long-term storage. –80°C is recommended.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

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 2.2.0 *General information* (diseases of crustaceans).

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 is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages 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 | 1. Surveillance of apparently  healthy animals | | | | 1. Presumptive diagnosis of  clinically affected animals | | | | 1. Confirmatory diagnosis1 of a suspect result from surveillance or presumptive diagnosis | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV | Early life stages2 | Juveniles2 | Adults | LV |
| Wet mounts |  |  |  |  |  |  |  |  |  |  |  |  |
| Histopathology |  |  |  |  |  | ++ | ++ | 1 |  |  |  |  |
| Cell culture |  |  |  |  |  |  |  |  |  |  |  |  |
| Real-time RT-PCR | ++ | +++ | +++ | 1 | ++ | +++ | +++ | 4 | ++ | +++ | +++ | 4 |
| Conventional RT-PCR | ++ | ++ | ++ | 1 | ++ | ++ | ++ | 1 |  |  |  |  |
| Conventional PCR followed by amplicon sequencing |  |  |  |  |  |  |  |  | ++ | +++ | +++ | 1 |
| *In-situ* hybridisation |  |  |  |  |  | ++ | ++ | 1 |  |  |  |  |
| Bioassay |  |  |  |  | + | + | + | 1 |  |  |  |  |
| ~~LAMP~~ Immunohistochemistry |  |  |  |  |  |  |  |  |  |  |  |  |
| Ab-ELISA |  |  |  |  |  |  |  |  |  |  |  |  |
| Ag-ELISA |  |  |  |  |  |  |  |  |  |  |  |  |
| ~~Other antigen detection methods~~~~3~~ |  |  |  |  |  |  |  |  |  |  |  |  |
| Other methods3 |  |  |  |  |  |  |  |  |  |  |  |  |

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.   
1For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). 2Susceptibility of early and juvenile life stages is described in Section 2.2.3.   
3Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

Fix the cephalothorax tissues of moribund shrimp suspected to be affected by YHV1 in Davidson’s fixative, prepare tissue sections and stain with Meyer’s haematoxylin and eosin (H&E) using standard histological procedures (Lightner, 1996). Examine tissues of ectodermal and mesodermal origin by light microscopy for the presence of moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2 µm in diameter or smaller (Chantanachookin *et al.,* 1993). Tissues of the lymphoid organ, stomach subcuticulum and gills are particularly informative.

Lymphoid organ spheroids are commonly observed in healthy *P. monodon* chronically infected with YHV1 or GAV and lymphoid organ necrosis often accompanies disease (Spann *et al.*, 1997). However, spheroid formation and structural degeneration of lymphoid organ tissue also result from infection by other shrimp viruses (Lightner, 1996).

4.3. Cell culture for isolation

Although primary shrimp cell culture methods are available, they are not recommended to isolate and identify YHV1 as a routine diagnostic method. No continuous cell lines suitable for YHV1 culture are available.

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.

4.4.1. Real-time PCR

~~Not available.~~

The protocol for the real-time RT-PCR for detection of YHV1 has been developed by the WOAH Reference Laboratory for YHV1. This assay is specific for genotype 1. Validation data are provided in the submitted validation report for diagnostic tests and confirm suitability to be recommended for inclusion in the *Aquatic Manual* (ADD LINK OR AGREED REFERENCE).

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen/  target gene | Primer/probe (5’–3’) | Concentration | Cycling conditions(a) |
| YHV1 /ORF1 | YHV1-12-qF: AGT-CTA-CAG-TGC-TCT-GAT-CT YHV1-12-qR: GAT-TCT-TGA-AGC-GCA-TGA-GT YHV1-12-qPr: FAM-TCT-CAT-GTG/ZEN/TCA-TGA-TAT-TCT-CAA-GCG-AGT-IABkFQ | 900 nM of each primer 250 nM of probe | Reverse transcription at 48°C/30 min 1 cycle 95°C/10 min 45 cycles of 95°C/15 sec and 60°C/60 sec |

(a)A denaturation step prior to cycling has not been included.

4.4.2. Conventional RT-PCR

Three RT-PCR protocols are described. For all conventional RT-PCR protocols, assignment to YHV genotype 1 can be achieved by nucleotide sequence analysis of the RT-PCR amplicon. Reference sequences for YHV1 include:

Protocol 1 is a 1-step RT-PCR that can be used to detect YHV1 in affected shrimp. The protocol is as described by Mohr *et al.* (2015) and adapted from Wongteerasupaya *et al.* (1997). This protocol will detect YHV1 but not GAV or any of the other genotypes currently recognised.

Protocol 2 is a more sensitive multiplex nested RT-PCR that can be used to differentiate YHV1 from GAV. The protocol is as described by Mohr *et al*. (2015) and adapted from Cowley *et al.* (2004). The first stage of the multiplex nested RT-PCR (primary RT-PCR) was designed to detect YHV1 and GAV but has been reported to also detect YHV7 (Mohr *et al*., 2015). Both the primary RT-PCR and the nested PCR detected the novel YHV genotype from China (People’s Rep. of) (Liu *et al*., 2014). In the second PCR step, a 277 bp product indicates detection of YHV and a 406 bp product indicates detection of GAV. The presence of both 406 bp and 277 bp products indicates a dual infection with GAV and YHV1. The nested PCR can be run as two separate assays specific for YHV1 or GAV by omitting either the G6 or Y3 primer, respectively. **NOTE:** Due to reported problems with primer specificity for some emerging strains, all PCR products generated using protocol 2 should be sequenced to confirm the virus genotype.

Protocol 3 is a multiplex nested RT-PCR protocol that can be used for screening shrimp for any of the seven genotypes of the yellow head complex of viruses. The protocol is as described by Mohr *et al*. (2015) and adapted from Wijegoonawardane *et al.* (2008b). Two primers were designed to each site, one accommodating sequence variations amongst YHV1 isolates and the other variations amongst isolates of the other genotypes (Wijegoonawardane *et al.*, 2008b). It is not known whether this assay will detect ~~the~~ YHV8 ~~genotype recently detected in China (People’s Rep. of)~~ (Liu *et al*., 2014).

***Primer sequences***

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen / target gene | Primer (5’–3’) | Concentration | Cycling parameters(a) |
| Protocol 1 (Wongteerasupaya *et al.,* 1997; GenBank Accession No.: [FJ848675.1](https://www.ncbi.nlm.nih.gov/nucleotide/FJ848675.1?report=genbank&log$=nuclalign&blast_rank=1&RID=FB5MGCFC01R); amplicon size: 135 bp) | | | |
| YHV1 / ORF1b | 10F: CCG-CTA-ATT-TCA-AAA-ACT-ACG 144R: AAG-GTG-TTA-TGT-CGA-GGA-AGT | 180 nM 180 nM | 40 cycles of 94°C/30sec, 58°C/45 sec, 68°C/45 sec, |
| Protocol 2 (Cowley *et al.,* 2004; GenBank Accession No.: [FJ848675.1](https://www.ncbi.nlm.nih.gov/nucleotide/FJ848675.1?report=genbank&log$=nuclalign&blast_rank=1&RID=FB5MGCFC01R)) | | | |
| YHV1 and GAV / ORF1b | Primary (Amplicon size: 794 bp) GY1: ~~5~~GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG GY4: GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG  Nested for detection of YHV1 (Amplicon size: 277 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA Y3: ACG-CTC-TGT-GAC-AAG-CAT-GAA-GTT  Nested for detection of GAV (Amplicon size: 406 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA G6: GTA-GTA-GAG-ACG-AGT-GAC-ACC-TAT | 180 nM 180 nM  360 nM 360 nM  360 nM 360 nM | 35 cycles of 95°C/30 sec, 66°C/30 sec, and 68°C/45 sec |
| Protocol 3 (Wijegoonawardane *et al.,* 2008b; GenBank Accession No.: [FJ848675.1](https://www.ncbi.nlm.nih.gov/nucleotide/FJ848675.1?report=genbank&log$=nuclalign&blast_rank=1&RID=FB5MGCFC01R)) | | | |
| YHV1 to YHV7 / ORF1b | Primary (amplicon size: 359 bp) YC-F1ab pool:  ATC-GTC-GTC-AGC-TAC-CGC-AAT-ACT-GC ATC-GTC-GTC-AGY-TAY-CGT-AAC-ACC-GC  YC-R1ab pool: TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC  Nested (amplicon size: 147 bp) YC-F2ab pool: CGC-TTC-CAA-TGT-ATC-TGY-ATG-CAC-CA CGC-TTY-CAR-TGT-ATC-TGC-ATG-CAC-CA  YC-R2ab pool: RTC-DGT-GTA-CAT-GTT-TGA-GAG-TTT-GTT GTC-AGT-GTA-CAT-ATT-GGA-GAG-TTT-RTT Mixed base codes: R(AG), Y(CT), M(AC), K(GT), S(GC), W(AT), H(ACT), B(GCT), V(AGC), D(AGT), N(AGCT). | 180 nM 180 nM    180 nM 180 nM    180 nM 180 nM    180 nM 180 nM | 35 cycles of 94°C/45 sec, 60°C/45 sec, 68°C/45 sec,          35 cycles of 94°C/45 sec, 60°C/45 sec, 72°C/45 sec; |

(a)A denaturation step prior to cycling has not been included.

The Protocol 2 Y3 primer contains a mismatch for GAV but is specific for YHV1. The mismatch can cause false-positives with GAV in the nested PCR of protocol 2 where GAV generates an amplicon of very similar size to the expected size of the YHV1 amplicon. ~~For GAV, the 7th base from left (T) is substituted for C so that the primer sequence for GAV should be 5’-CAT-CTG-CCC-AGA-AGG-CGT-CTA-TGA-3’, according to the sequence data of the GAV genome (database accession numbers: NC\_010306.1 and AF227196.2).~~

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

4.4.3. Other nucleic acid amplification methods

Not available.

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

The *in-situ* hybridisation (ISH) protocol of Tang *et al.* (2002) is suitable for detecting YHV1 or GAV (Tang & Lightner, 1999). To preserve viral RNA accessibility, fix tissues sampled from live shrimp in neutral-buffered, modified Davidson’s fixative without acetic acid (RF-fixative) (Hasson *et al.*, 1997). To achieve good tissue preservation whilst also preserving RNA accessibility, normal Davidson’s fixative can be used as long as the fixation time is limited to 24 hours (maximum of 48 hours). Detailed methods can be found in Tang *et al.* (2002) YHV-infected cells give a blue to purple-black colour against the brown counter stain. Positive controls of YHV-infected tissue and negative controls of uninfected shrimp tissue should be included. The digoxigenin-labelled DNA probe can be prepared by PCR labelling using the following primers:

YHV1051F: 5’-ACA-TCT-GTC-CAG-AAG-GCG-TC-3’

YHV1051R: 5’-GGG-GGT-GTA-GAG-GGA-GAG-AG-3’

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

The bioassay procedure is based on that described by Spann *et al.* (1997), but similar procedures have been described by several other authors (e.g. Lu *et al.,* 1994). The bioassay should be conducted in susceptible shrimp that have been determined to be free from YHV complex viruses.

Suspect YHV1-infected samples should be maintained at 4°C or on ice. If necessary, the whole shrimp or the retained cephalothorax may be snap-frozen and stored at –80°C or in liquid nitrogen until required. Viral inoculum should be prepared as described by Spann *et al.* (1997).

Juvenile shrimp of a known susceptible species are injected with viral inoculum. Negative controls (buffer injected) and positive controls (known YHV1 positive material) treatment groups are required. Shrimp should be maintained separately to prevent cross-contamination between treatments. Observe the shrimp and record mortalities for at least 21 days or until the test and positive control groups reach 100% mortality.

Dead shrimp can be processed for PCR and sequence analysis. The surviving shrimp are processed for gross signs, histopathology, PCR and sequence analysis. A positive result is indicated by the detection of gross signs and characteristic histological lesions, and by PCR and amplicon sequence analysis. The negative control shrimp must remain negative for at least 21 days for gross or histological signs of infection with YHV1.

4.9. Antibody~~-~~ or antigen-based detection methods (ELISA, etc.)

None has been successfully developed.

4.10. Other methods

None at present.

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

~~Nested~~ Real-time RT-PCR ~~(Protocol 3)~~ is recommended for demonstrating freedom from YHV1 in ~~an~~ apparently healthy populations. ~~Sequencing of any amplified PCR products is required to determine the YHV genotype. Two-step PCR negative results are required for YHV1.~~

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 a suspect and confirmed case 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 WOAH 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.

6.1. Apparently healthy animals or animals of unknown health status[[1]](#footnote-2)

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 YHV1 shall be suspected if the following criterion is met:

i) Positive result by ~~a recommended~~ conventional RT-PCR ~~detection test~~

ii) Positive result by real-time RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with YHV1 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

ii) ~~A positive result by conventional RT-PCR and identification of YHV1 by sequence analysis of the amplicon from each of two different RT-PCR methods followed by sequence analysis of the amplicons to identify YHV1~~

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 YHV1 shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs consistent with YHV1 infection

ii) Histopathology consistent with YHV1 infection

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result by ISH

vi) Positive result by bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with YHV1 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

i) ~~A positive result from each of two different RT-PCR methods targeting non-overlapping parts of the genome followed by sequence analysis of the amplicons to identify YHV1~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with YHV1 are provided in Tables 6.3.1. and 6.3.2 (no data are currently available for either). ~~This information can be used for the design of surveys for infection with YHV1, 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 (*n*) | DSp (*n*) | Reference test | Citation |
| YHV1 RT-qPCR | Diagnosis | Infected by co-habitation or feeding | Pleopods | *Penaeus monodon, P. merguiensis* | 100% (n=130) | 100% (n=130) | Real-time PCR | [Validation report](https://www.woah.org/app/uploads/2024/03/validation-report-template-yhv1.pdf) |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation 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 (*n*) | DSp (*n*) | Reference test | Citation |
|  |  |  |  |  |  |  |  |  |

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

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\* \*

**NB:** There is a WOAH Reference Laboratory for infection with yellow head virus genotype 1  
(please consult the WOAH web site:   
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).  
Please contact the WOAH Reference Laboratories for any further information on   
infection with yellow head virus genotype 1

**NB:** First adopted in 1995 as Yellowhead disease. Most recent updates adopted in 2019.

1. For example transboundary commodities. [↑](#footnote-ref-2)