**USA COMMENTS IN RED FONT**

**Annex 57. Item 8.2.2. – Chapter 2.4.1. Infection with abalone herpesvirus**

Chapter 2.4.1.

infection with abalone herpesvirus

1. Scope

Infection with abalone herpesvirus means infection with the pathogenic agent *Aurivirus haliotidmalaco1* (~~commonly~~ previously known as *Haliotid herpesvirus 1*, and *abalone herpesvirus* [AbHV~~-1~~]) of the genus *Aurivirus* and the Family *Malacoherpesviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

*Aurivirus haliotidmalaco1* ~~AbHV-1~~ is the aetiological agent of abalone viral ganglioneuritis (AVG); for the purpose of this chapter, the agent will be referred to as AbHV. AVG is a contagious disease of abalone species in Australia (Ellard *et al.,* 2009; Hooper *et al.,* 2007), China (People’s Rep. of) (Gu *et al*., 2019; Wang *et al.,* 2004) and Chinese Taipei (Chang *et al.,* 2005). Comparison of nucleotide sequences of the Victorian isolate of AbHV~~-1~~ and ostreid herpesvirus-1 (Davison *et al.,* 2009; Le Deuff & Renault, 1999) over common coding regions identified similarities ranging from 19% to 53%, indicating that these viruses share a low level of sequence similarity (Savin *et al.,* 2010). AbHV~~-1~~ has been assigned as a second member of the *Malacoherpesviridae* (ICTV, 2022). Complete genome sequences of isolates demonstrated that there are at least five genetic variants of AbHV~~-1~~ within Australia (Cowley *et al*., 2012; Corbeil *et al*., 2016) and one Chinese Taipei strain (Chang *et al*., 2005). More recent analysis demonstrated that the Chinese strain represents a further variant (Bai *et al*., 2019b).

Purified AbHV~~-1~~ particles (Tan *et al.,* 2008) observed by transmission electron microscopy are enveloped and icosahedral with electron dense cores and 100–110 nm in diameter. The intranuclear location of AbHV~~-1~~ particles, their size and ultrastructure are characteristic of members of the *Herpesviridae*. Isopycnic gradient centrifugation (in potassium tartrate and caesium chloride density gradients) indicated a virus particle buoyant density of 1.17–1.18 g ml–1 (Tan *et al.,* 2008).

2.1.2. Survival and stability in processed or stored samples

Virus derived from tissue obtained from experimentally infected abalone that had been homogenised in sterile EMEM Gibco) containing 10% fetal bovine serum, centrifuged (1500 **g** for 20 minutes at 4°C), filtered (0.22 µM) and stored as 250 µl aliquots in liquid nitrogen remains infectious for at least 21 months (Corbeil *et al*., 2012b).

2.1.3. Survival and stability outside the host

Experimental studies (Corbeil *et al*., 2012b) demonstrated that AbHV~~-1~~ remained infectious for up to 5 days when held in seawater at 4°C and for only 1 day at 15°C.

2.2. Host factors

Acute disease was first reported in farmed *Haliotis diversicolor supertexta* in Chinese Taipei (Chang *et al*., 2005). Subsequently, disease outbreaks occurred in both farmed and wild abalone populations in Australia in all age classes of *H. rubra*, *H. laevigata*, and their hybrids (Hooper *et al.,* 2007). AbHV~~-1~~ is also suspected to be the aetiological agent of an epizootic disease that devastated the abalone aquaculture industry in southeastern China (People’s Rep. of) starting in 1999 and continuing through the early 2000s (Gu *et al*., 2019; Wei *et al*., 2018; Wu & Zhang, 2016). Interestingly, New Zealand päua (*H. iris*) was highly resistant to experimental infection (Corbeil *et al*., 2017).

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with AbHV~~-1~~ according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~small abalone (~~*~~Haliotis diversicolor~~*~~), Greenlip abalone (~~*~~Haliotis laevigata~~*~~), Blacklip abalone (~~*~~Haliotis rubra~~*~~) and hybrids of Greenlip × Blacklip abalone (~~*~~Haliotis laevigata~~* ~~x~~ *~~Haliotis rubra~~*~~).~~

|  |  |  |
| --- | --- | --- |
| **Family** | **Scientific name** | **Common name** |
| Haliotidae | *Haliotis diversicolor* | small abalone |
| *Haliotis laevigata* | greenlip abalone |
| *Haliotis rubra* | blacklip abalone |
| *Haliotis laevigata × H. rubra* | hybrid of greenlip × blacklip abalone |

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 AbHV~~-1~~ according to Chapter 1.5 of the *Aquatic Code* are: none ~~Japanese abalone (~~*~~Haliotis discus~~*~~) and Rainbow abalone (~~*~~Haliotis iris~~*~~)~~.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: ~~none.~~

|  |  |  |
| --- | --- | --- |
| **Family** | **Scientific name** | **Common name** |
| Haliotidae | *Haliotis discus* | Japanese abalone |
| *Haliotis iris* | rainbow abalone |

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

All age classes of *H. diversicolor, H. rubra*, *H. laevigata*, and hybrids of *H. rubra* × *H. laevigata* appear to be highly susceptible to disease (Corbeil 2020; Gu *et al.,* 2019).

2.2.4. Distribution of the pathogen in the host

The major histopathological lesion identified in abalone affected with AVG is ganglioneuritis: inflammation confined to neural tissue. The cerebral, pleuropedal and buccal ganglia can be affected as well as the cerebral commissure and associated peripheral nerves (Bai *et al*., 2019a; Chang & Handlinger, 2022; Hooper *et al.,* 2007). The Chinese variant is also able to infect and replicate in haemocytes of *H. diversicolor* (Bai *et al.,* 2020)

2.2.5. Aquatic animal reservoirs of infection

No information available.

2.2.6. Vectors

No information available.

2.3. Disease pattern

Outbreaks of AVG in both farmed and wild abalone populations in Australia are associated with the rapid onset of high mortality rates (up to 90%) in all age classes (Corbeil *et al.,* 2010). Similarly, in Chinese Taipei, during the epizootic in cultured abalone (the water temperature was 16–19°C), both adult and juvenile abalone suffered from the disease, with cumulative mortalities of 70–80%. It was reported that death of all of the abalone in a pond could occur within 3 days of the onset of clinical signs (Chang *et al.,* 2005). A similar disease pattern occurred with experimental infections (Chang *et al.,* 2005; Crane *et al.,* 2009).

2.3.1. Mortality, morbidity and prevalence

In on-farm epizootics in Australia cumulative mortality in all age classes can reach >90%. In experimental trials, 100% mortality can occur within 5 days post-exposure. Most abalone that display gross signs are likely to die within 1–2 days.

In Australia, and similarly in Chinese Taipei, an outbreak of AVG is associated with a rapid rise in mortality rate (up to 90% or more). Affected abalone demonstrating clinical signs (e.g. curling of the foot) are likely to die within 1 day of showing these signs. Ganglioneuritis is observed in sections of neural tissue by light microscopy and confirmation of the presence of AbHV~~-1~~ is obtained by real-time PCR or *in-situ* hybridisation (Crane *et al.,* 2016). The precise prevalence of AVG in wild abalone populations in Australian waters is unknown. The first epidemiological study undertaken in China (People’s Rep. of), using real-time PCR (Gu *et al*., 2019), revealed a detection rate of 27–30% in abalone (*H. diversicolor* and *H. discus hannai*) farms with both healthy and diseased abalone.

2.3.2. Clinical signs, including behavioural changes

AVG outbreaks in both farmed and wild abalone were associated with high mortality rates (up to 90% on farm). Clinically, abalone may demonstrate one or more of the following signs: irregular peripheral concave elevation of the foot; swollen and protruding mouth parts; eversion of the radula; minimal movement of the pedal muscle; excessive mucus production; absence of the marked extension of the foot shown in the righting reflex when healthy abalone are turned onto their backs; reduced pedal adhesion to the substrate. In Tasmania, abalone affected by AVG in processing plants exhibited ‘hard foot’ or tetany, excessive mucus production, abnormal spawning and ‘bloating’ (Ellard *et al.,* 2009). These facilities also experienced much lower morbidity and mortality rates than reported on farms or in wild abalone in Victoria, Australia. Similar signs have been reported for an abalone disease epizootic in Chinese Taipei (Chang *et al.,* 2005).

AVG is normally an acute disease, with abalone dying within 1–2 days of demonstrating gross signs of the disease. Wild harvested abalone held in live-holding facilities in Tasmania have previously exhibited slower onset of clinical signs and mortality. Some Tasmanian wild caught abalone have previously tested positive for AVG using real-time PCR without overt clinical or histological signs.

2.3.3 Gross pathology

Abalone that are loosely attached to the substrate owing to weakness or abnormalities of the pedal muscle should be selected for sampling. If this gross pathology is caused by acute AVG, it is likely that these abalone will die within 1–2 days.

2.3.4. Modes of transmission and life cycle

Horizontal transmission (Bai *et al.,* 2019a; Chang *et al.,* 2005; Crane *et al.,* 2009) has been demonstrated experimentally by:

1. exposing healthy abalone to water containing diseased abalone in the same tank without direct contact between the diseased and healthy abalone;

2. placing healthy abalone in water that was previously inhabited by diseased abalone.

In all cases, 100% mortality was observed with a preclinical period of 1–2 days following exposure and then mortality commenced until 100% mortality occurred within 2–5 days post-infection.

2.3.5. Environmental factors

In Australia, the initial outbreak of AVG occurred on a farm during summer 2005/2006 and subsequently appeared to spread to wild populations, which experienced mortality throughout the following year i.e. during all seasons. All experimental infections to date have been carried out in the temperature range 15–18°C. In Chinese Taipei, during the reported epizootic, the water temperature was 16–19°C, and experimental infections were carried out at 17–20°C. In China (People’s Rep. of), natural infections were only detected at water temperatures below 23°C (Gu *et al*., 2019). How temperature affects viral replication and onset of disease has yet to be determined. The possible effects of changes in other environmental factors such as salinity and dissolved oxygen are unknown.

2.3.6. Geographical distribution

Reported in Asia-Pacific.

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.

2.4.2. Chemotherapy including blocking agents

No data available.

2.4.3. Immunostimulation

No data available.

2.4.4. Breeding resistant strains

No data available.

2.4.5. Inactivation methods

AbHV~~-1~~ was inactivated by treatment with 50 ppm of the iodophor Buffodine® as well as a 1% solution of the non-ionic surfactant Impress®. Calcium hypochlorite (1.5 ppm) treatment also inactivated the virus (Corbeil *et al*., 2012b).

2.4.6. Disinfection of eggs and larvae

No data available.

2.4.7. General husbandry

To date, experimental data indicates that AbHV~~-1~~ is highly virulent. Practices that could be implemented to reduce the severity of the disease have not been identified. It is interesting to note that, in contrast to the situation in Victoria, Australia, clinical disease has not been reported in wild abalone populations in Tasmania, Australia. Disease outbreaks in processing plants in Tasmania suggest that stress factors may influence expression of subclinical infection.

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

At the first signs of increased numbers of abalone appearing to be weak or behaving abnormally, or sudden onsets of unexplained mortality, live moribund individuals should be selected for sampling. If moribund or freshly dead abalone are not available, samples of overtly normal abalone from all parts of the farm, and representing all age classes, should be selected for sampling.

3.2. Selection of organs or tissues

Neural tissue that includes the cerebral, pleuropedal and buccal ganglia.

3.3. Samples or tissues not suitable for pathogen detection

To date, lesions have not been detected consistently in non-neural tissues.

3.4. Non-lethal sampling

Not available.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.4.0 *General information* (*diseases of molluscs*).

3.5.1. Samples for pathogen isolation

The results of bioassay depend 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 (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.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

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.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

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 such as larvae 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 |  |  |  |  |  |  |  |  |  |  |  |  |
| Imprints |  |  |  |  |  |  |  |  |  |  |  |  |
| ~~Histopathology~~ |  | ~~+~~ | ~~+~~ | ~~NA~~ |  | ~~++~~ | ~~++~~ | ~~2~~ |  | ~~++~~ | ~~++~~ | ~~2~~ |
| Transmission electron microscopy |  |  |  |  |  | + | + | NA |  | ~~+~~ | ~~+~~ | ~~NA~~ |
| Real-time PCR |  | +++ | +++ | 2 |  | +++ | +++ | 2 |  | +++ | +++ | 2 |
| Conventional PCR |  |  |  |  |  | ++ | ++ | 2 |  |  |  |  |
| Conventional PCR followed by amplicon sequencing |  |  |  |  |  |  |  |  |  | +++ | +++ | 2 |
| *In-situ* hybridisation |  |  |  |  |  | ++ | ++ | NA |  | ++ | ++ | NA |
| Bioassay |  |  |  |  |  | + | + | NA |  |  |  |  |
| 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.   
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.   
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Electron microscopy/cytopathology

Transmission electron microscopy is not a routine diagnostic method but can be used to confirm the presence of viral particles in infected ganglia. AbHV~~-1~~ particles are icosahedral with electron dense cores and a diameter of 100–110 nm. The intranuclear location of the particles and their ultrastructure are characteristic of members of the *Herpesviridae* (Tan *et al.,* 2008).

Tissue samples (containing pleuropedal ganglion) for examination by electron microscopy should be fixed using 2.5% (v/v) glutaraldehyde and 2–4% (v/v) paraformaldehyde in 0.1 M cacodylate buffer and post-fixed in 1% (w/v) osmium tetroxide, washed in reverse osmosis water (3 × 5 minutes), dehydrated in a graded series of ‘analytical grade’ ethanol (70%, overnight at 4°C; 95%, 20 minutes; 100%, 3 × 20 minutes), infiltrated in 100% Spurr’s resin (overnight) and then embedded in Spurr’s resin.

4.3. Histopathology

Neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) is the prime target and should be sampled, fixed ~~(using 10% formalin)~~ and processed using standard procedures, and stained with haematoxylin and eosin for histological examination as specified in Chapter 2.4.0.

Abalone affected with AVG demonstrate inflammation (increased infiltration by haemocytes) and necrosis confined to neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) as observed in histological sections of neural tissue stained with haematoxylin and eosin and examined by light microscopy (Chang & Handlinger, 2022; Ellard *et al.,* 2009; Hooper *et al.,* 2007).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* of Chapter 2.4.0 *General information* (diseases of molluscs). An 18S rDNA real-time PCR can be used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane *et al.,* 2016). 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

Following validation of the real-time PCR test targeted to ORF49 (Corbeil *et al.,* 2010), the discovery of genotypic variants in Australia not recognised by this test necessitated other real-time PCR tests to be developed based on more conserved regions of the viral genome. Real-time PCR tests targeted to ORF49 and ORF66 have been used extensively in disease investigations and the accumulated data have been used in test validation (Caraguel *et al*., 2019). For the detection of all genetic variants, the ORF49 and ORF66 real-time PCR tests should be run in parallel, and infection with AbHV can be confirmed by a positive result from either of the two tests. Each of these tests can be multiplexed with an 18S rDNA real-time PCR test, used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane et al., 2016).

***Primers and probes* (*sequences*)**

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen/ target gene | Primer/probe (5’–3’) | Concentration | Cycling parameters(a) |
| Crane *et al.,* 2016; GenBank Accession No.: MW412419.1 | | | |
| AbHV ORF49 | ORF49F1: AAC-CCA-CAC-CCA-ATT-TTT-GA ORF49R1: CCC-AAG-GCA-AGT-TTG-TTG-TT 49Prb1: 6FAM-CCG-CTT-TCA-ATC-TGA-TCC-GTG-G-TAMRA | 300 nM 300 nM 100 nM | 50 cycles of: 95°C/3 sec and 62°C/30 sec |
| Crane *et al.,* 2016; GenBank Accession No.: MW412419.1 | | | |
| AbHV ORF66 | ORF66F1: TCC-CGG-ACA-CCA-GTA-AGA-AC ORF66R1: CAA-GGC-TGC-TAT-GCG-TAT-GA 66Prb1: 6FAM-TGG-CCG-TCG-AGA-TGT-CCA-TG-TAMRA | 300 nM 300 nM 100 nM | 50 cycles of: 95°C/3 sec and 60°C/30 sec |

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

4.4.2. Conventional PCR

Conventional PCR may also be used for detection of AbHV~~-1~~ in tissue samples. Nucleic acid is extracted as described above. The AbHV1617 PCR has been shown to generate amplicons of various length (522bp to 588bp) depending on the AbHV~~-1~~ isolate. Thus, it is potentially useful for epidemiological studies and to confirm positive real-time PCR results (Crane *et al*., 2016). A second PCR targeting the Taiwanese AbHV~~-1~~ DNA polymerase gene has also been developed (Chen *et al*., 2012). The primer sequences for the two tests are detailed below.

***Primer sequences***

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen / target gene | Primer (5’–3’) | Concentration | Cycling parameters(a) |
| Method 1: Crane *et al.,* 2016; GenBank Accession No.:MW412419.1 amplicon size: 522–588 bp (depending on genetic variant) | | | |
| AbHV | AbHV-16: GGC-TCG-TTC-GGT-CGT-AGA-ATG AbHV-17: TCA-GCG-TGT-ACA-GAT-CCA-TGT-C | 360 nM 360 nM | 40 cycles of: 94°C/30 sec and 52°C/30 sec |
| Method 2: Chen *et al*., 2012; GenBank Accession No.: HQ317456; amplicon size: 606 bp | | | |
| AbHV | 40f: TCC-ATC-GAG-ATT-CCC-AGT-TC  146r: ACG-CCA-CCC-TGT-ATA-ACG-AG | 400 nM 400 nM | 35 cycles of: 94°C/60 sec and 52°C/60 sec |

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

4.4.3. Other nucleic acid amplification methods

A loop-mediated isothermal amplification assay for rapid and sensitive detection of AbHV~~-1~~ has been developed that is 100-fold more sensitive than conventional PCR (Chen *et al*., 2014) but is not widely used because of false positive and false negative results.

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

*In-situ* hybridisation localises AbHV~~-1~~-infected cells within the neural tissue which, on histological examination, demonstrates ganglioneuritis typified by an inflammatory change with increased cellularity involving mainly haemocytes and glial cells, and cell necrosis in the affected nerves (Mohammad *et al.,* 2011).

The *in-situ* hybridisation (ISH) procedure uses a digoxygenin (DIG)-labelled DNA probe to detect AbHV~~-1~~ in formalin-fixed, paraffin-embedded (FFPE) tissue sections and is described in Crane *et al.* (2016).

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

A bioassay is not normally required for routine diagnosis. However, when there is a suspect case due to the presence clinical signs and/or histopathology but molecular tests yield negative results, a bioassay (Corbeil *et al*., 2012a) can be used for confirmation of the presence of a previously unknown genetic variant. Homogenised and clarified neural tissue is used as inoculum and injected (i.m.) into the foot of known uninfected susceptible abalone host species. The inoculated abalone are monitored for clinical signs such as loss of adhesion to the substrate and then samples taken for histology, molecular analyses and electron microscopy. If presence of a herpesvirus is confirmed by electron microscopy further investigation such as whole genome sequencing should be initiated.

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

None currently available.

4.10. Other methods

None.

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

The real-time PCR assays targeting ORF49 and ORF66 performed in parallel is recommended for surveillance to demonstrate freedom in apparently health populations (Caraguel *et al*., 2019).

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

i) Positive result by a real-time PCR

ii) Positive result by conventional PCR

~~Histopathological changes consistent with the presence of the pathogen~~

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of Infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

1. Positive results by real-time PCR and positive result by conventional PCR followed by sequence analysis of the amplicon

ii) Positive results by *in-situ* hybridisation and positive result by conventional PCR followed by sequence analysis of the amplicon

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 abalone herpesvirus 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 a real-time PCR

iii) Positive result by conventional PCR

iv) Histopathological changes consistent with the presence of the pathogen or the disease

v) Positive result of a bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

1. Positive results by real-time PCR and by conventional PCR followed by sequence analysis of the amplicon
2. Positive results by *in-situ* hybridisation and by conventional PCR followed by sequence analysis of the amplicon

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with abalone herpesvirus are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with abalone herpesvirus, 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 |
| Real-time PCR | Diagnosis | Clinically diseased abalone from the wild and processing plants | Pleuropedal ganglion or pedal nerve cords | *Haliotis rubra* | 100 (48) | 100 (48) | Histopathology | Corbeil  *et al*., 2010 |
| Conventional PCR |  |  |  |  |  |  |  |  |
| ~~Histopathology~~ |  |  |  |  |  |  |  |  |

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

**QUESTION:** Can the Commission please clarify whether “n” means individual animals were tested or whether there was pooling of animals for testing?

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 |
| Real-time PCR | Surveillance | Naturally AbHV~~-1~~ infected wild and farmed populations; AbHV~~-1~~ free populations | Pleuropedal ganglion or pedal nerve cords | *Haliotis laevigata*; *H. rubra*; *H. laevigata* x *H. rubra* hybrids | 90.1 (1452) | 97.7 (1452) | Histopathology | Caraguel  *et al.,* 2019 |
| Histopathology | Surveillance | Naturally AbHV-~~1~~ infected wild and farmed populations; AbHV~~-1~~ free populations | Pleuropedal ganglion or pedal nerve cords | *Haliotis laevigata*; *H. rubra*; *H. laevigata* x *H. rubra* hybrids | 6.3 (1452) | 100 (1452) | real-time PCR | Caraguel  *et al.,* 2019 |

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

**QUESTIONS:**

1. For the Article 6.3.2. For surveillance of apparently healthy animals, can the Aquatic Code Commission please explain why “infected” populations were included in the source populations for “apparently healthy animals”?
2. The change from “AbHV-1” to “AbHV” was missed here and should be changed for consistency throughout this chapter.
3. Can the Commission please clarify whether “n” means individual animals were tested or whether there was pooling of animals for testing?

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

**NB:** First adopted in 2012.

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