CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

1. Scope

For the purpose of this chapter, infection with abalone herpesvirus (AbHV) is considered to be infection with the herpesvirus known to cause disease in abalone.

2. Disease information

2.1. Agent factors

AbHV is the aetiological agent of abalone viral ganglioneuritis (AVG), a contagious disease of abalone species in Australia (Ellard *et al*., 2009; Hooper *et al*., 2007) and possibly of species in other countries (Chang *et al*., 2005; Wang *et al*., 2004). Comparison of nucleotide sequences of AbHV VIC and ostreid herpesvirus-1 (Davidson *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), and it has been assigned tentatively as a second member of the Malacoherpesviridae.

Purified AbHV 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 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).

2.1.1. Aetiological agent, agent strains

Herpesviruses associated with abalone disease have been reported from Chinese Taipei and Australia. Comparison of nucleotide sequences of AbHV VIC and AbHV Taiwan over three common regions identified similarities of 92.4, 96.4, and 96.6%, indicating that these virus share a high level of sequence similarity. Recent genome sequence analyses have indicated that a number of genotypic variants are present in Australia (Cowley *et al*., 2011). Whether these AbHV genotypes vary in phenotype and virulence has yet to be determined.

2.1.2. Survival outside the host

Under investigation.

2.1.3. Stability of the agent

Under investigation.

2.1.4. Life cycle

Not applicable.

2.2. Host factors

2.2.1. Susceptible host species

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

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 abalone herpesvirus according to Chapter 1.5 of the *Aquatic Code* are: none known.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Japanese abalone (*Haliotis discus*) and rainbow abalone (*Haliotis iris*).

2.2.3. Species or subpopulation predilection (probability of detection)

No data.

2.2.4. Target organs and infected tissue

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 (Hooper *et al.,* 2007).

2.2.5. Persistent infection with lifelong carriers

No data.

2.2.6. Vectors

No data.

2.2.7. Known or suspected wild aquatic animal carriers

No data.

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. Transmission mechanisms

Horizontal transmission (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; and
3. intramuscular injection of healthy abalone with a filtered tissue homogenate from 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.

2.3.2. Prevalence

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 is obtained by quantitative polymerase chain reaction (qPCR) and/or *in-situ* hybridisation (Crane *et al.,* 2009). Using these methods there have been very few false-positive or false-negative results reported. The precise prevalence of AVG in wild abalone populations in Australian waters is unknown.

2.3.3. Geographical distribution

Australia (Victoria and Tasmania), Chinese Taipei.

2.3.4. Mortality and morbidity

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.

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. 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.

2.4. Control and prevention

In the absence of effective anti-viral treatments, implementing high levels of on-farm and live-holding facility biosecurity and regional movement restrictions is recommended. Following an on-farm outbreak, the destruction of infected stock, disinfection of water and equipment, and fallowing procedures appear to be effective at preventing reinfection. Sentinel abalone can be used to test the status of the previously infected premises prior to restocking.

2.4.1. Vaccination

No vaccines available.

2.4.2. Chemotherapy

No data.

2.4.3. Immunostimulation

No data.

2.4.4. Resistance breeding

No data.

2.4.5. Restocking with resistant species

No data.

2.4.6. Blocking agents

No data.

2.4.7. Disinfection of eggs and larvae

No data.

2.4.8. General husbandry practices

To date, experimental data indicates that AbHV 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, clinical disease has not been reported in wild abalone populations in Tasmania. Disease outbreaks in processing plants in Tasmania suggest that stress factors may influence expression of subclinical infection.

3. Sampling

3.1. Selection of 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. Preservation of samples for submission

Samples should be collected for examination by: i) histology and should be fixed in 10% formalin in filtered seawater; ii) electron microscopy (fixed in 2.5% glutaraldehyde in filtered seawater); iii) PCR (fixed in PCR preservative such as 95% ethanol). If fixatives are not available, samples should be kept chilled (on ice) and transported to the laboratory within 24 hours. Alternatively, samples can be sent frozen. Frozen samples are not suitable for histology or electron microscopy but can be analysed by PCR.

3.3. Pooling of samples

Tissues from moribund or freshly dead abalone should be collected according to age class and pond/farm/geographical location. To allow comparison between different tests, tissues from abalone should not be pooled.

3.4. Best organs or tissues

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

3.5. Samples/tissues that are not appropriate

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

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

In, 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. Similar signs have been reported for an abalone disease epizootic in Chinese Taipei (Chang *et al.,* 2005).

4.1.2. Behavioural changes

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 qPCR without overt clinical or histological signs.

4.2. Clinical methods

4.2.1. 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.

4.2.2. Clinical chemistry

No data.

4.2.3. Microscopic pathology

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 (Ellard *et al.,* 2009; Hooper *et al.,* 2007).

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

In-situ hybridisation localises AbHV-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).

4.2.7. Electron microscopy/cytopathology

Transmission electron microscopy can be used to confirm the presence of viral particles in infected ganglia. AbHV 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).

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

Direct detection methods developed, to date, for detection and identification of AbHV include microscopic methods (examination of tissue sections for typical lesions and electron microscopy for detection of herpesvirus particles), conventional and real-time PCR, and *in situ* hybridisation (ISH).

4.3.1.1. Microscopic methods

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

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.

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable

4.3.1.1.3. Fixed sections

Neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) is the prime target and examination of histological sections reveals ganglioneuritis – increased cellularity involving mainly haemocytes and glial cells, and cell necrosis.

4.3.1.2. Agent isolation and identification

AbHV is identified using methods that specifically detect AbHV nucleic acid (PCR, sequencing, qPCR and *in situ* hybridisation).

4.3.1.2.1. Cell culture/artificial media

To date, attempts to culture the virus in both vertebrate and invertebrate cell lines have been unsuccessful.

4.3.1.2.2. Antibody-based antigen detection methods

Not available.

4.3.1.2.3. Molecular techniques

Neural tissue samples for either conventional or real-time PCR should be fixed in preservative (80% reagent grade ethanol; 19.75% glycerol; 0.25% β-mercaptoethanol) or, alternatively, 95% ethanol.

The pleuropedal ganglion and/or pedal nerve cords are dissected from the fixed tissue and placed in 2.0 ml tubes for DNA extraction. Nucleic acid from AbHV-infected and uninfected abalone tissues (approximately 20 mg of muscle and neural tissue) are extracted using a commercial kit, e.g. QIAamp DNA mini kit (QIAGEN) or equivalent, according to the manufacturer's instructions. Nucleic acid, bound to minicolumns, is eluted and resuspended in a final volume of 100 µl buffer (~100 ng μl^{-1}) provided in the kit. For large sample numbers, tissues digested according to the relevant QIAamp® DNA Mini Kit (QIAGEN) protocol and nucleic acid extraction using the MagMAX-96 Viral RNA Isolation Kit (which extracts total nucleic acid from samples) and an AB MagMAX Express-96 Magnetic Particle Processor (Applied Biosystems)[1] is an alternative option.

qPCR (*TaqMan*)

Following validation of the qPCR test targeted to ORF49 (Corbeil *et al.,* 2010), the discovery of genotypic variants in Australia not recognised by this test necessitated other qPCR tests to be developed based on more conserved regions of the viral genome. qPCR tests targeted to ORF66 and ORF77 (see Table 4.1) have been used extensively in disease investigations and, while formal validation has not been undertaken, they have sensitively and generically detected all AbHV variants identified to date, including the isolate from Chinese Taipei.

Table 4.1. Nucleic acid sequences of primers and probes for AbHV qPCR tests

Primers or probes	Sequence (5' → 3')
ORF66 Primers (300 nM)	
AbHV ORF66F1	TCC-CGG-ACA-CCA-GTA-AGA-AC
AbHV ORF66R1	CAA-GGC-TGC-TAT-GCG-TAT-GA
ORF66 Probe (100 nM)	
AbHV 66Prb1	**6FAM**-TGG-CCG-TCG-AGA-TGT-CCA-TG-**TAMRA**

1 Reference to specific commercial products as examples does not imply their endorsement by OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

Primers or probes	Sequence (5' → 3')
ORF77 Primers (300 nM)	
AbHV ORF77F1	CAA-CCA-CTT-GTT-CGG-GTT-CT
AbHV ORF77R1	CAG-GGT-GAT-TAA-TGC-GGA-GT
ORF77 Probe (100 nM)	
AbHV 77Prb1	**6FAM**-TCC-GTA-CGC-GGG-ATC-TTC-GT-**TAMRA**
18S rRNA gene Primers (100nM)	
18SF1	CGG-CTA-CCA-CAT-CCA-AGG-AA
18SR1	GCT-GGA-ATT-ACC-GCG-GCT
18S rRNA gene Probe (100nM)	**6VIC**-TGC-TGG-CAC-CAG-ACT-TGC-CCT-C-**TAMRA**

qPCR testing of extracted DNA is performed using 96-well plates. Reactions contain 12.5 µl TaqMan® Fast Universal PCR Master Mix (2×), 2 µl (~100 ng μl^{-1}) extracted DNA sample and the reaction mixture is made up to 25 µl using deionised water after primers and probes have been added at the appropriate concentrations (see Table 4.1). The following thermal cycling conditions are used: 95°C for 59 seconds followed by 45 cycles of 95°C for 3 seconds and 60°C for 30 seconds.

The ORF66 and/or ORF77 qPCR tests are multiplexed with a qPCR test using primers and probe specific for the 18S ribosomal RNA gene (Applied Biosystems) is used to validate the nucleic acid extraction procedure and the absence of PCR inhibitors (see Table 4.1).

All samples (including positive and negative controls) are tested in duplicate or triplicate. The results of a TaqMan qPCR assay are expressed in the form of software-generated characteristic amplification curves. Amplification curves from positive and negative (no template controls) should be compared with the test sample. A sample is considered above the test background level when the change in fluorescence (ΔR_n) of FAM or VIC, relative to that of ROX (internal reference dye), exceeds the threshold value that is set at the upper end of the linear range of the amplification plots (normally 0.1 but this may be dependent on various factors such as equipment, reagents, host species). Results of a TaqMan assay can also be, and often are, expressed as cycle threshold (C_T) values. The cycle threshold (C_T) is defined as the cycle number at which a statistically significant increase in fluorescence output above background is detected.

At the completion of the TaqMan qPCR assay, the presence of AbHV DNA is demonstrated by the presence of specific amplicons, identified by software-generated characteristic amplification curves and cycle threshold values (C_T). No-template controls must have no evidence of specific amplicons.

If the test is deemed valid, the results for the test sample wells may be interpreted using the following criteria:

- Positive test results are defined as the presence of specific amplicons expressed as a characteristic amplification.
- Negative test results are defined as the absence of specific amplicons expressed by a characteristic amplification curve.

In addition to a no-template control, negative controls should include nucleic acid extracted from known uninfected abalone to determine that these do not yield a C_T value.

Positive controls can include nucleic acid extracted from known infected abalone and/or a plasmid DNA standard (Corbeil *et al*., 2010).

Conventional PCR

Conventional PCR may also be used for detection of AbHV 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 isolate. Thus it is potentially useful for epidemiological studies and to confirm positive qPCR results. The primer sequences are detailed below.

Primer	Sequence
AbHV-16	5'-GGC-TCG-TTC-GGT-CGT-AGA-ATG-3'
AbHV-17	5'-TCA-GCG-TGT-ACA-GAT-CCA-TGT-C-3'

Cycling conditions are as follows: one cycle at 95°C for 15 minutes, 40 cycles of 94°C for 30 seconds/52°C for 30 seconds/74°C for 45 seconds, followed by one cycle at 72°C for 7 minutes and then hold at 4°C.

In-situ hybridisation

The *in-situ* hybridisation (ISH) procedure described here uses a digoxygenin (DIG)-labelled DNA probe to detect AbHV in formalin-fixed, paraffin-embedded (FFPE) tissue sections.

Reagents

20× standard saline citrate (SSC) pH7 (store at room temperature)
175.32 g litre^{-1} NaCl
88.23 g litre^{-1} Sodium citrate

100× Denhardt's solution (store at –20°C)
2 g (100 ml)$^{-1}$ Bovine serum albumin (Fraction V)
2 g (100 ml)$^{-1}$ Ficoll 400
2 g (100 ml)$^{-1}$ Polyvinylpyrollidone

Hybridisation buffer (store at –20°C)
25 ml Formamide
10 ml 20× SSC
2.5 ml 100× Denhardt's solution
10 ml 50% dextran sulphate in distilled water
500 µl 10 mg ml^{-1} herring sperm DNA
Make up to 50 ml with MilliQ water

10× Tris-buffered saline (TBS) (store at room temperature)
23.6 g litre^{-1} Tris base
127 g litre^{-1} Tris/HCl
87.66 g litre^{-1} NaCl

Preparation of DIG-labelled probes

Perform PCR on purified AbHV DNA or a sample known to contain AbHV using a PCR DIG Probe Synthesis Kit (Roche Cat. No. 11 636 090 910) according to the manufacturer's instructions. The primers to be used are:

Primer Designation	Primer sequence	Amplicon (probe) size
AbHV_ORF66f1	5'-TCC-CGG-ACA-CCA-GTA-AGA-AC-3'	
AbHV_ORF66r2	5'-GCC-GGT-CTT-TGA-AGG-ATC-TA-3'	848bp

Use the following thermocycling profile: 95°C for 5 minutes followed by 30 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 60 seconds. Complete the PCR with a final elongation at 72°C for 10 minutes.

Preparation of sections

i) Section paraffin-embedded tissue at 3 µM thickness and place onto Superfrost plus slides (Menzel Catalogue No. SF41296SP) and allow to dry.

ii) Heat sections at 65°C for 30 minutes and deparaffinise in two stages of xylene.

iii) Rehydrate by placing slides in absolute ethanol for 2 minutes followed by 90% ethanol for 2 minutes, 70% ethanol for 2 minutes and then into distilled water.

iv) Place slides in 0.2 N HCl for 20 minutes and rinse in distilled water for 5–10 minutes.

v) Apply 50–100 µl of 100 µg ml^{-1} proteinase K in Tris-buffered saline (TBS; 0.1 M Tris 0.15 M NaCl pH 7.5) and incubate at 37°C for 30 minutes.

vi) Rinse with 0.2% glycine for 2 minutes.

vii) Wash in running water for 10 minutes.

viii) Dehydrate sample in 70% ethanol for 2 minutes followed by 90% ethanol for 2 minutes and 100% ethanol for 2 minutes.

x) Allow slides to air-dry.

Hybridisation procedure

i) Make 100 µl hybridisation solution per tissue section (4× SSC, 5× Denhardt's solution, 10 mg ml^{-1} herring sperm DNA, 10% dextran sulphate, 50% formamide, approximately 5 ng μl^{-1} probe).

ii) Heat the hybridisation solution to 95–100°C for 5 minutes to denature the probe and place on ice until ready for use.

iii) Apply sufficient hybridisation solution to cover the section (approximately 50 µl) and cover with a cover-slip.

iv) Heat the slides to 95°C for 5 minutes to denature the nucleic acid in the specimen. To heat the slides to 95°C a PCR heating block can be used or a purpose built hybridisation block such as the Invitrogen SPoT hybridiser.

v) Place the slides into a humidified chamber that has been preheated to 37°C and incubate at 37°C overnight (12–16 hours).

Post-hybridisation procedure

i) Remove cover-slips by immersing slides in 2× SSC at room temperature.

ii) Place slides in a rack and immerse in 2× SSC at room temperature.

iii) Wash, with gentle rocking/shaking, in 0.5 × SSC (pre-warmed to 37°C) at 37°C for 15 minutes.

iv) Wash slides briefly in TBS buffer at room temperature.

v) Incubate slides in blocking solution (0.5% skim milk powder in TBS) for 30 minutes at room temperature.

vi) Cover sections with 100–200 µl of sheep anti-DIG antibody conjugated to alkaline phosphatase (Roche Cat. No. 1093274) diluted 1 in 100 in blocking solution and incubate at room temperature for 1 hour.

vii) Wash in TBS buffer three times for 3 minutes each.

viii) Equilibrate in solution II (0.1 M Tris pH 8, 0.5 M NaCl, 0.1 M $MgCl_2$ pH 9 or 0.1 M Tris pH 8, 0.05 M $MgSO_4$,$7H_2O$, pH 9.5) for 3 minutes at room temperature.

Colour development

i) Incubate slides in the dark in NBT/BCIP diluted in solution II (25 µl/ml).

ii) Cover the sections with the staining solution and place a cover-slip over them. Incubate in the dark for 3–4 hours in a humidified container, making sure that the slides do not dry out.

iii) Monitor the colour development by periodically checking the slides under a light microscope.

iv) If required the slides can be incubated, in the dark at room temperature, overnight.

v) Stop the reaction and remove the cover-slip by immersing the slides in distilled water.

vi) Wash the slides in running water for 5 minutes.

vii) Slides can be counterstained for 1 minute with 0.5% Bismarck brown or equivalent.

viii) Mount the slides with mounting medium (DAKO Cat. No. S3023) and a cover-slip.

Interpretation of results

Specific dark blue-black intra-cellular staining is indicative of the presence of viral DNA.

4.3.1.2.4. Agent purification

No data.

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of AVG are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category A or B have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	d	d	d	c
Direct LM	d	d	d	d	d	d
Histopathology	d	d	b	b	a	a*
Transmission EM	d	d	d	d	d	c
Antibody-based assays	d	d	d	d	d	d
***In-situ* DNA probes**	d	d	c	c	d	a*
PCR	d	d	a	a	a	a
PCR and sequence	d	d	d	d	d	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction; *Histopathology can be confirmed using *in-situ* hybridisation (ISH).

6. Test(s) recommended for targeted surveillance to declare freedom from infection with abalone herpesvirus

The test recommended for targeted surveillance is qPCR on extracted nucleic acids from neural tissue of abalone.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

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

i) Presence of high mortality rates (up to 90%) associated with clinical signs of AVG as described in this chapter.

ii) Histopathology (ganglioneuritis) observed in neural tissue sections of a single abalone sample.

iii) Positive result by qPCR or conventional PCR on at least one abalone sample.

7.2. Definition of confirmed case

The presence of AbHV is considered to be confirmed if, in addition to the criteria in Section 7.1, one or more of the following criteria are met:

i) Positive result by qPCR on one or more abalone where positive histopathology (7.1.i) and/or high mortality with clinical signs consistent with AVG (7.1.ii) also occurs.

ii) Positive result by *in-situ* hybridisation on neural tissue section.

iii) Positive result by conventional PCR on neural tissue section followed by sequence analysis of the amplicon to confirm AbHV nucleic acid sequence.

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

NB: FIRST ADOPTED IN 2012. MOST RECENT UPDATES ADOPTED IN 2022 (SECTIONS 2.2.1 AND 2.2.2).