CHAPTER 2.3.8.

INFECTION WITH SALMONID ALPHAVIRUS

1. Scope

Infection with salmonid alphavirus (SAV) means infection with any genotype of the pathogenic agent SAV, Genus *Alphavirus* and Family *Togaviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

SAV is an enveloped, spherical, single-stranded, positive-sense RNA virus, approximately 60–70 nm in diameter, with a genome of ~12 kb. The genome codes for eight proteins: four capsid glycoproteins (E1, E2, E3 and 6K) and four nonstructural proteins (nsP1–4). Glycoprotein E2 is considered to be the site of most neutralising epitopes, while E1 contains more conserved, cross-reactive epitopes (McLoughlin & Graham, 2007). SAV is considered to belong to the Genus *Alphavirus* of the Family *Togaviridae* based on nucleotide sequence studies of SAV isolates, and supported by biological properties of the virus, including cross-infection and neutralisation trials. In addition, four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD), characteristic of alphaviruses, are present in the SAV genome (McLoughlin & Graham, 2007).

SAV has been divided into six genotypes (SAV 1–SAV 6) based solely on nucleic acid sequences for the proteins E2 and nsP3 (Fringuelli *et al.,* 2008). The level of antigenic variation among genotypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV genotype are likely to cross react with other SAV isolates (Graham *et al.,* 2014; Jewhurst *et al.,* 2004).

Infection with SAV causes pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), common dab (*Limanda limanda*), rainbow trout (*Oncorhynchus mykiss*) (McLoughlin & Graham, 2007) and Arctic char (*Salvelinus alpinus*) (Lewisch *et al*., 2018). The genotypes SAV 1 and SAV 2 cause disease in fish both in freshwater and seawater, while the four genotypes SAV 3 – SAV 6 have only been reported from disease outbreaks in seawater.

2.1.2. Survival and stability in processed or stored samples

There are no published scientific data specifically on the survival and stability of SAV in processed or stored samples. The OIE Reference Laboratory has found that SAV in serum/plasma samples and virus isolated from cell culture can be stored for many years at –80°C without significant decline in virus titre. This observation is consistent with research on other alphaviruses.

2.1.3. Survival and stability outside the host

Laboratory tests suggest that SAV would survive for extended periods in the aquatic environment. In these tests, virus could be detected at the end of the test period of 65 days in a majority of the trials. Virus survival was inversely related to temperature; at 20°C virus was not detectable beyond 35 days, and at 4°C was still present after 65 days.

The half-life of SAV in serum has been found to be inversely related to temperature, being up to 7 times longer at 4°C than at 20°C, emphasising the need for rapid shipment of samples at 4°C to laboratories for virus isolation. For long-term conservation of SAV-positive samples and cultured virus, storage at –80°C is recommended (Graham *et al.,* 2007b).

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 SAV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name	Genotype
Pleuronectidae	*Limanda limanda*	Common dab	SAV 5
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	SAV 1, 2, 3
	Salmo salar	Atlantic salmon	SAV 1, 2, 3, 4, 5, 6
	Salvelinus alpinus	Arctic charr	SAV 2

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the *Aquatic Code* are: long rough dab (*Hippoglossoides platessoides*), plaice (*Pleuronectes platessa*) and Ballan wrasse (*Labrus bergylta*).

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:

Family	Scientific name	Common name
Clupeidae	*Clupea harengus*	herring
Cottidae	*Myoxocephalus octodecemspinosus*	longhorn sculpin
Gadidae	*Melanogrammus aeglefinus*	haddock
	Trisopterus esmarkii	Norway pout
	Pollachius virens	saithe
	Merlangius merlangus	whiting
	Gadus morhua	Atlantic cod
Merlucciidae	*Merluccius hubbsi*	Argentine hake
Pleuronectidae	*Platichthys flesus*	European flounder
Salmonidae	*Salmo trutta*	brown trout

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

Atlantic salmon and rainbow trout are the species with the highest likelihood of infection with SAV. Experimental studies have demonstrated that all life stages are susceptible to infection (Taksdal & Sindre, 2016). SAV 1–SAV 6 have been detected in Atlantic salmon. SAV 1, SAV 2 and SAV 3 have been detected in rainbow trout.

For the purposes of Table 4.1, Atlantic salmon alevins and fry (e.g. up to approximately 1 g in weight) may be considered early life stages, parr and smolts can be considered as juveniles and all fish post smoltification as adults.

2.2.4. Distribution of the pathogen in the host

The heart and the pancreas are main target organs for infection with SAV. Necrosis and loss of exocrine pancreatic tissue, myocarditis and skeletal myositis are typical histopathological findings. During the viraemic stage, substantial amounts of virus are also found in serum, and during the infection virus can also be found in brain, kidney, spleen, gills, mucous and faeces (Taksdal & Sindre, 2016).

2.2.5. Aquatic animal reservoirs of infection

There is evidence that some survivors of outbreaks will become long-term carriers of the virus (Graham *et al*., 2010) and thus farmed Atlantic salmon and rainbow trout can be considered the main reservoir of SAV (Taksdal & Sindre, 2016). Infection with SAV has been detected in some wild flatfish species in Scotland (Bruno *et al.,* 2014; Snow *et al.,* 2010) which could also act as a reservoir of infection.

2.2.6. Vectors

Although most alphaviruses are transmitted by arthropod vectors, vector transmission of SAV has not yet been demonstrated. SAV has been detected by reverse-transcription (RT) PCR in salmon lice (*Lepeophtheirus salmonis*) collected during acute outbreaks of pancreas disease in Atlantic salmon, but transfer to susceptible fish species has not been reported (Petterson *et al.,* 2009).

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality rates due to infection with SAV may vary with genotype, season, year, use of biosecurity measures and species of fish (Bang Jensen *et al.,* 2012; Graham *et al.,* 2011; Rodger & Mitchell, 2007; Stormoen *et al*., 2013). The cumulative mortality at the farm level ranges from negligible to over 50% in severe cases (Bang Jensen *et al.,* 2012; Graham *et al.,* 2003; Rodger & Mitchell, 2007; Ruane *et al.,* 2008; Stene *et al.,* 2014). Experimental studies have demonstrated that SAV 2 infection in marine fish causes lower mortality than SAV 3 (Taksdal *et al.*, 2015).

Duration of disease outbreaks, defined as the period with increased mortality, may vary from 1 to 32 weeks (Jansen *et al.* 2010a; 2014; Ruane *et al.,* 2008).

The prevalence of infection with SAV is variable. During disease outbreaks, the prevalence is usually high; prevalences of 70–100% have been reported in Atlantic salmon farming sites (Graham *et al.,* 2010). Prevalences in wild fish are largely unknown. SAV has been detected by RT-PCR in some marine flatfish species in Scottish waters at prevalences ranging from 0% to 18%, depending on species and location (Snow *et al.,* 2010). A serological survey of wild salmonids in fresh water river systems in Northern Ireland did not detect virus neutralisation antibodies against SAV in any of 188 sera tested, whereas the majority of sera from farmed salmon in sea water in the same area tested positive (Graham *et al.,* 2003).

2.3.2. Clinical signs, including behavioural changes

A sudden drop in appetite may be observed 1–2 weeks before the detection of elevated mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak ("sleeping") fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed. However, it is important to note that clinical signs are not pathognomonic.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish ('runts') with poor body condition are typically observed. However, the presentation of long, slender fish can be caused by factors other than SAV.

2.3.3 Gross pathology

Yellow mucoid gut content is a usual post-mortem finding, typically seen in inappetant fish. Occasionally, signs of circulatory disturbances, such as petechial haemorrhages, mild ascites or reddening of the pancreatic region between the pyloric caeca may be seen. Some diseased fish may have pale or ruptured hearts. It is important to note that post-mortem findings are not pathognomonic.

2.3.4. Modes of transmission and life cycle

Horizontal transmission of SAV is demonstrated by a range of evidence including: phylogenetic studies, successful transmission among cohabiting fish, proven transmission between farming sites, studies on survival of SAV in sea water and the spread via water currents (Graham *et al.,* 2011; Jansen *et al.,* 2010a; Kristoffersen *et al.,* 2009; Stene *et al.,* 2013; Viljugrein *et al.,* 2009).

Long-distance transmission of SAV into a previously uninfected area is most likely due to movement of infected live fish (Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007). SAV has been detected in fat leaking from dead fish which accumulates at the sea water surface, contributing to spread of the virus by water currents (Stene *et al.,* 2016). Once SAV has been introduced into an area, farm proximity and water currents influence local transmission (Aldrin *et al.,* 2010; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Vertical transmission of SAV has been suggested (Bratland & Nylund, 2009), but not demonstrated (Kongtorp *et al.,* 2010; McLoughlin & Graham, 2007). The Norwegian Scientific Committee for Food Safety, (2010), carried out a risk assessment and concluded that the risk of vertical transmission of SAV is negligible.

2.3.5. Environmental factors

Clinical outbreaks and mortality are influenced by water temperature and season (McLoughlin & Graham, 2007; Rodger & Mitchell, 2007; Stene *et al.,* 2014; Stormoen *et al.,* 2013).

2.3.6. Geographical distribution

Infection with SAV has been reported from several countries in Europe. For recent information on distribution at the country level consult the WAHIS interface (https://wahis.oie.int/#/home).

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

DNA-based and cell-culture-based virus-inactivated vaccines against SAV are both commercially available.

2.4.2. Chemotherapy including blocking agents

No chemotherapy is available.

2.4.3. Immunostimulation

No immunostimulation is available.

2.4.4. Breeding resistant strains

Differences in susceptibility among different family groups of Atlantic salmon have been observed in challenge experiments and in the field, indicating the potential for breeding for resistance (Norris *et al*., 2008; Gonen *et al.*, 2015). Breeding programmes in Ireland and Norway have successfully produced fish with increased resistance to disease caused by SAV, which are now commercially available.

2.4.5. Inactivation methods

SAV is rapidly inactivated at pH 4 and pH 12, and after heating to 60°C (Graham *et al.,* 2007b). The virus is also readily inactivated by UV-light (Anon). A range of commercially available disinfectants have been tested for efficacy against salmonid alphavirus under different conditions, all being found to be effective under at least some of the conditions tested. The presence of organic matter was shown to decrease the effectiveness of disinfectants in some cases (Graham *et al.* 2007a).

2.4.6. Disinfection of eggs and larvae

Standard disinfection procedures are considered sufficient to prevent surface contamination of eggs by SAV (Graham *et al*., 2007a).

2.4.7. General husbandry

Stressing the fish by movement, crowding or treatment may initiate disease outbreaks on infected farms. Risk factors for outbreaks on a farming site include a previous history of infection with SAV, high feeding rate, high sea lice burden, the use of autumn smolts and previous outbreaks of infectious pancreatic necrosis (Bang Jensen *et al.,* 2012; Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007).

To avoid infection with SAV, good husbandry practices should be applied such as use of appropriate sites for farming, segregation of generations, stocking with good quality fish, removal of dead fish, regular cleaning of tanks and pens, control of parasites and other pathogens, as well as careful handling of fish. Once an outbreak has started, mortality may be reduced by minimising handling and ceasing feeding.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Extremely weak ('sleeping') fish may be found at the bottom of a tank or net-cages. If the number of clinically diseased fish is low, samples from long, thin fish ('runts') may be added. If moribund or thin fish or runts are sampled, the probability of detecting SAV is higher than if randomly selected, apparently healthy fish are sampled (Jansen *et al.,* 2010b).

Fish to be sampled are selected as follows:

i) Susceptible species should be sampled proportionally or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown disease status).

ii) If more than one water source is used for fish production, fish from all water sources should be included in the sample.

iii) Weak, abnormally behaving or freshly dead (not decomposed) fish should be selected. If such fish are not present, selected fish should include apparently healthy fish collected in such a way that all parts of the farm, as well as all year classes, are proportionally represented in the sample.

3.2. Selection of organs or tissues

Heart and mid-kidney are the recommended organs for detection of SAV either by molecular biological methods or by cell culture. During an outbreak, the heart usually contains more SAV than other tissues and should always be sampled. After disease outbreaks, gill and heart tissue (Graham *et al.,* 2010) and pools of heart and mid-kidney tissue (Jansen *et al.,* 2010b) remained positive by real time RT-PCR for months after initial detection.

For vaccinated fish, the heart should be sampled, and mid-kidney, spleen or other internal organs should not be sampled, because opening the abdominal cavity may cause contamination with viral RNA/DNA from the vaccine (See Section 2.4).

During the initial viraemic phase, serum samples are also suitable for detection of SAV either by molecular biological methods or by cell culture, which can provide an early warning of disease outbreaks (Graham *et al.,* 2010). From approximately 3 weeks after SAV infection, blood serum or plasma is suitable for a virus neutralisation test (Graham *et al.,* 2003).

Tissues suitable for histological examinations include gill, heart, pyloric caeca with attached pancreatic tissue, liver, kidney, spleen and skeletal muscle containing both red (aerobic) and white (anaerobic) muscle. Skin with associated skeletal muscle should be sampled at the lateral line level and deep enough to include both red and white muscle.

3.3. Samples or tissues not suitable for pathogen detection

Pancreas, although a target organ for the virus, is not suitable for RT-PCR detection of SAV, as it is impossible to separate this organ from the intestine of the fish during sampling, and in addition loss of pancreas is common in infected fish. Organs other than those recommended in Section 3.2 should not be used for the detection of SAV, as the sensitivity of the diagnostic methods might be reduced.

3.4. Non-lethal sampling

There are investigations into using non-lethal sampling methods for surveillance of SAV in fish farms, including detection of virus in water (Bernhard *et al.,* 2021). However, no validated methods are currently available. Serum samples may be collected via non-lethal sampling methods and considered suitable for some SAV test methods as described in Section 3.2.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.3.0.

3.5.1. Samples for pathogen isolation

For recommendations on transporting samples for virus isolation to the laboratory, see Section B.2.4 of Chapter 2.3.0 *General information* (diseases of fish).

3.5.2. Preservation of samples for molecular detection

Samples can be taken from the fish in accordance with the procedure described in Section 3.5.1, using a sterile instrument, and transferred to a sterile plastic tube containing transport medium.

Alternatively, tissue samples for RT-PCR testing should be preserved in an appropriate medium for preservation of RNA. Samples in RNA stabilising reagents can be shipped on ice or at room temperature if transport time does not exceed 24 hours.

For further storage, the samples should be kept below –20°C.

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

Tissue samples for histopathology should be fixed in 10% neutral buffered formalin immediately after collection. The recommended ratio of fixative to tissue is 10:1.

3.5.4. Samples for other tests

Blood samples should be centrifuged for the collection of serum or plasma as soon as possible after sampling, to avoid lysis of the red blood cells. Serum or plasma samples should be shipped on ice to the laboratory to ensure virus viability.

3.6. Pooling of samples

The reliability of a virus isolation and real-time RT-PCR for detecting SAV in pooled samples from apparently healthy and clinically diseased populations of Atlantic salmon has not been evaluated thoroughly. Results suggest that the use of individual samples rather than pools are more appropriate when testing for freedom from, or for confirmatory diagnosis of, infection with SAV (Hall *et al.,* 2014).

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy populations), ii) presumptive and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage. The designations used in Table 4.1 indicate:

Key:

+++ =	Recommended method(s) validated for the purpose shown and usually to stage 3 of the OIE Validation Pathway;
++ =	Suitable method(s) but may need further validation;
+ =	May be used in some situations, but cost, reliability, lack of validation or other factors severely limits its application;
Shaded boxes =	Not appropriate for this purpose.

The selection of a test for a given purpose depends on the analytical and diagnostic sensitivities and specificities repeatability and reproducibility. OIE Reference Laboratories welcome feedback on diagnostic performance for assays, in particular PCR methods, for factors affecting assay analytical sensitivity or analytical specificity, such as tissue components inhibiting amplification, presence of nonspecific or uncertain bands, etc., and any assays that are in the +++ category.

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

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology[3]					++	++	++	2				
Cytopathology[3]												
Cell culture					+	+	+	2	+	+	+	2
Real-time RT-PCR	+++	+++	+++	2	+++	+++	+++	2				
Conventional RT-PCR					++	++	++	1	++	++	++	1
Amplicon sequencing[4]									+++	+++	+++	1
***In-situ* hybridisation**												
Bioassay												
LAMP												
Ab ELISA												
Ag ELISA												
Immunohistochemistry										+	+	2
Serum neutralisation assay		+	++	1	++	++	++	2				

LV = level of validation, refers to the stage of validation in the OIE Pathway (chapter 1.1.2); RT-PCR = reverse transcription-polymerase chain reaction methods;
LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6).

[2]Early and juvenile life stages have been defined in Section 2.2.3.

[3]Histopathology and cytopathology can be validated if the results from different operators has been statistically compared.

[4]Sequencing of the PCR product.

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

The pathological changes most commonly found in clinically diseased fish are severe loss of exocrine pancreatic tissue, cardiomyocytic necrosis and inflammation, red (aerobic) skeletal muscle inflammation and white (anaerobic) skeletal muscle degeneration or inflammation. A less frequent but supporting finding is the detection of cells with many cytoplasmic eosinophilic granules along kidney sinusoids.

As the disease progresses, the development of these changes is not simultaneous in all organs: in a very short, early phase, the only lesions present might be necrosis of exocrine pancreatic tissue and a variable inflammatory reaction in the peripancreatic fat. Shortly thereafter, heart muscle cell degeneration and necrosis develop before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear, and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Subsequently, skeletal muscle degeneration, inflammation and fibrosis develop. In a proportion of fish, severe fibrosis of the peri-acinar tissue may occur, and in these cases, the pancreas does not recover (runts) (Christie *et al.,* 2007; Kerbart Boscher *et al.,* 2006; McLoughlin & Graham, 2007; Taksdal *et al.,* 2007).

Cytopathology is not relevant for diagnostic use.

4.3. Cell culture for isolation

Isolation of field isolates of SAV in cell culture may be challenging (Christie *et al.,* 1998; Graham *et al.,* 2007b; Petterson *et al.,* 2013).

CHSE-214 cell cultures are commonly used for primary SAV isolation. Nevertheless, variation in cell line susceptibility among different SAV field isolates has been reported (Graham *et al.,* 2008; Herath *et al.,* 2009). Therefore other susceptible cell lines such as BF-2, FHM, SHK-1, EPC, CHH-1 should be tested for initial cell culture isolation of SAV. Cell lines should be monitored to ensure that susceptibility to targeted pathogens has not changed.

The CHSE-214 cells are grown at 20°C in Eagle's minimal essential medium (EMEM) with non-essential amino acids and 0.01 M HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) buffer, or Leibovitz's L-15 cell culture medium, both supplemented with fetal bovine serum (FBS) (5% or 10%) and L-glutamine (4 mM).

For virus isolation, cells are grown in tissue culture flasks or multi-well cell culture plates. SAV-positive controls are inoculated in parallel with the tissue samples as a test for cell susceptibility to SAV. When positive controls are included, measures must be taken to avoid contamination.

Use the procedure for sample preparation and inoculation described in Chapter 2.3.0 *General information* (on diseases of fish), Section A.2.2.2.

Inoculated cell cultures are incubated at 15°C for at least 14 days and examined at regular intervals for the occurrence of cytopathic effect (CPE). Typical CPE due to SAV appears as plaques of pyknotic, vacuolated cells. However, Norwegian SAV field isolates (both SAV3 and SAV2) usually do not produce CPE in low passages, and this is also reported for other SAV genotypes (Graham *et al.*, 2008; Petterson *et al.*, 2013). If no CPE has developed after 14 days, subculture to fresh cell cultures. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures should always be examined for the presence of SAV by immunofluorescence (indirect fluorescent antibody test [IFAT]) or conventional RT-PCR or real-time RT-PCR as virus replication may occur without development of apparent CPE.

4.4. Nucleic acid amplification

4.4.1. Real-time RT-PCR

The primers described below for real-time RT-PCR and RT-PCR with sequencing will detect all known genotypes of SAV.

RT-PCR may be used for detection of SAV from total RNA (or total nucleic acids) extracted from recommended organs or tissues (see Section 3.4). Real-time RT-PCR for the detection of SAV is recommended as it increases the specificity and the sensitivity of the test.

For genotyping, RT-PCR with subsequent sequencing of fragments from the E2 gene is recommended.

The primer and probe sequences for real-time RT-PCR from the nsP1 gene, as well as primers for genotyping, are listed in Table 4.2. For RNA extraction, automatic and semi-automatic nucleic acid extractors can be used. In addition, a variety of manual RNA extraction kits can also be used successfully to extract SAV RNA. Various RT-PCR kits and real-time PCR machines can be used. The PCR programme depends on the kit and real-time PCR equipment used in the laboratory. The conditions for performing the real-time RT-PCR in the OIE Reference Laboratory is as follows: 50°C for 10 minutes, 95°C for 3 minutes, and 40 cycles of (95°C for 10 seconds, 60°C for 20 seconds).

Table 4.2. *Primer and probe sequences for RT-PCR and real time RT-PCR*

Primer and probe sequences	Test type	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3' (Taqman®probe)	Real-time RT- PCR	QnsP1	107 bp	Hodneland *et al*., 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-3'	RT-PCR	E2	516 107 bp	Fringuelli *et al*., 2008

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

4.4.2. Conventional RT-PCR

See Section 4.4.1 for comments on conventional PCR kits and PCR machines.

The E2-primers stated in Table 4.2 may be used for conventional RT-PCR detection of SAV, if necessary.

For the conventional RT-PCR, the following programme is used: 50°C for 30 minutes, 95°C for 15 minutes, and 45 cycles of (94°C for 60 seconds, 55°C for 45 seconds, 72°C for 60 seconds).

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

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

Nucleotide sequence analysis of the RT-PCR amplicon (Section 4.4.2) is recommended as one of the final steps for confirmatory diagnosis. SAV-specific sequences will share a higher degree of nucleotide similarity to one of the published reference sequences for SAV.

4.6. *In-situ* hybridisation

Not applicable.

4.7. Immunohistochemistry

Immunohistochemical testing (Taksdal *et al.,* 2007) is only recommended for samples from fish with acute necrosis of exocrine pancreatic tissue.

4.7.1. Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 3 µm thick sections (for immunohistochemistry sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for histopathology and immunohistochemistry as described below.

4.7.2. Staining procedure for immunohistochemistry

All incubations are carried out at room temperature and all washing steps are done with Tris-buffered saline (TBS).

i) Nonspecific antibody binding sites are first blocked in 5% bovine serum albumin (BSA) in TBS for 20 minutes. The solution is then poured off without washing.

ii) Sections are incubated with primary antibody (monoclonal mouse antibody 4H1 against E1 SAV glycoprotein [Todd *et al.,* 2001]), diluted 1/3000 in 2.5% BSA in TBS and then incubated overnight, followed by two wash out baths lasting a minimum of 5 minutes.

iii) Sections are incubated with secondary antibody (biotinylated rabbit anti-mouse Ig) diluted 1/300 for 30 minutes, followed by wash out baths as in step ii above.

iv) Sections are incubated with streptavidin alkaline phosphatase conjugate (1/500) for 30 minutes followed by wash out baths as in step ii above.

v) For detection of bound antibodies, sections are incubated with Fast Red[1] (1 mg ml^{-1}) and Naphthol AS-MX phosphate (0.2 mg ml^{-1}) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) and allowed to develop for 20 minutes followed by one wash in tap water before counterstaining with Mayer's haematoxylin and mounting in aqueous mounting medium.

SAV-positive and SAV-negative tissue sections are included as controls in every setup (Taksdal *et al.,* 2007).

4.8. Bioassay

Not applicable.

4.9. Antibody or antigen-based detection methods

4.9.1. Antibody-based verification of SAV growth in cell culture

This technique should not be used as a screening method. All incubations below are carried out at room temperature unless otherwise stated.

i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well plates) or on cover-slips, depending on the type of microscope available (an inverted fluorescence microscope is necessary for monolayers grown on tissue culture plates). The necessary monolayers for negative and positive controls must be included.

ii) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 9–11 days.

iii) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. If necessary, the fixed cell cultures may be stored dry for 14 days at 4°C until staining.

iv) Incubate the cell monolayers with anti-SAV antibodies in an appropriate dilution in phosphate-buffered saline (PBS) for 1 hour and rinse three times with PBS with 0.05% Tween 20.

v) Incubate with fluorescein isothiocyanate (FITC)-conjugated species-specific immunoglobulin antibody for 1 hour, according to the instructions of the supplier. To increase the sensitivity of the test, FITC-conjugated anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-

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

labelled streptavidin with rinsing as in step d) in between the steps. The nuclei can be stained with propidium iodide (100 µg ml^{-1} in sterile distilled water). Add PBS (without Tween 20) and examine under fluorescence microscope. To avoid fading, the stained plates should be kept in the dark until examination. To reduce photobleaching of FITC due to the exposure to excitation light during microscopy, a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.10. Other methods

4.10.1. Serum neutralisation assay

Experimental studies have shown that neutralising antibodies can first be detected 10–16 days post-infection (Graham *et al.,* 2003), and serum neutralisation (SN) assays can be used as a diagnostic tool for the detection of SAV antibodies. SN assays are based on the presence or absence of detectable virus growth in cultured cells following incubation with serum that may contain neutralising antibodies. In addition, the assay allows detection of virus in serum or plasma, if present, as control wells of samples without added SAV are always included in the assay to assess presence of virus in the samples.

CHSE-214 cells are grown as described in Section 4.3.1. A suspension of trypsinised cells, diluted 1/3 in growth medium (10% FBS) is prepared for the SN assay.

i) 1/20 and 1/40 dilutions of each test serum are prepared in maintenance medium (2% FBS), and transferred to two duplicate wells (15 µl per well) on a flat-bottomed tissue culture grade microtitre plate. An equal volume of virus (100 $TCID_{50}$ [median tissue culture infective dose]) is added and the plate is incubated for 2 hours at room temperature.

ii) 70 µl of maintenance medium, and 50 µl of the CHSE-214 cell suspension is added to each well, and the plates are incubated for 3 days at 15°C.

iii) The cell monolayer is then fixed and stained as described in Section 4.9.1 *Antibody-based verification of SAV growth in cell culture*, or using the following procedure: monolayers of CHSE-214 cells are fixed for 30 minutes at room temperature in 10% neutral buffered formalin. Following two washes with 0.01 M PBS, a MAb against SAV is added to the monolayers in an appropriate dilution. Bound MAb is visualised using a labelled streptavidin–biotin system according to the manufacturer's instructions.

iv) SN titres (ND_{50}) are then calculated according to the method of Karber (1931), with titres ≥ 1:20 being considered positive. Both known negative serum controls and a control well for each sample (without virus added), and a virus control (without serum added) must always be included in the assay, to ensure valid results. During viremia (as indicated by detection of SAV in the sample control wells) a SN titre cannot be assessed.

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

The recommended test to be used in surveillance of susceptible fish populations for declaration of freedom from SAV is real-time RT-PCR as described in Section 4.4.1.

6. Corroborative diagnostic criteria

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

The case definitions for 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. It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case. If a laboratory does not have the capacity to undertake the necessary diagnostic tests it should seek advice from the appropriate OIE Reference Laboratory.

6.1. Apparently healthy animals or animals of unknown health status[2]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic 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 SAV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR;

ii) Detection of neutralising activity against SAV in serum or plasma.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with SAV is considered to be confirmed if one or more of the following criteria is met:

i) A positive result on tissue preparations by conventional RT-PCR and sequencing of the amplicon;

ii) SAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon;

iii) A positive result on tissue preparations by immunohistochemistry, and by conventional RT-PCR and sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

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

i) Gross pathology or clinical signs associated with infection with SAV;

ii) Histopathology consistent with SAV infection;

iii) SAV-typical CPE in cell culture;

iv) Positive result by real-time RT-PCR;

v) Positive result by conventional RT-PCR;

vi) Detection of neutralising activity against SAV in serum or plasma.

6.2.2. Definition of confirmed case in clinically affected animals

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

i) A positive result on tissue preparations by conventional RT-PCR and sequencing of the amplicon;

ii) SAV-typical CPE in cell culture followed by virus identification by conventional RT-PCR and sequencing of the amplicon;

iii) A positive result on tissue preparations by immunohistochemistry, and by conventional RT-PCR and sequencing of the amplicon.

Reference Laboratories should be contacted for specimen referral when testing laboratories cannot undertake any of the recommended test methods and testing is being undertaken that will result in notification to the OIE.

2 For example, transboundary commodities.

6.3. Diagnostic sensitivity and specificity for diagnostic tests: under study

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with SAV is provided in Table 6.3.1. This information can be used for the design of surveys for infection with SAV, 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 two of the validation pathway described in Chapter 1.1.2 and the information is available within published diagnostic accuracy studies.

Table 6.3.1. *Diagnostic performance of tests recommended for surveillance or diagnosis on field studies*

Test type	Test purpose	Test populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Surveillance Diagnosis	Infected	Kidney	Atlantic salmon	0.39 (598)	>0.99 (598)	n/a (Bayesian probability model)	Hall *et al.*, 2014
Real-time PCR	Surveillance Diagnosis	Infected vs assumed SAV free	Heart and mid-kidney	Atlantic salmon	0.978 (268)	0.831 (268)	n/a (Bayesian latent class analysis)	Jansen *et al.*, 2019
Isolation of SAV in cell culture	Diagnosis	Infected	Heart ventricle and head-kidney	Atlantic salmon	0.50 (598)	>0.99 (598)	n/a (Bayesian probability model)	Hall *et al.*, 2014
Isolation of SAV in cell culture	Diagnosis	Infected vs assumed SAV free	Heart and mid-kidney	Atlantic salmon	0.950 (268)	0.993 (268)	n/a (Bayesian latent class analysis)	Jansen *et al.*, 2019
Detection of neutralising activity against SAV	Surveillance	Infected vs assumed SAV free	Serum or plasma	Atlantic salmon	0.085 (268)	0.744 (268)	n/a (Bayesian latent class analysis)	Jansen *et al.*, 2019
Histopathology	Diagnosis	Infected vs assumed SAV free	Heart and mid-kidney	Atlantic salmon	0.637 (268)	0.967 (268)	n/a (Bayesian latent class analysis)	Jansen *et al.*, 2019

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

7. References

ALDRIN M., STORVIK B., FRIGESSI A., VILJUGREIN H. & JANSEN P.A. (2010). A stochastic model for the assessment of the transmission pathways of heart and skeleton muscle inflammation, pancreas disease and infectious salmon anaemia in marine fish farms in Norway. *Prev. Vet. Med.*, **93**, 51–61.

ANON. Development and optimization of an environmentally friendly water purification system for fish transport water based on UV technology. Project report, Norwegian Institute for Water Research. Project no: 245494/E40. 8pp. (In Norwegian).

BANG JENSEN B., KRISTOFFERSEN A.B., MYR C. & BRUN E. (2012). Cohort study of effect of vaccination on pancreas disease in Norwegian salmon aquaculture. *Dis. Aquat. Org.*, **102**, 23–31.

BERNHARD L.-V., MYRMEL M., LILLEHAUG A., QVILLER L. & WELI S.C. (2021). A filtration method for concentration and detection of Salmonid alphavirus in seawater during a post-smolt Atlantic salmon (*Salmo salar* L.) cohabitant challenge trial. *Dis. Aquat. Org.,* DOI: https://doi.org/10.3354/dao03572

BRATLAND A. & NYLUND A. (2009). Studies on the possibility of vertical transmission of Norwegian salmonid Alphavirus in production of Atlantic salmon in Norway. *J. Aquat. Anim. Health*, **21**, 73–78.

BRUNO D.W., NOGUERA P.A., BLACK J., MURRAY W., MACQUEEN D.J. & MATEJUSOVA I. (2014) Identification of a wild reservoir of salmonid alphavirus in common dab *Limanda limanda*, with emphasis on virus culture and sequencing. *Aquacult Environ Interact* 5:89-98. https://doi.org/10.3354/aei00097.

CHRISTIE K.E., FYRAND K., HOLTET L. & ROWLEY H.M. (1998) Isolation of pancreas disease virus from farmed Atlantic salmon, *Salmo salar* L., in Norway. *J. Fish Dis*., **21**, 391–394.

CHRISTIE K.E., GRAHAM D.A., MCLOUGHLIN M. F., VILLOING S., TODD D. & KNAPPSKOG D. (2007). Experimental infection of Atlantic salmon *Salmo salar* pre-smolts by i.p. injection of new Irish and Norwegian salmonid alphavirus (SAV) isolates: a comparative study. *Dis. Aquat. Org.*, **75**, 13–22.

FRINGUELLI E., ROWLEY H.M., WILSON J.C., HUNTER R., RODGER H. & GRAHAM D.A. (2008). Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences. *J. Fish Dis*., **31**, 811–823.

GONEN S., BARANSKI M., THORLAND I., NORRIS A., GROVE H., ARNESEN P., BAKKE H., LIEN S., BISHOP S.C. & HOUSTON R.D. (2015). Mapping and validation of a major QTL affecting resistance to pancreas disease (salmonid alphavirus) in Atlantic salmon (*Salmo salar*). *Heredity,* **115**, 405–414. https://doi.org/10.1038/hdy.2015.37

GRAHAM D.A., CHERRY K., WILSON C.J. & ROWLEY H.M. (2007a). Susceptibility of salmonid alphavirus to a range of chemical disinfectants. *J. Fish Dis*., **30**, 269–277.

GRAHAM D.A., FRINGUELLI E., WILSON C., ROWLEY H.M., BROWN, A., RODGER H., MCLOUGHLIN M.F., MCMANUS C., CASEY E., MCCARTHY L.J. & RUANE N.M. (2010). Prospective longitudinal studies of salmonid alphavirus infections on two Atlantic salmon farms in Ireland; evidence for viral persistence. *J. Fish Dis*., **33**, 123–135.

GRAHAM D.A., FROST P., MCLAUGHLIN K., ROWLEY H.M., GABESTAD I., GORDON A. & MCLOUGHLIN M.F. (2011). A comparative study of marine salmonid alphavirus subtypes 1–6 using an experimental cohabitation challenge model. *J. Fish Dis*., **34**, 273–286.

GRAHAM D.A., JEWHURST V.A., ROWLEY H.M., MCLOUGHLIN M.F. & TODD D. (2003). A rapid immunoperoxidase-basd neutralization assay for salmonid alphavirus used for a serological survey in Northern Ireland. *J. Fish Dis*., **26**, 407–413

GRAHAM D.A., ROWLEY H.M. & FROST P. (2014). Cross-neutralization studies with salmonid alphavirus subtype 1–6 strains: results with sera from experimental studies and natural infections. *J. Fish Dis*., 37, 683–691.

GRAHAM D.A., STAPLES V., WILSON C.J., JEWHURST H., CHERRY K., GORDON A. & ROWLEY H.M. (2007b). Biophysical properties of salmonid alphaviruses: influences of temperature and pH on virus survival. *J. Fish Dis*., **30**, 533–543.

GRAHAM D.A., WILSON C., JEWHURST H. & ROWLEY H. (2008). Cultural characteristics of salmonid alphaviruses – influences of cell line and temperature. *J. Fish Dis*., **31**, 859–868.

HALL L.M., MUNRO L.A., WALLACE I.S., MCINTOSH R., MACNEISH K. & MURRAY, A.G. (2014). An approach to evaluating the reliability of diagnostic tests on pooled groups of infected individuals. *Prev. Vet. Med*. **116**, 305–312. https://doi.org/https://doi.org/10.1016/j.prevetmed.2014.01.021

HERATH T., COSTA J., THOMPSON K., ADAMS A. & RICHARDS R. (2009). Alternative cell line for the isolation of salmonid alphavirus-1. *Icelandic Agricultural Sci.,* **22**, 19–27.

HODNELAND K. & ENDRESEN C. (2006). Sensitive and specific detection of salmonid alphavirus using real-time PCR (TaqMan). *J. Virol. Methods,* **131**, 184–192.

JANSEN M.D., GUARRACINO M., CARSON M., MODAHL I., TAKSDAL T., SINDRE H., BRUN E. & TAVORNPANICH S. (2019). Field evaluation of diagnostic test sensitivity and specificity for *Salmonid alphavirus* (SAV) infection and pancreas disease (PD) in farmed Atlantic salmon (*Salmo salar* L.) in Norway using Bayesian latent class analysis. *Frontiers Vet. Sci.,* doi: 10.3389/fvets.2019.00419

JANSEN M.D., TAKSDAL T., WASMUTH M.A., GJERSET B., BRUN E., OLSEN A.B., BRECK O. & SANDBERG M. (2010a). Salmonid alphavirus (SAV) and pancreas disease (PD) in Atlantic salmon (*Salmo salar* L.) in freshwater and seawater sites in Norway from 2006 to 2008. *J. Fish Dis*., **33**, 391–402.

JANSEN M.D., WASMUTH M.A., OLSEN A.B., GJERSET B., MODAHL I., BRECK O., HALDORSEN R.N., HJELMELAND R. & TAKSDAL T. (2010b). Pancreas disease (PD) in sea-reared Atlantic salmon, *Salmon salar* L., in Norway; a prospective, longitudinal study of disease development and agreement between diagnostic test results. *J. Fish Dis*., **33**, 723–736.

JEWHURST V.A., TODD D., ROWLEY H.M., WALKER I.W., WESTON J.H. MCLOUGHLIN M.F & GRAHAM D.A. (2004). Detection and antigenic characterization of salmonid alphavirus isolates from sera obtained from farmed Atlantic salmon, *salmo salar* L., and farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*., **27**, 143–149.

KERBART BOSCHER S., MCLOUGHLIN M., LE VEN A., CABON J., BAUD M. & CASTRIC J. (2006). Experimental transmission of sleeping disease in one-year-old rainbow trout *Onchorhynchus mykiss* (Walbaum), induced by sleeping disease virus. *J. Fish Dis*., **29**, 263–273.

KONGTORP R.T., STENE A., ANDREASSEN P.A., ASPEHAUG V., GRAHAM D.A., LYNGSTAD T.M., OLSEN A.B., OLSEN R.S., SANDBERG M., SANTI N., WALLACE C. & BRECK O. (2010). Lack of evidence for vertical transmission of SAV 3 using gametes of Atlantic salmon, *salmo salar* L., exposed by natural and experimental routes. *J. Fish Dis*., **33**, 879–888.

KRISTOFFERSEN A.B., VILJUGREIN H., KONGTORP R.T., BRUN E. & JANSEN P.A. (2009). Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003–2007. *Prev. Vet. Med.*, **90**, 127–136.

LEWISCH E., FRANK T., SOLIMAN H., SCHACHNER O., FRIEDL A. & EL-MATBOULI M. (2018). First confirmation of salmonid alphavirus infection in Arctic char Salvelinus alpinus and in Austria . *Dis. Aquat. Org*., **130**, 71–76.

MCLOUGHLIN M.F. & GRAHAM D.A. (2007). Alphavirus infections in salmonids – a review. *J. Fish Dis*., **30**, 511–531. https://doi.org/https://doi.org/10.1016/B978-0-12-801573-5.00023-1

NORRIS A., FOYLE L., RATCLIFF J. (2008). Heritability of mortality in response to a natural pancreas disease (SPDV) challenge in Atlantic salmon, *Salmo salar* L., post-smolts on a West of Ireland sea site. *J. Fish Dis.*, **31**, 913–920.

PETTERSON E., SANDBERG M. & SANTI N. (2009). Salmonid alphavirus associated with *Lepeoptheirus salmonis* (Copepoda: Caligidae) from Atlantic salmon, *Salmo salar* L. *J. Fish Dis*., **30**, 511–531.

PETTERSON E., STORMOEN, M., EVENSEN O., MIKALSEN A.B. & HAUGLAND O. (2013). Natural infection of Atlantic salmon (*Salmon salar*) with salmonid alphavirus 3 generates numerous viral deletion mutants. *J. Gen. Virol.,* **94**, 1945–1954.

RODGER H. & MITCHELL S. (2007). Epidemiological observations of pancreas disease of farmed Atlantic salmon, *Salmo salar* L., in Ireland. *J. Fish Dis*., **32**, 477–479.

RUANE N., GRAHAM D. & RODGER H. (2008). Pancreas disease in farmed salmon – health management and investigations at Irish farm sites 2005–2008. Marine Environments and Health Series, No. 34, Marine Institute. Available at http://oar.marine.ie/handle/10793/267

SNOW M., BLACK I., MCINTOSH R., BARETTO E., WALLACE I.S. & BRUNO D.W. (2010). Detection of salmonid alphavirus RNA in wild marine fish: implications for the origin of salmon pancreas disease in aquaculture. *Dis. Aquat. Org.*, **91**, 177–188.

STENE A., VILJUGREIN H., YNDESTAD H., TAVORNPANICH S. & SKJERVE E. (2013). Transmission dynamics of pancreas disease (PD) in a Norwegian fjord: aspects of water transport, contact networks and infection pressure among salmon farms. *J. Fish Dis*., **37**, 123–134.

STENE A., BANG JENSEN B., KNUTSEN Ø., OLSEN A. & VILJUGREIN H. (2014). Seasonal increase in sea temperature triggers pancreas disease in Norwegian salmon farms. *J. Fish Dis*., **37**, 739–751.

STENE A., HELLEBO A., VILJUGREIN H., SOLEVAG S.E., DEVOLD M. & ASPEHAUG V. (2016). Liquid fat, a potential abiotic vector for horizontal transmission of salmonid alphavirus? *J. Fish Dis*., **39**, 531-537.

STORMOEN M., KRISTOFFERSEN A.B. & JANSEN P.A. (2013). Mortality related to pancreas disease in Norwegian farmed salmonid fish, *Salmo salar* L. and *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*., **36**, 639–645.

TAKSDAL T., BANG JENSEN B., BÖCKERMAN I., MCLOUGHLIN M.F., HJORTAAS M.J., RAMSTAD A. & SINDRE H. (2015). Mortality and weight loss of Atlantic salmon, *Salmo salar* L., experimentally infected with salmonid alphavirus subtype 2 and subtype 3 isolates from Norway. *J. Fish Dis.*, **38**, 1047–1061. https://doi.org/10.1111/jfd.12312

TAKSDAL T., OLSEN A.B., BJERKAAS I., HJORTAAS M.J., DANNEVIG B.H., GRAHAM D.A. & MCLOUGHLIN M.F. (2007). Pancreas disease in farmed Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), in Norway. *J. Fish Dis*., **30**, 545–558.

TAKSDAL T. & SINDRE H. (2016). Chapter 23 - Togaviruses of Fish. In: Aquaculture Virology. Eds: Kibenge, F. & Godoy, M. Academic Press, San Diego, pp. 357–364. Paperback ISBN: 9780128015735, eBook ISBN: 9780128017548.

THE NORWEGIAN SCIENTIFIC COMMITTEE FOR FOOD SAFETY (VITENSKAPSKOMITEEN FOR MATTRYGGHET) (2010). Risikovurdering – stamfiskovervåking og vertikal smitteoverføring. **01**, 1-44. Available at:

HTTPS://VKM.NO/DOWNLOAD/18.A665C1015C865CC85BDFC47/1500464589864/VURDERING%20AV%20SANNSYNLIGHET%20FOR%20OG%20RISIKO%20VED%20VERTIKAL%20OVERF%C3%B8RING%20AV%20SMITTE.PDF

TODD D., JEWHURST V.A., WELSH M.D., BORGHMANS B.J., WESTON J.H., ROWLEY H.M., MACKIE D.P. & MCLOUGHLIN M.F. (2001). Production and characterisation of monoclonal antibodies to salmon pancreas disease virus. *Dis. Aquat. Org.*, **46**, 101–108.

VILJUGREIN H., STAALSTRØM A., MOLVÆR J., URKE H.A. & JANSEN P.A. (2009). Integration of hydrodynamics into a statistical mode on the spread of pancreas disease (PD) in salmon farming. *Dis. Aquat. Org.*, **88**, 35–44.

*
* *

NB: There is an OIE Reference Laboratory for infection with salmonid alphavirus
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with salmonid alphavirus

NB: FIRST ADOPTED IN 2014. MOST RECENT UPDATES ADOPTED IN 2021.