Best practice for tissue and tests for screening for PD/SAV?

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Diagnostic criteria SAV/PD

(from the OIE manual)

A suspected case of infection with SAV is defined as:

- Clinical signs consistent with infection with SAV or
- Gross and microscopically pathology consistent with the disease or
- Detection of antibodies against SAV (Section 4.3.2.1) or detection of SAV or
- If epidemiological information of infectious contact with suspected or confirmed case(s) appears.

Definition of confirmed case:

Evidence for the presence of SAV from two independent laboratory tests

- microscopic pathology
- cell culture
- RT-PCR
- serology

Sampling for diagnostics

- Focus on delivering the correct diagnosis for the diseases fish
- Sampling of tissue from target organs
- A small number of moribund fish sampled (5-10)
- detection of virus/pathology throughout the course of the disease
- Rule out differential diagnosis
- Sampling of tissues suitable for testing for other diseases
- Based on these criteria sampling from more than one tissue is recommended (heart and kidney in OIE manual)

Sampling for screening purposes

- Focus is on early detection of SAV in a farm
- Selection of target organs with highest amount of virus early in the disease development
- Ensure high sensitivity of the laboratory test
- Sampling in the field must be simple to perform because of the large amount of fish sampled
- Time- and cost-efficient laboratory analysis

Screening – OIE manual

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance		
	Fry	Juveniles	Adults
Gross signs	d	d	d
Histopathology	c	c	c
Immunohistochemistry	d	d	d
Isolation in cell culture	d	d	d
Serum neutralisation assay	d	c	b
Real-time RT-PCR	b	b	b
RT-PCR with sequencing	d	b	b

RT-PCR = Reverse-transcriptase polymerase chain r

(Real-time) RT-PCR is identified as the recommended test in the OIE manual.

Sampling for detection of SAV

- Andersen et al, 2007: large study on tissue tropism of SAV1 and SAV3
  - heart ventricle and pseudobranch contained the highest amounts of virus at peak and also the highest prevalence of positive samples in total.
  - The data also indicated that in very late stages of disease, head kidney had a relative high frequency of positives.

- The heart as a suitable organ is supported by Jansen et al 2010a,b and other available data showing that virus can be detected in the heart by real-time RT-PCR from shortly after infection until slaughtering.

Real-time RT-PCR for screening

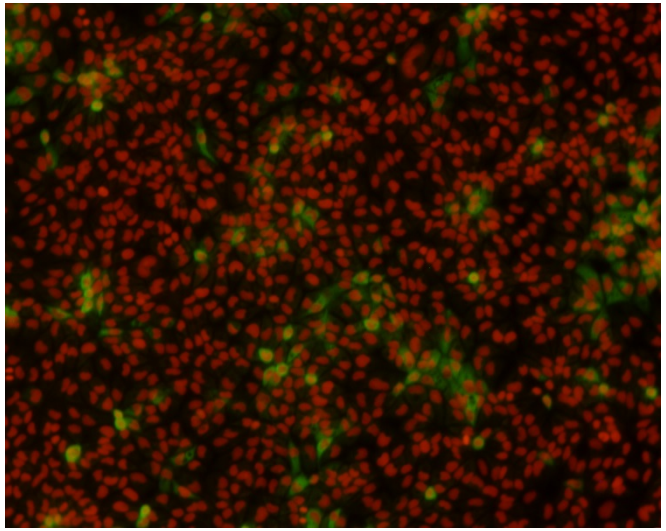
- important factors for choice of tissue
  - Easy to sample in the field and process in the laboratory
  - High laboratory sensitivity of the test
  - Sensitivity:
    - target tissue with high amount of positives early in disease development
      - Heart ventricle the tissue of choice:

      The spongious muscle tissue in the ventricle contains endothelial cells well exposed to SAV from the blood.
      - Sampling from the heart tip, especially from larger fish, may predominantly consist of compact muscle tissue, thus reduce the sensitivity of the analysis for SAV.
  - Specificity:
    - Risks connected to contamination during sampling and analysis
      - Vaccine residues may cause contamination when sampling for internal organs by opening the fish and exposing the peritoneum for a time period after vaccination.
      - Care has to be taken to reduce risks of laboratory contaminations leading to false positives

Serum neutralization test

- Detects the presence of neutralizing activity against SAV in serum/plasma
- Possible to test large amounts of samples relatively fast and cheap
- Well established method - used extensively in UK, Ireland
- Field isolates of SAV2 and SAV3 often does not exhibit CPE, necessary to perform IFAT
- Low sensitivity early in disease development
  - The onset of antibody production is delayed compared to virus detection

- Specificity
  - Cross-reactivity against other viruses?
    - Røsæg et al, 2017, reported that transient neutralizing activity against SAV could be measured in plasma following PRV-infection in a challenge trial.
    - We have also observed a case of possible cross-reactivity for plasma containing large amounts of antibodies against IPNV.
  - Effective vaccination likely to produce positive samples (dependent on vaccine type)



On-going research

- Comparison of different laboratory tests for the detection of SAV/PD
  - On-going project led by Dr. Atle Lillehaug at the NVI (PhD student Mario Guarracino)
  - Results will be presented by next year

Pancreas disease – diagnostic tests

– calculation of sensitivity and specificity

- On-going project led by Dr. Atle Lillehaug at the NVI (PhD student Mario Guarracino)
- Methods include:
  - Histopathology
  - Immunohistochemistry
  - PCR
  - Cell culture
  - Neutralizing antibodies
  - Serum precipitation test (Braceland et al, 2017)

Study design

- **Sampling:**

- One population of each category:
  - 100 fish each
  - SAV3 positive
  - SAV2 positive
  - Control – SAV negative

- Specificity and sensitivity to be calculated based on positive/negative test results of each test

On-going research

- Comparison of different laboratory tests for the detection of SAV/PD
  - On-going project led by Dr. Atle Lillehaug at the NVI (PhD student Mario Guarracino)
  - Results will be presented by next year
- Non-lethal detection methods
  - Detection of virus in water/eDNA-RNA
- Tissue tropism of less virulent strains?

Summary:

- The heart ventricle is the recommended tissue for screening purposes
- Care should be taken when sampling vaccinated fish (contamination issues)
- At present, real-time RT-PCR is the primary detection method for screening
- Non-lethal sampling procedures should be considered