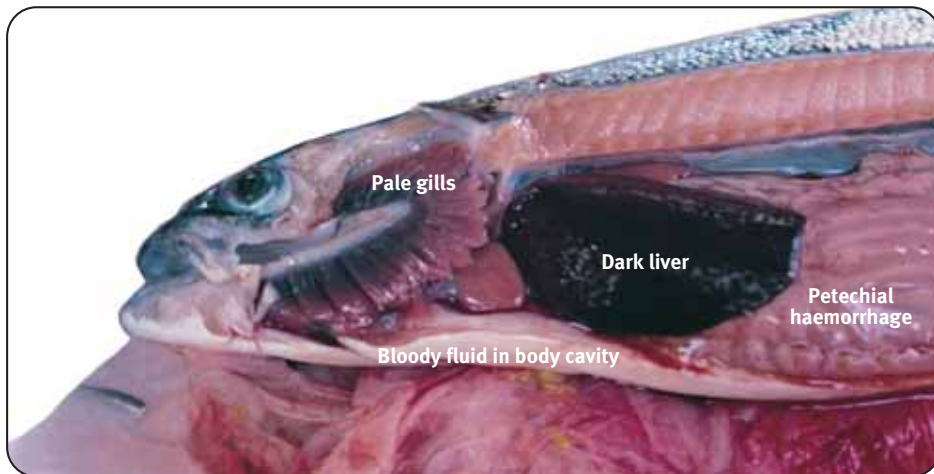


Diagnosis of Infectious Salmon Anaemia (ISA)



What is ISA?

ISA is a contagious viral disease of Atlantic salmon (*Salmo salar* L.) in sea water. The virus primarily affects endothelial cells lining the blood vessels of the fish, resulting in haemorrhage and severe anaemia. The disease can cause significant mortality, although within a farm this may spread slowly so that not all cages are affected simultaneously. The disease has been found in Norway, Canada, the USA, Faroe Islands, Scotland and Chile. ISA is a notifiable disease under UK and European legislation. If the presence of ISA is suspected on a farm, strict controls are put in place. If the disease is confirmed, affected stocks may have to be destroyed.

How is ISA diagnosed?

Diagnosis of ISA is achieved using a combination of clinical signs, histopathology and laboratory tests for evidence of viral infection.

Clinical signs may include:

- Lethargy
- Loss of appetite
- Gasping at water surface
- Pale gills (anaemia)

- Dark liver
- Accumulation of fluid in the body cavity
- Haemorrhage in internal organs
- High levels of mortality

Histopathology

Histopathology involves microscopic examination of fish tissues for signs of disease. In the case of ISA there can be serious damage to liver tissue (Fig. 2).

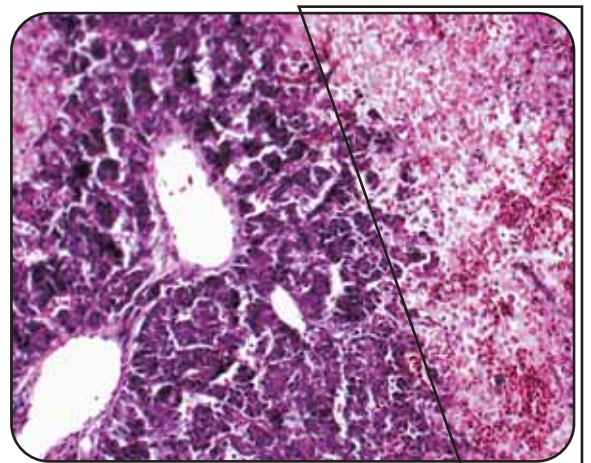


Figure 2.
Histological section of liver of an Atlantic salmon showing damage associated with ISA.

Version 1.00 | 8.1.09

IFAT

The indirect fluorescent antibody test (IFAT) detects an ISA virus (ISAV) protein in fish tissues. Following a specific staining procedure, cells of an infected salmon show a yellow-green fluorescence when viewed under the microscope (Fig. 3).

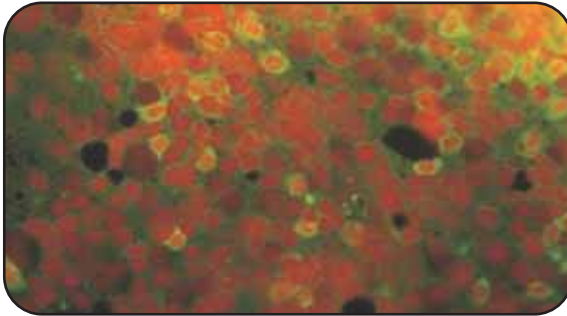


Figure 3.
IFAT of an Atlantic salmon kidney imprint. Positive cells show yellow-green fluorescence.

qRT-PCR

A real-time reverse transcriptase polymerase chain reaction (qRT-PCR) detects small quantities of RNA, the genetic material of the ISAV, in fish tissues. The assay is sensitive and specific and highly controlled to ensure confidence in the results obtained. qRT-PCR provides information on the relative amount of ISAV RNA present in the original sample (Fig. 4).

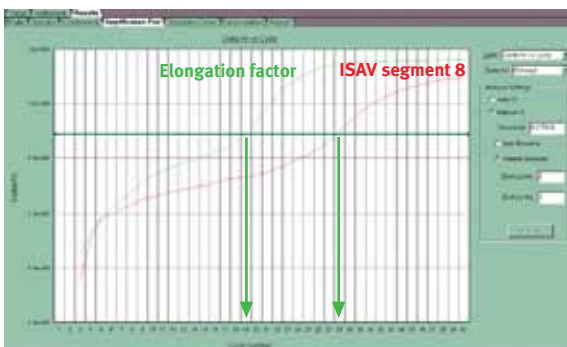


Figure 4.
Specific detection of ISAV using quantitative real-time reverse transcription PCR (qRT-PCR). An internal control reaction (ELF) conducted on each sample (elongation factor) verifies a high quality and quantity of RNA used in each assay. Appropriate negative and positive controls allow results to be interpreted with confidence.
(Screen shot for illustrative purposes only.)

IHC

The immunohistochemistry test (IHC) detects an ISAV protein in fish tissues.

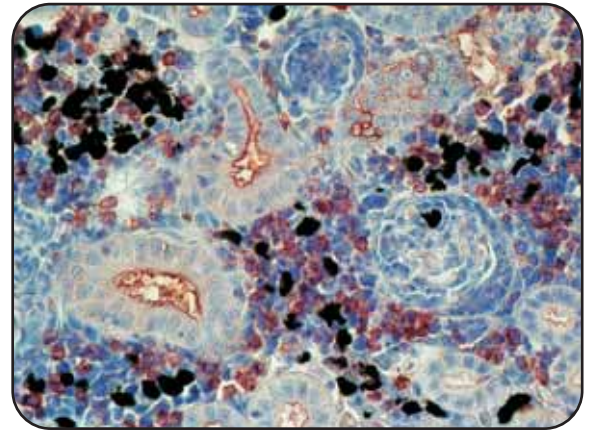


Figure 5.
Following a specific staining procedure, cells of an infected salmon show a pink/red colouration when viewed under the microscope.

Virus isolation

It is possible to isolate ISAV from the internal organs of an infected fish. The technique used is to place samples of heart, liver, kidney and spleen from the fish into sterile tissue cultures grown in the laboratory. ISAV, if present, will grow in the tissue cultures and cause a cytopathic effect indicated by cell rounding and death (Fig. 6). ISAV associated CPE is confirmed by specific antibody tests.

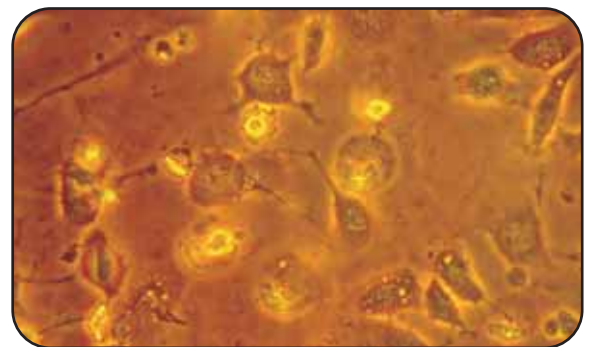


Figure 6.
Isolation of ISAV in tissue culture.

Accreditation

FRS participates in external ring tests, qRT-PCR and virus testing methods are accredited to ISO/IEC 17025.