DRAFT DOCUMENT
Interpretation of Infectious Salmon Anaemia (ISA) Positive Results Obtained Using Real-Time PCR

Real-time PCR test has limitations that have to be taken into consideration to appropriately interpret the results. Such interpretation should take into account the characteristics of the test, the agent identified, the sample tested, and the clinical presentation.

Clinical and Pathological features

There have been variations in the patterns of ISA clinical manifestations of the disease; these may depend upon the dose of virus, virus strain and pathogenicity, age and immune status of the salmon, and extraneous factors such as season and temperature. It is considered to be important to distinguish between ISA as a disease and infection by the ISA virus. The detection of virus by laboratory methods in the absence of clinical or pathological features does not necessarily indicate or predict ISA disease.

Laboratory Tests

The laboratory procedures are crucial for the reliability of a diagnostic test. All tests should be performed on a good quality sample. Quality control protocols must be in place to reduce the risk of erroneous findings with both false positive and false negative results that may arise from cross-contamination and cross-reaction.

Reverse transcriptase - polymerase chain reaction (RT-PCR)

This test detects specific sequences of the viral RNA genome, i.e. either whole viral RNA or particular fragments of degraded viral RNA, whether from live viruses or dead viruses.

The specificity of the primers used is of key importance. For example, primers amplifying a conserved region of the complementary DNA (cDNA) may detect all viruses within the orthomyxoviridae, not just ISA (resulting in false positive results), while primers amplifying variable regions of the cDNA may only amplify cDNA from a single virus isolate (possibly missing other ISA strains). Other important factors are:

- the choice of controls (negative, positive and/or internal controls) that run in parallel with the samples during each analysis;
- the organisation of the laboratory to avoid contamination between samples from different locations and from amplified products from earlier analysis;
- the laboratory skills of the persons performing the analysis;
- the procedures used for sampling tissue, in particular avoiding excessive degradation of viral RNA;
- the number of parallel samples tested separately;
- the number of PCR-positive fish from a single location required for confirmative diagnosis;
- the test sensitivity, specificity and predictive values. The test performance is essential for the analytic interpretation, such as the analytical sensitivity, the analytical specificity, i.e. the ability of the test to detect only the desired pathogen, and the intra-and interserial.