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BACKGROUNDER

INFECTIOUS SALMON ANEMIA (ISA) VIRUS – ACCEPTED TESTING METHODS

Searching for a Unique Genetic Fingerprint

Every virus has a unique genetic fingerprint. Polymerase chain reaction (PCR) is a technique that focuses on specific portions of this fingerprint so that they may be detected and identified. Detection of this small genetic target of viral RNA constitutes a presumptive positive test result.

The PCR is a highly sensitive test that sometimes produces false positive results; because of this, these presumptive positive samples require further confirmatory testing to ensure their validity.

Confirmatory testing can take two forms: First, there should be an attempt to isolate the virus from host tissues using cell culture. Cell culture allows the virus to infect the cells and multiply as it would in the host fish. It is possible to have a positive PCR test and ultimately a negative cell culture result. Cell culture also requires that a minimum dose of live virus be present in the test sample and can take up to four weeks to have results from this test.

And second, the virus needs to be properly identified and this is usually done using conventional PCR techniques to amplify larger and different portions of the viral genes which are then sequenced and compared to the unique ISA fingerprint.

To date, no attempts to isolate the suspect ISA virus in cell culture have been successful by any laboratory; nor has any sequencing data been produced. Thus, there have been no confirmed findings of ISA in the samples.

Tissue Quality

There are several factors which must be considered in the testing.

First, the nature of the PCR test requires the sample to be fresh or well preserved. Fish should be collected live, moribund, or as fresh mortalities (within 24 hours). Because both host (fish) and viral RNA degrades rapidly after death, virus detection can quickly become impossible by PCR or any other accepted test methods. Fish can be frozen to preserve the RNA, but tissue and virus degradation occurs even at -20 degrees Celsius. Storage at -70 degrees Celsius, or in a specialized storage preservative known as an "RNAlater," is required for long term preservation.

Second, because the virus is not distributed equally in all parts of the fish, the heart and kidney are the best organs to test. Gills can also be tested. However detection in gills indicates viral particles are in the environment. It does not mean infection of the host. Finally, sample size should be large enough for testing. A sample the size of a grain of rice allows for both PCR & molecular

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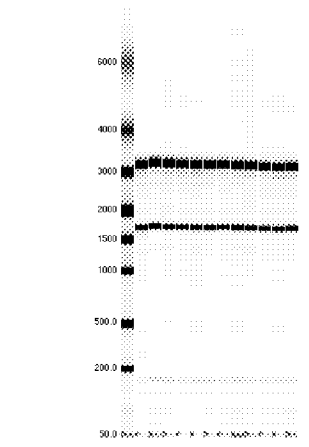
confirmatory tests (sequencing). Significantly larger amounts are needed for cell culture and archiving for future reference and testing.

Because RNA degrades rapidly, an extra test, called the "reference gene assay", is conducted on the original extract. The result of this assay indicates the level of degradation by comparing it to a well preserved sample of the same species. As mentioned, if the RNA has substantially degraded, neither a PCR nor any other approved testing method can determine the presence or absence of the virus with any degree of confidence.



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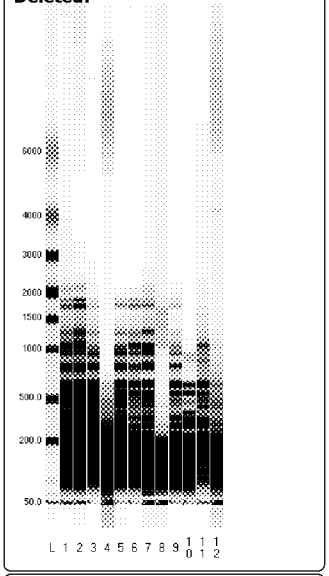

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