From: Marty, Gary D AGRI:EX <Gary.Marty@gov.bc.ca>
Sent: Friday, August 12, 2011 10:30 AM
To: Sheppard, Mark <Mark.Sheppard@dfo-mpo.gc.ca>; Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>
Subject: FW: Will ISAV PCR tests run by the BC Animal Health Centre detect all strain of ISAV?

Dear Mark and Kyle,

Here is some information (below) I provided CFIA about the PCR tests we have used and are using for ISAV testing at the Animal Health Centre. Since sending this e-mail Dr. Byrne also did a search to determine which of the GenBank sequences would be picked up by the qPCR recommended by the OIE manual the day he did his search. Dr. Byrne found that the OIE would pick up all the same strains as our test and, like our test, would not pick up strain Glesvaer/2/90, entered in 2001.

The other thing to note is that all ISAV tests done as part of our Fish Health Auditing and Surveillance Program are and were matched with histopathology by a board certified veterinary pathologist and analysis of farm mortality records by a fish health veterinarian.

Best regards,

Gary

Gary D. Marty, Fish Pathologist
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From: Marty, Gary D AGRI:EX
Sent: Thursday, May 12, 2011 11:21 AM
To: 'Kim Klotins'
Cc: Byrne, Sean AGRI:EX; Robinson, John H AGRI:EX; Kitching, Paul AGRI:EX
Subject: Will ISAV PCR tests run by the BC Animal Health Centre detect all strain of ISAV?

Dear Kim,

The British Columbia Animal Health Centre has been running PCR tests for ISAV for several years. We used a conventional PCR for all tests from 2006 - October 2009. This test was designed by our microbiologist Sean Byrne to target the RNA Polymerase (PB1) gene:

A2 (5'- GTC GAA TGA TGT GTC TTG TCT TTA C -3')
A4 (5'- ATA TGT ATC CTT TCA CTT CTT GTT TC -3'):

Since October 2009 we have been using a Real-time Assay for ISA which targets the matrix protein gene. This test was designed by a masters student that we had working here about 4 years ago (Lisa Wegener).

Primers:
ISA-rev-LW (5'- ACA GCA GGA TGC AGA TGT ATG C -3')
ISA-for-LW (5'- AGC GAC GAT GGC CTT TTC T -3')

Probe:
ISA-LW-prb (5'- 6-FAM - AGT TCG AAA GCC C - MGBNFQ -3')

Yesterday, Dr. Byrne performed a GenBank search for all ISAV entries for the relative segments of the ISAV genome. Of 34 sequences matching the A2 A4 primers (i.e., the conventional assay), three had a one-base variation compared to the A2 primer. These three variant strains included two (Gullesfjord/94 and ISAV11 (93/09/2264)) which had the variation close to the 5' end and probably would have still amplified, a third (Bremnes/98) probably would not have been amplified. All three of these strains would have been picked up by our real-time assay. Therefore, if a strain of ISAV was missed in British Columbia before October 2009, it would have been detected by at least one of the 159 ISAV PCR tests that we conducted as part of our Fish Health Audit and Surveillance program from October 2009 - March 2011.

Considering the probe used for the qPCR, two sequences in GenBank have one base difference which might have affected the ability to pick them up. One of these, Brekke/98, would have been detected by our conventional assay. The second, strain T0, was not in the RNA polymerase database, so we don't know if it would be detected by the conventional PCR. Genbank lists one sequence (strain Glesvaer/2/90, entered in 2001) that would not be picked up by our real time assay; however, two more recent entries from the same strain would be picked up by our assay (we think that the sequence entry from 2001 has errors).

Conclusion - we are confident that our PCR tests would have detected ISAV in our samples if ISAV was present in our samples.

Best regards,

Gary

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