From: Kaukinen, Karia <kauknenk@dfo.mpo.com>
Sent: Tuesday, October 25, 2011 2:16 PM
To: Miller-Saunders, Kristi <Kristi.Miller@dfo-mpo.gc.ca>
Subject: FW: ACRDP Creative salmon array information

FYI

From: Marty, Gary D AGRI:EX [mailto:Gary.Marty@gov.bc.ca]
Sent: October 25, 2011 2:15 PM
To: Kaukinen, Karia
Cc: 'Sonja Saksida'
Subject: RE: ACRDP Creative salmon array information

Dear Karia,

Thanks for sending the results. I apologize for the delay in responding, but I have had some other ISAV issues to deal with this past week. Here is some information we put together back in May 2011 for CFIA:

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).

Additional information:

Sensitivity - Our conventional assay (used from 2006 - 2009) detects 3 copies; the current assay (used for this case) detects 30 copies.

Positive control - the positive control we use for our qPCR test is extracted ISAV DNA obtained from Dr. Kibenge's laboratory at UPEI; it is a Canadian strain. Note, however, that the primers and probe in our qPCR test are designed to pick up all known strains. Veterinarians compare results from all of our qPCR tests with results from gross examination of the fish, mortality patterns on the farm, and many cases, with histopathology of multiple tissues from each tested fish.

Other available tests - We have all the primers and probes needed to run the PCR tests listed in the current OIE reference materials, but we did not use these tests in this case.

Earlier today I sent Sonja the converted case report (to spreadsheet format).

Best regards,

Gary

Gary D. Marty, Fish Pathologist
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604-556-3123

From: Kaukinen, Karia [mailto:Karia.Kaukinen@dfo-mpo.gc.ca]
Sent: Thursday, October 13, 2011 4:40 PM
To: Marty, Gary D AGRI:EX
Subject: FW: ACRDP Creative salmon array information

Hi Gary,

My name is Karia Kaukinen and I am working on an ACRDP project with Sonja. The fish in our project were sent to you for histology and I have summarized some of the results within our basic information file. As you can see below, Sonja was planning to have the data for the first 5 fish summarized similarly to the second batch of 22 fish examined. I have attached a shorten copy of the basic information file for your reference.

In your report you suggested that the samples be tested for VHSV. When I asked Sonja about this test, she forwarded the attached report. I was looking over the viral PCR results for 5 of the fish included in our project and I just wanted to confirm which primer/primer sets were used to determine the ISA results.
Also, do you know the detection limits to which these tests can detect? I am assuming that the testing lab uses positive controls for both the North American and European strains of ISA, is this correct?

Thank you, in advance, for your reply,
Karia

Ms. Karia Kaukinen

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