Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

DRAFT DOCUMENT

Infectious Salmon Anaemia (ISA) Laboratory Assessment:

NAAHLS Laboratory
Global Fisheries Center
Department of Fisheries and Oceans

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Canada

EXECUTIVE SUMMARY

The recent reports from the ISA OIE Reference Laboratory at the Atlantic Veterinary College (AVC) stating that the Infectious Salmon Anaemia virus (ISAV) has been found in British Columbia (BC) salmon have not yet been corroborated by federal officials through established processes. After initial investigations, concerns have been raised regarding the testing at the laboratory and subsequent reporting of these findings.

The Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO) are currently working together to assess the results based on scientifically sound and internationally recognized procedures. The Government of Canada is taking extensive actions to investigate claims about the presence of the ISA disease, the timeline of test results, and proper, science-based requirements for testing. As part of this process, the CFIA is leading an assessment of diagnostic laboratories involved in testing samples submitted by a third party that led to the original claim that Infectious Salmon Anaemia (ISA) infection has been detected in BC salmon.

The focus of this assessment is the ISAV testing carried out by DFO-Gulf Fisheries Center, the reference center for ISAV testing for the National Aquatic Animal Health Program. In parallel, an assessment of the Infectious Salmon Anaemia (ISA) OIE Reference Laboratory at the Atlantic Veterinary College (AVC, was also carried out and is documented in a separate report

This assessment will provide the background necessary to assist the CFIA National Emergency Response Team (NERT) in interpreting the test results reported by the ISA OIE Reference Laboratory at the AVC on samples submitted by a third party for ISAV testing, during the months of October and November 2011, from two sources in BC.

A working group (WG) was established to carry out the laboratory assessment. The Laboratory Assessment WG consisted of members from CFIA's Science, Operations and Policy and Programs Branches as well as a representative from DFO. Three people with technical expertise in diagnostic laboratory testing procedures and methodologies were identified to carry out the laboratory assessment. These included two individuals from CFIA's Science Branch and a one person from the Animal Health Laboratory, Laboratory Services Division, University of Guelph who acted as an independent third party expert.

The laboratory assessment included an assessment of both real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) and cell culture methodologies and consisted of a review of documentation on laboratory procedures and methodologies, followed by an on-site evaluation visit.

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Insert conclusions……

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INFECTIOUS SALMON ANAEMIA (ISA) LABORATORY ASSESSMENT

1. OVERVIEW

1.1. BACKGROUND

The recent reports from the ISA OIE Reference Laboratory at the Atlantic Veterinary College (AVC) stating that the Infectious Salmon Anaemia virus (ISAV) has been found in British Columbia (BC) salmon have not yet been corroborated by federal officials through established processes. After initial investigations, concerns have been raised regarding the protocols used in the testing at the laboratory and subsequent reporting of these findings.

The Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO) are currently working together to assess the results based on scientifically sound and internationally recognized procedures. The Government of Canada is taking extensive actions to investigate claims about the presence of the ISA disease, the timeline of test results, and proper, science-based requirements for testing. As part of this process, the CFIA is leading an assessment of diagnostic laboratories involved in testing samples submitted by a third party that led to the original claim that Infectious Salmon Anaemia (ISA) infection has been detected in BC salmon.

The focus of this report is the ISAV testing carried out by the ISA OIE Reference Laboratory at the AVC. An investigation into the collection, handling, transportation and storage of samples arriving at this laboratory has also been undertaken and is described in a separate report.

In parallel, an assessment of the ISA OIE Reference Centre at the AVC was also carried out and is documented in a separate report.

1.2. OBJECTIVE

This assessment will provide the background necessary to assist the CFIA National Emergency Response Team (NERT) in interpreting the test results reported by the ISA OIE Reference Laboratory on samples submitted by a third party for ISAV testing during the months of October and November 2011 from two sources in BC.

Specific Objectives

- To assess laboratory capability on: a) bio containment, b) quality assurance program, and c) validation of ISA test methods performed.

- To assess conformity of ISA testing with acceptable practices (e.g. OIE standards)

1.3. APPROACH

As part of the NERT, a working group (WG) was established to carry out the laboratory assessment. The Laboratory Assessment WG, chaired by a representative from CFIA's Science Branch, consisted of members from CFIA's Science, Operations and Policy and Programs Branches as well as a representative from DFO. Details about the WG membership, objectives, roles and responsibilities as well as a more detailed description of the assessment process are found in Appendix 1.

Three people with technical expertise in diagnostic laboratory testing procedures and methodologies were identified to carry out the laboratory assessment. These included two individuals from CFIA's Science Branch and one person from the Animal Health Laboratory, Laboratory Services Division, University of Guelph who acted as an independent third-party expert.(See Appendix1).

The laboratory assessment included an assessment of both real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) and cell culture methodologies. The process consisted of a review of the documentation of laboratory procedures and methodologies (Appendix 1 Annex 2), which was then followed by an on-site visit.

2. LABORATORY ASSESSMENT INFORMATION

Detailed information is documented in Appendix 2.

[Insert information – from Appendix 2]

2.1. LABORATORY ENVIRONMENT

2.1.1. Laboratory Space and Organization

2.1.2. Bio-Containment

2.1.3. Quality Management

2.1.4. Training and Qualification of Personnel

2.1.5. Proficiency Testing

2.1.6. Documentation

2.1.7. Records

2.2. SAMPLE INFORMATION

2.2.1. Sample Receipt

2.2.2. Processing – RNA Extraction

2.2.3. Internal Sample Control

2.3. REAL-TIME RT-PCR TEST METHODOLOGY

2.3.1. Primer and Probe Concentration

2.3.2. Test Validation

2.3.3. Positive Control

2.3.4. Classification of Test Results

2.4. CELL CULTURE

3. TECHNICAL AND TEST RESULT INFORMATION ON BC SAMPLES

3.1. Samples

See Appendix 3 – [Insert summary of information form Appendix 3]

4. Analysis of Data

5. ANALYSIS OF TEST RESULTS

There are a number of factors that lead can to either false positive or negative test results including cross-contamination, cross-reactions and errors in test procedures and/or interpretation.

5.1. Risk of Cross-Contamination

5.2. Risk of Cross-Reaction

5.3. Risk of Error in Test Procedure and/or Interpretation

5.4. Overall Interpretation of the DFO Test Results based on Lab Assessment Findings:

6. REPORTING OF TESTING RESULTS

ISAV is a reportable to the OIE as well as being reportable under the National Aquatic Animal Health Program (NAAHP) under the authority of the Health of Animal Act.

7. CONCLUSIONS

[Insert the findings of this assessment]

References

Appendices

Appendix 1 - Lab Assessment WG Process Document

Appendix 2 - Laboratory Assessment Detailed Data Table

Appendix 3 – DFO Test Results Report

APPENDIX 2: DFO-GFC: Detailed Table (To be Completed) *DRAFT*

Procedure	DFO	Comment (To be completed)
1. Sample receiving	Separate room used just for sample receiving and initial processing of samples (tissue harvesting, etc). Observed procedures which involved good use of aseptic technique during sample collection.	
2. RNA extraction	TRI Reagent or TRI-LS reagent - whole tissue or 10% tissue homogenates. RNA extraction was run in batches of 30 samples + 2 blanks. Reagents are separated from one batch to another. RNA template is added to PCR master mix in a separate room	
3. PCR targets	Segment 8 -developed by DFO with a predicted 179 bp product according to the draft protocol. However, I checked and it's a 169 bp product. The product overlaps that of the one described in the 2009 Manual of Diagnostic Tests for Aquatic Animals	Both Segment 8 protocols should in theory be similar with respect to analytic sensitivity, however, we have no direct comparison data
4. One-step or two-step RT-PCR?	Two-step RT-PCR.; RNA normalized prior to carrying out the RT step with High Capcity cDNA Kit (ABI)	In theory and in practice a two-step RT-PCR is usually more sensitive than a one-step RT-PCR
5. PCR chemistry utilized	TaqMan Universal PCR Master Mix Kit (ABI)	
6. Primer and probe concentrations	480 nM for primers, 200 nM for probe	Primer and probe concentrations in combination with the chemistries used can affect analytic sensitivity
7. Internal control	β-tubulin internal control was run with each assay as was measurement of OD_{260} and OD_{280} by MultiSkan to quantify the RNA. In addition, RNA integrity was assessed using the BIO-RAD Experion system. Results indicated RNA degradation in the samples received by DFO.	
8. Instrument platform used	Stratagene Mx3000P	Instrument platforms and associated analysis software can have some affect on analytic sensitivty/specificity and result interpretation
9. Cycling conditions	50°C x 2 minutes; 95°C x 10 minutes; 40 cycles @ 95°C x 30 sec, 60°C x 30 sec, 72°C x 30 sec	
10. Validation data?	Yes. Assay validated using infected and non-infected Atlantic Salmon kidney tissue	
11. Positive control	*in vitro* transcribed RNA from plasmid DNA that contains a 26 bp insert	

APPENDIX 3: Technical information for DFO Moncton, based on sample sets for lab assessment regarding ISA in BC salmon

	1A Smolts	1B Carcass	1B Kidney	1B Heart	1B Gill	1C Previously Necropsied Smolts	2 Hearts & Gills	3 5 sockeye heart & gill + 5 herring hearts
Tested at AVC				Y			Y	Y
Tested in Norway					Y			
Received at DFO Moncton Lab	Y	Y	Y(Extracts from PBS)	**N**	**N**	Y	Y (homogenates from AVC)	Y (homogenates from AVC)
Date received at DFO Moncton Lab	October 20	October 25	October 25	n/a	n/a	October 27	October 21 (1 homogenate) October 26(19 homogenates)	November 15
GFC # given	2011-14	2011-224	2011-227	n/a	n/a	2011-225	2011-215 (1) & 2011-226 (19)	2011-243
State at receipt	frozen	frozen	frozen	n/a	n/a	frozen	Frozen in L-15 medium	Frozen in L-15 medium
Necropsy in Moncton	1-96 Oct 20 97-299 Oct 21	1-48 Nov 04	N (extracts)	n/a	n/a	November 07	n/a	n/a
Condition of fish at necropsy	Poor – some fish rotten	Poor – only some gill left	n/a	n/a	n/a	Poor (a few already dissected)	n/a	n/a
Tissues collected during necropsy	Heart, Gill, Kidney (each in RNA-Later)	Gill only (each in RNA-Later)	n/a	n/a	n/a	Heart/Kidney/Gillif present (each in RNA-Later)	n/a	n/a
Material tested by PCR	297 hearts 168 gills	48 gills	48 extracts	n/a	n/a	None	2011-215 (1) & 2011-226 (19)	None
Testing period	Oct. 22- Nov 7	Nov 9 - 16	Oct 27- Nov 3	n/a	n/a	n/a	2011-215 Oct 22 2011-226 Nov 03-15	n/a
Test result	Negative	Negative	Negative	n/a	n/a	n/a	Negative	Not done
Reference gene result	Severely Degraded	Severely Degraded	Degraded	n/a	n/a	n/a	Severely Degraded	Not done
DFO Cell culture	Not done	n/a	n/a	n/a	n/a	n/a	Yes	Not done
Testing Period	n/a	n/a	n/a	n/a	n/a	n/a	2011-215 Oct 21 to Nov 18 2011-226 Nov 03 (on going)	n/a
Test result	n/a	n/a	n/a	n/a	n/a	n/a	2011-215 Negative 2011-226 (pending test)	n/a
Interpretation of DFO testing	Inconclusive	Inconclusive	Inconclusive	n/a	n/a	n/a	Inconclusive	n/a

	1A Smolts	1B Carcass	1B Kidney	1B Heart	1B Gill	1C Previously Necropsied Smolts	2 Hearts & Gills	3 5 sockeye heart & gill + 5 herring hearts
Notes	Kidneys + remaining gills not tested	Final report sent to PBS Nov 17	Final report sent to PBS Nov 17	n/a	n/a	Only test if requested by CFIA		Only test if requested by CFIA